PREFACE

The international symposium "**The Strategies of Animal Production in the Aspect of Environment Protection**" was held on 1-2 June 2006 at the Agricultural University in Lublin. It was organized to honour the jubilee of Prof. Czesława Lipecka, whose research work has rendered great service to Polish science. The symposium was organized by the Sheep and Goats Breeding Departments of the Agricultural Universities in Lublin, Warsaw and Poznań in acknowledgment and appreciation of Prof. Lipecka's outstanding achievements during many years of her work.

The symposium was attended by animal rearing specialists from Polish and foreign research centres, whose 32 original papers are published in this special issue of Archives of Animal Breeding. The published papers concern mainly research on small ruminants – sheep and goats. It is small ruminants, in particular sheep, that have been the main object of interest of Prof. Lipecka. The issue also includes papers on other species of farm animals – cattle, poultry and swine – as well papers on production technology and assessment of animal food products, which are thematically connected with the motto of the symposium.

We acknowledge and thank all the authors for their contributions to this special issue. In particular, we would like to express our gratitude to the editor-in-Chief and the editorial board of Archives of Animal Breeding for the opportunity to publish the contributions of this interesting symposium.

On behalf of the organizers:

Prof. Dr. Jacek Wójtowski, Agricultural University of Poznań

Prof. Dr. Dr. h. c.
Czesława Lipecka,
on she's 45 years
of scientific activity

Professor Czesława Lipecka is a scientist at the Agricultural University of Lublin, from which she graduated in 1960. She began her scientific career in 1961 at the Department for Sheep Breeding after finishing a one-year practical internship with the Sheep Breeders' Association. In 1967, she defended her doctoral dissertation, which was titled "Inheritance of Antigenic Factors in Sheep Blood". In 1975, she was awarded a post-doctoral degree on the basis of colloquium (colloquia), scientific achievements and a dissertation titled "Genetic Polymorphism in Transferrins in Sheep Selected for Useful Traits".
In 1983, she was invested as granted a professorship. Since 1985, she has been the director of the Department for Sheep and Goat Breeding.
Over the course of her 45-year-long career at the Agricultural University of Lublin, Professor Lipecka has focused on unraveling the mysteries of raising and breeding livestock, especially sheep and goats.
Her most recent scientific achievements include:

- Implementing systematic research on blood groups and blood cell antigens in sheep;
- Elucidating polymorphism in sheep serum proteins such as transferrin, hemoglobin, and albumin;
- Identifying blood protein cell markers in order to distinguish different sheep populations;
- Participating in a program to breed one new Polish Lowland Sheep breed, Uhrusk type;
- Participating in a program to create two new, synthetic, highly fertile meat sheep lines, BCP and SCP;

- Elaborating methods for sheep selection and breeding;
- Defining environmental conditions for increasing fertility in sheep, and for decreasing mortality in lambs without compromising breedingand production value;
- Elaborating methods for sheep selection and breeding;
- Long-term observations on physiological and production adaptations in meat breeds such as Suffolk and Berrichon du Cher, which have been introduced from France into eastern Poland;
- Initiating research on physiological and production reactions in sheep testing positive for Maedi Visna Virus.

Prof. Lipecka has written altogether 290 papers which have been published in Polish and foreign journals. Throughout her career, she has fervently fulfilled her administrative and educational duties.
During the course of her tenure, two of the people in her department were invested as professors, three were awarded post-doctoral degrees, eight were awarded doctoral degrees, and 130 were awarded masters degrees. She held lectures, practical courses and seminars, and also worked on many educational programs for students. She also reviewed more than 300 papers of various sorts.
Since 1991, she has been the team leader of the Sheep and Goat Breeding and Production Division of the *Polish Zootechnological Association*. She also actively takes part in work organized by the *Polish Association for Genetics.*
From 1993 to 1996, Professor Lipecka served as the prorector in charge of personnel and organization at the Agricultural University of Lublin. From 1999 to 2005, she was the representative for agricultural sciences in the Polish Central Council for Higher Education.
On October 21, 2006, Professor Lipecka was awarded an honorary doctoral degree from the Agricultural University of Wrocław.
On the basis of her broad knowledge, her creativity and her inspiring accomplishments, I can truly say that Professor Lipecka is a gifted and tireless research coordinator. On top of that, she always has found the time and energy to teach students and provide her younger scientific colleagues with ample opportunity to develop.

Prof. Dr. Tomasz M. Gruszecki
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The relations between breed and age associated susceptibility/resistance of sheep infection with meadi-visna virus (MVV)

Abstract

Our aim was to estimate the possible impact of genotype and age of sheep on susceptibility/resistance to infection with maedi-visna virus (MVV). Sheep genotype represented two synthetic fecund-meat lines (BCP and SCP), two groups of three-bred hybrids (LB and LS) as well as Suffolk and lowlands sheep breed. In this study 1875 sheep originating from flock in middle eastern part of Poland were serum sampled. The presence of specific antibodies to p25 and gp40 proteins of MVV was determined by ELISA assay. It was shown that 17.3% of the tested animals were serologically positive during three-year-study. The highest susceptibility to MVV infection was noted in Suffolk breed (23.6%) and its three-bred hybrids (31.7%). However, in the other genetic groups this index ranged from 11.3 to 20.6%. Further analysis showed that MVV seroprevalence increased with sheep age, mainly between 2 to 5 years of age. Along with the population aging a downwards tendency of infection increase was observed that may have resulted from the breeding practices followed by the sheep selection. These results provide evidence that susceptibility and resistance of sheep to MVV is associated with age and genotype of animals. The breeds or native lines are characterized by a higher resistance against MVV infection compared to the imported pedigrees.

Key Words: sheep, maedi-visna virus

Introduction

The maedi-visna disease cases are practically recorded all over the world, except for Austalia and New Zealand (BRODIE et al., 1998; KEEN et al.,1997; SIHVONENE et al., 1999). This disease causes the deterioration of animal physical condition, a decrease of body weight gains as well as increased mortality of lambs, which is associated with their low body weight at birth (ARSENAULT et al., 2003; CALAVAS et al., 1998). The MVV virus presence in a flock is also connected with the more frequent mastitis prevalence and some changes in joints. In order to prevent these effects, the prophylactic measures are undertaken that aim at the isolation and elimination of the infected sheep (SIHVONEN et al., 1999; STROUB, 2004). Another method to limit the MVV virus spread is search for some breeds, varieties or lines resistant against infections (KĘDZIORA et al., 2005). The studies also attempt to determine the age of sheep at the time of contraction. The obtained results may be helpful in the breeding works as their purpose is to eliminate this disease from flocks and thus to decrease the economic losses in the sheep farms.

The objective of the present investigations was to determine the susceptibility of animals of selected genotypes and the influence of sheep age on the maedi-visna prevalence in a flock maintained in the conditions of natural infections.

Material and Methods

The investigations were conducted in the years 2003-2005 in a sheep flock localized in the central-eastern Poland. They covered the sheep of two synthetic fecund-meat lines

BCP and SCP (938 units),two groups of three-bred hybrids LB and LS (348 units) and two purebreeds: Suffolk (S-220 units) and the Polish lowlands sheep (PON-369 units). During the experimental period, the age of sheep ranged from 4 months up to 7 years. The examined population was kept in the same breeding conditions and under the veterinary-zootechnical inspection. The animals were characterized with a different parturition number. The ewes of BCP and SCP brought forth their first offspring being aged one year, whereas the LB and LS and PON – 2 years.

Throughout the 3-year experimental period, blood for the examination was collected each year at two terms, i.e. April-May and November. The presence of specific antibodies in blood serum was determined using a commercial kit ELISA MVV (Institute Pourquier – France). The determinations were performed in compliance with the producer`s instructions and the read-out made with ELISA reading apparatus using 450 nm wavelength. Total 1875 samples of sheep blood serum were made for the animals that showed negative results of tests in the successive terms.

To determine a relation between the occurrence of a seropositive reaction towards MVV and a genotype and sheep age, a test chi^2 was applied.

Results

Table 1 gives the size of the examined population and percentage of sheep with the recognized positive result of ELISA test. It was proved that in the years 2003-2005, 17.3% sheep became infected with maedi-visna disease and the statistically significant differences were noted between the genetic groups. The Suffolk breed sheep and hybrids LS (50% Suffolk pedigree) exhibited the highest incidence and the seropositve reaction rate reached 23.6% and 31.7%,respectively. Among the PON animals, this value was 20.6%,while in the other genetic groups the percentage of sheep with a high concentration of MVV antibodies appeared substantially lower and oscillated between 11.3% up to 17.9%. The detected differences between the genetic groups were confirmed statistically ($p \leq 0{,}01$).

Table 1
Occurrence of MVV antibodies in the examined sheep population in 2003-2005(%)

Genetic group	Birth year	Number of examinations		Seropositive	
		total	mean for ewe	n	%
BCP	2001- 2005	505	2,46	57	$11{,}3^{ABCD}$
SCP	2001- 2005	4,33	2,34	54	$12{,}5^{EFG}$
LB	1997- 2003	184	2,33	33	$17{,}9^{AH}$
LS	1994- 2003	164	1,93	52	$31{,}7^{BEHI}$
PON	1994- 2005	369	1,14	76	$20{,}6^{CFI}$
Suffolk	1996- 2005	220	2,12	52	$23{,}6^{DG}$
Total		1875	1,91	324	17,3

^{A,B,C}—means denoted with the same letters within column are statistically significant ($P \leq 0.01$)

Analysing the age distribution of the infected population, it was found that to the end of the second year of life, a seropositive reaction was shown by 16% sheep. The highest percentage of seroreagents was recorded at the 2-5 years age interval. At that time, 57% sheep got infected (Fig.).

The occurrence of MVV antibodies within each genotype according to the age was differentiated (Table 2).It was demonstrated that in the BCP and SCP sheep, the infection intensification was reported at a younger age as compared to the other breed the groups. In the animals of the mentioned genotypes, 9% of the population showed a

seropositive response in the first year of life. This process enhanced in the second year, while the incidence peak was observed between the second and third year, when nearly 50% of individuals showed a seropositive reaction (Table 2).

Table 2
Rate of sheep with seropositive result for MVV subject to age and genetic group

Genetic group	n	Age in years							Significance of differences
		<1 (1)	1-2 (2)	2-3 (3)	3-4 (4)	4-5 (5)	5-6 (6)	6-7 (7)	
BCP	57	8,8	24,6^{ABCD}	49,1^{ABCD}	15,8^{A}	1,7^{AB}	-	-	3-1,5**
SCP	54	9,2	27,8^{EFGH}	51,9^{EFGH}	7,4^{BC}	3,7^{C}	-	-	3-1,4,5** 2-5**
LB	33	3,0	0,0^{AE}	3,0^{AE}	24,2	21,2^{A}	36,4	12,2	1-6** 2-4,6** 3-4,6**
LS	52	1,9	3,8^{BF}	7,7^{BF}	40,4^{ABD}	17,3	9,6	19,2	1-4,7** 2-4,7** 3-4** 4-6**
PON	76	9,2	0,0^{CG}	9,2^{CG}	14,4^{DE}	22,4^{B}	13,2	31,6	2-4,5,6,7**
Suffolk	52	1,9	3,8^{DH}	5,8^{DH}	30,8^{CE}	13,5^{C}	21,2	23,0	1-4,6,7** 2-7** 3-4**

^{A,B,C} means denoted with the same letters within columns are different significantly(P≤0.01)
** Differences significant statistically (P≤0.01) between age groups

Some different reactions were noted for LB and LS hybrids in which 6% and 13.4% infected sheep aged up to three years were recognized. A rise of infection intensity in these groups was noted between the third and fifth year of life. While growth in a seroreagents percentage up to the third year of life was recorded for the Suffolk sheep breed (11.5%) as well as for the Polish lowlands sheep (18.4%). Along with the population ageing, similar tendencies were observed in the range of the described index.

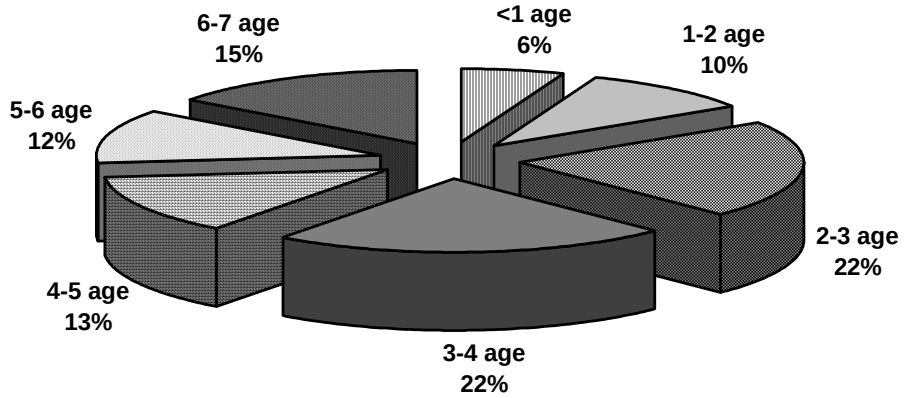

Figure: Percentage of sheep with seropositve reaction towards MVV subject to their age

Discussion

The first cases of maedi-visna disease in Poland were discussed by ZADURA et al., - in 1975. KOPACZEWSKI et al. (1987) proved the presence of specific antibodies MVV in 80% the Pomeranian sheep and 23% the Polish merino. KOŁODZIEJ et al. (1995) investigating the sheep from the Lower Silesia reported that the percentage of the infected animals oscillated from 30%-70% subject to a flock. The serological survey performed by KOZACZYŃSKA et al. (2002) showed a differentiated infection level (0.5%-96.1%).The preliminary assessment of the serological situation made by KĘDZIORA et al. (2005) implies that this disease entity is also localized in the flocks from the central-eastern Poland.

In the present studies, the analysis of sheep from different genetic groups exhibited that a seropositive MVV result averaged 17.3% in the examined population and the highest infection rate was recorded for the Suffolk breed sheep (23.6%)and their three-bred hybrids LS (31.7%). High sensitivity towards MVV in the early stage of sheep life was also noted for the animals of the synthetic lines SCP (25% Suffolk pedigree).

The domestic breeding of the Suffolk sheep was based on the material imported from England and France in the 80's of the last century. However, a severe problem of the Polish flocks of this breed appears to be a high death rate of lambs (LIPECKA et al., 1991). It is noteworthy that the reason for such a situation has not been recognized so far.

It is interesting, though, that the research work carried out by KEEN et al. (1997) gave different results. The authors claim that a percentage of seropositive Suffolk sheep was markedly lower (22.4%) compared to the Finnish sheep (77.4%) or teksel breed animals (65.4%).In the present studies, the analysis on susceptibility to infections induced by MV virus made on the native Polish lowlands sheep showed a seropositive reaction in 20.6% of animals, that is lower compared to the Suffolk or LS hybrids sheep. However, it should be emphasized that the mentioned above Polish lowland sheep were bred in the central-eastern region of Poland and based on the breeds like, the Polish merino, Leine and Kent.

According to WENDE and STRAUBE (quoted after ZADURA et al., 1975), merino exhibits low sensitivity towards infections as against teksel breed and eastern-Frisian sheep, whereas Leine pedigree has been recognized to possess remarkably high immunity to any disease (DOMAŃSKI et al., 1976). However, KOPACZEWSKI et al. (1987) proved that the merino sheep were characterized with sixfold lower death rate percentage as compared to the Pomeranian sheep.

The analysis of our own results and data available in the literature indicates that the native populations are more resistant to MVV than the imported breeds. It may be assumed that this fact is a result of the animal acclimatization in a new breeding environment.

On the basis of the conducted research, it was found that the animal ageing induces an increase of susceptibility towards MVV infection. In most of the examined genetic groups of sheep, the highest prevalence was recorded at the age of 3-5 years. Later, a smaller percentage of animals with seropositive reaction was observed and this fact, in the present authors` opinion, may arise from rejection of week animals from the basic flock.

Similar dependences were mentioned by KEEN et al. (1997), who reported the intensification of positive results of ELISA assay in the sheep aged 3-4 years (63.2-

62.6%), while the highest percentage in a group of 6-year and older animals (73.6%). The same relations were also depicted by BERRIATUA et al. (2003). Besides, a decreased level of specific antibodies against MVV is likely to arise from both short exposure time to infection by a vertical way and the fact of the retarded seroconversion occurrence (HOUWERS et al., 1989).

In the sheep from BCP and SCP lines, (compared to other groups), the elevated immunity towards MVV was recorded at the earlier stage of life. It seems that this effect may have resulted from their inclusion into reproduction as early as in the first year of life and not at the age of two years as in the other genetic groups.

Basing on the carried out research, the authors are of the opinion that sheep susceptibility towards maedi-visna infection grows with their age (to 4-6 years old). Moreover, interbreed variation is observed in respect of the discussed trait. Besides, it was found that the native breeds show far higher immunity towards MVV compared to the imported populations.

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Concentration of total cholesterol in milk of Polish White Improved goats during the whole lactation

Abstract

The aim of this study was to establish the changes of concentration of total cholesterol in goat milk during the whole lactation in two seasons of feeding (winter and summer feeding). The study was carried out in two years periods on 91 Polish White Improved goats, between 1st to 3rd lactation. During winter season of feeding, the diet consisted of maize silage, meadow hay, concentrates mixture with minerals and vitamins. In summer season of feeding, the hay was replaced by fresh green forage. The milk samples were analysed for basic chemical composition, somatic cell count and cholesterol content. There was significant influence of month of lactation, fat content and its amount in daily milk on concentration of cholesterol. The lowest concentration of cholesterol in milk was observed in second month of lactation, while the highest - in 5th month (July). It is supposed that the higher concentration of cholesterol in milk in July was caused by "flushing". There was significant influence of month of lactation, fat content and its amount in daily milk on concentration of cholesterol. The significant influence of month of lactation and milk yield on amount of total cholesterol of daily milk was found. There was significant impact of month of lactation, daily milk yield, fat content and amount of fat in daily milk on index CH/F. Correlation between cholesterol concentration and daily milk yield and its components yields were negative, while with its components content were positive. The amount of cholesterol, which was excreted to the milk, increased in lower level than the amount of fat in milk.

Key Words: goats, milk, cholesterol

Introduction

The amount of goat milk in total world milk production constitutes only 2.5% (FAO 2002). Both, in developing and developed countries all over the world the number of goats increased by around 60% during two last decades (MOHRAND-FEHR at al., 2004). But goat milk plays an important role in human nutrition in developing countries, especially in tropical regions. In developed countries, an increased interest in goat milk and milk products results from properties of this milk. Goat milk is regarded as a functional food in these countries because of its positive influence on human health. It has higher digestibility, alkaline properties, higher buffer capacity compared to cow milk and some therapeutic ability which is confirmed in human medicine (DAVENDRA and BRUNS, 1970; HAENLEIN and CACCESE, 1984; PARK and CZUKWU, 1988; PARK, 1994). About 40% of people with allergy to cow milk tolerate the protein of goat milk (HAENLEIN, 1993). Dietetics recommend the consumption of goat milk to people suffering from such illnesses as: hyperacidity, asthma, migraine, eczema, stomach and liver diseases (BABAYAN, 1981).

One of the controversial components of goat milk is the cholesterol. It is an important component of each animal and human cell, takes part in a lot of metabolic processes (hormones, fatty acids, vitamin D_3 precursor and other compounds). On the other hand, the consumption of too high amount of cholesterol can cause some perturbations in human health, because it provides for promoting arteriosclerosis (MURRAY et al., 1995).

Until today, information about concentration of cholesterol in fresh raw goat milk is negligible in scientific literature. According to POSATI and ORR (1976) the total

cholesterol concentration in goat milk is lower by 21% compared to human milk. The concentration of total cholesterol in goat milk presented in literature in occurus wide range eg.: 2 - 24.8 mg/100ml (RAPHAEL et al., 1975; AZIMA et al., 1969; AMER et al., 1999; BERNACKA and SIMIŃSKA, 2005); in the colostrum the concentration is higher – about 40mg/100ml (STEGER, 1961). According to many authors, the cholesterol concentration depends on year season, feeding system, stage of lactation and breed (JENNES, 1980; HAENLEIN and CACCESE, 1984; PARK and CHUKWU, 1988; PARK, 1990, 1991).

The cholesterol concentration in goat dairy products is related to its concentration in the milk. PIIRONEN et al. (2002) stated that the cholesterol concentration in milk with 1.5% of fat content contained 6.2 mg/100 ml of cholesterol, cream with 38% of fat – 77 mg/100 ml. While its concentration in cheese amounts to 33-82 mg/100g - depending on dry matter concentration. The content of cholesterol in commercial fluid milk, condensed milk, powder milk and in cheese was 11.0, 24.9, 119.5 and 91.7 mg/100g of product, respectively (PARK, 2000). The level of cholesterol in such products is also important, because during the storage period, especially when the contact with oxygen and exposure to the light is present, oxidised products, are created which have undesirable influence on human health (SIEBER, 2005).

Very interesting are the results of newest studies which indicated that goat milk has hypocholesterolemic properties (LOPEZ-ALIAGA et al., 2005). In the blood of rats fed diets with goat milk the level of cholesterol was lower by 17% compared to the cholesterol level in blood of rats' fed diets containing cow milk. This decrease was caused by higher concentration of middle length chain fatty acids in goat milk (34% and 21% in goat and cow milk, respectively in total fatty acid). The presence of these acids in the diet has influence on reduction of synthesis of endogenous cholesterol, as well as absorption in the small intestine (GARCIA and UNCITI, 1996). Hypocholesterolemic action shows also that in the fermented goat milk with different *Lactobacilli* strains, because it contributes to the decreasing of total cholesterol level, as well as its LDL fraction in blood plasma (ANDERSON and GILIARD, 1999; SANDERS, 2000; LIONG and SHAH, 2005).

There is a lack of experimental studies on cholesterol concentration in goat milk during the whole lactation, in connection with feeding and maintaining systems, in accessible papers. In this situation, the aim of this study was to establish the changes of concentration of total cholesterol in goat milk during the whole lactation in two seasons of feeding (winter and summer feeding).

Material and Methods

The study was carried out in two years periods on 91 Polish White Improved goats. The age of the animals was between 1st to 3rd lactation. The animals were kept in loose barn with possibility of outside running. During winter season of feeding, the diet consisted of very good quality maize silage (33-35% dry matter), meadow hay, as well as concentrates mixture with minerals and vitamins. In the winter diet was supplemented with carrot. In summer season feeding, the hay was replaced by fresh green forage, which was fed in the barn. The chemical analyses of feeds were carried out and the feeding values were calculated using INWAR computer package. On this basis, the compositions of diets were established according to INRA system. The basal diet covered the maintenance requirements and the production of 2 kg of milk. The

goats with higher production obtained additional amount of concentrates. The water was available in automatic watering trough. Before breeding season the goats obtained extra amount of concentrates ("flushing").
The goats were milked twice a day at 6 a.m. and 6 p.m. Milk was taken from individual goats once a month during evening and morning milking. The samples were taken proportionally from these two milking according, to the amount of milk. They were analysed for basic chemical composition and cholesterol content. The fat, protein and lactose content were checked on MilcoScan 140A, while the somatic cell count (SCC) on the Fossomatic apparatus. The samples with SCC more than 10^6/ml of milk were discarded. The extraction of total cholesterol and its saponification process were made according to the FLETOURISA et al. (1998) method, and then the quantity amount of cholesterol was determined in colorimetric reaction following the SEARCY and BERQUISTA (1960) method. On this basis the amount of cholesterol in daily milk (mg) was calculated and index: mg of cholesterol/g fat (CH/F) was determined.

Statistical analysis

The data were evaluated using GLM procedure of SAS v. 8e (SAS Institute, 1999-2000). The following statistical model was used:

$$y_{ijklm} = \mu + a_i + YS_j + P_k + ML_l + \beta_{1-3}(x_{ijklm}-x_{mean.}) + e_{ijklm}$$

where:

y_{ijklm} – investigated traits
μ – mean value of observations
a_i – random influence of goat (i = 1 to 91)
YS_j – fixed effect of year study (j=1, 2)
P_k – fixed effect of parity (k=1, 2, 3)
ML_l – fixed effect of month of lactation (l=1 to 10)
$\beta_{1-3}(x_{ijklm}-x_{mean.})$ – fixed effect of regression on milk yield, fat content and amount of fat excreted in volume of daily milk
e_{ijklm} – random error.

Additionally, the Pearson's correlation between cholesterol content and milk traits was calculated.

Results

Data in Table 1 show the influence of year of study, parity and month of lactation on daily milk yield and its chemical composition. There was no influence of the year of study and parity on the cholesterol concentration in individual milk samples (Table 2). While there was significant influence of month of lactation, fat content and its amount in daily milk on concentration of cholesterol. There was no significant influence of milk yield on total cholesterol concentration. The significant influence of month of lactation and milk yield on the amount of total cholesterol of daily milk was found. There was significant impact of month of lactation, daily milk yield, fat content and amount of fat in daily milk on index CH/F.
The lowest concentration of cholesterol in milk was observed in the second month of lactation i.e. at pick of lactation, while the highest - in 5th month (July) - Table 3. The difference between extreme values was 3.42 mg/100 ml. At the end of lactation the level of cholesterol in milk slightly increased. The amount of cholesterol in daily milk depended only on milk yield, which was connected with stage of lactation (Table 2). Index CH/F was determined by month of lactation, amount of fat in daily milk, fat

concentration and daily milk yield. The index value changed parallel to the cholesterol concentration in milk and to its amount in daily milk (Table 3).

Table 1
Daily goats milk yield and composition during lactation

Parametry Parameter	N	Daily milk yield, kg		Concentration in milk, %									
				fat		total protein		lactose		total solids		solids non fat	
Year		LSM	SE	LSM	SE	LSM	SE	LSM	SE	LSM	SE	LSM	SE
1	401	2.27	0.08	3.67	0.07	2.93	0.03	4.75	0.03	12.00	0.10	8.35	0.05
2	322	2.42	0.05	3.53	0.04	2.91	0.02	4.74	0.02	11.80	0.05	8.32	0.03
Lactation number													
1	321	2.37^{a}	0.10	3.34^{A}	0.09	2.88	0.04	4.66^{a}	0.04	11.55^{A}	0.12	8.21^{a}	0.06
2	157	2.50^{b}	0.08	3.48^{A}	0.07	2.88	0.03	4.72^{a}	0.03	11.75^{A}	0.09	8.27^{a}	0.05
3	245	2.17^{c}	0.18	4.00^{B}	0.16	2.99	0.07	4.87^{b}	0.07	12.54^{B}	0.22	8.53^{b}	0.10
Months of lactation													
1	23	1.91^{BC}	0.14	4.59^{A}	0.13	2.98^{A}	0.05	4.87^{A}	0.06	13.12^{AF}	0.17	8.53^{A}	0.08
2	54	2.61^{DE}	0.09	3.48^{CE}	0.08	2.71^{E}	0.03	4.78^{B}	0.04	11.52^{B}	0.11	8.05^{DG}	0.05
3	102	3.02^{G}	0.07	3.14^{D}	0.06	2.59^{G}	0.02	4.75^{B}	0.03	11.12^{C}	0.08	7.98^{G}	0.04
4	106	3.05^{G}	0.07	3.02^{D}	0.06	2.71^{E}	0.02	4.72^{B}	0.03	11.09^{C}	0.08	8.08^{D}	0.04
5	106	2.76^{E}	0.07	3.03^{D}	0.06	2.70^{E}	0.02	4.73^{B}	0.03	11.12^{C}	0.08	8.09^{D}	0.04
6	80	2.79^{E}	0.07	3.18^{D}	0.07	2.74^{E}	0.03	4.72^{B}	0.03	11.30^{C}	0.09	8.12^{D}	0.04
7	92	2.44^{D}	0.07	3.65^{EF}	0.06	2.90^{A}	0.03	4.87^{A}	0.03	12.10^{D}	0.09	8.45^{A}	0.04
8	51	2.06^{C}	0.09	3.58^{CF}	0.08	3.12^{D}	0.03	4.66^{B}	0.04	12.05^{DE}	0.11	8.48^{A}	0.05
9	68	1.77^{B}	0.08	4.13^{B}	0.07	3.28^{C}	0.03	4.72^{B}	0.03	12.84^{F}	0.10	8.71^{C}	0.05
10	41	1.05^{A}	0.10	4.31^{A}	0.09	3.43^{B}	0.04	4.72^{B}	0.04	13.19^{AG}	0.12	8.88^{B}	0.06

The value in the same column with different letters differ significantly:
a.b.c – p<=0.05; A.B.C.D – p<=0.01

Table 2
Analysis of variance

Effect	cholesterol concentration in milk	The amount of cholesterol in daily milk	Relation the amount of cholesterol to amount of fat
Goat	NS	NS	NS
Year	NS	NS	NS
Number of lactation	NS	NS	NS
Months of lactation	***	***	***
Daily milk yield	NS	***	*
Fat concentration in milk	***	NS	**
The amount of fat in daily milk	***	NS	***
Coefficients of determination (R^2)	0.33	0.70	0.50

* p<=0.05; ** p<=0.01; ***p<=0.001

In the present study (Table 4) higher correlation coefficients were observed between cholesterol concentration and protein content than between cholesterol concentration and fat (0.42 vs. 0.27). Correlations between cholesterol concentration and daily milk yield and its components yield were negative, while the correlation of cholesterol concentration with chemical components content were positive. The correlation between the amount of cholesterol in daily milk and yield of milk components was high and positive, while with concentration of components it was negative. The index CH/F was negatively correlated with fat concentration in milk. The negative relation

between these traits means that the amount of cholesterol, which was excreted to the milk, increased in lower level than the amount of fat in milk.

Table 3
Cholesterol concentration in goats milk

Effect		N	Concentration cholesterol in milk		Amount of cholesterol in daily milk		Relation the amounts of cholesterol to amounts of fat	
			LSM	SE	LSM	SE	LSM	SE
Year:	1	401	16.90	0.52	428.9	13	5.20	0.17
	2	322	18.09	0.29	460.0	8	5.54	0.10
Number of lactation :	1	321	17.30	0.65	438	17	5.23	0.22
	2	157	17.50	0.49	444	13	5.31	0.16
	3	245	17.70	1.15	450	30	5.55	0.39
Months of lactation:	1	23	16.46^{AD}	0.95	434^{AB}	25	5.25^{AB}	0.32
	2	54	15.68^{CD}	0.56	396^{B}	15	4.75^{B}	0.19
	3	102	17.29^{A}	0.43	449^{A}	11	5.29^{A}	0.15
	4	106	17.46^{A}	0.44	443^{A}	12	5.39^{A}	0.15
	5	106	19.10^{BE}	0.43	493^{D}	11	5.93^{D}	0.15
	6	80	16.87^{AD}	0.47	428^{AB}	12	5.14^{AC}	0.16
	7	92	17.24^{A}	0.45	441^{A}	12	5.41^{A}	0.15
	8	51	17.84^{A}	0.60	451^{A}	16	5.38^{A}	0.20
	9	68	18.42^{AE}	0.57	453^{A}	15	5.58^{AD}	0.19
	10	41	18.55^{AE}	0.78	455^{AD}	21	5.53^{AD}	0.26

The value in the same column with different letters differ significantly: A,B,C,D – $p<=0.01$

Table 4
Correlation coefficients of Pearson between the cholesterol concentration and daily goats milk yield and its chemical composition

Traits	Concentration of cholesterol in milk, mg/100 ml	Amounts cholesterol in daily milk, mg	Relation the amount of cholesterol to amount fat in milk, mg/g
Daily milk yield	-0.37^{***}	0.79^{***}	0.08^{*}
Daily FCM yield	-0.33^{***}	0.76^{***}	-0.12^{**}
Daily VCM yield	-0.29^{***}	0.77^{***}	NS
Daily amount of fat	-0.27^{***}	0.66^{***}	-0.29^{***}
Daily amount of protein	-0.29^{***}	0.78^{***}	NS
Daily amount of lactose	-0.35^{***}	0.78^{***}	NS
Daily SNF yield	-0.33^{***}	0.80^{***}	NS
Daily total solids yield	-0.32^{***}	0.79^{***}	NS
Gross energy in milk	0.32^{***}	-0.36^{***}	-0.50^{***}
Concentration of total protein	0.42^{***}	-0.36^{***}	-0.09^{*}
Concentration of fat in milk	0.27^{***}	-0.37^{**}	-0.56^{***}
Concentration of lactose in milk	NS	NS	-0.15^{***}
Concentration of total solids in milk	0.33^{***}	-0.35^{***}	-0.45^{***}
Concentration of non fat solids.	0.32^{***}	-0.23^{***}	-0.17^{***}
SCC	0.18^{***}	-0.11^{**}	0.07^{*}

*p<=0.05; **p<=0.01; ***p<=0.001

Discussion

It is difficult to compare our results with those obtained by other authors because the amount of information on this problem is rather negligible. The results obtained in most of the experiments concerned the concentration of cholesterol in commercial fresh liquid milk and milk products. There was found only one experiment concerning the cholesterol concentration in goat milk in respect to stage of lactation (BERNACKA and SIMIŃSKA, 2005). In contrast to our results BERNACKA and

SIMIŃSKA (2005) stated that the highest concentration of cholesterol was at the pick of lactation. Differences between extreme values were much higher in their study than in ours: i.e. 9.91 mg of cholesterol in 100 ml of milk - around 270% higher. It is difficult to establish the average cholesterol concentration in goat milk. The results showed in papers are in a very wide range, between 3 to 24.8 mg/100 ml of milk (AMER et al., 1999; AZIMA et al., 1969; BERNACKA and SIMIŃSKA, 2005; RAPHAEL et al., 1975). These big differences in cholesterol concentration in milk are reflected in milk products. PARK (1999) showed that cholesterol content in cheeses produced in America ranged between 80 and 147 mg/100g. According to many authors such big differences may be conditioned by various factors, among others: season of the year, stage of lactation, breed of animal and environmental factors (HAENLEIN and CACCESE, 1984; JENNES, 1980; PARK and CHUKWU, 1988; PARK et al., 1991).

The impact of method of analysis on cholesterol content in milk and its products is controversial. There are found very different opinions on this issue. PARK (1999, 2000) and LACROIX et al. (1973) pointed out that the value of cholesterol concentration determined by colorimetric method was almost two times higher compared to the gas chromatography method. Opposite opinion was presented by KUKIS et al. (1978) who stated that there are no significant differences between results obtained by colorimetric and gas chromatography methods. However, our results are similar to those presented by FLETOURISA et al. (1998), who used the gas chromatography method.

The most important factor, which has influence on cholesterol level in milk, is animal feeding. Considerably lower differences in cholesterol concentration in milk in our study compared to BERNACKA and SIMIŃSKA (2005) were the consequences of very good balanced diets, according to requirements and with small differences in feeding components between winter and summer diets. Only the highest concentration of cholesterol in milk in July was caused by increasing of concentration of energy in goat diets before breeding season (“flushing”). The high fat diets, when compared with the low fat diets, significantly raised plasma total cholesterol by 91% (BEYNEN et al., 2000). Similar results were observed with calcium salt supplementation (BALDI et al., 1992). In case of supplementation with plant oil, the cholesterol content in milk decreased even by 34.2% (REKLEWSKA et al., 2002). With the same type of diet the goats produced milk with higher level of monounsaturated fatty acids compared to cow milk (HAENLEIN, 2001), which was due to hypocholesterolemic properties (KRIS-ETHERSON and YU, 1997). In our study there was no impact of the year of study on the presented traits. These results are confirmed by other studies (VOUTSINAS et al., 1990).

On the basis of the results obtained in our study it is possible to suggest that considerably lower differences in cholesterol concentration in milk in our study were the consequences of very good balanced diets, according to requirements and small differences in feeding components between winter and summer diets.

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Effect of some factors on the yield and culinary quality of roasted and grilled lamb meat

Abstract

The effects of selected animal production factors on the yield of cuts and saleable meat and on the culinary parameters of roasted and grilled meat were investigated. Half-carcasses of 32 lambs, fattened intensively to 30-35 kg body weight, were studied. The experimental factors were: a) feeding – standard mixture (group C) or standard mixture with 10% rapeseed and linseed at a 2:1ratio (RL), b) breed group – Merino (M) and Ile de France × (F_1 Friesian × Polish Merino) ewes (If×FM) and c) sex of lambs. Half-carcasses were divided into primal cuts and saleable roast elements in accordance with Meat and Fat Research Institute procedures. After roasting or grilling, the samples were analysed for weight loss and evaluated organoleptically. Lamb fattening and sex did not result in large differences in the weight and proportion of the analysed cuts and saleable roast meat in half-carcasses. Commercial crossing of Polish Merino with the East Friesian dairy breed and the Ile de France meat breed increased the proportion of cuts and saleable meat in the front part of the half-carcass, as well as decreased the proportion of the back part. There was a significant effect of the cooking method on the loss of weight and results of organoleptic evaluation of saleable elements. During roasting, lower weight loss was shown by shoulder roll (26.1%) and greater weight loss by leg and neck roll (32.2%). During grilling, weight loss increased in the following order: brisket roll, loin chops and leg (17.0, 20.5 and 38.7%, respectively). Among roasted elements, higher total organoleptic scores were given to shoulder and brisket roll than leg (18.2 vs. 17.6 points on average). Among grilled elements, loin chops were given higher scores than leg and brisket roll (17.8 vs. 17.0 points). There was no significant effect of feeding, breed or sex on the grilling weight loss and on sensory traits of saleable elements. During roasting, a significant decrease in the meat scores of lambs receiving rapeseed and linseed was found. Compared to rams, the meat of ewes was given a lower total sensory score.

Key Words: lamb meat, live animal factors, culinary value, heat treatment

Introduction

Ever growing and changing consumer preferences for meat products, including lamb products, are an important reason for extending studies concerning the effect of breeding (genetic and environmental) practices on the quality of raw meat, as well as on the yield of saleable meat (carcass elements) and different aspects of its culinary suitability and organoleptic quality (ANDERSON, 2001; BORZUTA and STRZELECKI, 2001).

The present study was designed to determine the effect of selected animal production factors on the yield of cuts and saleable meat from lamb carcasses and on weight loss and organoleptic scores depending on the heat treatment of meat.

Materials and methods

Half-carcasses of 32 lambs, intensively fattened were investigated. The experimental groups of lambs were randomly created after weaning at 8 weeks of age and fattened for average of 40 days to 30-35 kg body weight. The experimental factors were: a) feeding – standard mixture (group C) or standard mixture with 10% rapeseed and linseed at a 2:1 ratio (RL), b) breed group – Merino (M) and Ile de France commercial crossbreds × (F_1 Friesian × Polish Merino ewes) (If×FM) and c) sex of lambs. Half-carcasses were divided into primal cuts and saleable roast elements in accordance with Meat and Fat Research Institute procedures (BORZUTA and STRZELECKI, 2001).

The weight of particular elements and their percentage in half-carcasses were determined. After adding 2% table salt and 24 h maturation at 4°C, selected elements of roast meat were roasted in an electric oven at 160°C until 80°C was reached inside the meat, or grilled in the form of 1.5 cm thick slices using an Expo Service toaster type GR100. After heat treatment and cooling, weight losses were determined and organoleptic evaluation was performed. The evaluation was made by a panel of 5 judges with proven sensory ability using a 5-point scale for evaluation of aroma, juiciness, tenderness and palatability (BARYŁKO-PIKIELNA, 1975).
The results were analysed statistically using three- or four-way analysis of variance (feeding, breed, sex, saleable element) and the ANOVA procedure of the Statistica 6.0 PL packet (STATISTICA, 2002). Significant differences between saleable elements were analysed using Duncan's test. Statistically significant first-degree interactions were used in the analysis of results.

Results

The proportion of oilseeds in the diet and the sex of lambs did not cause large differences in the weight and percentage of cuts and saleable elements in the half-carcass, saleable elements of roast meat and byproducts of carcass dissection (Tables 1-4). Statistically significant differences were only noted between rams and ewes in the weight and percentages of both knuckles with bone (Table 2) and shoulder without bone (Table 1), which were always higher in rams than in ewes. Numerically greater although non-significant differences were found in the weight and percentage of kidney fat, which was more abundant in the half-carcasses of RL than C lambs (by 33.3%, NS) and in ewes than in rams (by 17.6%) (Table 4).

Table 1
Weight and percentage of principal saleable elements with bone in half-carcass*

Item	Feeding		Breed		Sex		SEM
	C	RL	M	If×FM	♂♂	♀♀	
Number of lambs	16	16	16	16	18	14	
Weight of half-carcass; g	7569	7515	7414	7670	7581	7491	64,72
Shoulder with knuckle:							
- weight; g	1247	1229	1207A	1269A	1250	1222	10.99
- % in half-carcass	16.48	16.37	16.28	16.56	16.50	16.33	0.09
Front without shoulder:							
- weight; g	1563	1556	1494A	1624A	1590	1520	19.19
- % in half-carcass	20.64	20.70	20.16A	21.18A	20.98a	20.28a	0.16
Back - chop:							
- weight; g	1057	1051	1059	1048	1052	1056	11.98
- % in half-carcass	13.96	13.99	14.28a	13.67a	13.88	14.10	0.12
Ribs with brisket and flank:							
- weight; g	852	841	832	861	842	852	14.21
- % in half-carcass	11.24	11.18	11.21	11.21	11.10	11.36	0.13
Leg with knuckle:							
- weight; g	2604	2576	2569	2611	2596	2583	21.80
- % in half-carcass	34.42	34.29	34.65	34.06	34.25	34.49	0.15
Total saleable elements with bone: - weight; g	7322	7253	7162	7414	7331	7233	62.81
- % in half-carcass	96.74	96.53	96.59	96.68	96.69	96.56	0.10

* statistically non-significant interactions, Feeding: C - control, RL - rapeseed + linseed,
Breed: M - Merino, If×FM - Ile de France × (Friesian × Merino),
AA - P≤0.01; aa - P≤0.05, SEM – standard error of the arithmetic mean

Table 2
Weight and percentage of saleable elements in half-carcass*

Item	Feeding		Breed		Sex		SEM
	C	RL	M	If×FM	♂♂	♀♀	
Shoulder with bone:							
- weight; g	982	970	949A	1003A	982	968	9.72
- % in half-carcass	12.98	12.91	12.80	13.09	12.95	12.93	0.07
Front knuckle with bone:							
- weight; g	265	259	259	266	268A	254A	2.46
- % in half-carcass	3.50	3.46	3.49	3.47	3.55a	3.39a	0.04
Leg – roast with bone:							
- weight; g	1528	1506	1501	1532	1511	1524	16.66
- % in half-carcass	20.20	20.04	20.23	20.00	19.95	20.33	0.16
Rear knuckle with bone:							
- weight; g	340	339	342	338	345a	332a	3.09
- % in half-carcass	4.50	4.52	4.61a	4.41a	4.56	4.44	0.05
Leg – portion for stock:							
- weight; g	580	587	576	591	588	578	13.31
- % in half-carcass	7.66	7.83	7.78	7.71	7.74	7.75	0.18

* statistically non-significant interactions. For explanation of groups see Table 1, AA - P≤0.01; aa - P≤0.05

Table 3
Weight and percentage of saleable roast and stew meat in half-carcass*

Item	Feeding		Breed		Sex		SEM
	C	RL	M	If×FM	♂♂	♀♀	
Shoulder roll [P]:							
- weight; g	790	777	758A	810A	784	784	9.85
- % in half-carcass	10.44	10.34	10.22a	10.56a	10.33	10.47	0.08
Neck roll [P]:							
- weight; g	908	866	856	918	904	865	18.15
- % in half-carcass	11.98	11.51	11.53	11.96	11.91	11.53	0.18
Brisket roll [P] :							
- weight; g	615	583	605	593	595	604	14.02
- % in half-carcass	8.11	7.74	8.15	7.70	7.83	8.05	0.15
Leg [P]:							
- weight; g	1335	1312	1308	1339	1314	1335	15.54
- % in half-carcass	17.64	17.45	17.63	17.46	17.34	17.80	0.13
Portion for stock [G]							
- weight; g	326	333	326	333	331	327	9.94
- % in half-carcass	4.30	4.44	4.41	4.34	4.36	4.38	0.14
Rear knuckle [P]							
- weight; g	238	241	241	238	244	233	3.16
- % in half-carcass	3.15	3.21	3.25	3.11	3.23	3.12	0.04
Back – chop [P]							
- weight; g	1057	1051	1059	1048	1052	1056	11.98
- % in half-carcass	13.96	13.99	14.28a	13.67a	13.88	14.10	0.12
Front knuckle [P]							
- weight; g	265	259	259	266	268A	254A	2.46
- % in half-carcass	3.50	3.46	3.49	3.47	2.55a	3.39a	0.04
Total saleable meat							
- weight; g	5534	5422	5412	5545	5479	5458	62.81
- % in half-carcass	73.08	72.14	72.95	72.26	72.42	72.84	0.10

* statistically non-significant interactions. For explanation of groups see Table 1, AA - P≤0.01; aa - P≤0.05

Compared to Merino, the half-carcasses of If×FM crossbreds were characterized by a higher percentage of cuts and saleable meat in the front part of the half-carcass. Statis-

tically significant differences in the weight of shoulder with knuckle, front without shoulder, shoulder with bone and shoulder roll (by 5.1, 8.7 and 6.9%, respectively, all significant at P≤0.01, Tables 1-3) and marked but non-significant differences in the weight of neck roll (Table 3, by 7.2%) were partly due to the 3.5% higher (NS) weight of crossbred half-carcasses. A significantly higher percentage in the half-carcass of crossbreds than Merinos was only found for the front without shoulder (by 1.02 percentage units; P≤0.01) and for shoulder roll (by 0.34 percentage units; P≤0.05).

Table 4
Weight and proportion of less valuable dissection elements in half-carcass

Item	Feeding [F]		Breed		Sex [S]		SEM
	C	RL	M	If×FM	♂♂	♀♀	
Kidney:							
- weight*; g	54	55	55	54	55	53	0.93
- % in half-carcass*	0.71	0.74	0.75a	0.70a	0.73	0.71	0.01
Kidney fat:							
- weight; g	121	148	130	139	125	147	6.96
- % in half-carcass	1.60	1.97	1.76	1.81	1.65	1.96	0.09
Bones:							
- weight; g	1538	1577	1516a	1599a	1588	1518	18.15
- % in half-carcass	20.52	21.01	20.48	20.89	20.99	20.29	0.26
Other less valuable elements[1]:							
- weight; g	381	357	361	377	375	361	8.37
- % in half-carcass	5.02	4.75	4.87	4.91	4.95	4.81	0.09

For other explanations see Table 1, [1] – blood meat and fat, tail, spinal cord
* - F×S interaction significant at P≤0.05; aa - P≤0.05

Statistically significant differences depending on the breed of lambs were also found in the percentage of rear knuckle with bone and kidney (lower in crossbreds by 0.20 and 0.05 percentage units, respectively; P≤0.05), as well as in the weight of waste bones (higher in crossbreds by 5.5%; P≤0.05).

Table 5
Weight loss and sensory evaluation for roasted saleable elements*

Item	Saleable element			Feeding		Breed group		Sex		SEM
	L	SR	NR	C	RL	M	If×FM	♂♂	♀♀	
Amount	32	32	32	48	48	48	48	54	42	
Weight loss; g/100 g MES	31.9 B	26.1 AB	32.6 A	29.0 a	31.3 a	30.9	29.4	29.8	30.6	0.61
Sensory score:										
- aroma [max 5 pts]	4.44 AB	4.50 A	4.46 B	4.43	4.50	4.50	4.44	4.50 a	4.42 a	0.02
- juiciness [5]	4.31 AB	4.61 A	4.52 B	4.48	4.48	4.52	4.44	4.50	4.45	0.03
- tenderness [5]	4.40 A	4.64 Aa	4.52 a	4.51	4.52	4.57 a	4.47 a	4.55	4.48	0.03
- palatability [5]	4.46 Aa	4.62 A	4.56 a	4.52	4.57	4.58	4.52	4.57	4.51	0.02
- total score [20]	17.62 Aa	18.37 A	18.06 a	17.95	18.08	18.16 a	17.86 a	18.12 a	17.87 a	0.08

L - leg, SR – shoulder roll, NR – neck roll, For other explanations see Table 1
* statistically non-significant interactions, AA - P≤0.01; aa - P≤0.05

The highest sensory scores among roasted elements were given to shoulder roll, slightly lower to neck roll, and significantly the lowest to leg (Table 5). Lower scores

of roasted leg concerned all the organoleptic parameters, and differences in the total organoleptic score of this element were 2.4% (P≤0.05) when compared to neck roll, and 4.1% (P≤0.01) when compared to shoulder roll.
Among grilled elements, the highest scores for all organoleptic parameters were given to loin chops (kebabs), and the differences in relation to brisket roll and leg were statistically significant. Only the scores for juiciness of kebabs and shoulder roll were similar and significantly higher than the scores for leg, by 5.3% on average (P≤0.01). Total organoleptic score of grilled kebabs was significantly higher than that of the other elements, and in relation to leg and brisket roll it was higher by 4.2 and 4.8%, respectively (P≤0.01).
The breed and sex of lambs did not cause marked differences in the weight loss of saleable elements during roasting and grilling (Table 5 and 6). These factors had no significant effect on the sensory scores of grilled elements. During roasting, slightly higher scores were given to elements from Merino lambs than from crossbred lambs (total score being higher by 1.7%, significant at P≤0.05) and from rams than from ewes (by 1.4%; P≤0.05).

Table 6
Weight loss and sensory scores for grilled saleable elements*

Item	Saleable element			Feeding		Breed group		Sex		SEM
	L	LC	BR	C	RL	M	If×FM	♂♂	♀♀	
Weight loss; g/100 g MES	38.7 AB	20.5 BC	17.0 AC	25.2	25.6	24.9	25. 9	25.5	25.2	1.07
Sensory score:										
- aroma [max 5 pts]	4.37 B	4.45 A	4.18 AB	4.32	4.35	4.30	4.36	4.35	4.31	0.03
- juiciness [5]	4.12 AB	4.37 A	4.31 B	4.26	4.27	4.33 a	4.21 a	4.29	4.25	0.03
- tenderness [5]	4.19 A	4.37 Aa	4.23 a	4.27	4.26	4.29	4.24	4.27	4.26	0.02
- palatability [5]	4.37 Ba	4.58 AB	4.24 Aa	4.40	4.39	4.40	4.39	4.41	4.38	0.03
- total score [20]	17.06 B	17.77 AB	16.95 A	17.25	17.27	17.32	17.20	17.31	17.19	0.09

L – leg, LC – loin chops (kebabs), BR – brisket roll. For other explanations see Table 1
* statistically non-significant interactions, AA - P≤0.01; aa - P≤0.05

Discussion

In the present study, the fattening of lambs with oilseeds and the sex of lambs did not cause significant differences in the weight and percentage of cuts and saleable elements in lamb carcasses. The results obtained can be compared with those obtained using the same lamb carcass dissection method by STRZELECKI et al. (2005), in which no effect of whole rapeseeds and linseeds on the yield of saleable meat from lamb half-carcasses was found. GRZEŚKOWIAK et al. (2004), however, showed a favourable effect of using different particle sizes of linseed on the yield of saleable meat, and the best results were obtained when 50% oilseeds were ground. The lack of effect of sex of lambs, intensively fattened to 30-35 kg body weight, on the yield of cuts and saleable meat is confirmed by the study of STRZELECKI et al. (2003).
The effect of sex of lambs or commercial crossbreeding scheme on the yield of cuts and saleable elements from lamb carcasses varied according to breed, breed component, fattening method and weight standard of the lambs at slaughter. Favourable ef-

fects of crossing sheep from prolific populations with Texel and Charollais meat rams on the yield of cuts and saleable meat during intensive fattening to 30-35 kg were reported by GRZEŚKOWIAK et al. (2003), while STRZELECKI et al. (2003) did not find large effects for a similar commercial crossbreeding scheme and slaughter weight of lambs, when the lambs were fattened semi-intensively. A clear advantage of the meat breeds (Texel, Ile de France and Suffolk) over dairy and prolific breeds (Friesian, Finn) in this respect was reported by GRZEŚKOWIAK et al. (2003).

Overall, in terms of the yield of primal cuts and saleable elements, the lambs studied fell within the range of values obtained in a series of our earlier studies in this area (GRZEŚKOWIAK et al., 2003 and 2004; STRZELECKI et al., 2003 and 2005).

It is difficult to discuss the results obtained for weight loss and organoleptic score of roasted or grilled saleable elements because of the differences in the type and location of samples (cuts or single muscles) used by other authors and in heat treatment conditions. The applied roasting or grilling conditions caused clear differences in the weight loss of saleable elements. A significant increase in the weight loss of meat with increasing final temperature inside the heat-treated meat (by 27-39% at 60-85°C), was shown for roasted beef meat by PURCHAS et al. (2004). Significant differences also occurred between roasted and grilled cuts, and between roast meat of leg, which was the only element to be both roasted and grilled. Clearly higher weight loss from grilled than roasted meat of leg is confirmed by the findings of LOPES et al. (2003) for the *m. longissimus dorsi*. The grilling weight loss of kebabs from the *m. longissimus dorsi* (20%) was also reported by TOOHEY and HOPKINS (2006). The significantly lower grilling weight loss of loin chops than of meat of leg in the present study is confirmed by the findings of BADIANI et al. (1998) for roasted meat from the back and leg of lambs.

In the available literature there are no comparable results for the organoleptic score of whole saleable elements of lamb meat. It should be noted, however, that all the roasted and grilled saleable elements were given scores of 4.2-4.6 points for particular quality parameters, which is an evidence of their high organoleptic quality. At the same time, roasted elements were given higher scores than grilled elements, as confirmed by the scores for the roasted and grilled leg meat.

In general, the results of the present study have led us to the conclusion that feeding oilseeds and sex of lambs did not cause large differences in the weight and percentage in half-carcass of the primal cuts and roast saleable meat. The commercial crossing of Polish Merino with East Friesian dairy and Ile de France meat breed increased the proportion of cuts and saleable meat in the front part of the half-carcass, while decreasing the proportion of the back part.

Heat treatment was found to have a significant effect on the weight loss and organoleptic scores of saleable elements. During roasting, lower weight loss was shown by shoulder roll (26.1%) and greater weight loss by leg and neck roll (32.2%). During grilling, thermal losses increased in the following order: brisket roll, loin chops and leg (17.0, 20.5 and 38.7%, respectively). Among roasted elements, higher total organoleptic scores were given to shoulder and brisket roll than leg (18.2 vs. 17.6 points on average). Among grilled elements, loin chops were given higher scores than leg and brisket roll (17.8 vs. 17.0 points). Feeding, breed and sex had no significant effect on the loss of weight of grilled saleable cuts and their sensory scores. There was a statisti-

cally significant decrease in the scores for roasted cuts in lamb fed rapeseed and linseed-based diet and in ewes compared to rams.

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Composition of fatty acids of muscle tissue of lambs fed feedstuff supplemented with flax seeds

Abstract

The experiment was run in two replications, each included 24 lambs randomly assigned into two groups. The lambs remained with the mothers till 70 d of life, then were supplied a complete mixture solely till they obtained 35 kg body weight. The nutritive fodder was administered ad libitum from 14 d of life. The control group was given a standard rich mixture containing soy oil meal. In the experimental mixture, however, a part of soy meal was substituted by 10% crushed flax seeds, according to a protein level. The samples for quantitative and qualitative analysis of muscle fat were collected from musculus longissimus lumborum. A content of dry mass, protein and ash appeared to be comparable in both groups. A muscle tissue in the experimental group contained slightly more fat: 1.88% as compared to the control group :1.76% but the differences were statistically insignificant. The high significant differences (P<-0.01), though, were detected in a linolenic acid content (C 18:3 n3). This acid concentration in the muscle tissue of the control group reached 6.65 mg.100-1g, whereas in the experimental one it increased up to 25.95 mg.100-1g. Total polyunsaturated fatty acids (PUFA) was also shown significantly higher (P<-0.05) after mixture with flax supply. A content of PUFA in the control was 91 mg.100-1g and in the experimental group – 124 mg.100-1 g of muscle tissue.
No other statistically significant changes were recorded in the content of saturated and unsaturated fatty acids between the studied lamb groups.

Key Words: flax, lamb, fatty acids

Introduction

The quality of the consumed food exerts a direct impact on human health. Therefore, the research efforts have been performed all over the world to find the methods to improve the materials of animal origin BAS and MORAND – FEHR (2000), BODKOWSKI (2000), PONNAMPALAM et al. (2002). The dieticians draw special attention to the polyunsaturated fatty acids, especially essential unsaturated fatty acids (EUFA). It is believed that these compounds show the antiatherogenic, antineoplastic activity as well as play the key role in the counteraction against the metabolic disturbances.The mentioned above facts indicate the benefits of a diet rich in polyunsaturated fatty acids BARTNIKOWSKA and KULASEK (1994), PATKOWSKA-SOKOŁA and BODKOWSKI (2003).
The objective of the present work was an attempt to modify the chemical composition of lamb muscle tissue through some feedstuffs supplemented with crushed flax seeds included into fatteners diets.

Material and Methods

The experiment was made in two replications (in the years 2004-2005) in the Experimental Station of Young Ruminants in Bezek, a part of the University of Agriculture in Lublin. The experimental material was constituted by 24 young rams that were divided randomly into two groups equal in number; control and experimental. The lambs were raised with mothers up to 70d of life and then fattened till they gained 35kg body weight. During the rearing period, the lambs sucked their

mothers` milk and from 14 d of life the animals were fed nutritive feed ad libitum. Over the fattening period, the lambs from the control and experimental group were supplied with rich mixture, whose composition is given in Table 1.

Table 1
Composition of feed mixtures for fatteners

	Control	Experimental
Barley %	36.00	36.00
Oats/meal %	17.00	16.00
Wheat bran %	20.00	20.00
Beet pulp %	11.00	7.00
Flax seeds %	-	10.00
Soybean meal (45%) %	15.00	10.00
Fodder chalk %	0.50	0.50
Mineral mixture MM %	0.50	0.50
Dry mass %	87.48	88.08
MJ NEL/kg	6.50	6.80
Crude protein g/kg	157.50	156.00
PDI. g/kg	117.50	115.00
Crude fat %	2.66	5.45
SFA; % FA	20.32	24.51
UFA; % FA	79.68	75.49
MUFA; % FA	29.35	23.94
PUFA; % FA	50.29	51.55
SFA:UFA	0.26	0.32
C18:3; % FA	5.43	24.55

SFA -saturated fatty acids
UFA -unsaturated fatty acids
MUFA- monounsaturated fatty acids
PUFA -polyunsaturated fatty acids

After slaughter the carcasses were cooled at temperature about 4C for 24 h, next the samples of m. longissimus lumborum were collected to determine the basic chemical composition and a fatty acids content in a lipid fraction.

The analysis of the basic chemical composition of muscle tissue included the determinations of dry mass, crude ash, crude protein and crude fat according to the procedure AOAC (1999).

A fatty acids content in the muscle tissue was established by the gas chromatography technique. The extracted fat (FOLCH et al., 1951) underwent the process of saponification and esterification 13-15% BF2 in methanol, while margaric acid served as internal standard. The determinations were performed on a gas chromatograph Varian CP-3800; Varian column CP WAX 52CB;the length 60m; diameter 0.25 mm; detector FID; carrier gas helium, flow: 1,4ml min -1; temperature of the column 210 C, injector – 260 C ,detector – 260 C, amount dosage per column – 1 ul.

The obtained results were analysed statistically using two-factor variance analysis, including the influence of a year and a feeding group in the mathematical model. The Tables present only the characteristics concerning the experimental and control groups because the experimental year and the interactions proved statistically insignificant.

Results

The analysis of the chemical composition of lamb muscle tissue showed that the contents of dry mass, protein and ash were very similar in both studied groups (Table

2). The muscle tissue of the lambs from the experimental group contained slightly more fat (1.88 %) compared to the control (1.76%) and this difference also appeared to be statistically insignificant.

Table 2
Chemical composition of loin – lambs (% m.n)

	Control		Experimental	
	$\bar{x}$	S	$\bar{x}$	S
Dry mass	24.32	0.54	24.40	0.53
Ash	1.14	0.03	1.10	0.03
Protein	21.39	0.38	21.38	0.40
Fat	1.76	0.28	1.88	0.34

Table 3 gives the data characterizing the content of fatty acids of the lipid fraction in the longest dorsal muscle. The high significant statistically differences ($P<0.01$) between the groups were recorded only for the linolenic acid C 18:3n3. The muscle tissue of the control animals contained 6.65 mg.100-1g,whereas in the experimental group a markedly higher level of this acid was noted (29.95 mg. 100-1g). The content of the other fatty acids was similar in both groups.

Table 3
Content of fatty acids; mg.100-1g meat

	Control		Experimental	
	$\bar{x}$	S	$\bar{x}$	S
C12:0	1.75	0.42	2.30	0.51
C14:0	23.75	1.70	22.50	3.24
C14:1	0.80	0.14	no ditect	
C15:0	3.90	0.71	4.25	0.41
C16:0	224.00	33.90	220.00	36.89
C16:1	17.75	3.12	16.85	2.79
C18:0	161.00	26.20	169.00	33.75
C18:1	391.50	91.20	374.00	74.76
C18:2n6	65.10	11.40	78.65	19.82
C18:3n6	0.90	0.11	no ditect	
C18:3n3	6.65**	0.91	25.95**	4.99
C20:0	1.00	0.34	no ditect	
C20:1	1.05	0.24	no ditect	
C20:2	1.00	0.20	no ditect	
C20:3n6	2.40	0.69	1.75	0.27
C20:4n6	13.45	4.51	11.95	5.57
C20:5n3	2.45	0.82	5.75	1.43

** $P\leq0.01$;

The summary analysis of the studied compounds (Table 4) exhibited a slightly higher content of saturated fatty acids (SFA)in the muscle of the experimental animals (418 mg.100-1g) as against the control (415.5 mg.100-1g). Similar tendencies were recorded for the unsaturated fatty acids(UFA). In the experimental group this value

was 515 mg.100-1g,while in the control it appeared to be lower and reached 502,5 mg.100-1g muscle tissue.

A content of polyunsaturated acids (PUFA) was statistically significant higher ($P<0.05$) in the group supplied with dietary flax supplement (124 mg.100-1g) contrary to the control in which these acids content maintained at 91 mg.100-1g level in muscle tissue.

Table 4
Content of fatty acids groups; mg.100-1g meat

	Control		Experimental	
	$\bar{x}$	S	$\bar{x}$	S
SFA	415.5	83.00	418.00	91.69
UFA	502.5	103.00	515.00	112.78
MUFA	411.5	158.00	391.00	172.05
PUFA	91.00*	20.00	124.00*	26.09
SFA:UFA	0.80	0.03	0.85	0.05

* $P \leq 0.05$

Discussion

Application of dietary additives of crushed flax seeds in the nutrition of fattened lambs has not influenced significantly the basic chemical composition of muscle tissue. The content of dry mass, ash, protein and fat was similar in all the animals just as the values obtained by the other authors MAHGOUB et al. (2000), MANSO et al. (1998).The present authors claim that the situation described resulted from the balance of both mixtures with respect to energy and protein content as Table 1 explicitly presents.

Taking into account the dietetics, the qualitative composition of raw materials and food obtained is of primary importance and the key role is attributed to the polyunsaturated acids. The analyses performed revealed that in muscle tissue of the experimental lambs, a linolenic acid (C 18:3 n3) content has increased in a statistically significant way. This evident fact proves that it is the result of dietary supplement of crushed flax seeds, as all the other experimental factors were the same in both studied groups of animals. Similar tendencies were reported for the other acids of numerous unsaturated bonds, like C 18:2n6, C20:5n3 (Tab. 3). The detected differences, though, were not confirmed statistically and became manifest at the total analysis of fatty acids groups (Tab. 4). It was found out that, muscle tissues of the lambs given dietary flax seeds additive contain a significantly higher content of the polyunsaturated fatty acids in contrast to the lambs from the control.

Similar dependencies were noted by the other authors who investigated the influence of differentiated nutrition, particularly when feedstuffs were supplemented with seeds of high polyunsaturated acids level (BAS and MORAND-FEHR, 2000; PONNAMPALAM et al., 2002; PATKOWSKA-SOKOŁA et al., 1994, PATKOWSKA-SOKOŁA et al., 1995).

Summing up all the results obtained, it should be stated that feedstuff supplementation with 10% crushed flax seeds induces a significant improvement of the dietetic value of lamb muscle tissue.

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Estimates of maternal genetic and permanent environmental effects in sheep

Abstract

Body weight is determined by genetic and environmental factors. A number of reports indicate considerable maternal effects on the trait. The objective of this contribution was to estimate direct and maternal genetic variances as well as covariances between direct and maternal additive genetic effects. Maternal permanent environmental variances were also estimated. The following three traits were studied: birth weight [BW], fourth week weight [FWW] and average daily gain [ADG] until 28-th day. A total of 29317 recorded lambs (36494 pedigreed animals) were used to estimate genetic parameters by three single trait animal models. The material was classified according to: breed groups (4 levels), sex, birth year (17), flock (3), age of dam at lambing (10). These effects were treated as fixed. The random part of a full model (III) contains direct and maternal (omitted in model II) genetic effects as well as maternal permanent environmental effect (omitted in models I and II). Estimates of genetic parameters were obtained by the use of DFREML package programs. For all the traits studied, direct heritability estimates ranged between 0.2 to 0.3 as well as maternal heritabilies were also relative high (exceed 0.15). These highest heritabilities were estimated for BW. Negative covariances between direct and maternal effects were reported. Ratios of maternal permanent environmental variance to total variance (for each trait) were close to zero. It was suggested that inclusion of maternal effects into the model for the traits is necessary.

Key Words: maternal effects, lamb weights, variance components, REML method

Introduction

Body weight and growth traits in sheep are known to be influenced by direct and maternal genetic effects as well as by environmental effects. A number of reports indicate considerable maternal effects for these traits in sheep (HASSEN et al., 2003; NÄSHOLM, 2004; VAN VLECK et al., 2003). From the mother's perspective, maternal effects on progeny performance result from maternal traits controlled by her genotype and associated environmental factors. Therefore, these effects are divided into genetic and environmental components. However, from the side of the offspring, maternal effects are reflected as environmental. So, there are indirect genetic and environmental effects. In consequence, maternal genetic effects are defined as any influence from dam to progeny, excluding the effects of directly transmitted genes. Some authors (see e.g. LIGDA et al., 2000) reported negative relationships between direct and maternal genetic effects.

The objective of this paper was to estimate direct and maternal additive genetic variances as well as covariances between direct and maternal genetic effects. Maternal permanent environmental variances were also estimated.

Material and methods

Material

A population of 29317 lambs of both pure breeds and synthetic mutton lines born between 1976-1996 was studied. In that number 36494 animals were pedigreed. Relatively low inbreeding level (0.2%) was registered for the population hence it is omitted in further analysis. This contribution is a continuation of a paper by DOBEK

et al. (2004). Two lines (prolific-meat line and prolific-wool line) have been supplemented into present study. The structure of the "fixed part" of the models used, are the same as described in the earlier study. Four groups of breeds have been formed. They were included as linear covariable. The material was also classified according to: sex, birth year (17 years), flock (3 units), age of dam at lambing (10 age classes). The following three traits were studied: birth weight [BW], fourth week weight [FWW] and average daily gain [ADG] until 28-th day. More details on the data structure and flock management have been described in earlier studies by DOBEK et al. (2004), GUT (1994) and WÓJTOWSKI (1999). A brief description of the data set is given in Table 1.

Table 1
Description of the data sets

Trait	Average	Standard deviation
BW (in kilograms)	4.422	0.840
FWW (in kilograms)	12.354	2.514
ADG (in grams)	293	83

Methods

Three single trait linear animal models have been employed to estimate genetic parameters.

Model I:

$$\mathbf{y} = \mathbf{X\beta} + \mathbf{Z_1a} + \mathbf{e}$$

where: **y** is the *nx1* vector of observations;

β is a *p x1* vector of fixed effects (see DOBEK et al., 2004)

a is a *qx1* vector of random direct additive genetic effects;

e is a *nx1* vector of random errors;

X, $\mathbf{Z_1}$ are the *nxp*, and *nxq* design matrices, respectively.

Model II:

$$\mathbf{y} = \mathbf{X\beta} + \mathbf{Z_1a} + \mathbf{Z_2m} + \mathbf{e}$$

where: **m** is a *qx1* vector of random maternal additive genetic effects;

$\mathbf{Z_2}$ is a *nxq* design matrix connected observations with maternal additive genetic effects

y, **β**, **a**, **e**, **X**, $\mathbf{Z_1}$ – as above

Model III:

$$\mathbf{y} = \mathbf{X\beta} + \mathbf{Z_1a} + \mathbf{Z_2m} + \mathbf{Z_3c} + \mathbf{e}$$

where: **c** is a *rx1* vector of random maternal permanent environmental effects;

$\mathbf{Z_3}$ is a *nxr* design matrix connected observations with maternal permanent environmental effects

y, **β**, **a**, **m**, **e**, **X**, $\mathbf{Z_1}$, $\mathbf{Z_2}$ – as above

The first and second moments were assumed to be as follows:

$$E\begin{bmatrix}\mathbf{a}\\ \mathbf{m}\\ \mathbf{c}\\ \mathbf{e}\end{bmatrix}=\begin{bmatrix}\mathbf{0}\\ \mathbf{0}\\ \mathbf{0}\\ \mathbf{0}\end{bmatrix} \text{ and } D\begin{bmatrix}\mathbf{a}\\ \mathbf{m}\\ \mathbf{c}\\ \mathbf{e}\end{bmatrix}=\begin{bmatrix}\mathbf{A}\sigma_{\mathbf{a}}^2 & \mathbf{A}\sigma_{\mathbf{am}} & \mathbf{0} & \mathbf{0}\\ \mathbf{A}\sigma_{\mathbf{am}} & \mathbf{A}\sigma_{\mathbf{m}}^2 & \mathbf{0} & \mathbf{0}\\ \mathbf{0} & \mathbf{0} & \mathbf{I}_r\sigma_{\mathbf{c}}^2 & \mathbf{0}\\ \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{I}_n\sigma_{\mathbf{e}}^2\end{bmatrix}$$

where **A** is the *qxq* additive relationship matrix; $\mathbf{I_r}$ and $\mathbf{I_n}$ are the identity matrices, σ_a^2 is the direct additive genetic variance; σ_m^2 is the maternal additive genetic variance; σ_{am} is the covariance between direct and maternal additive effects, σ_c^2 is the maternal permanent environmental effects, σ_e^2 is the error variance

Hence, $\mathbf{y} \sim N(\mathbf{X\beta}, \mathbf{Z_1AZ'_1}\sigma_a^2 + \mathbf{Z_1AZ'_2}\sigma_{am} + \mathbf{Z_2AZ'_1}\sigma_{am} + \mathbf{Z_2AZ'_2}\sigma_m^2\ \mathbf{Z_3Z_3'}\ \sigma_c^2 + \mathbf{I_n}\sigma_e^2)$.

The following genetic parameters have been estimated:

- direct heritability ($h_a^2=\sigma_a^2/\sigma_p^2$),where σ_p^2 is the phenotypic variance,
- maternal heritability ($h_m^2=\sigma_m^2/\sigma_p^2$),
- covariance between direct and maternal effects as proportion to phenotypic variance ($d_{am}=\sigma_{am}/\sigma_p^2$),
- total heritability $h_T^2=(\sigma_a^2+0.5\sigma_m^2+1.5\sigma_{am})/\sigma_p^2$ according to the formula given by WILLHAM (1972)
- ratio of maternal permanent environmental variance to phenotypic variance (c^2).

Computing algorithm and comparison criteria

The average-information restricted maximum likelihood (AI-REML) algorithm (JOHNSON and THOMPSON, 1995) has been employed for estimation of these variance components. A value of 10^{-8} was used as the convergence criterion for the whole analysis. The following starting values were taken for each data set: 0.5 for h_a^2, 0.01 for h_m^2 and 0.001 for d_{am}. Residual variance estimate was used as comparison criterion (PTAK and ŻARNECKI, 2000). The computations were performed using the DFREML package programs of MEYER (2001).

Results

Figures 1-3 present the estimates of genetic parameters for each of the three traits obtained via the three models. For BW, the additive heritability estimates were influenced by the statistical model. In the case of the simplest model (without maternal effects) h_a^2 was the highest (0.29). After inclusion of the maternal genetic effects the value decreased negligibly (0.26). These relationships were not confirmed for FWW and ADG. Similar direct heritability estimates were obtained by all the three models. As expected, higher maternal heritability (0.27) was estimated for BW (h_m^2 exceeded h_a^2) whereas for the other two traits the estimates of h_m^2 were lower (0.18). For each traits a negative covariance between direct and maternal genetic effects were registered. This influenced the magnitude of total heritabilities, which ranged between 0.11-0.14. By contrast to genetic variances, the maternal permanent environmental

variances for all traits were close to zero. In consequence, estimates c^2 were negligible. Approximated standard errors of estimates of (co)variance component functions did not exceed 0.015.

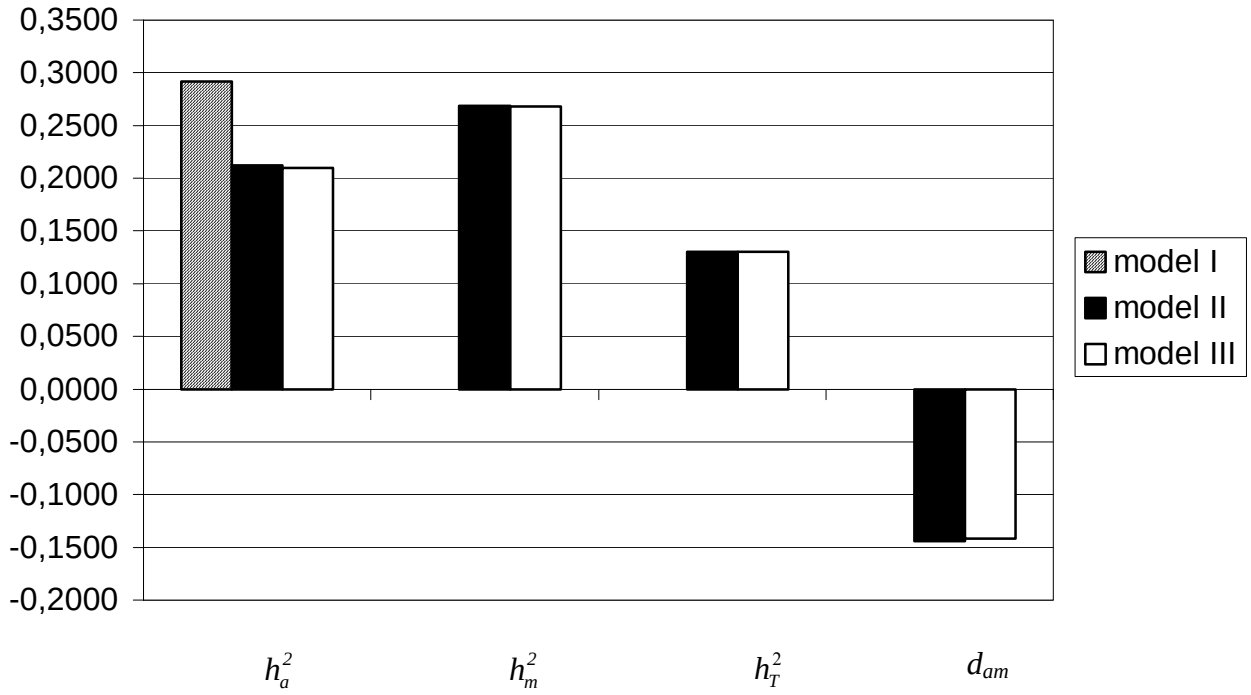

Fig. 1: Genetic parameters for birth weight

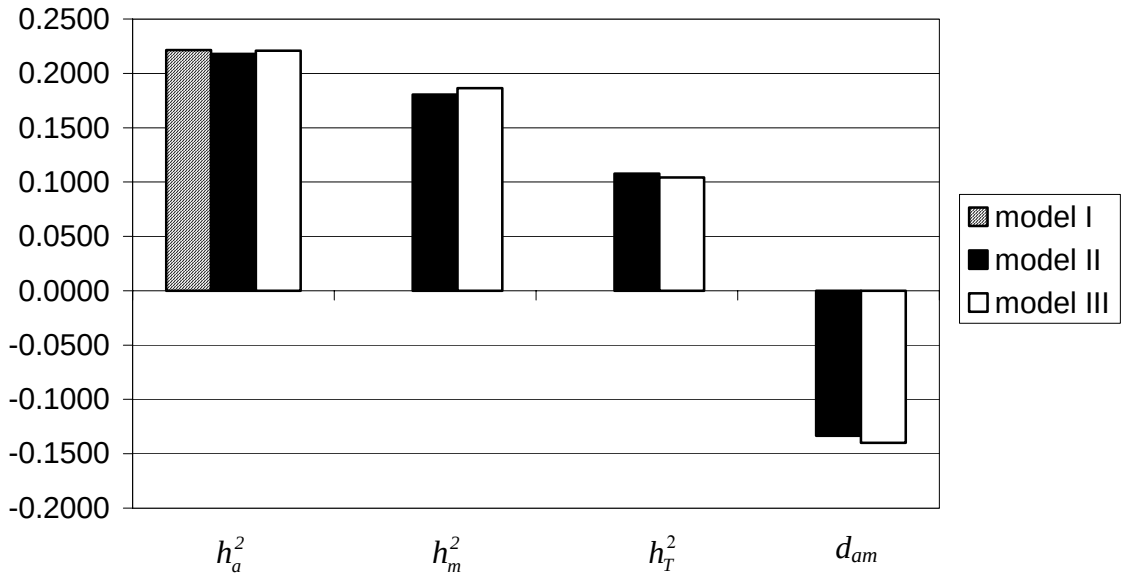

Fig. 2: Genetic parameters for fourth week weight

The obtained results indicate that maternal genetic effects should be included into the linear model for body weight of genetic evaluation in sheep. One of the most popular criteria of goodness of model fit is residual variance estimate. These error variance estimates for the traits studied are listed in Table 2. In each case, the smallest ones were obtained for a model with direct and maternal additive genetic effects. So, maternal permanent environmental effects should be omitted in the linear model.

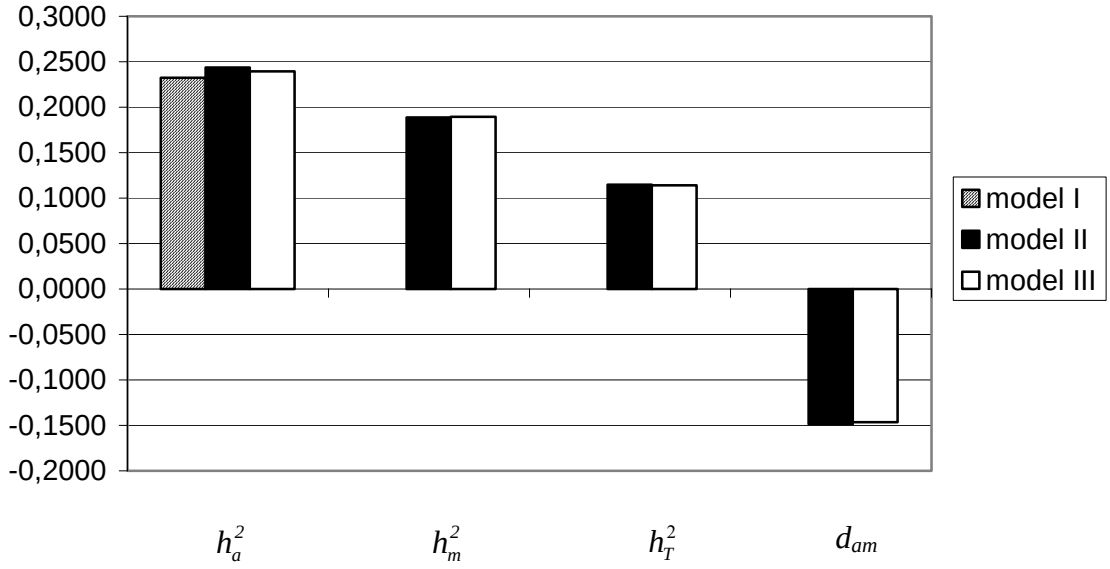

Fig. 3: Genetic parameters for average daily gain

Table 2
Estimates of residual variances for the models studied

Trait	Model I	Model II	Model III
BW	0.3542	0.3310	0.3315
FWW	3.653	3.513	3.532
ADG	4431	4146	4154

Discussion

Nowadays, body weight is one of the major selection traits in sheep population. As already mentioned this contribution is a continuation of work by DOBEK et al. (2004). Although the number of recorded and pedigreed individuals increased in comparison with the previous study, magnitudes of these estimates of fixed effects are very similar. Therefore, estimates of breed group, sex, birth year, flocks, and age classes of dam at lambing are not listed here.

It is well known that estimates of genetic parameters vary widely across authors, years, methods and genetic groups for the same traits. Comparison with estimates obtained via the sire model is difficult since the heritability is usually overestimated. On the other hand, direct and maternal heritability estimates obtained in the present study are in agreement with those reported for various populations by a number of authors. For instance, similar estimates from 15 various animal models (employed for 33 994 recorded individuals) were reported by VAN VLECK et al. (2003): direct heritabilities of birth weight were very stable (0.24-0.25), maternal heritabilities were equal as well (0.19-0.20). Higher direct heritabilities of BW were found by HASSEN et al. (2003) for joint analysis of pure and crossbred sheep in Ethiopia. As expected, smaller heritabilities were estimated for pure populations. For example, in the case of a multi-generation population of 1889 lambs of Polish Whiteheaded Mutton sheep the direct and maternal heritabilities for BW were 0.17 and 0.02, respectively (GUT et al., 2001). However, similar results were also reported by FOSSCECO and NOTTER (1995) for

crossbred sheep. This indicates the impact of size and structure of data on estimates of the parameter. In the study by GUT et al. (2001) the average number of offspring per dam was relatively small. The hypothesis was also confirmed in investigations carried out in species characterised by low fecundity, for instance horses (see e.g. TORZYŃSKI et al., 2005).

The heritability, especially maternal one was higher for BW compared to later body weights and their changes over time. From the biological perspective the birth body is strongly determined by maternal conditions during embryological development. This has been confirmed in empirical studies by many authors (AHUNU et al., 1997; LIGDA et al., 2000; VAN VLECK et al., 2003) as well.

Not very considerable differences in direct heritabilities for FWW and ADG obtained from the first and second models are somewhat difficult to interpret, since when maternal effects are large, the h_a^2 from a simple model is usually overestimated. This can indicate other genetic effects unidentified here, which should be included in the model in further studies.

If maternal genetic effects are important, a model that contains both direct and maternal genetic effects should provide more precise predictive ability of future progeny performance than would a model that contains only direct genetic effects.

As already mentioned, genetic antagonism between direct and maternal effects was obtained for each studied trait. It corresponds with many reports on sheep (HAGGER, 1998; VAN VLECK et al., 2003) and other livestock and poultry species. SWALVE (1993) suggested that negative covariance between direct and maternal genetic effects may be the result of management system. In field data the correlations are more negative compared to experimental data. However, an investigation conducted by DODENHOFF et al. (1999) on several breeds of beef cattle indicates that dependences between direct and maternal effects are determined by breed. Furthermore, LEWIS and BEATSON (1999) found different covariances between these effects within flocks for one sheep breed.

Conclusions

The study confirmed that genetic evaluation should be based on a model including maternal effects for two reasons. First, the maternal genetic variances for the traits studied are relatively large. Secondly, a negative covariance between direct and maternal genetic effects indicates different rankings of individuals when the maternal contribution is omitted in the evaluation procedure.

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Lipid profile of intramuscular fat in kids fattened to 60, 90 and 180 days of age

Abstract

The aim of the study was to determine the effect of age and breed of goat kids on the lipid profile of muscle tissue. *Longissimus dorsi* muscles of 72 Saanen kids and F_1 crosses with Anglo-Nubian bucks slaughtered at 60, 90 and 180 days of age were investigated. Cholesterol content of muscle tissue and fatty acid profile, including the level of conjugated linoleic acid (CLA) in intramuscular fat (IMF), were analysed. Cholesterol content was similar in kids slaughtered at 60 and 90 days (72.5 mg/100 g on average) and lower in 180-day-old kids (66.2 mg). Age did not result in differences in the total content of saturated fatty acids (SFA), with a significantly lowest C12:0 and C14:0 content in the IMF of 180-day-old kids. As the kids grew older, the content of monounsaturated fatty acids (MUFA), especially the dominant C18:1, increased significantly, while the content of all polyunsaturated fatty acids (PUFA) decreased. The content of Ω3 PUFA and the PUFA:SFA ratio decreased with age, with no marked differences in the other health parameters based on the fatty acid profile (DFA:OFA, Ω6:Ω3 PUFA, IA and IT). CLA was the most abundant in the muscles of 90-day-old kids (0.66 g/100 g tissue), being an average of 39% more than in the other age groups. The crossbreeding scheme studied did not affect the cholesterol content of muscle tissue and total SFA in IMF. Compared to Saanen kids, the fat of crossbreds was found to contain more MUFA and CLA, and less PUFA. Overall, the IMF of the crossbreds was characterized by an unfavourably lower Ω3 PUFA content and a lower PUFA:SFA ratio, with a favourably lower Ω6:Ω3 PUFA ratio. From the viewpoint of health-promoting quality, the meat of kids representing lower age and weight standards (60 and 90 days) had a generally more favourable lipid profile, while the effect of the analysed commercial crossbreeding scheme was fairly obvious but inconsistent.

Key Words: fattened kids, muscle tissue, lipid profile, age, breed

Introduction

The use of goats for meat production is of less interest to researchers than the use of goats for milk. However, it is believed that goat meat has special dietetic and taste attributes, as confirmed by review papers (BANSKALIEVA et al., 2000; PIENIAK-LENDZION, 2002) and comparative studies, in which the meat of kids and lambs was compared for quality (GRUSZECKI et al., 1999; NIEDZIÓŁKA et al., 2005; SHERIDAN et al., 2003). In light of the available findings and comparative data, goat meat, and kid meat in particular, is considered to be one of the most valuable meats in terms of current nutritional preferences, mainly thanks to its low fatness, favourable lipid profile, low cholesterol content, composition of fatty acids and CLA.

The quality of goat meat is significantly affected by genetic factors (breed and crossbreeding scheme) and environmental factors (age, weight standard, housing and fattening methods, type of feed). In the literature, especially that available in Poland, there are few studies investigating the effect of these factors on kid meat quality, especially in terms of its health quality. Therefore, the aim of the present study was to determine the effect of age and breed of goat kids on the cholesterol content of their muscle tissue and fatty acid profile of intramuscular fat, and the resulting parameters of health quality.

Materials and methods

The study was carried out in two replications (2002 and 2003) with 72 Saanen (S) kids (castrated at 14 days of age on average) and F_1 crosses of Saanen goats and Anglo-

Nubian bucks (AN × S).

Throughout the experiment, kids were kept in 2 breed groups under identical housing and feeding conditions. Kids sucked their mothers to 20 days of age, and were fed on the basis of milk replacer and Fernando mixture from weaning to 60 days of age. From 60 to 180 days of age, kids were fattened semi-intensively under the rationed feeding system, in accordance with National Research Institute of Animal Production standards (Normy żywienia, 1993). The diets contained hay, ensiled hay and concentrate mixtures Fernando and Dosche 360. Experimental slaughter was performed on kids randomly selected from the above breed groups at 60 (at an average weight of approx. 12 kg), 90 (approx. 20 kg) and 180 days of age (approx. 30 kg). Numbers of animals in particular groups are given in Table 1.

The *longissimus dorsi* muscle was assayed for cholesterol content, and fatty acid profile was determined in the intramuscular fat extracted from this muscle. Cholesterol content was determined colourimetrically at a wavelength of 570 μm, using a colour reaction of cholesterol with a 10% acetic acid solution ($FeCl_3$) diluted 100 times with sulphuric acid. Samples were prepared in accordance with the procedures used at the Main Laboratory of the National Research Institute of Animal Production, in which a muscle sample is homogenized in a mixture of chloroform and methanol (2/1), evaporated, saponified, extracted with hexane, re-evaporated, and determined colourimetrically. Fatty acid profile was analysed using gas chromatography by determining acids in the form of methyl esters. Samples were prepared in accordance with the method of FOLCH (1957), in which a sample is homogenized in a mixture of chloroform and methanol (2/1) and the solvent evaporated, followed by saponification (0.5 N NaOH in methanol) and esterification (BF_3 in methanol) of the evaporation residue. The resulting methyl esters of fatty acids were determined in hexane extracts using gas chromatography (VARIAN 3400), on a column filled with acid-modified polyethylene glycol (FFAP, CP-WAX 58), using an 8200CX autosampler.

In terms of the health-promoting parameters of fatty acids, the index of atherosclerosis (IA) and the index of thrombosis (IT) were calculated using the formulae developed by ULBRICH and SOUTHGATE (1991) (according to formulae given under Table 3).

The results were analysed statistically in a three-factorial design (age at slaughter, breed group, replication) using the ANOVA procedure of the Statistica 6.0 PL packet (STATISTICA, 2002). Significant differences between the age groups of the kids were estimated using Duncan's test. Tables and analysis of results only include statistically significant first-degree interactions.

Results

Age of kids. The cholesterol content of muscle tissue varied according to the age of kids. Cholesterol was the most abundant in the muscles of kids aged 90 day, followed by those aged 60 days (5.2% less, NS) and 180 days (11.0% less than in 90-day-old kids; $P \leq 0.05$) (Table 1).

The total SFA content of intramuscular fat in kids slaughtered at 60, 90 and 180 days did not differ significantly (Table 1). For this group of fatty acids, no marked differences were found in the content of dominant saturated acids C16:0 and C18:0, although there was a significantly lower content of C12:0 and C14:0 acids in the oldest kids (180 days) compared to younger kids – by 73.3 and 34.5% in relation to 60-day-old kids, and by 63.6 and 35.4% in relation to 90-day old kids, respectively.

Table 1
Cholesterol content of muscle tissue (mg /100 g tissue) and SFA content of intramuscular fat (g/100 g fat)

Trait	Age at slaughter, days [W]			Breed [R]		Replication [P]		SEM
	60	90	180	S	F_1 AxS	I	II	
Number	15	35	22	36	36	36	36	
Cholesterol	70.7	74.4a	66.2a	70.0	72.4	67.2a	75.2a	1.52
SFA[1, 6]	43.27	44.03	42.14	43.66	42.92	41.09A	45.50A	0.52
C 12:0[1, 6]	0.60A	0.44a	0.16Aa	0.50a	0.27a	0.23A	0.54A	0.06
C 14:0[2]	3.28B	3.33A	2.15AB	2.93a	3.00a	1.90A	4.03A	0.21
C 16:0	20.86	22.32	21.98	21.56a	22.26a	20.02A	23.80A	0.39
C 18:0[4, 6]	17.07	16.81	17.01	17.67	16.18	16.91	16.93	0.39

SFA - Σ: C8:0, C10:0, C12:0, C14:0, C15:0, C16:0, C17:0, C18:0, C20:0, C22:0
AA, BB - P≤0.01; aa - P≤0.05, Interactions: P≤0.01: [1] - W×R; [2] - W×P; P≤0.05: [4] - W×R; [6] - R×P

The total UFA content of intramuscular fat was not differentiated by the age of kids (Table 2). There was a statistically significant effect of age of kids on the MUFA and PUFA content. The content of MUFA increased with age, by 22.0% at 90 days and by 41.7% at 180 days of age in relation to the youngest kids slaughtered at 60 days of age. The differences resulted from, and corresponded with, the differences in the content of the dominant acid (oleic acid, C18:1) in the group of MUFA. In contrast, the PUFA content decreased significantly with the age of slaughtered kids. In relation to kids aged 60 days, intramuscular fat contained 38.8% and 61.6% less PUFA than in 90- and 180-day-old kids, respectively. This concerned the content of all PUFA acids analysed: C18:2, C18:3, C20:4, C20:5 and C22:6 (Table 2).

Table 2
The UFA content of intramuscular fat in kids (g/100 g fat)

Trait	Age at slaughter [W]			Breed [R]		Replication [P]		SEM
	60	90	180	S	F_1 A×S	I	II	
UFA	56.17	55.56	57.53	55.97	56.60	58.08A	54.50A	0.50
MUFA[1, 6]	34.83AB	42.50BC	49.36AC	41.04 A	44.94A	43.26	42.72	0.96
C 16:1[6]	1.88	2.22	2.14	1.88A	2.37A	2.83	1.97	0.08
C 18:1[1, 6]	32.10AB	39.55BC	46.64AC	38.54a	41.79a	39.58	40.75	0.91
PUFA[4]	21.34AB	13.06BC	8.20AC	14.93A	11.67A	14.82A	11.77A	0.82
C 18:2[4]	11.76AB	7.68BC	4.31AC	8.52A	6.48A	7.96	7.03	0.48
C 18:3	0.65a	0.63ab	0.49ab	0.57	0.61	0.54a	0.65a	0.03
C 20:4	4.98AB	2.79B	2.10A	3.56A	2.51A	3.14	2.93	0.24
C 20:5[1]	0.77AB	0.31B	0.18A	0.45A	0.28A	0.39	0.34	0.04
C 22:6	0.36AB	0.18Ba	0.09Aa	0.21	0.17	0.20	0.18	0.02
SKL[4, 3]	0.50a	0.66Aa	0.45A	0.46A	0.65A	0.47A	0.65A	0.03

MUFA = Σ C14:1, C15:1, C16:1, C17:1, C18:1, C20:1; C22:1;
PUFA = Σ C18:2, SKL, C18:3, C20:2, C20:3, C20:4; C20:5; C22:4, C22:5, C22:6
AA, BB, CC - P≤0.01; aa, bb - P≤0.05, Interactions: P≤0.01: [1] - W×R; [3] R×P; P≤0.05: [4] - W×R; [6] - R×P

Changes in the CLA content of intramuscular fat with advancing age of the kids followed a different pattern. CLA was the most abundant in the fat of 90-day-old kids,

being 32.0% higher than in 60-day-old kids (P≤0.05) and 46.7% higher than in 180-day-old kids (P≤0.01).

In terms of health quality parameters, calculated from the fatty acid profile of intramuscular fat (Table 3), the age of kids caused significant differences in the proportions of PUFA:SFA acids and the content of Ω3 PUFA. From the viewpoint of meat health quality, these parameters were the most favourable in the youngest animals and deteriorated with age; in relation to 60-day-old kids, PUFA:SFA decreased by 39.9 and 60.9%, and Ω3 PUFA by 48.4 and 67.1% in 90- and 180-day-old kids, respectively. In the youngest kids, there was also a non-significant tendency towards the more favourable Ω6:Ω3 PUFA ratio, which was 15.4 and 8.1% lower than in 90- and 180-day-old kids, respectively. The age categories of kids did not have a marked and characteristic effect on the UFA:SFA and DFA:OFA ratios, and on the indices of atherosclerosis (IA) and thrombosis (IT) - Table 3.

Table 3
Health parameters of fatty acid profile in the intramuscular fat of kids

Trait	Age at slaughter [W]			Breed [R]		Replication [P]		SEM
	60	90	180	S	F_1 AxS	I	II	
UFA:SFA[4, 6]	1.316	1.290	1.381	1.317	1.329	1.432A	1.215A	0.03
PUFA:SFA[4]	0.501AB	0.301BC	0.196AC	0.347A	0.274A	0.364A	0.257A	0.02
DFA:OFA	2.944	2.781	3.007	2.938	2.829	3.147A	2.621A	0.08
PUFA Ω3[4]; g/100 g fat	2.89AB	1.49Ba	0.95Aa	1.74a	1.49a	2.07A	1.16A	0.13
PUFA Ω6:Ω3[3]	6.960	8.227	7.576	8.659a	6.870a	6.101A	9.428A	0.39
IA[2]	0.449	0.478a	0.425a	0.458	0.454	0.385A	0.527A	0.01
IT[1]	0.746	0.785	0.725	0.774	0.743	0.679A	0.838A	0.02

UFA = MUFA + PUFA; DFA = UFA + C18:0; OFA = SFA - C18:0
PUFA Ω3 = Σ C18:3, C20:5; C22:5, C22:6; PUFA Ω6 = Σ C18:2, C20:2, C20:3, C20:4; C22:4,
IA (index of atherosclerosis) = Σ (12:0, C14:0, C16:0) : UFA
IT (index of thrombosis) = Σ (14:0, C16:0, C18:0) : Σ (MUFA + Ω3 and Ω6 PUFA + Ω3:Ω6 PUFA)
AA, BB, CC ** - P≤0.01; aa, * - P≤0.05,
Interactions: P≤0.01: [1] - W×R; [3] - R×P, [3] - R×P; P≤0.05: [4] - W×R; [6] - R×P

Breed of kids. Crossing Saanen goats with Anglo-Nubian bucks did not cause differences in the cholesterol content of *longissimus dorsi* muscle of the crossbred kids.

There were no significant differences in the total SFA content of intramuscular fat depending on the breed of kids, with statistically significant differences for most individual acids of this group. The fat of AN×S crosses contained more C14:0 and C16:0 acids (by 2.4 and 3.2% respectively, P≤0.05) and less C12:0 (by 46.0%, P≤0.05) and C18:0 acids (by 8.5%, NS).

The crossbreeding applied did not cause differences in the content of UFA in relation to the initial Saanen breed, although there were significant differences in the content of MUFA and PUFA (Table 2). Intramuscular fat of the crossbreds contained significantly more MUFA, both the sum of MUFA (by 9.5%) and the two acids of this group analysed: C16:1 and C18:1 (by 26.1 and 8.4%, respectively). In contrast, AN×S crossbreds had a lower content of PUFA, both the total content (by 21.8%, P≤0.01) and the content of individual acids of this group, with differences ranging from 19.0% (NS) for C22:6 to 23.9-37.8% for C18:2, C20:4 and C20:5. Only the content of C18:3 lino-

lenic acid did not differ markedly depending on the breed of kids.
Intramuscular fat of the crossbreds contained significantly more CLA than that of purebred Saanen kids (by 41.3%, P≤0.01).
In terms of the health quality parameters based on the fatty acid profile, the fat of crossbreds was characterized by an unfavourably lower PUFA:SFA ratio (by 21.0%, P≤0.01) and lower Ω3 PUFA content (by 14.4%, P≤0.05), with a favourably lower Ω6:Ω3 PUFA ratio (by 20.7%, P≤0.05) - Table 3. No greater differences were found between the breed groups in the UFA:SFA and DFA:OFA ratios and in the indices of atherosclerosis (IA) and thrombosis (IT).
Replication. The year of the study caused clear differences in the cholesterol content of muscle tissue and the fatty acid profile of intramuscular fat of the kids studied. The muscles of kids from replication II contained more cholesterol than those from replication I (by 11.9%, P≤0.05). The intramuscular fat of kids from replication II was characterized by a clearly poorer fatty acid profile than that of kids from replication I. It contained significantly more of all saturated acids except stearic acid C18:0 (by a total of 10.7%, P≤0.01). In terms of UFA, there was a lower total content of these acids in kids from replication II (by a total of 6.2%, P≤0.01), with no significant differences in the content of MUFA and a significantly lower content of PUFA – by a total of 20.6%, P≤0.01.
These differences were reflected in the health quality parameters of the analysed fat, which were calculated from the composition of fatty acids (Table 3). These were definitely more favourable for kids from replication I. Compared to replication II, their fat was characterized by a significantly higher (P≤0.01) UFA:SFA, PUFA:SFA and DFA:OFA ratios, and by a higher Ω3 PUFA content, with a concurrently lower Ω6:Ω3 ratio and lower indices of atherosclerosis (IA) and thrombosis (IT).
It is worth noting a significantly higher CLA content of intramuscular fat in kids from replication II than in kids from replication I (by 38.3%, P≤0.01).

Discussion

Cholesterol. The cholesterol content of muscle tissue of the kids ranged from 60 to 80 mg/100 g of fresh tissue, which is considered normal for the meat of farm animals (BAROWICZ and JANIK 1998). However, the values obtained (71.2 mg on average) were higher than those reported by PARK et al. (1991) for the muscles of weaned alpine and Nubian kids (57.8 mg/100 g tissue on average) and by KALINOWSKA and PUSTKOWIAK (2000) for Polish White Improved and its crosses with Boer and Anglo-Nubian (58.6 mg/100 g tissue on average).
The higher cholesterol content in the muscles of 60- and 90-day-old kids compared to 180-day-old kids could result from the naturally more intense cholesterol synthesis in the bodies of the youngest animals, resulting from their physiological needs. This is confirmed by the studies of other authors with lambs (ARSENOS et al. 2000, BORYS et al. 2003). The lowest cholesterol content in the oldest kids could be due to the differences in nutrition. This is suggested by the results of studies cited by HANCZAKOWSKI et al. (2000), which indicate that the presence of animal protein in the diet (mother's milk or powdered milk in milk replacer) increases the cholesterol content of animal tissues.
The lack of effect of the analysed breed components and the concurrent significant

effect of replication on the cholesterol concentration in the muscle tissue of kids confirm the view that environmental factors have a greater and more uniform effect on the concentration of this undesirable component in products of animal origin (BORYS and PISULEWSKI, 2001).

Overall, the effect of the main experimental factors (age and breed) on the cholesterol concentration in the muscle tissue of kids was limited, while the significant differences depending on age of kids and replication resulted from natural physiological differences connected with the age of kids and variation in environmental conditions that were beyond our control.

Fatty acid profile. The changes in the fatty acid profile of intramuscular fat of kids were probably the result of the effects of different factors, above all the natural changes in the function of kid bodies related to age and differences in nutrition. The present study showed that as the kids grew older from 60 to 180 days, the content of total SFA, including mainly saturated acids (C16:0 and C18:0), did not change significantly. This is not confirmed by the findings of ZYGOYIANNIS et al. (1992, quoted after: BANSKALIEVA et al., 2000), which found a decrease in the proportion of stearic acid C18:0 in depot fat of suckling kids as they grew older.

In terms of UFA, there was a significant increase in the content of MUFA (including the dominant oleic acid C18:1) and a concurrent decrease in the content of all PUFA with the age of the kids. These findings are not confirmed by the studies of BAS et al. (1987, quoted after BANSKALIEVA et al., 2000), who observed a decrease in the MUFA content of subcutaneous fat in weaned kids with age.

The unfavourable changes in the health properties of meat of the analysed kids with age are indicated by changes in the content of Ω3 PUFA and in the proportions of PUFA:SFA and Ω6:Ω3 PUFA. Clearly better uniformity of the majority of the parameters analysed in the oldest kids (180 days old) than in younger kids is considered favourable; this seems to have been connected with the feeding of younger kids with feeds that had more uniform components than mothers' milk, which was the basic feed of the youngest kids.

The lack of comparable studies concerning the fatty acid profile of intramuscular fat of kids does not allow for a more comprehensive discussion of the results obtained. However, by analogy with the results of studies on other species of ruminants, e.g. lambs (BORYS and BORYS, 2001; CIURYK and KACZOR, 2001), it is concluded that from the health quality point of view, the increased age of slaughtered animals is accompanied by unfavourable changes in the fatty acid profile of their meat.

The present study showed a statistically significant effect of the crossbreeding scheme on the content of most fatty acids analysed in the intramuscular fat of fattened goats. Overall, compared to Saanen, the muscles of Anglo-Nubian × Saanen kids were characterized by a less favourable profile of fatty acids. This resulted mainly from the significantly lower effect of PUFA, including Ω3 PUFA (except C18:3), and the related deterioration in the PUFA:SFA and Ω6:Ω3 PUFA ratios in the crossbreds.

The unfavourable effect of crossbreeding with the Anglo-Nubian breed on the fatty acid composition of muscle tissue of the crossbreds is confirmed by the findings of KALINOWSKA and PUSTKOWIAK (2000). The intramuscular fat of Anglo-Nubian crossed with Polish White Improved was characterized by a markedly lower proportion of PUFA and less favourable PUFA:SFA ratio (by 29 and 38%, respectively). The available literature provides a small number of studies concerning the effect of kid

breed or crossbreeding scheme on the lipid profile of their muscle tissue. Comparison of the relevant data available for purebred goats in a review by BANSKALIEVA et al. (2000) indicates large differences in the lipid profile of intramuscular fat of kids of different breeds, but these results were obtained under different conditions and with fat extracted from different muscles.

The significant effect of replication on the content of most fatty acids in the intramuscular fat of kids, and the health quality parameters of muscle tissue calculated on this basis, show a significant effect of other factors (mainly environmental factors) that were beyond our control.

Relatively many, statistically significant first-degree interactions (mainly age × breed and breed × replication) point to the mutual effect of these factors in terms of the analysed lipid profile parameters of the muscle tissue of the kids.

In general, the present study found a certain effect of age on the cholesterol content of muscle tissue of the fattened kids (lower in 180- than in 60- and 90-day-old kids), with no differences depending on the breed component.

The fatty acid profile of intramuscular fat of the kids changed unfavourably with their age, mainly because of the increased MUFA content and decreased PUFA content (including Ω3 PUFA).

The Anglo-Nubian × Saanen crossbreeding had a generally unfavourable effect on the fatty acid profile of muscle tissue fatty acids of the crosses, resulting mainly from the lower content of PUFA, including Ω3 PUFA, and the less favourable PUFA:SFA and Ω6:Ω3 PUFA ratios.

Both experimental factors caused significant differences in the CLA content of intramuscular fat of the investigated kids. The higher content of this component was found in 90-day-old kids than in 60- and 180-day-old kids and in AN × S than in Saanen kids.

From the viewpoint of health-promoting quality, the meat of kids representing lower age and weight standards (60 and 90 days) had a generally more favourable lipid profile, while the effect of the analysed commercial crossbreeding scheme was fairly obvious but generally inconsistent.

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Effect of lactation on the hygiene quality and some milk physicochemical traits of the Wielkopolska mares

Abstract

The objective of this research project was to ascertain the hygiene quality and some physicochemical properties of milk obtained from mares of the Wielkopolska breed at different periods of lactation. Colostrum and milk for analyses were collected on days: 1, 2, 3, 4 and 5 of lactation and afterwards at 15-day intervals up to day 150 of lactation. A significant influence of lactation on levels of the examined traits was found. Depending on the period of lactation, titrable acidity was found to be at the level of 4.6ºSH (from 8.6 to 2.5), the mean temperature of milk freezing was –0.541ºC, whereas electrical conductivity fluctuated in the interval of 3.8-4.6 mS x cm^{-1}. The mean somatic cell count during the lactation period amounted to 62 x $10^3/cm^3$ and ranged from 194 x 10^3 on the first day of lactation to 41 x 10^3 during the last month of lactation. The total count of microorganisms remained within the interval from 33 x $10^3/cm^3$ to 51 x $10^3/cm^3$.

Key Words: mare, milk, somatic cells, total microorganism count, physicochemical traits.

Introduction

Milking mares to obtain milk for consumption purposes is practically, unknown in Poland (DANKÓW et al., 2006). On the other hand, relatively large number of horses in our country create favourable conditions for horse milk production and utilisation as a dietary supplement for senior citizens, convalescents and children allergic to cow milk. The chemical composition of mare milk is similar to human milk (MALACARNE et al., 2002), whereas the twice higher content of lysozyme in comparison with human milk explains the effect of milk on thrush, aphthae, diseases of the upper airways as well on the acceleration of healing of wounds and postoperational scars (SEIDL, 2002). In Germany, mare milk is available on the market either as fresh liquid, deep frozen (-18ºC), powder dried with the assistance of spray-drying, or freeze-drying and fermented drink (KUY, 1998; SCHUBERT et al., 2005). Due to its low casein and fat content, mare milk is unsuitable for cheese and butter production (KALLIALA et al., 1951; NEUHAUS, 1960). Poor stability of mare milk above the temperature of 40ºC (FOX and HOYNES, 1976) makes it necessary to cool it immediately after milking and consume it in a liquid form not later than 6 – 9 hours after milking (SCHEPPER, 1988). No standards concerning the quality of raw mare milk have been established in Poland so far. All attempts to sustain high hygiene quality of this milk aims at the protection of human health, maintenance of the natural biological value of the raw material and assurance of the proper course of technological parameters during its processing. The considerable headage of Wielkopolka horses as well as absence of data referring to the hygiene quality of milk obtained from them encouraged the authors to undertake investigations whose main goal was to determine the cytological and microbiological quality as well as some selected physicochemical properties of this milk depending on the lactation period.

Material and methods

Experiments were carried out on milk obtained from 10 mares of the Wielkopolska breed which foaled during the period from January to March 2005 in one of the horse breeding stations in the region of Wielkopolska (Western Poland). The age of the experimental mares ranged from 6 to 9 years. All the experimental animals were raised in the same environmental conditions and were fed in accordance with the nutritional requirements of lactating mares (CHACHUŁOWA, 2004). Colostrum was collected twice; the first time during the period of 18 to 24 hours after foaling and the second – from 42 to 48 hours *post-partum*. Milk for analyses was collected daily in the amount of 100-150 cm^3 on days: 2, 3, 4 and 5 of lactation and then at 15-day intervals until the 150^{th} day of lactation. Mares were milked always at the same time of the day, i.e. between 8 and 9 o'clock a.m. one hour after weaning the foals from their mothers. During milking, foals remained in the visual and tactile contact with their mothers.

The following parameters were determined in the collected samples of colostrum (n=20) and milk (n=130): titrable acidity in ºSH (POLISH STANDARD, 1968), electrical conductivity (CC-315 microcomputer conductometer), freezing temperature (Combi Foss 5000), number of somatic cells (Combi Foss 5000) as well as the total microbial count (Bacto Scan 8000S). The obtained results were subjected to a single-factorial analysis of variance using for this purpose the STATISTICA® (StatSoft® 2003) statistical package. In order to obtain the normal distribution of traits, before carrying out the statistical analysis, data characterising milk cytological and microbiological quality (number of somatic cells and total microbial count) were transformed with the assistance of the natural logarithm.

Results and discussion

Table 1 shows physicochemical parameters in the milk of the experimental mares. The analysis of the results of titrable acidity revealed that the highest ºSH value was recorded in the colostrum in which a slight declining tendency of the acidity was observed. It was found to be by 0.4ºSH lower as early as on the second day after foaling. A distinct decline of the ºSH value was also found in the examined milk with the passage of the lactation period. The difference between the 3^{rd} and 60^{th} day of lactation amounted to 3.4 ºSH. On the other hand, during the period when, as a rule, milk for human consumption is obtained, i.e. between day 75 until the end of the lactating period, this decline was not so dramatic and amounted to 0.5 ºSH. The results obtained by the authors differ from those reported by KULISA (1970) who found the acidity of milk obtained from Arabian mares on day 15 of lactation at the level of 3.5ºSH. This author reported a decline of the acidity to the level of 2.9ºSH up to day 75 followed by its increase to 3.6ºSH on day 150 of the lactation period.

Milk electrical conductivity revealed the highest value on day 1 of the lactation and this value declined by 0.5 $mS \times cm^{-1}$ up to day 5. During the period from day 15 to 120, the electrical conductivity of the examined milk remained on the same level and it decreased only during the period from day 135 to day 150, and this difference was statistically significant ($p<0.05$). The mean recorded electrical conductivity of mare milk during the lactation period amounted to 4.0 $mS \times cm^{-1}$. Results of our investigations coincide with those obtained by KUY (1998) who reported values of mare milk electrical conductivity ranging from 1.9- to 4.3 $mS \times cm^{-1}$.

Table1
Physicochemical traits of Wielkopolska mares milk

lactation (days)	titratable acidity (ºSH)	electrical conductivity ($mS \times cm^{-1}$)	freezing temperature (ºC)
1	8.6a	4.6a	- 0.556a
2	8.2b	4.4b	- 0.551b
1-2	8.4	4.5	-0 0.553
3	7.6e	4.2b	- 0.547b
4	7.2e	4.2b	- 0.544ab
5	6.4d	4.1b	- 0.542a
15	4.5c	4.2b	- 0.540c
30	3.7b	4.2b	- 0.541c
45	3.5b	4.1b	- 0.540c
60	3.2ab	4.0b	- 0.539bc
75	3.1ab	3.9 b	- 0.538b
90	3.1ab	4.0b	- 0.540c
105	3.0ab	4.1b	- 0.537b
120	2.8a	4.0b	- 0.536b
135	2.7a	3.7a	- 0.536b
150	2.5a	3.7a	- 0.532a
3-60	5.2a	4.1	- 0.542a
75-150	2.9b	3.9	- 0.537b

a-e different small letters in columns differ at $p<0.05$

The values of the freezing temperature were also found the highest on the first day of lactation. Up to day 5 of lactation, the freezing point decreased by 0.014ºC (difference statistically significant). On the other hand, from day 15 to day 150, the freezing temperature showed a declining tendency. The mean freezing temperature for the 150-day lactation period was at the level of -0.541ºC and was by -0.008ºC higher than that reported by KUY (1998).

Table 2
Somatic cell count in mares milk during lactation

Lactation (days)	Somatic cell count ($10^3/cm^3$)			
	$\overline{X}$	max	min	$Log_e SCC$
1	194	269	124	12,15a
2	79	94	48	11,25b
1-2	136	269	48	11,81
3	75	92	42	11,21c
4	50	75	31	10,79b
5	62	96	42	11,03b
15	59	84	41	10.98c
30	49	57	39	10,79b
45	59	79	27	10,98c
60	50	70	38	10,79b
75	36	44	27	10,47a
90	38	56	28	10,52a
105	41	57	32	10,61a
120	43	51	35	10,66a
135	47	55	36	10,75b
150	41	54	33	10,61a
3-60	58	96	27	10,96a
75-150	41	57	27	10,61b

a-c different small letters in columns are differ at $p<0.05$

On the basis of the analysed results of measurements of numbers of somatic cells during the period of 150-day lactation period (Tab. 2), it was concluded that mare milk is characterised by a considerably lower number of these cells than the milk derived

from the healthy udder of a cow (about 100 x10^3/cm^3), goat (about 1 x10^6/cm^3) and sheep (about. 300 x 10^3/cm^3, DANKÓW et al., 2003). The highest mean number of these cells was recorded on day 1 after foaling - 194 x 10^3/cm^3 with the smallest quantity of 124 x 10^3/cm^3 and maximum - 269 x 10^3/cm^3. The above value is significantly lower than that reported by RIELAND (1997) which reached 377 x 10^3/cm^3. The mean level of somatic cells in our studies recorded during the first 5 days of lactation was at the level of 92 x 10^3/cm^3 (max 125 x 10^3/cm^3, min 57 x 10^3/cm^3). The mean number of somatic cells (NSC) in the period from day 15 to 150 was 46 x 10^3/cm^3 (max 61 x10^3/cm^3, min 34 x 10^3/cm^3), of which in 62% of the examined samples the NSC was below 50 x 10^3/cm^3 and only in 38% - the NSC ranged from 50 - 100 x 10^3/cm^3. It is also worth emphasising that the cytological milk quality was statistically significantly higher during the period of the potential commercial milk production (from day 75 of lactation) in comparison with the milk obtained during the first 10 weeks after foaling ($p<0.05$; Tab. 2).

Investigating the NSC in the milk of mares, KUY (1998) reported the mean content of somatic cells at the level of 39 x 10^3/cm^3 (max 436 x 10^3/cm^3, min 2 x 10^3/cm^3), whereas the proportion of milk in which the number of these cells was below 50 x 10^3/cm^3 - at 83.2%. Other researchers give different somatic cell counts in milk, for example OKADA (1960) quoted the content of somatic cells at the level of 100-300 x 10^3/cm, REMBALSKI (1979), on average – 7.5 x 10^3/cm^3 (from 1 to 31 x 10^3/cm^3), MANTEUFEL (1989) 16 x 10^3/cm^3 and REILAND (1997) from 17-52 x 10^3/cm^3. WENDT et al. (1994) maintain that the reported low NSC in mare milk is the result of the hidden udder of mares and, hence, smaller exposure to injury and infection associated with it and, additionally, the frequent emptying of the udder by the suckling foal, even up to 60 times per day.

Analysing the results of total microorganism counts (TMC) in milk samples collected during the 150-day lactation period, their quantities were very small indicating a very good health condition of the udder and hygiene of milking (Figure). The mean microbial count during the first 5 days of milking was at the level of 46 x 10^3/cm^3 (max 54 x 10^3/cm^3, min 42 x 10^3/cm^3), whereas during the period from day 15-150, it reached 37 x 10^3/cm^3 ($p<0.05$). During the above period, 60% of samples was characterised by the TBC of below 40 x 10^3/cm^3. Even lower numbers of microorganisms, namely about 35 x 10^3/cm^3 were determined during the period when mares are commonly milked, i.e. from week 8 to 10 of lactation. Such low content of microorganisms allows drinking mare milk without pasteurisation. MANTEUFEL (1989) maintains that the mean total bacterial count in the mares milk corresponds to their numbers in cows pasteurised milk. According to RIELAND (1997), this should be attributed to the fact that mare milk is exceptionally rich, much richer than in other types of milk, in lysozyme – enzyme possessing antibacterial properties. In his experiment, KUY (1998) found mean TMC at the level of 28.5 x 10^3/cm^3, with 8.2% of samples with TMC exceeding 50 x 10^3/cm^3, while in 90.2% of the samples the TMC was found to be below 30 x 10^3/cm^3. Other researchers give contradictory information about the TMC in the mare's milk. According to STROCH (1985), the TMC in pooled milk amounted to 4.015 x 10^3/cm^3, NEUHAUS (1960) gives the value of 100 bacteria to 10 x 10^3 /cm^3, REMBALSKI (1979) - from 8.5 to 41.4 x 10^3/cm^3 and MANTEUFEL (1989) -5.9 x 10^3/cm^3.

Recapitulating, it should be said that the period of the mare's lactation exerts influence on the physicochemical parameters of milk. In comparison with the milk of other mammals, the milk of mares is characterised by a very low content of somatic cells and bacteria. This problem, especially, the determination of the types of bacteria occurring in this milk requires further investigations.

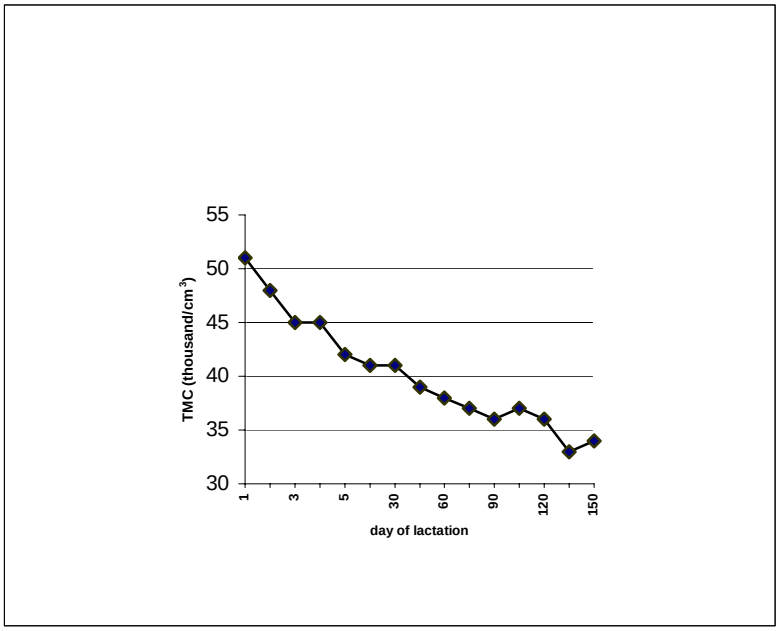

Figure: Total microorganism count in investigated mares milk

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Technological usefulness of milk from two local breeds maintained in the regions with great grassland share

Abstract

The objective of the present work was to evaluate the chemical composition and technological usefulness of milk obtained from the cows of two local breeds (Simental and Whiteback) kept in the regions of considerable grassland share. The control group was constituted by the milk gained from the cows of Polish Holstein-Frisian breed black-white variety maintained in the farms aimed at the intensive milk production. The investigations covered 657 milk samples, in that 296 in the spring-summer period and 361 in the autumn-winter season. From the Whitebacks, total 118 samples were collected, from Simentals 173 and Polish Holstein- Frisian black-white variety - 366. During cows selection efforts were made that in each of the evaluated breed there were animals in 2nd stage of lactation i.e. between 101 and 200 days, and at similar age. In each sample there was determined a content of fat, protein, casein, lactose and dry mass. Besides, milk acidity (pH and °SH), fat molecules content in each value interval, milk thermostability at 140°C temperature and rennet induced milk coagulation were established. It was found that only the milk from Whiteback breed cows showed in both seasons high content of big-sized fat molecules (>10μm),i.e. 9.72% in the spring-summer period and 10.43% in the autumn-winter season. In the milk from two other breeds, some substantial differences between two periods were recorded, that is Simental – 5.03% and 13.8%, while black-white – 2.35% and 13.61%. A beneficial effect of pasture feeding on curd formation rate was stated. In milk of the grazing cows, i.e. Whiteback and Simental, it appeared to be much shorter (average 5.18 and 5.43 min) as compared to milk from black-white cows (7.06 min) that were maintained in the alcove system. The obtained results indicate that in the regions of high natural values with a substantial grassland share the local cattle breeds should be maintained as these cows will produce milk of the advantageous chemical composition and appropriate technological usefulness.

Key Words: local breeds, milk, chemical composition, technological usefulness

Introduction

Having used up the limits of milk purchase (milk amount) in the UE member states and in Poland, the dairy industry takes an interest in a material of higher technological parameters. LITWIŃCZUK & SZULC (2005) claim that some breeds of cows yield material more useful for the concentrates production, i.e. condensed milk, consumable milk and UHT, whereas some other breeds for cheese or butter making. The limits imposed on the milk production in the UE make the producers search for some new solutions that could assure higher profits (at the same quantity of delivered material) resulting from among others, more advantageous chemical composition and higher technological usefulness of milk. One of such solutions is likely to be the better use of the local cattle breeds (LITWIŃCZUK & SZULC, 2005; BARŁOWSKA et al., 2005). Moreover, the appropriate nutrition may (to some extent) affect the chemical composition of milk, change its technological parameters as well as the content of the biologically active substances (REKLEWSKA et al., 2004; TYRISEVA et al., 2004).

Most of the recent studies (DZIUBA et al., 1999; CZAPLICKA et al., 2002) concerning the evaluation of the milk technological usability have been performed in Poland and dealt with the black-white cows with a varied genes share of the hf cattle. However, there are only few works treating this problem in other dairy pedigrees, whose percentage in Poland is low, yet considerable in the milk production in some regions (BARŁOWSKA et al., 2006).

The animals of these breeds adapted to the existing environmental conditions and are generally recognized as the local pedigrees (LITWIŃCZUK & SZULC, 2005). The regions where the local breeds are used are predominantly those of rich natural values, characterized with a substantial grassland share. Yet, it often happens that the land configuration appears to be unpropitious to the intensive milk production.
The studies performed lately (PISULEWSKI et al., 2001; REKLEWSKA et al., 2004) demonstrate that milk gained from grazing cows usually contains more biologically active substances. Still, there is scarcity of works treating the evaluation of technological usefulness of milk from cows of the local breeds together with the specificity of their nutrition.
The present paper aims at the evaluation of the chemical composition and technological usefulness of milk obtained from cows of two local breeds (Simental and Whiteback) maintained in the regions of high grassland share subject to a production season. The evaluations were referred to the control group constituted by the milk of the Polish Holstein - Frisian breed, black-white variety.

Material and Methods

The investigations covered the milk collected from the cows of two local breeds (Whiteback and Simental) kept in the farms situated in the regions of high grassland share. The control group was made by the milk obtained from the cows of the Polish Holstein-Frisian breed, black-white variety maintained in the farms engaged in the intensive milk production. The Whitebacks is an old native Polish breed that survived in the eastern Poland, mainly in Polesie, in the Bug and Biebrza river-basins (LITWIŃCZUK et al., 2004). The cows of this breed included in the studies were usually maintained in the small farms in which a number of milk cows don't exceed 10 units. Simental breed prevails in the Bieszczady region and the cows included in the investigations were kept in the farms with tens of animals each (20-40 most often).
The studies were performed at two production seasons (connected with different feeding system), i.e. spring-summer and autumn-winter. Over the spring-summer period the Whitebacks fed mainly green fodder composed of grasses and legumes supplemented with hay or straw. During the autumn-winter season the animals were supplied with mainly hay, fodder beets or potatoes. The Simentals grazed in the spring-summer period predominantly, yet in some farms they fed hay silage as well. Whereas over the autumn-winter season the animals consumed mainly hay silage and maize silage. The feeding of the Polish hf cows was based on (regardless a season) silage from maize, hay silage and possible additive of green fodder in some farms (at spring-summer season). In all the farms the dietary units were supplemented with a nutritive feed.
The investigations covered total 657 milk samples, in that 296 in the spring-summer season and 361 in the autumn-winter. From the Whitebacks total 118 milk samples were collected (39 – spring-summer and 79 – autumn-winter, from Simental 173 (55 – spring-summer and 118 – autumn-winter) and from the Polish Holstein-Friesian black-white variety 366 (202 – spring-summer and 164 – autumn-winter). The milk samples for analysis were collected, if it was possible, from the same cows throughout the experimental period, that is the spring-summer (V-VII) and autumn-winter (XI-III). During cows selection efforts were made that in each of the evaluated breed, there

were animals in 2nd stage of lactation i.e. between 101 and 200 days, and in similar age. In each milk sample, the chemical composition was determined, i.e. a content of fat, protein, lactose and dry matter with Milko-Scan 104 equipment. A casein content was established after the Walker method in compliance with PN-68/A-86122. There was also fixed milk acidity (pH and °SH) according to PN-68/A-86122 and fat molecules content in each values interval according to PN-75/A-86059. Milk thermostability at 140°C temp. (in the oil bath TEWES-BIS) and coagulation rate of milk induced by rennet (the Scherz method) were determined after the methodology described by JURCZAK (1999). The mentioned above analyses were made only in those milk samples, where the somatic cells count did not surpass 440 th/ml using Somacount 150 apparatus.

The obtained results were analysed statistically with StatSoft programme Inc. STATISTICA ver.6, on the grounds of two-factor variance analysis with interaction, giving the mean values and standard deviation. The difference significance was determined by the NIR Fischer test.

Results

From the data presented in Table 1 it follows that the mean milk performance of cows in the spring-summer period was higher by 1.9 kg with a protein content ($P \leq 0.05$) higher by 0.07% as compared to the autumn-winter season.

Table 1
Efficiency and chemical composition of cow milk in relation to a breed and production system

Specification		n		Daily efficiency (kg)	Content (%)					P/F#
					fat	protein	casein	lactose	dry matter	
Season	spring-summer	269	$\bar{x}$	18.8	4.36	3.52*	2.59	4.85	13.34	0.83
			SD	7.23	0.91	0.47	0.36	0.23	1.23	0.20
	autumn-winter	361	$\bar{x}$	16.9	4.38	3.45*	2.58	4.81	13.35	0.83
			SD	7.62	1.06	0.40	0.41	0.34	1.16	0.16
Breed	Whitebacks	118	$\bar{x}$	14.3^{A}	4.60^{c}	3.54	2.53^{a}	4.70^{A}	13.49	0.80^{A}
			SD	5.76	1.02	0.45	0.34	0.33	1.22	0.18
	Simental	173	$\bar{x}$	19.8^{B}	4.20^{a}	3.53	2.56^{ab}	4.85^{B}	13.23	0.87^{B}
			SD	7.37	0.85	0.47	0.40	0.30	0.98	0.19
	Polish Holstein-Frisian black-white variety	366	$\bar{x}$	22.2^{B}	4.38^{b}	3.47	2.62^{b}	4.86^{B}	13.36	0.82^{A}
			SD	7.68	1.00	0.42	0.40	0.28	1.26	0.17
	interaction season x breed			xx	xx	x	xx	xx	xx	xx

* - differences between seasons, * - differences significant at P≤0.05; a, b – differences between breeds;
a, b, c – differences significant at P≤0.05; A,B – differences significant at P≤0.01;
interactions: xx – at P≤0.01; x – at P≤0.05
- protein-fat ratio

The analysis of the basic indices of milk technological usefulness (Tab. 2) shows that at the autumn-winter period the cows produced milk of a significantly lower content (by 7.41%) of small-sized fat molecules (<6um) and higher (nearly twice) content of big-sized (>10μm).Milk gained during the autumn-winter feeding was characterized

with a longer thermostability time, that is 5.48 min as against the spring-summer season – 3.15 min (P≤0.01). Milk produced in the spring-summer period, however, exhibited more advantageous indices concerning curd formation time (P≤0.01).
The data given in Table 2 demonstrate that milk of cows of Whiteback and Simental breeds was characterized with a significantly higher (P≤0.01) content of big-sized fat molecules (10.2 – 10.4%) as compared to milk of black-white cows (7.68%). The milk from these cows showed a shorter time of the rennet induced coagulation (5.54 and 6.18 min), whereas in the black-white cows` milk this process occurred after 8 minutes (P≤0.01). It should be emphasized that the milk of Whitebacks obtained the best parameters of thermostability, that is, it proved resistant to the sustained thermal treatment at 140°C (5.34 min). The lowest thermostability, though, was recorded for the Simentals` milk (4.08 min). It was likely to arise from the highest acidity determined in this milk (Tab. 2).

Table 2
Technological usefulness indices of cow milk subject to a breed and season

Specification		n		acidity		Fat molecule share (%)			Thermal stability (min)	Clotting time (min)
				pH	°SH	small (<6μm)	medium (6 – 10μm)	big (>10 μm)		
Season	spring-summer	269	$\bar{x}$	6.69	7.12	68.65**	25.34	6.09**	3.15**	6.37**
			SD	0.17	0.80	6.27	6.54	3.83	1.53	4.01
	autumn-winter	361	$\bar{x}$	6.68	7.26	61.24**	26.47	11.93**	5.48**	7.48**
			SD	0.11	0.96	11.26	8.66	6.71	2.29	4.19
Breed	Whitebacks	118	$\bar{x}$	6.71^{B}	6.98^{A}	64.33	24.98	10.20^{b}	5.34^{C}	5.54^{A}
			SD	0.11	0.89	10.08	9.09	5.08	2.32	3.05
	Simental	173	$\bar{x}$	6.63^{A}	7.44^{B}	62.83	26.94	10.45^{b}	4.08^{A}	6.18^{A}
			SD	0.17	0.89	11.93	7.57	7.81	2.11	3.39
	Polish Holstein-Frisian black-white variety	366	$\bar{x}$	6.70^{B}	7.15^{A}	65.12	27.18	7.68^{a}	4.36^{B}	8.09^{B}
			SD	0.12	0.87	8.60	3.80	6.93	2.40	4.34
interaction season x breed				xx	x	xx	x	xx	xx	ns

*, ** - differences between seasons, * - differences significant at P≤0.05; **– differences significant at P≤0.01;
a, b – differences between breeds; a, b – differences significant at P≤0.05; A,B, C – differences significant at P≤0.01; interactions: ^{xx} – at P≤0.01; ^{x} – at P≤0.05; ns – no statistic

The analysis on the simultaneous effect of two factors, i.e. a cow breed and season, on milk performance and its chemical composition (Tab. 1) revealed some significant interactions (P≤0.01) between all the considered parameters, except for a protein content, where the significance level was P≤0.05. A similar analysis performed for the indices of milk technological usefulness, some significant interactions were reported for the active acidity (pH), small- and big-sized fat molecules and milk thermostability (P≤0.01). Besides, the significant dependences (P≤0.05) were stated for the milk potential acidity and a content of fat molecules of 6-10 μm diameter. No significant interaction was recorded between a season and cow breed and the curd formation rate (Tab. 2).

Discussion

The results from our studies regarding the chemical composition of milk from three analyzed cow breeds, i.e. Whiteback, Simental and Polish hf, are in conformity with those mentioned by other authors (BARŁOWSKA et al., 2006), who proved that milk gained from the local pedigrees usually showed a higher concentration of the basic components. As far as Simental breed is concerned, the most beneficial protein-fat ratio was recorded (in the present investigations as well).

The measurable indices determining the technological milk usability for cheese making appear to be the curd formation rate and dispersion level of fat molecules. According to WIKING et al. (2004) and ZIAJKA (1997) milk of a high content of big-sized fat molecules proves more useful for the cheese and butter production. Our investigations revealed that milk gained over the autumn-winter season contained nearly twice as large molecules of over 10 μm diameter. GREGA et al. (2000) evaluating the milk obtained from Simentals showed a slightly higher content of big-sized fat molecules (by 0.06%) in the summer season than in the winter.

The recognized significantly higher (by ca 3%) fat molecules content in the milk of the Whiteback and Simental breed (as compared to milk from the Polish hf breed black-white variety cows) confirm the results obtained by other authors (BARŁOWSKA et al., 2006) made on the milk collected (from the cows of these pedigrees) only over the spring-summer. A content of big-sized fat molecules was 9.49% - Whiteback, 5.37% - Simental and 2.96% - Polish hf black-white variety. CZERNIEWICZ et al. (2006) analyzing the dispersion of milk fat of cows Holstein-Frisian and jersey showed that mean diameter of fat molecules reached 6.19 and 7.68 μm, respectively.

A main index determining the usability of milk for cheese making is still the curd formation rate induced by rennet. MISTRY et al. (2002) combine the rate of rennet coagulation with the amount of milk protein and casein and micelles size. It is assumed that the appropriate milk coagulation time should last 4 – 10 minutes. A sustained curd forming time implies that the milk may have come from a diseased udder. TYRISEVA et al. (2003) claim that milk from the cow heifers undergoes the enzymatic coagulation readily at the lactation outset and end as well as those fed green fodder. DZIUBA et al. (1999) assessing the rennet induced coagulability of milk from hf cows imported from France and Germany and Polish black-white obtained the values 2.24 - 2.37 min for this index. A similar analysis made by CZAPLICKA et al. (2002) revealed that the milk obtained from hf cows got coagulated after 2.39 min, whereas that produced by black-white after 2.46 min.

DZIUBA (1984) reports that milk thermostability is affected by the total casein content, that is a κ-casein level. According to this author, the increased rennet induced coagulation time brings about the milk thermostability decrease. This decline also depends on numerous factors, in that the changes resulting in milk pH drop. Our studies exhibited that a longer rennet coagulation time was connected with lower milk thermostability. Milk of the Whitebacks was characterized with the best (the longest) thermostability and the shortest time of the enzymatic coagulation.

It is noteworthy that solely the milk from Whitebacks in both seasons showed a high content of big-sized fat molecules (>10 μm), i.e. 9.72% during spring-summer and 10.43% in autumn-winter. In the milk of two other breeds there were stated substantial differences concerning this index between the seasons, that is Simental – 5.03% and 13.87%, whereas black-white – 2.35% and 13.61%. It is likely to arise from the

genetic conditioning of the Whitebacks for this index. This fact has been confirmed to some extent by the studies conducted by ŻURKOWSKI et al. (2004) where 24 loci of the DNA microsatellites were analyzed and about 50 alleles more in the Whitebacks were detected compared to Black-White breed. This hypothesis needs some further studies.

It should be also emphasized that pasture grazing exerts a beneficial effect on the value of the main index determining usability of milk for cheese production, that is a coagulation rate. In milk of cows of both grazing breeds, i.e. Whiteback and Simental it appeared to be remarkably shorter (average 5.18 and 5.43 min) than milk of black-white cows (7.06 min), that were maintained in the alcove system ($P \leq 0.01$).

Summing up, the present authors` studies showed that in the regions of high natural values, with a substantial share of grasslands the local cattle breeds should be maintained because the cows grazing the regional pastures (mainly in summer) will yield milk of the beneficial chemical composition and very high technological usefulness.

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Effect of packaging systems on the quality and shelf-life of the Rokpol type mould cheese from goat milk

Abstract

The material for experiments comprised 4 batches of the Rokpol type mould cheese manufactured at 25-day intervals. The raw material used to manufacture the cheese was goat milk of extra class (TBC - total bacterial count – less than 100 x 10^3/cm^3 and number of somatic cells - 400 x 10^3/cm^3). The experimental Rokpol cheese was packed in aluminium foil as well as in modified atmosphere and in vacuum. The packaging material used to pack cheeses in the modified atmosphere was a barrier foil (oriented polyethylene polyamide). The performed microbiological investigations revealed that, in the case of packaging of the experimental cheese in aluminium foil, proliferation of the *coli* type of bacteria occurred. In the remaining cheeses packed in the modified atmosphere, the number of bacteria from the *coli* group remained stable from the moment of their production until the end of their shelf-life, irrespective of the type of the applied gas mixture. The performed organoleptic evaluation showed that cheeses packed in the aluminium foil were characterised by highest consumer overall acceptability.

Key Words: goat milk, mould cheese, packaging systems, quality

Introduction

Rokpol belongs to rennet, soft, ripening cheeses and it can be manufactured from both cows' and goats' milk (BOYAZOGLU et al., 2001). This type of cheese is characterised by the occurrence in its entire volume of bluish-green veins and marbling which is caused by the fruiting vegetation of the penicillium, *Penicillium roqueforti*. The mould which is developing in the entire volume of these cheeses gives the ripe cheeses their characteristic sharp and piquant taste (RUBINO et al., 1999). The majority of mould cheeses are manufactured in France but, due to their exceptional taste value mould cheeses, they became popular all over the world (TEUBNER et al., 1997). Also in Poland, the interest in ripening cheeses has been growing in recent years and, quite naturally, this growing interest has been accompanied by increasing production. At present, Rokpol types of cheeses with blue (Lazur), green (Turcus), white (Pełrowy) and black (Aksamit) moulds are manufactured in two dairy plants in Poland.

For many years, the majority of soft mould cheeses have been packed in aluminium foil which can easily be damaged. The ever-changing life-style gathering pace in recent years has forced manufacturers to offer their customers "easy" food. This refers, among others, to the ease of opening and the method of serving cheeses, re-packing them with the aim for further storage as well as ensuring their high quality standards during the extended shelf-life. In order to meet the expectations of the consumers, manufacturers and distributors new food packaging techniques are introduced, for example vacuum packaging or packaging in modified atmosphere.

Vacuum packaging consists in the removal of air from the package prior to its sealing. This system of packaging requires absolute air tightness of the packaging which should provide appropriate barrier in relation to oxygen. Even so, carbon dioxide is generated inside the package by developing anaerobic microorganisms but also as a

result of respiration processes taking place in the product (SUBRAMANIAM, 1998; PIKUL, 2003, 2005). So far, this type of packaging has been employed to pack raw meat, sausages, hard cheeses and cottage cheeses.
In the case of packaging in the modified atmosphere, the air from the package is replaced by a mixture of O_2, CO_2 and N_2 gases whose composition is determined in the course of packing. The employed mixture should delay chemical putrefaction processes and inhibit the development of bacteria responsible to putrefaction ((FIK, 1995). Despite the fact that the initial atmosphere composition undergoes continuous changes during storage, as a result of respiration processes, biochemical transformations in the food itself as well as slow permeation of gases through the packaging material, nevertheless these changes do not usually have a significant impact on the food quality within well-defined and prolonged shelf-life of the products.
The aim of this research project was to determine the effect of the storage time and the system of packaging on the physicochemical, microbiological and organoleptic changes in the Rokpol type of cheese manufactured from goat milk.

Material and methods

The experimental material included four batches of mould cheese of the Rokpol type manufactured at 25-day intervals by the "Lazur" Dairy Cooperative in Nowe Skalmierzyce. The raw material used to manufacture cheeses was goat milk of extra class (TBC - total bacterial count – less than 100 x $10^3/cm^3$ and NSC – number of somatic cells - 400 x $10^3/cm^3$). The experimental Rokpol cheese was packed in aluminium foil in a modified atmosphere with the following proportions of gases: 100%N_2; 30%CO_2 and 70%N_2; 50%CO_2 and 50%N_2; 70%CO_2 and 30%N_2; 100%CO_2; 30%CO_2, 60%N_2 and 10%O_2; 70%CO_2, 20%N_2 and 10%O_2 as well as in vacuum.
The packaging material employed to pack the experimental cheeses in the modified atmosphere was barrier foil (oriented polyethylene polyamide) which was characterised by the following permeability for the employed gases: O_2 – 16-19 $cm^3/m^2/24h$; CO_2 – 100-130 $cm^3/m^2/24h$; N_2 – 3-5 $cm^3/m^2/24h$; water vapour – 2-3$g/m^2/24h$.
Cheeses packed in aluminum foil and using the modified atmosphere were stored at the temperature of approximately 4ºC for the period of 50 days. Appropriate analyses were carried out directly after the period of ripening and on day 25 and 50 of their storage (shelf-life – 45 days). The following parameters were determined: active acidity, titrable acidity , water content, protein and fat (POLISH STANDARD 1973). Microbiological investigations included the determination of bacteria from the *coli* group (POLISH STANDARD 1993a), coagulase positive staphylococci (POLISH STANDARD 1993b) and Salmonella (POLISH STANDARD 1993c). The performed organoleptic assessment included such indices as: colour, taste, smell, consistency and overall desirability. On the basis of the results of the performed sensory evaluation carried out on the 1st and 2nd batches of the cheese, vacuum packaging as well as the following gas mixtures were rejected: 100%N_2; 100%CO_2; 50%CO_2 and 50%N_2; 30%CO_2 and 70%N_2. The performed evaluation employed a linear 10 cm scale with the following boundary designations: for the colour – "white - creamy", for the taste – "piquant, mushroom – sour, mild, light bitter – strongly bitter", for the smell -"typical - strange", for the consistency – "soft, slightly short - too soft, too short, hard". The

assessment was carried out by a 6-person trained panel. The obtained results were subjected to statistical analysis using for this purpose the Statistica 6.0 PL program (STATSOFT® 2003) and the two-factorial analysis of variance in two replications was conducted. The variability factors included: storage time and packaging method.

Results and discussion

The Table present to comparison of the composition of selected physicochemical indices in Rokpol cheeses packed in the aluminium foil and with the participation of modified atmosphere during their cold storage.

Analysing the active acidity of cheeses packed in the aluminium foil the pH value was found to increase from 6.65 directly after the period of ripening to 6.84 and 7.21, respectively after 25 and 50 days of storage. During the cold storage of the same cheeses which were packed in modified atmosphere, the pH value after 25-day storage was found to drop and after 50 days to increase irrespective of the composition of the employed gas mixture. In the case of the cheese batch packed in the atmosphere of 70% CO_2 and 30%N_2, the pH value after 25 days of storage decreased from 6.65 to 5.84 and then, after 50 days, increased to 5.93. Similarly, the pH value in the cheese packed in the atmosphere containing 10% O_2, 70% CO_2, 20% N_2 decreased after 25 days of storage from 6.65 to 5.68 and after 50 days it increased to 5.70. Finally, in the case of the cheese packed in the atmosphere containing 10% O_2, 30% CO_2, 60%N_2, the pH value declined from 6.65 to 5.62 after 25 days of storage and later increased to 5.95.

Table
Effect of packaging systems on some physicochemical traits of Rokpol type mould cheese during refrigerated storage

Systems of packaging		Time of refrigerated storage (days)	Active acidity (pH)	Titratable acidity (^oSH)	Water (%)	Protein (%)	Fat (%)
Directly before packaging		0	6.65 c	43.0 c	39.21 b	17.42 a	38.61 a
Wrapped in aluminium foil		25	6.84 d	38.7 b	38.51 b	17.61 a	38.92 a
Modified atmosphere	70% CO_2 30% N_2		5.84 b	58.5 d	39.16 b	17.52 a	38.59 a
	10% O_2 70% CO_2 20% N_2		5.68 a	59.0 de	39.07 b	17.38 a	38.65 a
	10% O_2 30% CO_2 60% N_2		5.62 a	61.7 e	38.95 b	17.45 a	38.72 a
Wrapped in aluminium foil		50	7.21 e	31.8 a	37.81 a	17.72 a	39.16 a
Modified atmosphere	70% CO_2 30% N_2		5.93 b	56.9 d	38.82 b	17.55 a	38.72 a
	10% O_2 70% CO_2 20% N_2		5.70 a	56.5 d	38.81 b	17.46 a	38.85 a
	10% O_2 30% CO_2 60% N_2		5.95 b	61.4 e	38.75 b	17.52 a	38.90 a

a-e different letters in the column represent statistically significant differences at the level of p=0.05

In the case of the cheese packed in the aluminium foil, with the passage of storage time, a systematic decline of the total acidity was observed from 43.0ºSH recorded directly after the ripening process to 38.7ºSH and 31.8ºSH after 25 and 50 days of storage, respectively. In the case of the cheese packed in the modified atmosphere, irrespective of the applied gas mixture, the authors observed an increase of the titrable acidity on day 25 of storage and its decline on day 50. In the cheese packed in the atmosphere of 70%CO_2 and 30%N_2; 10%O_2, 70%CO_2 and 20%N_2; 10%O_2, 30%CO_2 and 60%N_2 titrable acidity on the 25th day of storage was determined at the level of 58.5ºSH; 59.0ºSH; 61.7ºSH, whereas on the 50th day of investigations – at 56.9ºSH; 56.5ºSH and 61.4^{o}SH.

As the time of storage of the cheeses packed in the aluminium foil and in modified atmosphere passed, the recorded decrease in the water content was only slight. The highest loss of the content of water was observed in the cheese packed in the aluminium foil and it dropped from 39.21% directly after ripening to 37.81% following 50 days of storage. The percentage proportion of protein and fat during 50 days of storage changed only slightly and appeared to be independent of the packaging system, ranging from 17.42% for protein and 38,61% for fat determined directly after the ripening process to 17.72% for protein and 39.16% for fat on day 50 of storage.

Analysing the results of microbiological investigations conducted directly after ripening and on the 50th day of storage, only a slight increase in the amount of the *Coli* type of bacteria was found (from 10 cfu/g to 14 cfu/g) in the aluminium foil packed cheeses. On the other hand, in the case cheeses packed in the modified atmosphere, the count of C*oli* bacteria remained on the same level throughout the experiment.

Bacteria from the *coli* group, which are treated as an indicator of the product hygiene quality, indicate the ineffectiveness of the pasteurisation of the raw material or secondary contamination (STEINKA and PRZYBYTOWSKI, 1998).

The number of bacteria from the genus of *Staphylococcus aureus* remained on the initial level (below 10 cfu/g) both in the case of cheeses packed in the aluminium foil and in the modified atmosphere. No *Salmonella* were found in 25 g samples of the examined cheese.

The performed organoleptic evaluation of the Rokpol type of cheeses packed in aluminium foil and modified atmosphere it was found that, both at the initial and final stages of storage, cheeses packed in the aluminium foil were given the highest general scores and scores for their overall desirability. Cheeses packed in the modified atmosphere received higher scores only for their colour.

Summing up of results and conclusions

1. Storage time and the applied packaging system exert a significant influence on active and titrable acidity.
2. Water percent content in cheeses was significantly influenced by the packaging system, whereas the storage time failed to have a significant influence on this parameter.
3. No significant impact of the storage time and packaging system was found on the percentage content of protein and fat.
4. The performed microbiological studies proved that in the course of storage bacteria from the *Coli* group proliferated in the cheese packed in the aluminium foil. In the case of the remaining cheeses packed with the participation of modified atmosphere, irrespective of the applied gas mixture, the number of the

Coli bacteria remained on the same level from the moment of their production until the end of the storage time.

5. The performed organoleptic evaluation revealed higher overall acceptably of cheeses packed in the aluminium foil,
6. An attempt to replace the traditional aluminium foil to pack soft mould cheeses by packing them in the atmosphere of modified gasses need more investigations.

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Effects of electromagnetic fields on the meat performance and wool performance of sheep

Abstract

The effects of pulsed electromagnetic fields (PEMF) on ewes were determined in two experiments. The ewes were exposed to the fields twice daily, for 12 minutes, in a pen covered with flat coils generating a PEMF at a frequency of 33 Hz, with magnetic induction ranging from about 3.5 μT at the floor to about 92 μT on the surface of the coils. Experiment 1 was performed on ewes aged 4 to 9 months. The following parameters were analysed: body weight, daily gains, cross-section measurements of the dorsal muscle (*musculus longissimus dorsi* - m. l. d.), thickness of the fat layer over the loin eye, greasy wool production, staple length, staple thickness and staple strength, as well as hematological and biochemical blood indices and acid-base equilibrium parameters. Experiment 2 was conducted on adult ewes during 70-day lactation, and their wool performance was determined, like in experiment 1. It was found that PEMF had a significant and positive effect on the body weights and daily gains of growing ewes, and on the depth and cross-section area of m. l. d. This could be a consequence of the stabilizing effect of PEMF on metabolic processes, as suggested by lower AST activity, lower concentrations of creatinine and urea, as well as increased oxygen supply to cells confirmed by a lower oxygen saturation percentage (O_2SAT). No direct correlations were found between exposure to PEMF and wool performance, staple length and wool quality traits.

Key Words: sheep, electromagnetic fields, meat performance, wool performance, blood parameters.

Introduction

External pulsed electromagnetic fields (PEMF) affect the biological functions of the body (BASSET, 1993; KAFKA, 1998, 2000, 2001; MICHAELIS, 1999). The biological response is evoked particularly by pulsed electromagnetic fields whose frequency and induction resemble those of the Earth's magnetic field (KAFKA, 2000; MICHAELIS, 1999). Modulated electromagnetic signals with a wide frequency spectrum are the most effective since they permit selective activation of metabolic processes (KAFKA, 2000, 2001; MICHAELIS, 1999). The application of such fields to farm animals produces desirable results, e.g. in the treatment of joint and tendon injuries in horses (RAMEY, 1999), or mastitis in cows (MURAUEU et al., 1994). NIEDZIÓŁKA et al. (2001) demonstrated a positive effect on PEMF on the egg hatching rate. The studies conducted so far on sheep suggested that their productivity may be stimulated with specific PEMF (MILEWSKI et al. 2001, 2003, 2005; MILEWSKI, 2004). PEMF were found to have no negative effects on ewes, and to positively influence milk yield and quality, the slaughter value of lambs and reproductive performance traits.

The aim of the present study was to determine the effects of pulsed electromagnetic fields on the meat performance, wool performance and blood parameters of growing ewes, as well as on the wool performance of adult ewes.

Materials and Methods

Experiment 1 was performed on growing ewes, and experiment 2 – on adult ewes. In experiment 1 the experimental materials comprised 22 F_1 Kamieniecka crossbreeds by Charolaise sires. At four months of age they were divided into two groups, control (I) and experimental (II), identical in terms of body weight. The ewes of group II were

exposed to pulsed electromagnetic fields (PEMF) twice daily for 150 days, between 8.00 and 10.00 a.m. and between 4.00 and 6.00 p.m., in a 2 x 4 m pen covered with four openwork flat coils generating PEMF. The time of each exposure was 12 min. A modulated signal with a peripheral frequency of 33 Hz was applied. Magnetic induction B = 0 was applied for 1/2 of the period, an then it was increased linearly to the maximum value, with the frequency increasing gradually from 500 Hz to 2 kHz. Signal polarization was changed every minute. The signal-emitting modules were coupled to the coils installed 80 cm above the floor. Mean values of B of the field in the pen were 3.57 μT at the floor and 91.75 μT on the surface of the coils.

Analysis of meat performance included: body weight, cross-section measurements of the dorsal muscle (*musculus longissimus dorsi* - m. l. d.) - depth, width and surface area, and the thickness of the fat layer over the loin eye. These parameters were determined three times: at the beginning of the experiment, and after 60 and 150 days of exposure to PEMF, i.e. when the ewes were four, six and nine months of age. The measurements of m. l. d. and fat thickness were determined in vivo, behind the last rib, using a SSD-500 Aloka ultrasonograph with a 7.5 MHz linear probe.

Analysis of wool performance included: greasy wool production and staple length per year, and staple thickness and staple strength in 9-month old ewes. Wool characteristics were determined by traditional methods: staple thickness with a MP 3 lanameter, staple strength with a DSz 3 dynamometer.

Immediately after the completion of the experimental period blood was collected from the jugular vein for analysis of hematological and biochemical indices and acid-base equilibrium parameters. Hematological analyses included: white blood cell count (WBC), red blood cell count (RBC), hematocrit (HCT), hemoglobin (HBG), platelet count (PLT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC). All determinations were made by universally accepted methods, using a Vet ABC 18 hematological analyzer (Animal Blood Counter). Biochemical analyses included: glucose, total protein, activity of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP), and concentrations of creatinine, urea, cholesterol, triacylglycerols, inorganic P, Ca, Mg, Na^+, K^+ and Cl^-. They were determined by standard methods, using an EPOLL 200 spectrophotometer. The CORMAY diagnostic kit was used for glucose, and the ALPHA DIAGNOSTICS kits for the other parameters. The ionogram (Na^+, K^+, Cl^-) was determined by the potentiometric method, using an Easy Lyte PLUS ion-selective analyzer (Medica, USA). The parameters of acid-base equilibrium, i.e. partial pressure of carbon dioxide, (pCO_2) and partial pressure of oxygen (pO_2), bicarbonate concentration (HCO_3^-), base excess (BE), oxygen saturation of hemoglobin (O_2SAT), concentration of carbon dioxide ($ctCO_2$), were determined with a pH and blood gas analyser (Ciba-Corning C-248, Bayer).

In experiment 2 the experimental materials comprised 22 Kamieniecka ewes divided into two groups, control (I) and experimental (II), identical in terms of age, body weight and number of suckling lambs. The ewes of group II were exposed to pulsed electromagnetic fields (PEMF) every day during a 70-day lactation period, as described in experiment 1.

Analysis of wool performance included: greasy wool production and staple length per year, staple thickness and staple strength at 2, 28 and 70 days of lactation. The parameters were determined by the methods used in experiment 1.
In both experiments the ewes were fed as recommended by the Institute of Animal Husbandry (ed. RYŚ, 1998). Over the entire experimental period the control and experimental groups received the same diets, i.e. concentrate CJ, haylage composed of grasses and legumes, and meadow hay.
The results were analysed statistically in one- and two-factor orthogonal designs, and the significance of differences between groups was verified by the Student's t-test and the Duncan test.

Results and discussion

The characteristics of the meat and wool performance of growing ewes are given in Table 1.

Table 1
Traits of meat performance and wool performance

Specification	Group			
	I		II	
	$\bar{x}$	s	$\bar{x}$	s
Traits of meat performance:				
Body weight (kg) at the age of:				
4 months	32.26	1.90	32.11	1.54
6 months	37.73	2.43	38.34	2.29
9 months	43.93^{b}	2.46	45.89^{a}	2.08
Daily gains (g) at the age of:				
4-6 months	91.17	17.13	103.83	14.35
6-9 months	68.88^{b}	14.89	83.88^{a}	9.47
4-9 months	77.80	15.11	91.87	10.14
M. L. D. USG scanning:				
- depth (cm) at the age of:				
4 months	2.16	0.31	2.30	0.12
6 months	2.31	0.43	2.41	0.13
9 months	2.66^{b}	0.18	2.88^{a}	0.08
- width (cm) at the age of:				
4 months	5.82	0.19	5.97	0.28
6 months	6.25	0.18	6.40	0.24
9 months	6.81	0.15	6.97	0.14
- surface area (cm^2) at the age of:				
4 months	9.93	0.99	10.20	1.23
6 months	11.05	0.85	11.57	0.58
9 months	15.59^{b}	0.62	16.98^{a}	0.58
Fat thickness (mm) at the age of:				
4 months	2.09	0.03	2.08	0.03
6 months	3.01	0.06	2.98	0.04
9 months	3.37	0.04	3.19	0.17
Traits of wool performance:				
Greasy wool production per year (kg)	4.35	0.68	4.33	0.80
Staple length per year (cm)	9.65	1.90	9.51	1.80
Staple thickness (µm)	27.03	2.10	27.26	2.81
Staple strength (km)	5.75	1.44	6.06	1.47

A, B - $P \leq 0.01$; a, b - $P \leq 0.05$

It was found that the experimental ewes had a faster growth rate than the control ewes, and at nine months of age the body weights of the former were significantly higher ($P \leq 0.05$). This was a consequence of differences in daily gains, which between six and

nine months of age were significantly higher ($P \leq 0.05$) in the experimental ewes than in the control ones. Over the analysed period changes were also observed in the measurements of m. l. d. Until six months of age all ewes were characterized by similar parameters of m. l. d. Then a faster rate of changes was observed in the ewes of group II, and at nine months of age the depth and surface area of m. l. d. were significantly greater ($P \leq 0.05$) in this group. These results showed a beneficial effect of PEMF on the meat performance of ewes and correspond to those obtained in experiments on growing lambs between 2 and 70 days of age (MILEWSKI 2004, MILEWSKI et al. 2005), with an identical PEMF exposure program. Greasy wool production and staple length per year, as well as staple thickness and staple strength, were similar in both groups. The exposure to PEMF did not cause significant changes in hematological indices, but such changes were recorded in biochemical indices and acid-base equilibrium parameters (Table 2).

Table 2
Hematological and biochemical blood indices and acid-base equilibrium parameters (Hämatologische und biochemische Indikatoren sowie Indikatoren des Säure-Basen-Haushalts des Blutes)

Specification	Group			
	I		II	
	$\bar{x}$	S	$\bar{x}$	S
WBC (10^9/l)	11.83	3.30	11.53	2.95
RBC (10^{12}/l)	11.82	1.07	12.03	1.03
HBG (g/l)	119.16	8.14	118.63	7.69
HCT (l/l)	0.365	0.031	0.359	0.028
PLT (10^9/l)	245.58	70.13	312.26	139.49
MCV (fl)	30.68	1.20	29.95	1.22
MCH (pg)	10.06	0.58	9.89	0.40
MCHC (g/l)	337.47	13.99	330.47	9.90
Glucose (mmol/l)	3.29	0.42	3.61	0.38
Total protein (g/l)	72.00	4.59	69.87	3.14
AST (IU/l)	87.13^{a}	23.39	70.51^{b}	24.63
ALT (IU/l)	3.23	3.42	11.43	5.16
ALP (IU/l)	171.83	59.18	196.74	94.13
Creatinine (µmol/l)	128.96^{A}	12.39	115.87^{B}	9.02
Cholesterol (mmol/l)	1.19	0.15	1.11	0.15
Triglycerides (mmol/l)	0.14	0.05	0.22	1.07
Urea(mmol/l)	8.44^{A}	1.39	7.24^{B}	1.02
Ca (mmol/l)	2.54	0.08	2.55	0.19
Inorg. P (mmol/l)	2.03^{B}	0.41	2.37^{A}	0.22
Mg (mmol/l)	0.89^{B}	0.05	0.98^{A}	0.06
Na^+ (mmol/l)	142.61^{B}	2.73	146.40^{A}	2.63
K^+ (mmol/l)	4.63	0.28	4.86	0.48
Cl^- (mmol/l)	106.25	2.15	106.34	2.31
pH	7.42^{a}	0.03	7.39^{b}	0.04
pCO_2 (kPa)	5.70	0.62	5.61	0.46
pO_2 (kPa)	6.68	0.55	6.22	0.48
HCO_3 (mmol/l)	25.78	2.41	25.00	1.90
BE (mmol/l)	2.37^{B}	2.22	2.64^{A}	2.03
O_2 SAT (%)	75.77^{a}	6.25	71.06^{b}	5.19
$ctCO_2$ (mmol/l)	26.01	2.51	25.14	1.94

A, B - $P \leq 0.01$; a, b - $P \leq 0.05$

In comparison with the control ewes, the experimental ones showed lower AST activity ($P \leq 0.05$), lower concentrations of creatinine and urea ($P \leq 0.01$), and lower pH ($P \leq 0.05$), O_2SAT and BE ($P \leq 0.01$), as well as higher concentrations of inorganic P, Mg and Na^+ ($P \leq 0.01$).

It seems that this could result from the stabilizing effect of PEMF of the animal body, as suggested by a comparison of the biochemical blood indices in the ewes of both groups. In group II all parameters except for Na^+ concentration remained within the physiological range (KULETA et al. 1993, WINNICKA 2002), whereas in group I urea concentration exceeded the upper limit of normal and Na^+ concentration - the lower limit of normal. The high urea concentration, accompanied by elevated levels of creatinine and AST activity, may indicate that greater strain was put on the kidneys and liver of the control ewes. A similar stabilizing effect of PEMF was observed in the case of suckling ewes (MILEWSKI 2004). One of the effects exerted by PEMF on metabolic activity may be an increase in the degree of oxygen saturation, due to vasodilatation effects (KAFKA 1998, MICHAELIS 1999) and changes in the hemodynamic properties of blood (MICHAELIS 2001, SPODARYK 2001). This is proved by substantially lower oxygen saturation of hemoglobin (O_2SAT) in the experimental ewes, indicating easier movement of oxygen into cells. Such an influence of PEMF on O_2SAT was observed in ewes during lactation (MILEWSKI 2004) and during period before mating (MILEWSKI et al. 2005).
The characteristics of the wool performance of adult ewes are presented in Table 3.

Table 3
Traits of wool performance (Merkmale der Wolleleistung)

Specification	Statistics	Group		Day of lactation			Interactions
		I	II	2nd	28th	70th	
Greasy wool production per year (kg)	$\bar{x}$	5.45	5.47	-	-	-	-
	s	0.58	0.97	-	-	-	-
Staple length per year (cm)	$\bar{x}$	11.56	12.22	-	-	-	-
	s	1.42	1.56	-	-	-	-
Staple thickness (µm)	$\bar{x}$	29.54	28.85	29.61	29.39	29.14	-
	s	3.90	3.02	3.34	3.68	3.09	-
Staple strength (km)	$\bar{x}$	7.07	6.68	7.87^{Aa}	6.59^{b}	6.16^{Bb}	xx
	s	1.47	1.71	1.62	1.39	1.44	-

A, B - $P \leq 0.01$; a, b - $P \leq 0.05$
xx - $P \leq 0.01$

The ewes of both groups were characterized by similar levels of annual greasy wool production and staple length. No significant differences were found in staple thickness and staple strength, either. However, significant changes in wool quality took place during lactation, and concerned primarily staple strength, which decreased gradually. From 2 to 28 days of lactation staple strength reduced by 1.28 km ($P \leq 0.05$), and at the second stage of lactation, between 28 and 70 days, by 0.43 km and finally the difference between day 2 and day 70 was found to be highly significant. There was an interaction between these factors ($P \leq 0.01$). During lactation significant changes in staple strength were observed in the experimental group only; the changes recorded in the control group were statistically non-significant. Our study showed that pulsed electromagnetic fields had no direct effects on annual greasy wool production, staple length and wool quality, which might have resulted from the fact that the experimental ewes were exposed to PEMF during a part of the year only. Reduced staple strength during lactation/suckling is quite normal and results from intense pressure put on the ewe's body over this period. Although this effect was more noticeable in the experimental ewes, it should not be associated with exposure to PEMF. Such a

possibility was excluded in experiment 1. The reason for this phenomenon is a considerably higher yield of milk and milk components in this group of ewes, as demonstrated by MILEWSKI (2004).

Conclusions

The investigations showed that PEMF had a significant and positive effect on the body weight and daily gain of growing ewes, and on the depth and cross-section area of m. l. d. This could be a consequence of the stabilizing effect of PEMF on metabolic processes, as suggested by lower AST activity, lower concentrations of creatinine and urea, as well as increased oxygen supply to cells confirmed by a lower oxygen saturation percentage (O_2SAT). No direct correlations were found between exposure to PEMF and wool performance, staple length and wool quality traits.

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The effect of early colostrum collection on selected performance traits in sheep

Abstract

Colostrum for the needs of pharmaceutical industry was collected from Corriedale ewes at 1, 3 and 6 h after lambing, yielding from 0.17 to 0.20 kg raw material annually. The effect of management system on maternal nursing was assessed on the basis of survival rate until day 7 and lamb rearing rate until day 100 from dams of the experimental and control groups. Lamb growth and development were assessed on the basis of body weight at birth, at day 28 and 56, as well as body weight gains between day 1 and 28, 1 and 56, and 28 and 56. No negative effect was found for the early collection of colostrum on lamb survival and rearing rates and lamb body weight at the age of 8 weeks. Only in the period until week 4 growth rates of lambs from the experimental group were lower than those recorded in their age mates from the control group.

Key Words: sheep colostrum, collection, lamb nursing and growth

Introduction

The collection of sheep colostrum is advisable due to the high content of proline–rich polypeptides (PRP) (GEORGIADES and FLEISCHMAN, 1996; JANUSZ and LISOWSKI, 2000; JANUSZ et al., 1987). They are used in the production of Colostrinin®, a drug used in human medicine. Colostrinin® inhibits the formation of amyloid senile plaques in the brain, characteristic for Alzheimer's disease (LESZEK et al., 1999). Sheep colostrum obtained during the first six hours after lambing, containing considerable amounts of PRP, is especially valuable (NIŻNIKOWSKI et al., 2006). The development of a method for the early collection and preservation of sheep colostrum may also facilitate the generation of an additional source of income from sheep production, which at present is not very profitable in Poland.

The aim of the study was to assess the effect of early collection of sheep colostrum on survival rate, as well as growth and development of nursed lambs.

Material and methods

Investigations were conducted in the years 2005 - 2006 on 250 sheep of the native Corriedale breed kept in a herd in the Łódź province. Dams were fed according to the feeding standards of the Institute of Animal Science (OSIKOWSKI et al., 1993), appropriately for the body weight and month of gestation based on feeds produced at the farm. Colostrum was collected from 97 (50 in 2005 and 47 in 2006) randomly selected ewes at 1, 3 and 6 h after lambing, following the methodology described by NIŻNIKOWSKI et al. (2006). The bulk amount of colostrum collected from the whole group within a year was calculated, which was further used to calculate the mean amount per 1 ewe. Basic chemical composition was assayed in the collected colostrum, i.e. contents of protein, fat, lactose, solids and nonfat solids (Milco-Scan). Somatic cell counts (SCC) in 1 cm^3 were determined using a Fossomatic apparatus (ZARZYCKI et al., 1983).

The effect of management system on lamb nursing was assessed on the basis of lamb survival rates until day 7 and rearing rates until day 100 from dams of the experimental group (97 head) and the control (153 ewes). Lamb growth and development was assessed on the basis of body weight at birth, at day 28 and 56, as well as body weight gains between days 1-28, 1-56 and 28-56.
Statistical analysis of results was performed using an SPSS software package (2004) taking into consideration the following factors: year of experiment (replications) (Y), time of sample collection (T), the number of lambs at birth (N), the age of the ewe (A), management system of the ewe (M), the number of nursed lambs (L) and their sex (S), as well as interactions: T x N , T x A, T x Y, Y x N, S x M and L x M. The significance of the effect of analyzed factors and interactions was assessed using the F test, while differences between levels of a given factor were estimated using the Duncan's test (RUSZCZYC, 1981). In order to obtain the normal distribution of the somatic cell count in colostrum, the SCC values were transformed using Briggs' logarithm prior to the statistical analysis.

Results and discussion

When collecting colostrum within the first 6 h of lactation in the course of two years of the experiment on average 185 g colostrum was collected annually from 1 ewe (Fig.).

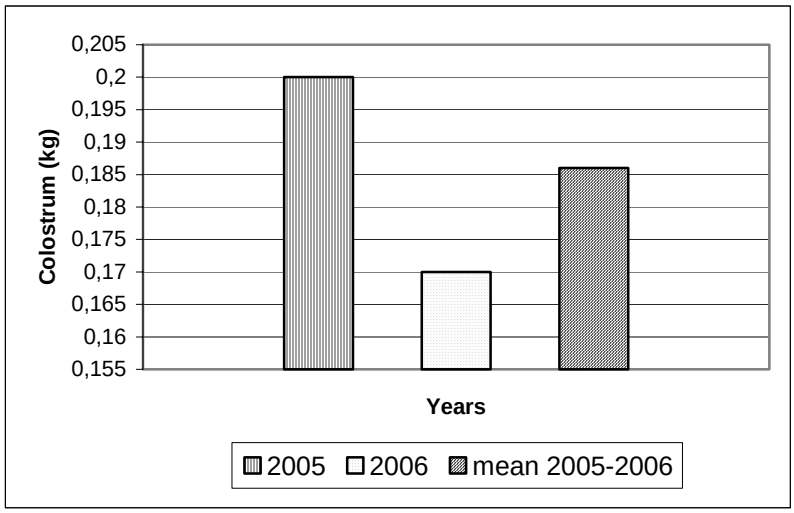

Figure: Colostrum production in the first 6 hours after lambing

The variation in the amount of produced colostrum, i.e. 200 g in 2005 and 170 g in 2006, caused by the effect of environmental conditions (the effect of calendar year), is a reliable prognostic for the amount of this raw material, which may possibly be obtained from Corriedale sheep. This result is lower in comparison to the amount of colostrum obtained from the Pomerania sheep (NIŻNIKOWSKI et al., 2006), which may be considered a characteristic trait for the sheep breed analyzed in this study.
The assessment of the effect of colostrum collection time on the basic chemical composition is presented in Table 1. The lowest contents of both protein and nonfat solids were obtained at the 6th hour of lactation in comparison to the higher amounts and uniform periods at birth and at the 3rd hour of lactation. Similar dependencies were also obtained in Polish studies conducted on Heath sheep, and Żelazne and Pomerania sheep (LUBASZEWSKA and NIŻNIKOWSKI, 2002; NIŻNIKOWSKI et al., 2006).

Table 1
The effect of collection time on basic chemic composition and somatic cell count in collected colostrum

Traits		Colostrum collection time after lambing (h)		
		1	3	6
no. of animals	head	97	97	97
Fat content (%)	LSM	14.04	16.42	16.66
	SE	1.48	1.48	1.48
Protein content (%)	LSM	21.24	18.97	15.06
	SE	1.38	1.38	1.38
	*	C	c	A.c
Lactose content (%)	LSM	3.26	3.44	3.84
	SE	0.25	0.25	0.25
Solids content (%)	LSM	46.95	47.94	44.39
	SE	1.81	1.81	1.81
Nonfat solids content (%)	LSM	32.91	31.38	27.73
	SE	1.18	1.18	1.18
	*	C	c	A.b
Somatic cell count in 1 cm^3 colostrum (log_{10})	LSM	2.69	2.54	2.62
	SE	0.24	0.24	0.24

* a,b,c - P≤ 0.05; A,B,C - P≤0.01

Table 2
The effect of colostrum collection from lambs on lamb survival and nursing rates

Traits	Lambs of dams, from which colostrum was milked			Lambs of dams of the control group		
	n	LSM	SE	n	LSM	SE
Lamb survival rate until day 7 (head)	97	0.92	0.03	226	0.89	0.02
Lamb rearing rate (head)	97	0.89	0.04	226	0.86	0.03

In terms of the other characteristics of chemical composition and somatic cell count per 1 cm^3 colostrum no significant effect was shown of collection time on the levels of these traits (Tab. 1). This result differs slightly from the dependency found in the Pomerania sheep in relation to contents of fat and lactose (NIŻNIKOWSKI et al., 2006), showing a statistically significant variation at a similar period of lactation in relation to these characteristics of colostrum chemical composition traits. In case of Żelazne sheep (LUBASZEWSKA and NIŻNIKOWSKI, 2002) observed trends were similar to those of Corriedale sheep. The shown dependencies indicate that in Corriedale sheep, apart from contents of protein and nonfat solids, no statistically significant changes were found in colostrum chemical composition and cytological quality (SCC/cm^3 colostrum). This facilitates the collection of a relative uniform raw material for the production of Colostrinin®. This finding indicates high quality of colostrum obtained from Corriedale sheep, perfectly suitable for the production of this valuable drug.

The effect of colostrum collection on lamb development and growth is presented in Tables 2 and 3. No significant differences were shown in lamb survival and rearing rates in relation to dams from which colostrum was milked in comparison to the control group (Tab. 2). This indicates a possibility of rational lamb rearing and lamb losses were probably dependent on environmental factors, which were not included in the experiment. In relation to lamb survival rate until day 7 this result remains fully consistent with other studies (NIŻNIKOWSKI et al., 2006). A lack of the effect of dam management system on lamb rearing rates indicates a limited effect of early colostrum collection on lamb rearing. Similarly as in a study by NIŻNIKOWSKI et al. (2006), significant or highly significant higher lamb body weights were found at the age of 28 days and higher daily body weight gains in the period from birth to day 28 and to day 56 in the control group not subjected to colostrum milking (Tab 3). However, daily weight gain in the period from day 28 to day 56 did not differ significantly between the analyzed groups of lambs, which indicates that early colostrum collection has an adverse effect on lamb growth and development only in the period of the first 28 days of lambs' lives. In combination with lamb rearing results and taking into consideration the compensation of their growth found until day 56, ewes may be used for that purpose with no limitations.

Table 3
The effect of ewe management method on body weight traits and daily weight gains

Trait	Ewes from which colostrum was milked		Ewes from which colostrum was not milked	
	$\overline{x}$	SD	$\overline{x}$	SD
n	97		153	
Body weight at birth (kg)	4.36	0.1	3.77	0.05
Body weight at age of 28 days (kg)	9.7	0.32	10.85^{XX}	0.17
Body weight at age of 56 days (kg)	19.13	0.45	19.8	0.24
Daily weight gain from birth to day 28 (g)	190.1	11.5	217.00^{XX}	6
Daily weight gain from day 1 to 56 (g)	263.6	8	286.30^{X}	4.1
Daily weight gain from day 28 to 56 (g)	336.7	13.8	355.6	7.2

X-P≤0.05; XX-P≤0.01

Conclusion

On the basis of the conducted investigations:

- the effect of the period of lactation on a decrease in contents of protein and nonfat solids was observed starting from 6 h *post partum*, which suggests a uniform product may be obtained within that time period,
- no negative effect of early colostrum collection on lamb survival and nursing rates and lamb body weight at the age of 8 weeks was found in the experiment,
- Corriedale ewes were found suitable for the production of colostrum at the early lactation stage, amounting annually to 0.17 – 0.20 kg colostrum per sheep.

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Ultrasound image of morphological changes of teat end in sheep caused by machine milking

Abstract

A total of 29 ewes with an approx. 90% share of East Friesian dairy sheep genes in their genotype were investigated. Ultrasound images of teats were recorded 4 times daily: before and immediately after the morning milking, and next 4 and 10 h after milking. The diameter and length of the teat canal and the thickness of the teat wall were measured on recorded images. The adopted method of ultrasound teat diagnostics made it possible to effectively monitor changes in internal teat structures caused in sheep by mechanical milking. The reaction of the teat tissue to milking was manifested in a significant increase in length, diameter or thickness of the analyzed structures ($P<0.01$; $P<0.05$). The most marked morphological changes were observed 4 h after milking. The effect was found of successive lactation and udder type on the levels of analyzed morphological traits of the teat. Both the thickness of the teat wall and the length of the teat canal increased with the age of animals and successive lactations ($P<0.01$ and $P<0.05$).

Key Words: sheep, teat, machine milking, ultrasonography

Introduction

The teat of farm animals used for milk production is an important part of the udder, onto which a milking cluster is attached and which serves the role of both a valve regulating the outflow of milk and of a natural barrier for exogenous infections (HAMANN and MEIN, 1995). Studies on the reaction of the teat to mechanical milking have so far been conducted mainly using traditional research methods describing the health state of the teat (HAMANN and MEIN, 1996; JANKUS and BAUMANN, 1986; KATONA and MESZAROS, 1971; MCDONALD, 1968; SCHULZ et al., 1999). Some of these methods, such as e.g. histological examination of the epithelium, due to the invasive nature of the procedure, are limited in their scope (LUDEWIG, 1998). Most studies on the subject have been conducted on dairy cattle (HAMANN and MEIN, 1996; JANKUS and BAUMANN, 1986; MCDONALD, 1970; SHEARN and HILLERTON, 1996). Studies using ultrasound equipment in teat diagnostics have been carried out for only a few years now and they generally focus on the development of a methodology to obtain an image of intramammary structures (FRANZ et al. 2001). Very little attention is paid to this problem in small ruminants, especially sheep (FAHR et al., 2001; FRANZ et al., 2001). Increasing economic role of sheep and goat milk, to an increasing degree collected through mechanical milking, as well as growing requirements of consumers concerning hygienic quality of milk, justify the need to undertake studies on this subject.

The aim of this study was to assess morphological changes in teats of sheep, occurring as a reaction to mechanical milking.

Materials and methods

Investigations were conducted on 29 ewes of a milk line with an approx. 90% share of genes of the East Friesian milk sheep in their genotype, kept at the Experimental Station of the Department of Sheep and Goat Breeding, the Agricultural University, in

Złotniki near Poznań. Animals aged 2 - 5 years (1st to 4th lactation) were in their fourth month of lactation and the mean milk yield of the investigated animals during the experiment was approx. 1 kg. Milking was performed in a milking parlour with 14 side by side units equipped with standard clusters for sheep milking by Westfalia Separator with pulsation rate of 90 ± 5 pulses per minute and a vacuum unit with milking vacuum of 44 kPa.

Ultrasound images of the longitudinal cross-section of teats from a Hitachi EUB 405+ scanner coupled with a 10 MHz linear probe were taken on animals immobilized in milking units four times a day at the following time intervals:

- Before the morning milking,
- Immediately after the morning milking,
- 4 h after the morning milking,
- 10 h after the morning milking, immediately before the evening milking.

An ultrasound probe was placed in a plastic cup filled with water of 35 - 40^0C, into which teats were immersed (Figs. 1 and 2). Images were recorded in real time on a VHS cassette, next transmitted onto an INDEO® Fast Frame Grabber cart to a computer and archived in the form of disc files (a bit map). The diameter and length of teat canals and the thickness of teat walls at Furstemberg's rosette (Fig. 3) were measured on images recorded in a MultiScan 12.5 computer system (Computer Scanning Systems Ltd.). The experiment was conducted in three replications at the interval of 16 days with a total of 348 teat measurements taken.

Results were verified statistically using a analysis of variance (SAS 9.1.3.), taking into consideration the effect of time interval between measurements (1...4), replications of the experiment (1...3), successive lactation (1...3) and the type of the udder (1,2).Due to the small number of sheep in their fourth lactation they were included in the group of sheep in their third lactation.

Results and discussion

Descriptive statistics of the analyzed traits are presented in Table 1. A very good uniformity of internal mammary structures needs to be emphasized. Both wall thickness and the length and diameter of the teat canal in both analyzed teats were very similar. A high coefficient of variation (V%), depending on a given trait amounting up to 25%, resulted from a reaction of the teat (tumescence, swelling) to partial vacuum and pulsation during mechanical milking. Obtained values of internal mammary structures were higher than results of measurements recorded by FRANZ et al. (2003) on sheep of four breeds: East Friesian, Tiroler Steinschaf, Bergschaf and German Blackheaded sheep. In that study the length of the teat canal ranged from 5.7 to 10.3 mm and its diameter ranged from 1.8 to 3.1 mm, whereas mean values for these traits were 8.6 (SD=1.3 mm) and 2.3 (SD=0.4 mm), respectively (FRANZ et al. 2003).

Table 1
Descriptive statistics of analyzed traits (n=348)

Index	Left teat [mm]			Right teat [mm]		
	Wall thickness	Canal length	Canal diameter	Wall thickness	Canal length	Canal diameter
mean	5.70	9.82	0.85	5.72	9.49	0.86
Minimum	2.50	4.50	0.40	2.70	4.10	0.40
Maximum	10.30	16.90	1.50	9.50	15.00	1.60
V%	20.80	19.19	24.43	23.31	19.61	24.63

Fig. 1: A teat measuring unit: an ultrasound apparatus with a 10 MHz linear probe attached in a container with water and a video cassette recorder (Photo P. Ślósarz)

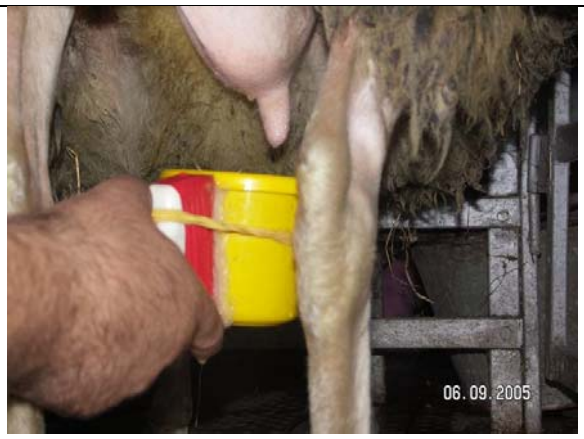

Fig. 2: Water bath ultrasound teat diagnostics of sheep (Photo J. Wójtowski)

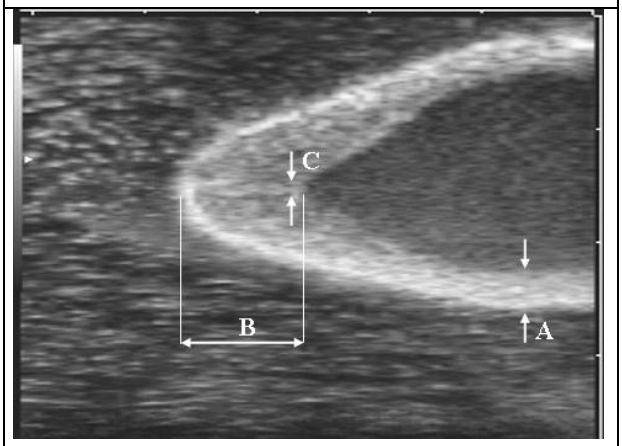

Fig. 3: The location of measurement sites: A – teat wall thickness, B – teat canal length, C – teat canal diameter at Furstenberg's rosette (Photo P. Ślósarz)

Results of ultrasound measurements of teats taking into consideration experimental factors are presented in Table 2. Reaction of the teat tissue to mechanical milking, except for the diameter of the left teat canal, was manifested in the ranges of all the other traits in a significant increase of length, diameter or thickness of analyzed structures ($P<0.01$; $P<0.05$). The most marked morphological changes occurred 4 h after milking. The thickness of the wall of the left and right teats in relation to the measurement taken immediately before milking increased by 17 and 16%, respectively, and the length of the teat canal by 10 and 8%, respectively ($P<0.01$). It is well-documented by ultrasound images of teats of one of the examined sheep (Fig. 4ef). Gradually disappearing tumescence and swelling, recorded 10 h after milking may also be observed on these images (Fig. 4gh). The short-term effect of milking on teats is swelling. Under vacuum, strain is generated in the teat wall, which induces of blood vessels and expandable compartments in the peri-vesicular tissue. This results in an accumulation of fluid in the teat: blood and lymph. Such swelling of the teat may influence the resistance of the teat canal to bacterial invasion during the recovery period after milking.

Table 2
Results of ultrasound teat measurements in sheep

Factor	n	Left teat [mm]			Right teat [mm]		
		Wall thickness LSM ± SE	Canal length LSM ± SE	Canal diameter LSM ± SE	Wall thickness LSM ± SE	Canal length LSM ± SE	Canal diameter LSM ± SE
Measurement (M):		**	**	ns	**	*	*
- before milking	87	5.40 ± 0.14 AB	9.30 ± 0.23 AB	0.83 ± 0.02	5.30 ± 0.15 AB	9.16 ± 0.22 a	0.85 ± 0.03
after milking:							
- immediately	87	6.04 ± 0.14 AC	10.15 ± 0.23 A	0.87 ± 0.02	6.18 ± 0.15 AC	9.66 ± 0.22	0.89 ± 0.03 a
- 4 h after	87	6.33 ± 0.14 BD	10.20 ± 0.23 B	0.82 ± 0.02	6.14 ± 0.15 BD	9.93 ± 0.22 ab	0.81 ± 0.03 ab
- 10 h after	87	5.52 ± 0.14 CD	9.90 ± 0.23	0.86 ± 0.02	5.58 ± 0.15 CD	9.26 ± 0.22 b	0.89 ± 0.03 b
Replication (R):		ns	ns	**	**	ns	*
1	116	5.70 ± 0.12	9.98 ± 0.19	0.91 ± 0.02 AB	5.48 ± 0.13 A	9.64 ± 0.18	0.90 ± 0.02 a
2	116	5.86 ± 0.12	9.59 ± 0.20	0.82 ± 0.02 A	6.07 ± 0.13 A	9.39 ± 0.19	0.85 ± 0.02
3	116	5.91 ± 0.12	10.08 ± 0.21	0.80 ± 0.02 B	5.85 ± 0.14	9.47 ± 0.20	0.83 ± 0.02 a
Lactation (L):		*	**	ns	**	**	*
1	180	5.61 ± 0.09 a	9.42 ± 0.16 aA	0.85 ± 0.02	5.61 ± 0.10 A	8.88 ± 0.15 AB	0.85 ± 0.02 a
2	84	5.78 ± 0.13	10.03 ± 0.21 a	0.84 ± 0.02	5.39 ± 0.14 B	9.71 ± 0.20 A	0.82 ± 0.02 b
≥ 3	84	6.08 ± 0.13 a	10.21 ± 0.22 A	0.84 ± 0.02	6.39 ± 0.15 AB	9.91 ± 0.21 B	0.91 ± 0.02 ab
Type of udder (T):		*	ns	ns	ns	**	ns
- round	100	5.98 ± 0.12	9.78 ± 0.19	0.83 ± 0.02	5.81 ± 0.13	9.09 ± 0.18	0.84 ± 0.02
- oval	248	5.67 ± 0.08	9.99 ± 0.13	0.86 ± 0.01	5.79 ± 0.09	9.91 ± 0.12	0.88 ± 0.01
Interactions		ns	ns	ns	ns	RxT*	ns

A,B… (a,b…) – means denoted with identical capital (small) letters differ significantly at P≤0.01 (P≤0.05)
ns – difference statistically non-significant

The effect was observed of successive lactation and the type of the udder on the level of analyzed morphological traits of the teat. Both the thickness of the teat wall and the length of the teat canal increased with the age of animals and successive lactation ($P<0.01$ and $P<0.05$). The effect of the type of the udder on the size of internal mammary structures was less evident. Sheep with round udders had longer teat canals in comparison to ewes with oval teats, while in case of the thickness of the teat wall this dependence was opposite ($P<0.01$ and $P<0.05$). Moreover, a smaller diameter of the teat canal was also found in the third replication of the study in comparison to the result from the first replication, i.e. 16 days earlier.

So far few studies have been conducted on the assessment of the effect of milking on the state of the udder and teats using ultrasound equipment. In a study on the effect of mechanical milking on changes in the thickness of the teat in dairy cattle using a cutimeter (a calliper instrument which measures the teat end width) HAMANN and MEIN (1990) found a tumescence of the teat after milking amounting to 10 - 20% in relation to the condition before milking. In turn, much bigger changes amounting to 26 - 50% (teat wall) and 19-28% (canal length) were observed by NEIJENHUIS (2000). According to that author changes in these structures of the teat caused by sucking of milk by the calf are much smaller and amount to 6 and 7%, respectively (NEIJENHUIS, 2000). Studies with the use of ultrasound equipment for the diagnostics of teats in the Hungarian population of Simmental cattle were carried out e.g. by HÚTH et al. (2001), who found increasing values of somatic cell counts in milk of cows with the longer than average length of the teat canal. A similar dependence was observed by FRANZ et al. (2003) in sheep of four different breeds kept in Austria. In a study conducted on milk goats diagnosed using ultrasonography before and 1 h after milking FAHR et al. (2001) found a thickening of the teat walls amounting up to 30% and a lengthening of the teat canal by approx. 20%. According

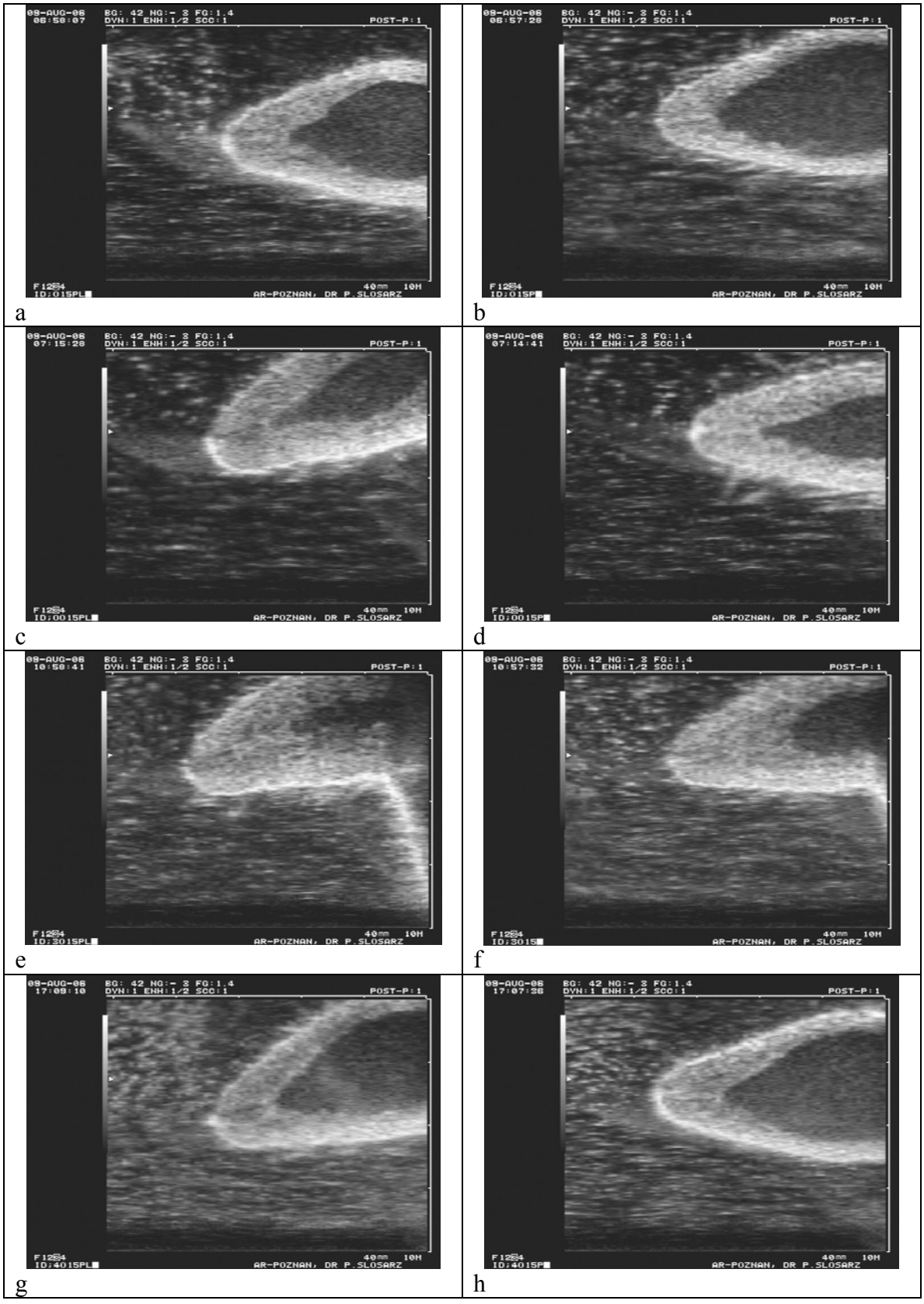

Fig. 4: a, b – left and right teats of sheep no. 15 before morning milking; c, d – left and right teat immediately after morning milking; e, f – left and right teat 4 h after milking; g, h – left and right teat 10 h after milking (a Hitachi EUB 405B apparatus, 10MHz). Photo P.Ślósarz

to the above mentioned authors these changes in the form of congestion and stagnation oedema were observed for a period longer than 1 h after milking. This study confirmed those findings also in sheep. Even 10 h after milking internal structures of the teat did not reach the size from before milking. However, the biggest, statistically highly significant differences in their size were recorded 4 h after milking. The experiment conducted in this study showed the significance of the problem and confirmed the advisability of the conducted investigations. The applied method of ultrasound mammary gland diagnostics facilitated effective monitoring of changes in the internal structures of the teat in sheep caused by mechanical milking. However, it is advisable to extend the scope of such studies to include the assessment of the interrelation between the anatomical structure of the teat and microbial and cytological quality of the collected milk.

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Cytological quality of milk from cows kept in different types of pens, according to the season of the year

Abstract

A total of 1947 farms from the Pomerania and Kujawy regions were analysed for the effect of type of pen (with or without litter) on the cytological quality of milk, taking into account the season of the year in the statistical calculations. It was found that in most barns, cows were kept in shallow litter stalls. Regardless of the season of the year, milk of the highest cytological quality was obtained from cows kept in pens on litter. Regardless of type of pen and type of litter, the highest proportion of samples with the SCC indicating clinical or subclinical mastitis was found from September to November.

Key Words: cow milk, somatic cells, housing with or without litter

Introduction

Mastitis is the most common and the most expensive disease of cows (KOSSAIBATI and ESSLEMONT, 1997; RAJALA-SCHULTZ et al., 1999; MALINOWSKI and KŁOSOWSKA, 2000). One of the indicators of udder health is milk SCC (MALINOWSKI, 2001), which is also an important criterion of grading the milk purchased from farmers. Changes in the SCC are affected by season of the year, management and milking conditions, age of cows or stage of lactation to a much lower extent than by mastitis. The results of many studies (DORYNEK and KLIKS, 1998; BRZOZOWSKI et al., 1999; BORKOWSKA and JANUŚ, 2001; SAWA and PIWCZYŃSKI, 2002; STENZEL et al., 2002; STENZEL et al., 2003) have shown that milk SCC increases in summer and autumn compared to spring and winter, when it is the lowest. BRZOZOWSKI et al. (1999) attribute this phenomenon to strong seasonality of calvings and greater susceptibility of cows to udder inflammation during the summer period. MALINOWSKI (1996) reports that udder inflammations in summer are favoured by high air temperature and heavy rainfall. The latter encourages the breeding of flies, which prefer hairless skin of the teats when attacking cattle, thus spreading pathogens. In the studies of GRODZKI et al. (1998) and BARŁOWSKA et al. (2003), the spring-summer season proved more favourable in terms of the SCC. The authors attributed this to better hygienic conditions of summer management when cattle spend most of the day on pastures, in contrast to indoor housing in the winter.

When discussing the hygienic quality of milk, focus is made on the technological aspects of dairy cow management, including the type of stall and litter used. Litter is the main breeding ground for fungi, which may be a cause of mastitis. They can easily proliferate in old, moist straw and in sawdust (MALINOWSKI, 1997). In the studies by GRODZKI et al. (2002) in 109 analysed farms, shallow litter pens accounted for 89.8%, shallow pens without litter for 8.3%, and deep litter pens for 1.9%. In the studies by LUDWICZUK (2001) conducted on 100 pens, there were 24% deep litter pens and 76% shallow litter pens. The same author states that it is much more laborious to maintain cow hygiene in buildings with deep litter pens than in buildings with shallow litter pens. In the former Zamojskie province in the years 1997/1998, most pens (90%) were shallow, with only 5% of deep litter pens and 5% of deep-litter loose pens (5%) (BORKOWSKA et al., 1998). The studies of GÓRSKA et al. (1999),

in which the percentages of particular management types were more proportional, indicate that in shallow pens there were more (80%) cows giving milk with less than 500,000 somatic cells per 1 ml than in deep litter pens (75%). The findings of GÓRSKA et al. (1999) show that the proportion of cows giving milk with less than 500,000 somatic cells per ml was higher in shallow pens than in deep-litter pens (80% vs. 75%).

The aim of the present study was to analyse the cytological quality of cow's milk depending on the season of the year and management conditions in pens with or without litter, based on large material concerning milk SCC that was routinely collected as part of the milk testing scheme.

Material and Methods

The study involved 1947 farms in which cows were evaluated for milk performance. Using the questionnaire method, animal specialists gathered housing conditions data on shallow litter pens with straw, shallow litter pens with sawdust, deep litter pens with straw, pens with sand and without litter, and pens with mattresses and without litter. The SYMLEK system provided data on the SCC in 214 225 samples of milk from test milkings carried out from June 2001 to May 2002. SCCs were transformed using the natural logarithm (LnSCC). In the statistical analysis, the average LnSCC value was calculated in relation to management conditions. The significance of differences between means was calculated using Duncan's test.

The chi^2 test [SAS/STAT 1995] was used to analyse, depending on the above management conditions and seasons of the year (December-February, March-May, June-August, September-November), the frequency of milk samples in which the SCC per ml of milk was ≤100,000, 100,001-200,000, 200,001-400,000, 400,001-500,000, 500,001-1,000,000 and >1,000,000. The above classification of milk samples served as a basis for evaluating udder health status (according to RENNER, 1975, with our own modifications) as very good, good, at risk, latent changes, subclinical changes, and clinical changes.

Results

Analysis of data in Table 1 shows that shallow litter stalls were the most common in Pomerania and Kujawy farms, with 9% of deep litter pens and only 0.6% of stalls without litter. This shows a significant disproportion in the number of analysed farms with cows housed in different systems. Therefore, the results obtained are considered preliminary despite the statistically significant effect of the type of pen on the cytological quality of milk. The type of stall was shown to have a significant effect on LnSCC. Highest quality milk was obtained when the cows were kept in shallow litter pens with sawdust (LnSCC=11.9) and straw (LnSCC=12.2). The cytological quality of milk from cows in deep litter pens was significantly poorer compared to shallow litter pens. The SCC was clearly the highest in the milk of cows kept in pens without litter. It was also found that the type of stall resulted in statistically significant differences ($chi^2 = 516.09^{xx}$) in the proportion of milk samples, especially those with the lowest (<100,000) and highest (>1,000,000) SCC per ml of milk. The highest (39%) proportion of milk samples with the low SCC was obtained when cows were kept in shallow litter pens with sawdust, and the lowest (24%) in pens without litter and with

sand. In the pens in which straw was used as litter, the proportion of milk samples indicative of very good udder health was 30%, regardless of whether the pen was shallow or deep. The proportion of milk samples indicative of subclinical or clinical mastitis was the lowest in shallow litter pens, slightly higher in deep litter pens, and the highest in pens without litter.

Analysis of the effect of season of the year on the frequency of milk samples with particular SCCs showed that the worst situation occurred in autumn (only 28% of the samples indicative of very good udder health and almost 14% of the samples indicative of clinical mastitis) (Tab. 2). In summer, the proportion of milk samples with SCC of <100,000/ml was the highest (32%), with a relatively high (13%) proportion of samples with more than 1,000,000 somatic cells per ml milk. The results obtained for samples taken in spring were similar to those found in winter and indicate that very good udder health was found in 30% of the cows, with 12% of the cows having clinical mastitis.

Table 1
Effect of stall and litter type on the proportion of cow's milk samples with low and high somatic cell counts

Type of stall and litter	No. of cow-houses	No. of milk samples	LnSCC	Percentage of milk samples with SCC (thous./ml) of:					
				≤100	100-200	200-400	400-500	500-1000	>1000
Shallow litter – straw	1915	214225	12.24 A	30.4	21.1	19.4	5.0	11.5	12.6
Shallow litter – sawdust	4	1556	11.89 A, B, C, D	39.4	23.6	18.9	3.7	7.8	6.6
Deep litter – straw	17	5577	12.32 A, B	30.4	20.2	17.4	5.4	12.0	14.6
Without litter – sand	7	5540	12.54 A, B, C	23.9	18.0	20.4	5.6	14.4	17.7
Without litter – mattress	4	2379	12.58 A, B, D	25.0	19.1	18.9	5.4	12.3	19.3
Total	1947	229277		30.2	21.0	19.4	5.1	11.5	12.8

A, B, C... – values marked with the same letters differ highly significantly at P≤0.01

Analysis of the results concerning the effect of season of the year and cow management system on the frequency of milk samples with particular SCCs shows that the greatest variation occurred in the autumn (chi2=202^{xx}) and the lowest in the winter (chi2=123^{xx}) (Tab. 2). Compared to the other seasons, in the autumn there was the highest proportion of milk samples indicative of clinical mastitis. This particularly concerned shallow litter pens with sawdust (21.3%) and pens without litter and with sand (21.3%). A similar situation occurred for the proportion of milk samples indicative of subclinical mastitis. It is hard to interpret the high proportion of milk samples with more than 1,000,000 somatic cells per ml, found in the autumn period in shallow pens in which sawdust was used as litter, especially since milk of the highest cytological quality was obtained in these pens in the other seasons of the year. Probably, this was due to a small number of shallow-litter pens with sawdust, and thus a small number of milk samples analysed. Analysis of the milk samples with the lowest SCC showed that the best situation occurred in pens in which straw was used as a bedding. When evaluating the effect of type of stall on the cytological quality of milk in winter, spring and summer, the worst conditions were found in pens without litter (the lowest proportion of milk samples from cows with healthy udders and the highest proportion of milk samples from cows with clinical mastitis). In the above seasons of the year, the most desirable proportion of milk samples (the greatest number of high quality samples and the lowest number of poor quality samples) was from cows kept in shallow litter stalls.

Discussion

Our results confirmed those of the other authors who reported that in the majority of Polish farms, cows are kept in shallow litter buildings (LUDWICZUK, 2001; GRODZKI et al., 2002; GÓRSKA et al., 2003). WINNICKI et al. (2004) estimate that in Poland 97.1% of the barns have shallow litter stalls, 19.2% have deep litter stalls, and only 1.8% have no litter. The results of the above analyses indicate that because of the potential for production of milk of high cytological quality, shallow litter stalls are the most desirable, followed by deep litter stalls and stalls without litter. Studies by MAJCHRZAK and PEŁCZYŃSKA (1997), MAJEWSKI and TIEZTE (1990) and GÓRSKA et al. (2003) indicate that cows kept in shallow stalls were characterized by better udder health and their milk contained less bacteria and somatic cells. GULIŃSKI et al. (2002) showed that housing cows in shallow stalls caused a marked increase in the percentage of extra class milk. According to KARRER (2001), udder hygiene is better when using a mattress rather than a mat for lying.

Table 2
Effect of season of the year and type of stall and litter on the proportion of samples of cow's milk with low and high somatic cell counts

Season of the year	Type of stall and litter	No. of samples	Percentage of milk samples with SCC (thous./ml) of:					
			≤100	100-200	200-400	400-500	500-1000	> 1000
XII-I	Total	60160	30.4	21.0	19.5	5.1	11.7	12.3
	Shallow litter – straw	55878	30.5	21.1	19.6	5.1	11.6	12.1
	Shallow litter – sawdust	443	37.2	22.1	20.8	5.0	8.8	6.1
chi^2= 123^{xx}	Deep litter-straw	1570	31.4	21.8	16.6	4.6	11.6	14.0
	Without litter – sand	1568	24.4	18.5	21.0	5.4	14.3	16.4
	Without litter – mattress	701	27.5	19.7	17.1	4.6	12.7	18.4
III-V	Total	48667	30.7	21.5	19.7	5.0	11.2	11.9
	Shallow litter-straw	45322	30.9	21.7	19.6	4.9	11.1	11.8
	Shallow litter – sawdust	379	32.3	26.5	20.6	4.2	8.2	8.2
chi^2= 147^{xx}	Deep litter – straw	1332	32.3	20.3	17.7	5.6	11.9	12.2
	Without litter – sand	1179	26.0	18.4	21.4	6.5	14.8	12.9
	Without litter – mattress	454	17.7	17.3	22.1	6.8	12.0	24.1
V-VIII	Total	56133	32.2	20.6	18.4	4.8	11.0	13.0
	Shallow litter –straw	52707	32.5	20.7	18.4	4.8	10.9	12.7
	Shallow litter – sawdust	273	45.8	24.9	15.8	1.8	7.7	4.0
chi^2= 190^{xx}	Deep litter – straw	1066	28.8	20.1	17.3	5.7	12.8	15.3
	Without litter – sand	1475	23.0	16.9	21.2	5.5	13.8	19.6
	Without litter – mattress	612	30.7	20.4	15.2	5.4	11.3	17.0
IX-XI	Total	64317	28.1	20.8	19.9	5.4	12.1	13.7
	Shallow litter – straw	60317	28.2	20.9	20.0	5.4	12.0	13.5
	Shallow litter – sawdust	461	22.4	18.1	17.9	5.2	15.1	21.3
chi^2= 202^{xx}	Deep litter – straw	1609	28.8	18.8	17.9	5.9	12.1	16.5
	Without litter – sand	1318	22.4	18.1	17.9	5.2	15.1	21.3
	Without litter – mattress	612	21.7	18.5	22.1	5.5	13.1	19.1

It was shown that the cytological quality of milk was differentiated by the season of the year, with the poorest quality milk obtained in the autumn regardless of the housing conditions. The results of studies by other authors (DORYNEK and KLIKS, 1998; GRODZKI et al., 1998; KWAŚNICKI et al., 2003) on the effect of season of the year on milk SCC are inconclusive. The lower SCC (DORYNEK and KLIKS, 1998; BRZOZOWSKI et al., 1999) found in the winter and the higher SCC found in the summer probably result from the increased incidence of mastitis in the summer months, because high temperature stress can increase the susceptibility of cows to

infection, while humidity related to high temperature makes it easier for microorganisms to proliferate (SMITH and HOGAN, 1996). Our study confirms the findings of GRODZKI et al. (1998), who showed that the spring-summer season was more favourable in terms of milk SCC.

Conclusions

Most cows were kept in shallow litter stalls. These stalls had a favourable effect on the cytological quality of milk. Regardless of the season of the year, milk of the highest cytological quality was obtained from cows kept in pens on litter. Regardless of type of pen and type of litter, the highest proportion of milk samples with SCC indicative of clinical or subclinical mastitis was found from September to November.

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Effect of lin seeds supplement in mixtures on chemical composition and fatty acids profile in muscular tissue of male kids

Abstract

Male kids of White Improved breed divided into two groups were used as an experimental material. Kids of the 1st group (control) were fed with standard mixture, while those of the 2nd group (experimental) were fed with mixtures containing 10% of lin seeds. Basic chemical composition in muscular tissue was analysed, and fatty acids profile in intramuscular fat of m. longissimus dorsi was tested by means of gas chromatography. It was found that the insertion of lin into mixtures for kids significantly affected dry matter and fat contents in muscular tissue. Lower values of the components were proved in the experimental group. Moreover, changes in fatty acids profile were stated. The changes mainly referred to the larger content of unsaturated fatty acids, especially $C_{18:1}$, $C_{18:2}$, $C_{20:1}$, $C_{20:3}$, $C_{20:4.}$ in kids fed with mixture containing lin seeds. The results showed favourable effects of lin seeds on chemical composition and fatty acids profile in muscular tissue during the kids fattening.

Key Words: male kids, fatty acids, chemical composition

Introduction

Present dietetics prefers products of animal origin with limited fat content but rich with polyunsaturated fatty acids. The circumstances necessitate seeking ways in order to improve meat dietetic value. Inserting oil plants into feed mixtures for animals could considerably affect fatty acids profile in their muscular tissue (BAS and MORAND-FEHR, 2000; BOROWIEC et al., 2004; PATKOWSKA- SOKOŁA and BODKOWSKI, 2003)

Scientific research of many authors (JONHSON et. al., 1995; BODKOWSKI et. al., 1999; GRUSZECKI et. al., 1999; KALINOWSKA and PUSTKOWIAK, 2000; BANSKALIEVA et. al., 2000; PIENIAK-LENDZION et al., 2001; PIENIAK-LENDZION, 2004; WOOD et al., 2004) indicated that chemical composition and fatty acids profile in muscular tissue of ruminants, among others, depended on species, age, fatty tissue position and kinds of mixture.

The aim of the study was to analyse the effect of lin seeds supplement in mixtures on chemical composition and fatty acids profile in muscular tissue of male kids.

Material and Methods

The experimental material consisted of 20 male kids of white improved breed. After weaning in about the 60th day of life the kids were divided into two groups. The animals of the control group were fed with standard mixture (CJ), whereas those of the experimental group were fed with mixture containing: 37% of barley, 17% of oat, 25% of wheat bran, 10% of soybean meal, 10% of lin seeds, 1% of mineral mixture. Chemical analysis of the mixtures and fat acids composition were presented in Table 1. The kids were slaughtered at the age of 150 days and their average body weight in the control group amounted to 34.1 kg while in the experimental group 39.1 kg, according to the method presented by the Animal Science Institute. The carcasses were cooled for 24 hours at 4°C, and then samples of longissimus dorsi and those of muscle adductor from the left half carcasses were taken. The basic chemical composition of

adductor muscle was tested. The analyses comprised: dry matter content carried out by means of the drying oven method, crude protein content by Kjeldahl method, intermuscular fat content by Soxhlet method and crude ash content by the burning method. The W/B index was calculated on the grounds of water (W) and protein (B) contents.

The fatty acids profile in intermuscular fat of muscle longissimus dorsi was estimated by means of Soxhlet method. Fatty acids composition was tested by gas chromatography method using apparatus CHROM 5 in the following conditions: flaming and ionizing detector (FID), glass and spiral column with 10% phase SILAR 5CP of 4 mm in inner diameter and of 2.5 m in length, carrying gas – nitrogen – influx 30 ml min $^{-1}$, temperature: column 200^0C, feeder 250^0C, detector 250^0C.

The results were statistically analysed by Statistica test 6.0 PL (2002).

Results

The analysis of the results presented in Table 2, which referred to the effect of feeding on chemical composition of muscular tissue in kids, indicated similar contents of crude protein and ash in both groups. Significant differences (P≤0,01), however, in dry matter and fat contents were found. Lower fat content (by 1.82%) and dry matter content (by 1.40%) in the experimental group were proved. The W/B index was, in a way, an indicator of aged meat for particular animal species (PROST, 1975). In the study the W/B index oscillated from 3.66 to 3.78 and no significant differences between groups were found.

Table 1
Chemical composition of mixtures

Specyfication	Groups	
	Control	Experimental
Dry master (%)	89.61	88.26
Crude protein (g/kg)	164.73	165.28
Fat (%)	3.45	5.23
Ash (%)	2.89	2.45
SFA (%)	22.74	18.53
UFA (%)	77.26	81.47
MUFA (%)	32.08	31.21
PUFA (%)	45.18	50.26

Table 2
Chemical composition of male kids meat

Specyfication	Groups			
	Control		Experimental	
	$\bar{x}$	SD	$\bar{x}$	SD
Dry master (%)	25.15**	0.39	23.75**	0.49
Crude protein (%)	19.79	0.40	20.84	0.46
Fat (%)	3.60**	0.15	1.78**	0.09
Ash (%)	1.08	0.14	1.09	0.03
Index (water/protein)	3.78	0.13	3.66	0.12

** - P ≤ 0,01

Data regarding fat acids content in intermuscular fat of kids were shown in Table 3. Significantly lower content of saturated fatty acids (40.34%) by 7.7% in kids fed with mixture containing lin (P≤0,05) was found. Among saturated fatty acids statistically

significant differences in palmitic acid ($C_{16:0}$) and stearic acid ($C_{18:0}$) were proved and they amounted to 24.84 and 19.90% in kids of the control group as well as 21.11 and 15.72% in kids fed with lin supplement, respectively. Monounsaturated acid content oscillated from 52.97 to 59.66%, and the differences between groups were statistically significant. The analysis of the acid composition showed larger by 6.79% content of $C_{18:1}$ and by 0.16% of $C_{20:1}$, whereas lower by 0.14% of $C_{14:1}$ in kid meat fed with mixture with lin supplement; significance of differences was statistically proved. Differences in the rest of monounsaturated fatty acids were statistically non significant. Lin supplement in mixtures caused significant ($P \leq 0,05$) increase in total number of polyunsaturated acids by 1.88% in intermuscular fat of kids, in comparison with the control group. Significant or highly significant differences in $C_{18:2}$, $C_{20:3}$ and $C_{20:4}$ were found. From the dietetic point of view, relations between unsaturated and saturated acids are equally important for a consumer, and ratio 2 is thought to be the optimum one (NESTEL, 1987). In the study the ratio amounted to 1.10 in the control group, and 1.47 in the experimental group.

Table 3
Fatty acid profile of male kids *m. longissimus dorsi* (%)

Specification	Group			
	Control		Experimental	
	$\bar{x}$	SD	$\bar{x}$	SD
$C_{12:0}$	0.04	0.02	0.37	0.05
$C_{12:1}$	0.04	0.01	0.05	0.01
$C_{14:0}$	1.88	0.18	1.87	0.09
$C_{14:1}$	0.27*	0.05	0.13*	0.04
$C_{15:0}$	0.27	0.04	0.24	0.05
$C_{15:1}$	0.56	0.13	0.30	0.11
$C_{16:0}$	24.84*	0.73	21.11*	0.80
$C_{16:1}$	2.72	0.19	2.14	0.11
$C_{17:0}$	0.65	0.07	0.86	0.15
$C_{17:1}$	0.88	0.13	0.71	0.11
$C_{18:0}$	19.90**	0.53	15.72**	1.80
$C_{18:1}$	43.22**	0.42	50.01**	2.77
$C_{18:2}$	3.26*	0.59	4.55*	0.67
$C_{18:3}$	0.47	0.09	0.30	0.07
$C_{20:0}$	0.25	0.04	0.17	0.03
$C_{20:1}$	0.15*	0.04	0.31*	0.05
$C_{20:2}$	0.10	0.03	0.14	0.05
$C_{20:3}$	0.09*	0.04	0.25*	0.10
$C_{20:4}$	0.21**	0.08	0.46**	0.09
SFA	48.03*	1.09	40.34*	3.77
UFA	52.97**	1.08	59.66**	3.70
MUFA	47.84**	0.78	53.65**	2.55
PUFA	4.13*	0.68	6.01*	0.77

* - $P \leq 0,05$; ** - $P \leq 0,01$

Discussion

It was found that the insertion lin seeds into mixtures resulted in decreasing fat and dry matter contents in chemical composition of kid meat. No significant differences, however, in chemical composition of muscular tissue were found in scientific research carried out on lambs fattened and fed with mixtures containing lin seeds (BOROWCA et al., 2004; MARCIŃSKIEGO et al., 2003; GRZEŚKOWIAK et al., 2004).

Lin seeds supplement in mixtures for kids had a highly significant influence on the increase in unsaturated fatty acids content, including polyunsaturated fatty acid content and on the decrease of saturated fatty acid content in intermuscular fat, in comparison with the control group. Similar results were presented by KESAVA RAO et al. (2002), who conducted research on the effect of introduction remains after extracting oil from Azadirachta indica in kids fattened till 5 – 6 weeks of age. In the studies the authors found the decrease of total number of saturated fatty acids and the increase in the number of unsaturated acids. The detailed analysis showed the decrease of palmitic acid content ($C_{16:0}$) and the increase in oleic acid content ($C_{18:1}$). MARCINSKI et al. (2003) carried out experiments, in which they introduced lin seeds into mixtures (10%) for fattened lambs. The authors found that lin supplement in mixtures for lambs affected the changes of fatty acid profile in fat of muscle longissimus dorsi. The increase in $C_{18:1}$ and $C_{18:3}$ contents in animals fed with Opal lin seeds, while the increase in $C_{18:2}$ content in animals fed with Linola lin seeds were proved. Significantly larger $C_{18:1}$, $C_{18:2}$ and $C_{20:4}$ contents in muscular tissue of lambs fed with mixtures containing 10% of fatty preparation Megalac were also found by GRUSZECKI et al. (2004).
Similar relations in the similar kind of studies conducted on lambs fattened with mixtures with 15% of lin seeds were found by KACZOR et al. (1999). Significantly larger by 4.5% unsaturated fatty acid content, including polyunsaturated fatty acids by 2.5% was proved. Moreover, larger unsaturated fatty acid contents, and especially $C_{18:1}$ and $C_{20:4}$, in lambs fed with mixtures containing lin were stated by BOROWIEC et al. (2004). Additionally, lin species differentiated fatty acid content in muscular tissue.

Conclusion

Studies presented in the paper showed that lin seeds supplement in mixtures for kids significantly affected dry matter and fat contents in muscular tissue. Lower values of the components in the experimental group were found. In addition, changes of fatty acid profile were also proved. The changes especially referred to larger unsaturated fatty acid content, particularly $C_{18:1}$, $C_{18:2}$, $C_{20:1}$, $C_{20:3}$ and $C_{20:4}$ in kids fed with mixtures containing lin seeds. The results proved favourable effect of lin seeds on chemical composition and fatty acid profile in muscular tissue of fattened kids.

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The effect of a disinfectant on the ammonia concentration on the surface of litter, air and the pathomorphological picture of kidneys and livers in broiler chickens*

Abstract

The study was conducted in order to determine the effect of a disinfectant on the level of ammonia on the surface of litter and air of henhouses as well as the pathomorphological picture of broiler chicken livers and kidneys. 240 meat-breed broilers (Cobb 500) were used in the study and divided into two identical groups, control and experimental – in which a disinfectant was used on the surface of litter (trade name Lubisan®). The study showed a significantly higher ($p \leq 0.01$) concentration of ammonia on the surface of the control litter in comparison to the litter treated with the disinfectant. In addition, despite the absence of significant differences, the level of ammonia in the air in the control group was slightly higher. A pathomorphological examination revealed that there were 49% less deviations from standard in the internal organs of the experimental group chickens, with 55% less of such disorders in the kidneys and 39% less in the livers. It is noteworthy that there were significantly less (by 63%) regressive changes in the livers of chickens kept on the treated litter and that no inflammations occurred in the examined organs, which were recorded in the control group both in the kidneys and the livers. To summarise the results of this study, it can be said that the application of a disinfectant helps to reduce the concentration of ammonia on the surface of litter for poultry and to restrict the occurrence of morphological disorders in the livers and kidneys of broiler chickens.

Key Words: broiler chickens, litter, disinfectant, ammonia, liver, kidneys.

Introduction

The ammonia which is emitted from poultry farms is an environmental problem everywhere in the world (SAPEK, 1995; KURVITS and MARTA, 1998; WATHES, 1998). Apart from that, it is considered to be one of the major stress causing microclimatic factors in poultry production. Excessive amounts reduce the birds' productivity and deteriorates their health (AL MASHHADANI and BECK, 1985; TYMCZYNA and SABA, 1987). WATHES (1998) observed that when ammonia concentration was higher than 25 ppm, the growth of chickens was considerably slower. Other authors (AL HOMIDAN et al., 2003) found that health disorders might occur in birds when they are exposed to concentrations lower than 25 ppm NH_3. WATHES et al. (2004) suggest that in order to improve birds' welfare the acceptable concentration of 20 ppm of ammonia in poultry farms should be reduced to 10 ppm.

Research has been conducted for many years into ways to reduce the amount of ammonia emitted for poultry litter. However, many of the preparations used in the past for reducing the amount or neutralising ammonia proved toxic or carcinogenic (TYMCZYNA, 1993; MOORE et al., 1996). Currently, there is a tendency to apply preparations which can be safely used in the presence of animals, without harming the environment. Among the range of additives, such as human preparations, aluminosilicates, saponins or microbiological substances (DOBRZAŃSKI et al., 2000), there are also disinfectants. One of specific products, containing chloramine T and inorganic compounds together with essential oils, is a sanitising and disinfecting preparation with the trade name of Lubisan®. It is intended for disinfecting rooms

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where animals are kept and for disinfecting litter during the rearing of animals. According to the producers, the preparation should not only reduce the number of microbes and parasites but also reduce the concentration of noxious gases in the buildings where animals are kept.
The aim of this study was to assess the effect of the disinfectant preparation by the trade name of Lubisan® on the level of ammonia on the litter surface and in the air, and the pathological picture of the broilers' livers and kidneys.

Materials and Methods

240 meat-breed broiler chickens (Cobb 500) were used for the experiment and were divided into two identical groups: control and experimental (for which the Lubisan® disinfectant was applied on the litter). The birds were kept for 6 weeks on litter of cut rye straw, in isolated, separated rooms with similar microclimatic conditions. There were 13 birds on each square metre. All the birds were fed *ad libitum*, on Starter, Grower and Finisher complete feed, according to the Dossche company feeding programme.
Ammonia concentration (ppm) on the litter surface and in the air (on the level of the birds' heads) was measured throughout the rearing period, daily at 7.00, 1.00 p.m. and 9.00 p.m., with a MiniTOX 3 multigas meter.
Directly after slaughter, the livers and kidneys of 12 randomly- chosen broilers from each group were examined macroscopically; following this, a section was taken from the left lobe of the liver (*lobus hepatis sinister*) and from the left kidney in order to conduct a microscopic examination. The material was preserved in 10% neutralised formalin and subjected to histopathological treatment. Paraffin sections were stained with haematoxylin-eosin (HE), and the frozen liver (in order to visualise their being covered with fatty tissue) was stained with Sudan III, according to the method developed by Lillie Ashburn.
The results were analysed statistically with *Statistica 7.0* computer software, with the use of single-factor analysis of variance, in orthogonal design. The significance of differences between the average values of the features under study was determined with Duncan's test.

Results

No ammonia was found on the surface of the analysed litter during the first week of the experiment; in the second week 5.5 ppm of ammonia was found on the control litter while on the litter optimised with the disinfectant – not even traces of the gas were found (Table 1). In the fourth week, statistically significantly higher ($p \leq 0.05$) concentration of NH_3 (21.7 ppm) was found on the control litter than on the experimental (12.0 ppm), while in the fifth week, the difference was highly significant (control – 22.7 ppm, experimental – 12.0 ppm). In the last week of the bird rearing, the highest concentrations of ammonia were found, but the difference between the control (31.2 ppm) and experimental litter (22.3 ppm) was not significant. The analysis of the average concentration of ammonia on the litter surface throughout the period of bird rearing revealed a highly significant difference between the rooms in which the chickens were kept, with the ammonia concentration on the control litter higher by 49% and amounting to 16.0 ppm, while on the experimental litter it was only 8.1 ppm. The highest ammonia concentration during the whole period of experiment was found

on the surface of the litter on which the disinfectant was not applied. A very high concentration of 95 ppm was recorded in the control group in the last week of the bird rearing, while it was lower by 42% (46 ppm) in the experimental group.

Table 1
Ammonia concentration (ppm) on the litter surface and in the air in the chicken rooms

Week of rearing	Statistical measures	Litter		Air	
		Control	Experimental	Control	Experimental
1	$\bar{x}$	0.0	0.0	0.0	0.0
	min	0.0	0.0	0.0	0.0
	max	0.0	0.0	0.0	0.0
	s	0.0	0.0	0.0	0.0
2	$\bar{x}$	5.5	0.0	0.0	0.0
	min	0.0	0.0	0.0	0.0
	max	8.0	0.0	0.0	0.0
	s	1.4	0.0	0.0	0.0
3	$\bar{x}$	13.0	6.5	0.0	0.0
	min	11.0	4.0	0.0	0.0
	max	17.0	9.0	0.0	0.0
	s	3.3	2.3	0.0	0.0
4	$\bar{x}$	21.7*	12.0*	0.7	0.7
	min	20.0	11.0	0.0	0.0
	max	26.0	14.0	1.0	1.0
	s	3.1	1.7	0.6	0.6
5	$\bar{x}$	22.7**	12.0**	2.8	2.5
	min	7.0	6.0	1.0	0.0
	max	54.0	33.0	5.0	5.0
	s	13.5	6.1	1.2	1.6
6	$\bar{x}$	31.2	22.3	5.8	5.5
	min	7.0	4.0	3.0	2.0
	max	95.0	46.0	9.0	6.0
	s	23.8	13.6	1.7	1.9
Total	$\bar{x}$	16.0**	8.1**	1.6	1.5
	min	0.0	0.0	0.0	0.0
	max	95.0	46.0	9.0	6.0
	s	15.4	11.0	2.5	2.5

No ammonia was found in the air where the birds were kept for the first three weeks of the experiment (Table 1). Only during the fourth week did the first traces of ammonia start to appear in both henhouses, at a maximum of 1.0 ppm. In the fifth week, the gas concentration in the control room was recorded at an average level of 2.8 ppm, with the minimum value of 1.0 ppm. In the room where the disinfectant was applied, there were times during the same week when no ammonia was recorded, although its average concentration (2.5 ppm) was close to that recorded in the control room. The ammonia concentration in the air of both rooms grew in the sixth week, but it was similar in both (control – 5.8 ppm, experimental – 5.5 ppm). However, the maximum value of the NH_3 concentration in the control room was higher (9 ppm) than in the one where the litter was optimised with the disinfectant (6 ppm).

The macroscopic examination revealed that the morphological picture of the analysed organs was consistent with the standards (ROTKIEWICZ et al., 1995). The microscopic examination of the livers and kidneys of the chickens showed the presence of regressive changes, blood circulation disorders, inflammation and progressive changes (Table 2). Among the regressive changes, accumulation of fat in hepatocytes was observed (Fig. 1, 2). These disorders were observed in all the 12

chickens in the control group and in three in the experimental one. The changes were considerably less intense in the birds from the experimental group (Fig. 1, 2). In the liver of three chickens from the control group and of two from the experimental group, degenerative changes of parenchyma were observed. Additionally, in each group one case of degenerative vacuole was observed. The regressive changes in livers were usually accompanied by various degrees of congestion. This was observed in 7 birds in the control group and in 4 in the experimental group (Table 2). In some blood vessel walls in three birds from the control group, swelling of endothelium cells was observed. No such changes were observed in the experimental group (Table 2). Among the progressive changes in the liver, proliferation and hypertrophy of stellate cells were observed in stellate cells in one chicken in both groups. In 4 birds from the control group, proliferation of connective tissue in the blood vessel walls was recorded. Additionally, in 2 of the control chickens and in 4 experimental ones, infiltration of mononuclear phagocytes of monocytic occurred (Table 2).

Table 2
Number and type of microscopic lesions in the liver and kidneys of 42-day-old broiler chickens Cobb 500

Type of microscopic lesions	Liver		Kidneys		Total	
	Control	Experimental	Control	Experimental	Control	Experimental
Regressive lesion	16	6	6	3	22	9
Circulation disorders	7	4	6	6	13	10
Inflammations	3	0	2	0	5	0
Progressive lesions	7	5	4	2	11	7
Total	33	15	18	11	51	26

In the kidneys, parenchymatous degeneration of renal tubulae epithelium was frequently observed (Fig. 3, 4). This was found on small areas in three control chickens and in 3 experimental ones. In 3 chickens from the control group, single epithelium cells of renal tubulae were damaged by necrosis (Fig. 3, Table 2). Congestion in the kidneys usually accompanied regressive changes (Fig. 3) and inflammation – it was observed in 5 control and 4 experimental birds. Sometimes near the blood vessels, widened and filled with morphotic elements (the experimental group - 2), and less frequently away from them (control – 1), point-like extravasations were visible (Table 2). In two birds from the control group, glomerulitis was observed (Fig. 4). The range of the process was limited, the inflammation was only visible in a few of the glomeruli in each preparation from a given chicken. In such cases, the change was accompanied by congestion and sometimes extravasation of the organ (Fig. 4, Table 2). In birds of both groups there were sporadic infiltrations of mononuclear cells (control – 2, experimental – 1). Sometimes proliferation of the connective tissue was observed - 2 cases in the control group and one in the experimental group (Table 2).

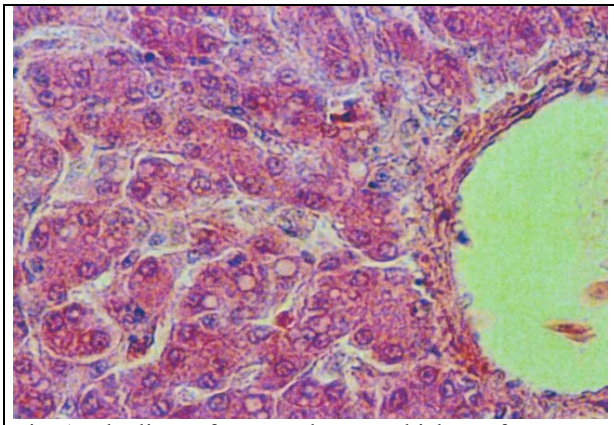

Fig. 1: The liver of a control group chicken - fat accumulation in hepatocytes. Stained with HE, magnified 520×

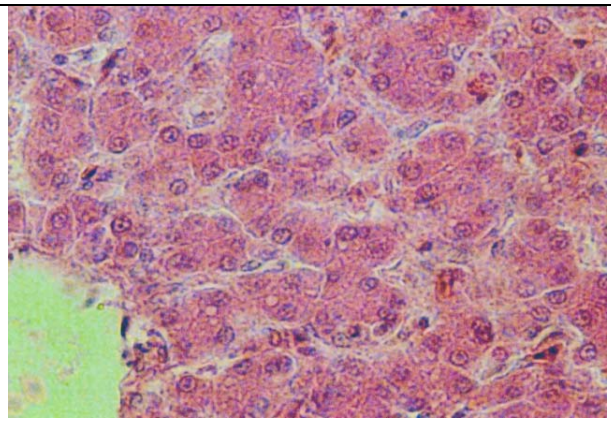

Fig. 2: The liver of an experimental group chicken - low degree of fat accumulation in hepatocytes. Stained with HE, magnified 520×

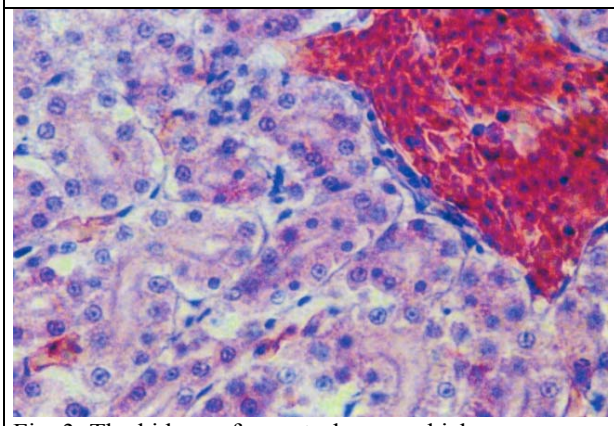

Fig. 3: The kidney of a control group chicken – parenchymatous degeneration of renal tubulae epithelium, necrosis of single epithelium cells, congestion. Stained with HE, magnified 520×

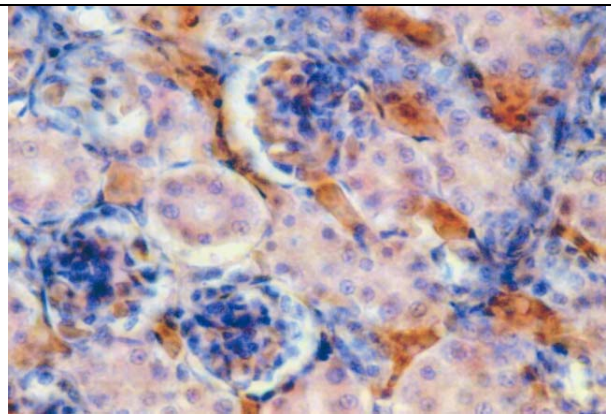

Fig. 4: The kidney of a control group chicken – parenchymatous degeneration of renal tubulae epithelium, extravasations, glomerulitis. Stained with HE, magnified 520×

Discussion

In the experiments conducted for this study, the presence of ammonia on the litter surface was found starting with the second week of the rearing, but this was only in the control group. In subsequent weeks, the concentration of ammonia increased; however, it was lower on the litter which was treated with the disinfectant. It must be stressed that compared to the findings of other authors (TYMCZYNA et al., 1995; RUDZIK, 1998) concerning the average ammonia concentrations on the surface of the litter under study, the maximum concentration of the gas in the control group was twice as high (95 ppm) as in the experimental group (46 ppm). Studies conducted by many authors also confirm the effectiveness of various agents in reducing ammonia concentration in litter. RUDZIK (1998) studied the use of kaolin and zeolite in a poultry farm with 13.5 thousand chickens and proved their effectiveness in reducing the concentration of ammonia in litter, and consequently in the air above it. Other authors analysed such additives as lignite (DOBRZAŃSKI et al., 1989), a humus preparation under the name of Humokarbowit (DOBRZAŃSKI et al., 1994), natural aluminosilicate – bentonite (TYMCZYNA et al., 1995), a microbiological preparation called Cobio-litiere (DOBRZAŃSKI et al., 2000), organic acids (IVANOV, 2001) and

found higher ammonia concentrations on the surface of litter which was not treated with any additives.

In numerous studies on the effectiveness of various additives in reducing the amount of ammonia in the air, different results were obtained by various researchers. AMON et al. (1997) applied a zeolite preparation by the name of Clinoptiolite and did not observe any positive action, recording a much higher concentration of ammonia in the room where the zeolite was used. The authors found that litter properties could bring opposite effects, inhibiting the absorption and causing ammonia to escape. RUDZIK (1998) applied aluminosilicates on a straw litter during a seven-week period of bird rearing and found the agents to be effective in reducing ammonia concentration, recording average values from 14.50 ppm in the control sector to 11.30 ppm in the sector with kaolin and 9.35 ppm in the sector where zeolite was applied. NÁVAROVÁ et al. (2004) also proved the effectiveness of Oxyhumolite (humus sorbing agent). On day 22 of that particular experiment, the authors recorded an ammonia concentration of 5.22 ppm in the control room and 3.08 ppm in the experimental one, while on day 28 the values were 9.36 ppm and 6.42 ppm, respectively. DOBRZAŃSKI et al. (2000), analysed the effect of the Cobio-litiere preparation, which contains a complex of bacteria, on the emission of ammonia from litter on which laying hens were kept for 5 months, and recorded the average from 14 ppm of ammonia in the air in the experimental room to 26.72 ppm in the control environment. Probably due to a larger number of birds, and in some cases due to a longer period of rearing, the research of the cited authors found higher concentrations of ammonia than recorded in the current study. However, it should be stressed that despite the low average concentration in the room where the litter was left untreated, the maximum ammonia concentration was as high as 9 ppm (Table 1), which is above 7 ppm - the value considered by some authors to be harmful to the human respiratory system (DOBRZAŃSKI et al., 2000).

During the pathomorphological examination, morphological changes lesions were observed both in the control chickens and in those kept on the disinfectant treated litter. The changes concerned the liver more often than kidneys. According to SZAREK et al. (2000), the liver of quickly growing chickens is particularly prone to morphological changes. 49% more morphological disorders were found in the organs of the control chickens. Compared to the group of chickens kept on the disinfectant treated litter, 55% more changes were found in the livers of those birds and 39% more in the kidneys. In addition, in the livers of the birds kept on the optimised litter, much fewer regressive changes (by 63%) were found than in the control group. It is noteworthy that the differences were also qualitative. The kidneys of 3 control birds contained microfocuses of necrosis, which were not observed in the experimental group. No inflammation focuses were found in the birds kept on the optimised litter, while in the control birds 3 inflammation focuses were found in the livers and 3 - in the kidneys. Particularly intense accumulation of fat in liver hepatocytes was also observed in the group (Fig. 1, 2). Fat drops, pushing the nuclei outwards in the cytoplasm of parenchymal cells, were in some birds larger than in the experimental chickens. Although fat accumulation in cells is usually a reversible process, (SZAREK et al., 1997), when the extent of damage to a cell structure is substantial, such changes may be a form of cell death (ŻULIŃSKI et al., 1981). TYMCZYNA and SABA (1987) quote other authors who claim that besides the typical effects produced by ammonia, such as changes in the respiratory system and eyeball, fatty degeneration of

liver also occurs. The environmental origin of the changes observed in the study may be confirmed by their location – near blood vessels (SZAREK et al. 1995).
To summarise the study results, it can be said that application of a disinfectant reduces the concentration of ammonia on the surface of litter and decrease the occurrence of morphological irregularities in the livers and kidneys of chickens.

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Polymorphism in the melatonin receptor gene MT1 (locus *MTNR1A*) in sheep

Abstract

The aim of the study was to identify the polymorphism at the melatonin receptor gene (locus *MTNR1A*) in sheep breeds: prolific Olkuska sheep, Polish Mountain sheep, Suffolk and in sheep F1 crosses (Merino-Romanov). A high frequency of the + allele was found in sheep with seasonal sexual activity: prolific Olkuska sheep (0.643), Polish Mountain sheep (0.684) and Suffolk (0.6). In aseasonal F1 (Merino-Romanov) sheep, a higher proportion of the – allele was found (0.795). The frequencies of +/+ genotype was 0.529, 0.474, 0.6, 0.205 in prolific Olkuska sheep, Polish Mountain sheep, Suffolk and F1 (Merino-Romanov) crosses respectively. Analysis of genotype and its relationship with litter size and blood concentration of melatonin in prolific Olkuska sheep showed that genotype had no effect on the parameters studied.

Key Words: MT1 melatonin receptor gene, polymorphism, litter size, sheep

Introduction

Identification of major genes or QTL (Quantitative Trait Loci) affecting the control of seasonal reproduction in sheep may be an important factor in understanding those neurophisiological processes. One of them is MT-1 melatonin receptor gene**,** the hormone that plays an important role in reproductive processes. (HERNANDEZ et al., 2005). Melatonin regulates a number of physiological processes such as the diurnal rhythm (WITT-EDENBRY et al., 2003), the proliferation and function of many tissues, the maturation and function of the reproductive system (CLEMENS et al., 2001) and seasonal reproduction (MALPAUX et al., 1996; BLUMENAU et al., 2001). Melatonin affects the body mainly thanks to the presence of specific protein receptors, MT-1 and MT-2. Subtypes Mel1a, Mel1b and Mel1c were identified among receptors (MT-1) that show high affinity to melatonin. Receptors with low affinity to melatonin (Mel2) were included in the MT-2 group (REPPERT et al., 1996). The gene coding for the melatonin receptor protein MT1 (*MTNR1A* locus), localized to chromosome 26 in sheep, is formed by two exons interrupted by an intron (REPPERT et al., 1994), and the differences occurring in the structure of receptors result from changes in the second exon (BARRETT et al., 1997). Within this segment, the presence of polymorphism detected by *Mnl*I and *Rsa*I restrictive enzymes is observed (MESSER et al., 1997). There are 8 sites identified by the *Mnl*I enzyme within the amplified sequence (BARRETT et al., 1997; MESSER et al., 1997; NOTTER et al., 2003). Thanks to the implied relationship between allelic versions of the gene and reproductive performance of sheep, the genotype at the *MTNR1A* locus can become one of the markers used in studying the sexual activity of sheep (NOTTER et al., 2003).

The aim of the study was to identify *MTNR1A/Mnl*I polymorphism in prolific Olkuska sheep, Polish Mountain sheep, Suffolk and F1 Merino-Romanov crosses, and to show a relationship between the polymorphism studied and the litter size at three, first lambings and melatonin concentration in the plasma of prolific Olkuska sheep ewes.

Material and Methods

The experiment was carried out using material taken from 168 sheep: the prolific Olkuska sheep; Polish Mountain sheep originating from the Experimental Station of the Agricultural University in Kraków and from private farms; Suffolk; and F1 (Merino-Romanov) crosses kept at experimental stations of the National Research Institute of Animal Production in Grodziec Śląski and Pawłowice, Poland.

DNA isolation was performed using a MasterPure Genomic DNA Purification Kit (Epicentre Technologies, USA). Polymorphism *MTNR1A/Mnl*I was identified using the PCR-RFLP method as described by MESSER et al. (1997). A fragment of 824 bp was amplified in a PTC-200 Engine thermocycler (MJ Research, Watertown, MA, USA). The PCR reactions included: 1 x PCR buffer, 250μM dNTP, 1.5mM $MgCl_2$, 0.6 U of Taq DNA polymerase (MBI Fermentas), 0.2μM of each primer: MT1-5'TGTGTTTGTGGTGAGCCTGG3'; MT2-5'ATGGAGAGGGTTTGCGTTTA3' in a 25-μL reaction volume. 10 μl of the PCR reaction product was digested with the *Mnl*I enzyme (0.2U) (MBI Fermentas). Digestion products were analysed on 3.5% agarose gel (NuSieve GTG, BMA, USA) in the presence of the Gene Ruler^{TM} 100bp DNA Ladder (MBI Fermentas). The results were analysed and documented using UVI-KS 4001/Image PC (Syngen, Biotech).

Melatonin concentration was determined in the blood of 10-month-old Olkuska ewes (n=12) with different genotypes at the *MTNR1A* locus. Blood was collected once during short day (December), starting from the sunset and continuing for the next 6 hour at 20-minute intervals. Blood was centrifuged and the plasma obtained was stored at -20°C. Melatonin concentration was determined in 500 μl of plasma using RIA according to a method described by FRASER et al. (1983) at the Institute of Animal Physiology and Nutrition of the Polish Academy of Sciences in Jabłonna. Ewes were kept indoors and fed conventional diets based on ensiled hay and supplemental concentrate.

For 40 ewes of the prolific Olkuska sheep (at the age of 3-6 years and originating from the Balice farm) with different genotypes at the *MTNR1A* locus, reproductive performance data were collected on the number of lambings and litter size in three first, successive years of utilization. Those dates were used for the calculation of the influence of the genotypes in locus *MTNR1A* on the average litter size per ewes.

In the studied herds the frequencies of alleles and genotypes in the locus *MTNR1A* were calculated. The significance of differences between the breeds within individual genotypes and deviations of genotype frequencies from Hardy-Weinberg equilibrium were tested using a Chi-square test (FALCONER, 1974). The degree of heterozygosity was assessed for the populations according to the method of NEI and ROYCHOUDHURY (1974).

To estimate the effect of genotype at the *MTNR1A* locus on melatonin concentration in blood and litter size of Olkuska ewes an analysis was performed using the GLM procedure of the SAS packet (SAS Ver. 8.2, 2001). Because the data obtained for litter size in successive years of reproductive use showed no normal distribution, they were transformed ($\sqrt[4]{}$). The following linear model was used: $Y_{ijk} = \mu + g_i + m_j + \varepsilon_{ijk,}$ where: Y_{ij} – mean value of the trait, μ – general mean, g_i – effect of *i* genotype (i=1,2,3), m_j – effect of *j* litter (j= 1,2,3), ε_{ijk} – random error. Preliminary analysis showed that interaction between genotype and successive litter was not statistically significant. The

data were presented as the mean ±SE. Differences were considered to be significant at P≤0.05.

Results

The use of the PCR-RFLP method enabled the identification at the *MTNR1A* locus of 3 genotypes designated as +/ +, +/ – and –/– , and 2 alleles designated as + and – (Table 1).

Table 1
Frequency of genotypes and degree of heterozygosity at the *MTNR1A* locus in sheep

Breed	h	Frequency of genotypes					
		n	+/+	n	+/-	n	-/-
Prolific Olkuska sheep	0,613	37	0.529A	16	0.228a	17	0.243aA
Polish Mountain sheep	0,587	9	0.474aA	8	0.421b	2	0.105bA
Suffolk	0,480	24	0.600bA	0	0.000	16	0.400B
Merino-Romanov sheep	0,326	8	0.205B	0	0.000	31	0.795C

n – observed number of animals; Frequency genotypes in columns with different letters (A, B) differ significantly (P≤0.01), (a, b) differ significantly (P≤0.05)
h – degree of heterozygosity in locus *MTNR1A*

In prolific Olkuska sheep and Polish Mountain sheep, all three genotypes were identified and the highest frequency of +/+ homozygotes was found (0.529 and 0.474, respectively). In the above herds, the + allele was found to dominate (0.643 and 0.684, respectively). In aseasonal Merino-Romanov sheep, the reverse distribution of genotype and allele frequencies was found and no heterozygous animals were identified (Table 2). In this breed group, animals with the –/– (0.795) genotype were identified more often (P≤0.01) than in the Suffolk, prolific Olkuska and Polish Mountain sheep (0.4, 0.243 and 0.105, respectively). The degree of heterozygosity in the populations is shown in Table 1. Both Prolific Olkuska and Polish Mountain sheep were characterized by a high degree of heterozygosity in locus *MTNR1A* of 0.613 and 0.587, respectively. Only genotypes frequency in the Polish Mountain sheep was in accordance with the Hardy-Weinberg distribution.

Table 2
Allelic frequencies for the *MTNR1A /MnlI* polymorphism in sheep

Breed	Allele	
	+	–
Prolific Olkuska	0.643	0.357
Polish Mountain sheep	0.684	0.316
Suffolk	0.600	0.400
Merino-Romanov sheep	0.205	0.795

Analysis relationship between genotypes in locus *MTNR1A* and the litter size at three, first lambings of ewes prolific Olkuska sheep were stated, that ewes with genotypes +/+ characterised the highest fecundity: 2.87±0.87 lambs/litter, but ewes of the other genotypes were lambed less (about 0.4 lambs/litter) The differences were statistically non-significant (P=0.523, Table 3).

Table 3
Least squares means for litter size in three first lambings of prolific Olkuska ewes

Trait	P-value	Genotype		
		+/+ (n=25)	+/– (n=8)	–/– (n=7)
Litter size (lambs/litter)±SE	0.052	2.87±0.87	2.44±0.95	2.44±0.88

The results obtained for melatonin concentration in the blood of the Olkuska ewes, which showed genotype differences at the *MTNR1A* locus, showed the highest the hormone with the +/+ genotype (175.5±20.53pg/ml), heterozygous and homozygous –/– ewes had a little lower values for that hormone: 152±30.57 pg/ml; 147.5±32.46 pg/ml, respectively. The differences in melatonin concentration were statistically non-significant (P≥0.05) in the ewes with particular genotypes.

Discussion

Analysis of allele distribution at the investigated locus showed that breeds with clear seasonality of sexual activity (Suffolk, Polish Mountain sheep, Olkuska sheep) had a high proportion of the + allele, while a high proportion of the – allele was observed in a seasonal Merino-Romanov sheep. A similar distribution of alleles as in the population of the Olkuska, Mountain and Suffolk sheep was observed in Suffolk, Coopworth sheep (MESSER et al., 1997), Columbia (WRIGHT, 2000), Soay (BARRETT et al., 1997) and Small-tailed Han sheep (CHU et al., 2003). The frequency of the + allele in these sheep was 0.75, 0.67, 0.84, 0.75 and 0.75, respectively. The meat breeds of the Hampshire and Ile-de-France sheep were characterized by a high frequency of the – allele: 0.61 and 0.55, respectively (WRIGHT, 2000; PELLETIER et al., 2000). Comparison of source data and the present study for allele frequency shows large breed variation. However, both alleles were identified in all the analysed breeds, which is evidence that the investigated mutation appeared early during evolution (NOTTER and COCKETT, 2005). While analysing the distribution of genotypes, three genotypes were identified in Olkuska and Mountain sheep and the presence of only both homozygotes was found in Merino-Romanov sheep. No heterozygotes were determined in 39 crossbreds analysed.

The prolific Olkuska sheep and Polish Mountain sheep are the breeds characterized by marked seasonality of breeding but differ in terms of reproductive parameters. The Olkuska sheep are a prolific breed, with prolificacy exceeding 220%, while Mountain sheep have a prolificacy of approximately 130%. The frequency of two homozygous genotypes in the a seasonal Merino-Romanov sheep differed from that observed in sheep with seasonal sexual reproduction, which were characterized by a high frequency of –/– homozygous animals.

A considerable effect of the *MTNR1A/MnlI* polymorphism on the incidence of spontaneous ovulation in sheep outside the reproductive reason was confirmed (PELLETIER et al., 2000). In Merino d'Arles sheep with repeated oestrus outside the typical reproductive period for this breed, the frequency of the +/+ genotype was high and no –/– homozygotes were identified within this breed. In lines of sheep selected for the extended season of sexual activity (Virginia Tech OOS), the frequency of + and – alleles was 0.42 and 0.58, respectively (NOTTER and COCKETT, 2005). In Tisdale Polypay sheep selected for shorter lambing interval, the + allele had a frequency of 0.47, and in seasonal Suffolk and Soay breeds, the frequency was 0.67 and 0.75, respectively (BARRETT et al., 1997; MESSER et al., 1997). The relationship between genotype and parameters of reproductive performance in ewes was observed when analysis was restricted to the group of mature (at least three-year-old) sheep. Sheep with the +/+ genotype were characterized by 11.2% higher fertility in the early spring period (NOTTER et al., 2003).

Reports on the effect of genotype at the *MTNR1A* loci on litter size indicate that genetic factors have no significant effect on this productive trait despite the fact that slightly larger litters were observed in sheep with one copy of the + allele (NOTTER et al., 2003). Studies with aseasonal and highly prolific Han sheep showed that the –/– genotype identified using the RsaI enzyme was related to litter size of ewes at second lambing (CHU et al., 2003). In the present study with prolific Olkuska ewes, effect of genotype on litter size was not found but compared to the other genotypes, +/+ homozygous ewes were characterized by a greater number of lambs born in the first three lambings (0.4 lambs/litter).

The present study showed that during the dark phase (December), average melatonin concentration in the investigated animals with different genotypes at the *MTNR1A* locus did not differ. The lack of effect of polymorphism at the MT1 melatonin receptor gene on melatonin concentration was also reported by HERNANDEZ et al. (2005) in seasonal Ile de France sheep. The above authors also showed that the MT1 polymorphism has no effect on prolactin secretion and the function of hair sheaths. Therefore it seems that the MT1 polymorphism can be a small part of genetic variation that determines the seasonality of reproduction in sheep and can largely depend on breed and environmental factors.

In summary, identification of polymorphism *MTNR1A/MnlI* in the sheep of Polish breeds showed a high frequency of the + allele in: prolific Olkuska sheep (0.643), Polish Mountain sheep (0.684) and Suffolk meat sheep (0.6). In aseasonal Merino-Romanov sheep, a high proportion of the – allele was observed (0.795). Analysis of the MT1 polymorphism and its relationship with litter size and blood melatonin concentration of prolific Olkuska sheep did not show the effect of genotype on the parameters studied.

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The content and retention of some major and trace minerals in sheep's milk and cheese

Abstract

The aim of the study was to determine the content and retention of selected elements of sheep's milk in 5 types of sheep's milk cheeses: curd rennet (bundz – Bu), soft (bryndza – Br), brine (feta type – Ft), scaled-smoked (oscypek type – Os) and maturing cheese (semi-hard cheese - Pt). The heavy metal and arsenic (Pb, Cd, Hg and As) content of milk and cheeses was below the limit of quantification. Milk contained the major elements Ca – 2.47, Mg – 0.21, Na – 0.56, K – 1.49 and P – 1.67 g/kg, and the trace elements Cr – 0.024, Zn – 6.66, Fe – 0.69 and Cu – 0.09 mg/kg. Average major element content of Bu, Br, Ft, Os and Pt cheeses was 6.6, 7.1, 5.9, 10.3 and 9.0 g/kg for Ca; 0.39, 0.39, 0.33, 0.50 and 0.48 g/kg for Mg; and 3.9, 4.6, 4.5, 6.5 and 6.1 g/kg for P, respectively. Average trace element content of Bu, Br, Ft, Os and Pt cheeses was 0.050, 0.048, 0.045, 0.083 and 0.084 mg/kg for Cr; 18.77, 21.22, 17.95, 35.35 and 27.77 mg/kg for Zn; 2.11, 2.00, 1.94, 3.28 and 2.34 mg/kg for Fe; and 0.18, 0.24, 0.22, 0.35 and 0.31 mg/kg for Cu. The highest potassium content was characteristic of Br cheese, followed by Pt, Ft, Bu and Os cheeses (2.0, 1.8, 1.6, 1.4 and 1.2 g/kg, respectively). The Ca : P ratio in the analysed cheeses ranged from 1.31 for Ft cheese to 1.68 for Bu cheese. The retention of most elements in the cheeses was similar and averaged 76.2% for Ca, 47.9 for Mg, 73.4 for P, 59.9 for Cr, 86.8 for Zn, 79.0 for Fe and 68.8% for Cu. Potassium retention was the lowest and showed significant differences; it was the highest in Br cheese (37.3%), intermediate in Bu, Ft and Pt cheese (28.7% on average), and the lowest in Os cheese (14.0%).

Key Words: sheep, milk, cheese, elements, retention

Introduction

Milk and milk products are a source of many valuable nutrients and minerals for humans. The mineral content of ruminant milk depends on many factors such as the type of feed, stage of lactation, breed of animals and milk protein polymorphism (KRZYŻEWSKI et al., 2002; BIS-WENCEL, 2003; SABA et al., 2003). On the one hand, the determination of major and trace elements makes it possible to define the degree to which a product can meet the human requirements for particular elements (KUNACHOWICZ et al., 2005). On the other hand, the determination of heavy metals in animal products may indirectly show the degree to which the animal's environment is polluted (WĘGLARZY, 2005). There are relatively few studies on the determination of minerals in sheep's milk and sheep's milk products.

The present study was aimed to determine the content of some elements (including heavy metals) in sheep's milk and the degree of their retention in sheep's milk cheeses.

Materials and methods

The experiment was based on the milk obtained from a flock of 60 Coloured Merino sheep. The milk was processed into 5 types of cheese:

- curd rennet cheese (bundz type - Bu)
- soft cheese (bryndza - Br)
- brine cheese (feta type - Ft)
- scalded-smoked cheese (Koluda oscypek - Os)

- maturing Koluda cheese (semi-hard - Pt)

The experiment was carried out from February to May. The milked ewes were kept and fed indoors. The diet contained silage, hay and concentrate until the second decade of April, and rye and grass forage, hay and concentrate from the third decade of April to the end of milking. Sheep were milked mechanically twice daily after weaning of lambs at 2 months of age.

The observations were made on bulk milk and different types of cheese obtained from this milk. Milk was processed into cheese starting from the 3rd month of lactation (1st month of milking) at intervals of 2-3 weeks (4 batches). All cheese types were made from heat-treated (pasteurized) milk and produced in accordance with the Experimental Station Koluda Wielka standards. After pasteurization, the processed milk was supplemented with anhydrous calcium chloride ($CaCl_2$) at a ratio of 0.2 g/l. During the production of soft, brine and maturing cheeses, pasteurized milk was acidified with appropriate bacteria cultures prior to rennet addition. Various salt doses were added: 1-2 g to curd cheese (Bu) and 2.5-3.0% to soft cheese (Br). Brine cheese (Ft) matured in whey with 11-12% salt addition, while scalded-smoked (Os) and maturing cheeses (Pt) were salted for 24 h in a 13 and 16% brine solution, respectively.

The milk and cheese samples were assayed for the content of major elements (Ca, Mg, Na, K and P) and trace elements (Cu, Fe, Zn and Cr). Tests for the presence of heavy metals (Cd, Hg, Pb) and arsenic (As) were carried out.

The content of elements (except mercury) in milk and cheese was determined using atomic emission spectrometry (ICP-AES) on a Jobin Yvon spectrometer type 138 Ultrace. The mercury content was determined using atomic absorption spectrometry on an Altec device type AMA-254.

The content of major and trace elements in the processed milk was used as the basis for estimating their retention in the cheeses made.

The results were analysed statistically using one-way analysis of variance.

Results

During the period when silage diets were fed, the average content of the feeds was the following – heavy metals and arsenic: 0.52 Pb, 0.060 Cd, 0.006 Hg and 0.07 As; trace elements: 0.640 Cr, 76.86 Zn, 603.9 Fe and 11.1 Cu mg/kg; major elements: 7.51 Ca, 2.15 Mg, 0.98 Na, 33.11 K and 5.6 P g/kg of ration dry matter. In the forage diets, the content of As and Hg did not change, while the content of the other elements was 0.56 for Pb, 0.068 for Cd, 0.501 for Cr, 70.48 for Zn, 686.0 for Fe, 12.02 mg/kg for Cu, 7.85 for Ca, 2.47 for Mg, 1.16 for Na, 28.31 for K and 7.59 g/kg d.m. for P.

The levels of heavy metals and arsenic (Pb < 0.07, Cd <0.009, Hg <0.001 and As < 0.07) in milk and cheese were below the limit of quantification.

In the milk intended for the production of particular types of cheese, there were only slight differences in the content of major and trace elements (Table 1). It is worth noting the favourable Ca : P ratios of 1.69 in Bu, 1.55 in Br, 1.31 in Ft, 1.59 in Os and 1.49 in Pt cheeses.

The content of major and minor elements obtained in our study in different types of cheese made from Merino milk showed some variation (Table 2). The greatest differences between particular cheeses were observed in the content of sodium (Na), which was related to different degrees of their salting during the technological process.

The lowest content of Na was found in curd cheese (Bu), intermediate in scalded-

smoked (Os) and semi-hard cheeses (Pt), and much higher in soft (Br), and especially brine cheese (Ft). Scalded-smoked cheese (Os) had the highest content of Ca, Mg, P, Zn, Fe and Cu. The content of chromium (Cr) was also high, but similar as in semi-hard cheese (Pt). In relation to other cheeses, semi-hard cheese (Pt) was characterized by a higher content of all the elements studied except potassium (K), the level of which was the highest in soft cheese (Br).

Table 1
Average content of metals in the milk processed into different types of cheese

	Milk for cheese production:				
	Curd Bu	Soft Br	Brine Ft	Scalded-smoked Os	Maturing Pt
N	4	4	4	4	4
Major elements (g/kg):					
Ca*	2.49	2.53	2.49	2.50	2.53
Mg	0.22	0.22	0.22	0.22	0.21
Na	0.56	0.54	0.56	0.56	0.56
K	1.50	1.51	1.50	1.53	1.52
P	1.74	1.72	1.74	1.69	1.66
Trace elements (mg/kg):					
Cr	0.024	0.026	0.024	0.026	0.026
Zn	6.74	6.81	6.74	6.95	6.74
Fe	0.75	0.74	0.75	0.76	0.67
Cu	0.090	0.098	0.090	0.093	0.098

* - total calcium content of milk + CaCl

Table 2
Content of major and trace elements, heavy metals and arsenic in cheeses made from the milk of Merino ewes

	Milk for cheese production:				
	Curd Bu	Soft Br	Brine Ft	Scalded-smoked Os	Maturing Pt
Major elements (g/kg):					
Ca	6.61^{C}	7.11^{BCb}	5.95^{C}	10.29^{A}	9.03^{ABa}
Mg	0.39^{b}	0.39^{b}	0.33^{B}	0.50^{Aa}	0.48^{Aa}
Na	4.83^{Dc}	13.94^{ABa}	16.08^{A}	9.85^{BCb}	8.89^{CDb}
K	1.41^{BCcd}	2.01^{Aa}	1.61^{bc}	1.19^{Cd}	1.70^{ABab}
P	3.93^{B}	4.60^{B}	4.53^{B}	6.46^{A}	6.05^{A}
Trace elements (mg/kg):					
Cr	0.050^{B}	0.048^{B}	0.045^{B}	0.083^{A}	0.084^{A}
Zn	18.77^{C}	21.22^{Ca}	17.95^{Cb}	35.35^{A}	27.77^{B}
Fe	2.11^{B}	2.00^{B}	1.94^{B}	3.28^{A}	2.34^{B}
Cu	0.18^{Bc}	0.24^{bc}	0.22^{bc}	0.35^{Aa}	0.31^{ab}
Heavy metals (mg/kg):					
As	<0.07	<0.07	<0.07	<0.07	<0.07
Pb	<0.07	<0.07	<0.07	<0.07	<0.07
Cd	<0.009	<0.009	<0.009	<0.009	<0.009
Hg	<0.001	<0.001	<0.001	<0.001	<0.001

A, B, C, D - ≤0.01, a, b, c, d - ≤0.05

The retention of particular major and trace elements in the milk of the cheese types analysed showed some variation (Table 3). For trace elements, the highest retention was found for Ca and P, and very low for K. The sodium (Na) content of cheese was found to increase many times in relation to milk (Table 1 and 2). This was due to the use of different salt supplements during the production of different types of cheese. For this reason, the retention of sodium in cheeses from milk was not analysed.

Table 3
Estimated retention of some major and trace elements in sheep's milk cheeses (%)

	Type of cheese				
	Curd Bu	Soft Br	Brine Ft	Scalded-smoked Os	Maturing Pt
N	4	4	4	4	4
Major elements:					
Ca	80.3	77.7	72.0	73.8	77.1
Mg	54.0	49.7	46.2	40.9	48.5
K	28.4^{Ab}	37.3^{Aa}	32.3^{A}	14.0^{B}	25.5^{Ab}
P	68.7	74.2	78.7	68.3	78.6
Trace elements:					
Cr	63.5	51.3	57.3	57.5	69.8
Zn	84.9	86.7	81.0	92.0	89.3
Fe	86.1	76.1	77.0	78.7	77.1
Cu	60.8	69.8	75.2	68.0	70.0

A, B - ≤0.01, a,b - ≤0.05

Among trace elements, more favourable retention results were obtained for Zn and Fe than for Cr and Cu. The type of cheese produced had a significant effect on potassium (K) retention only. Very low retention of K was obtained in the scalded-smoked cheese (Os).

Discussion

In our study, the content of major elements (Ca, Mg, Na, K and P) and Fe in the milk of Merino ewes was higher than the average values reported for sheep's milk by other authors (KUNACHOWICZ et al., 2005; HAENLEIN, 1995), as well as higher (except Na) than in the milk of Sarda sheep (PIRISI et al., 1999). Of the trace elements, the content of Zn was at a similar level, Cu was much lower, and Fe about twice that in the study of SZYMANOWSKA et al. (1997). In the milk of the Blackheaded sheep from Pomerania (SABA et al., 2003), the content of most minerals was lower except K, which was higher and Mg, which was at a comparable level to that in the Merino ewes. The content of major elements (Ca, P and Mg) in sheep's curd cheese (and in the other cheeses) was higher than in the cheeses of comparable types (curd, brine and maturing) from cow's milk (KUNACHOWICZ et al., 2005), and at a similar level as in whey cheeses obtained from cow's milk (SURAŻYŃSKI et al., 1977). As reported by SURAŻYŃSKI et al. (1977), the calcium content of cheeses (480-750 mg/100 g of product) allows them to be classified as high-calcium products; therefore, all the cheeses analysed meet this requirement.

In sheep's milk and in curd cheese obtained from sheep's milk, WĘGLARZY (2005) obtained Pb levels of 0.04-0.06 mg/kg and Cd levels of 0.002-0.006 mg/kg, i.e. below the values assumed in our study as the limit of quantification for these elements.

The lack of available studies investigating the retention of milk minerals in sheep's cheeses makes it difficult to discuss the results obtained. SURAŻYŃSKI et al. (1977) obtained calcium retention of 53.1-65.5% in whey cheeses from cow's milk, which is less than in our study (72-80%). Overall, the milk of the Merino sheep and the cheeses made from this milk were characterized by a relatively high content of major and trace elements, but they did not contain heavy metals (Pb, Cd and Hg) and arsenic (As) at a level exceeding their limit of quantification.

Significant differences were found in the content of trace and major elements (except

K) depending on the type of cheese. Compared to curd, soft and brine cheeses, scalded-smoked and maturing cheeses contained more Ca, Mg, P, Cr, Zn, Fe and Cu. The type of cheese had no effect on the retention of Ca, Mg, P, Cr, Zn, Fe and Cu from milk, but caused differences in the content of potassium (lowest in scalded-smoked cheese and highest in soft cheese).

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Effects of crossbreeding and different feeding systems on slaughter value and meat quality of lambs reared in natural pastures of the Beskid Sądecki Mountains

Abstract

Polish Mountain Sheep (PMS) lambs and their crossbreds with Bergschaf and Weisses Alpenschaf rams (F_1 and F_2), fed extensively on pasture or semi-intensively on pasture and indoors, were investigated. The crossbreeding of PMS lambs with alpine rams had a positive effect on both live and slaughter traits. Superior live and slaughter traits were found in semi-intensively fed lambs, while their extensive grazing resulted in their lower fatness. Water holding capacity (centrifugal drip), the content of intramuscular fat and the fatty acid profile were considerably differentiated by the crossbreeding scheme used. Compared to semi-intensive feeding, extensive feeding had a significant effect on higher drip loss, lower level of unsaturated acids and higher level of saturated acids, and lower level of polyunsaturated fatty acids. The meat of the lambs was characterized by high sensory scores.

Key Words: crossbreeding, lamb, feeding, slaughter value, meat quality

Introduction

Thanks to their unique natural diversity, the Beskid Sądecki Mountains have been included in the Natura 2000 European network of nature conservation sites on a transfrontier scale. A mountain lamb produced under these conditions should be associated with the "origin and quality guaranteed" trademark (DROŻDŻ and GÓRA-DROŻDŻ, 1999) and given an "eco-" or "bio-" label (VLACIL and MARGETIN, 1999).

The Polish Mountain Sheep (PMS) is the only native breed of sheep for which mountains are the natural habitat. The breed has poorly developed meatiness traits, which makes this sheep hardly suitable for fattening (CIURUŚ and DROŻDŻ, 1995).

This resulted in the need to import the Aalpine breeds Bergschaf (BF) and Weisses Alpenschaf (WAS) into Poland. These breeds are characterized by good meatiness, aseasonality, and resistance to harsh environmental conditions.

The aim of the study was to determine the slaughter value and meat quality of The Polish Mountain Sheep and their F_1 and F_2 crosses with the Bergschaf and Weisses Alpenschaf rams, kept in different feeding systems (extensive on pasture or semi-intensive on pasture and indoors).

Material and Methods

The experiment was carried out in the Sheep Research and Implementation Centre in Piorunka n. Krynica Zdrój, in the years 2003-2004, using 50 PMS lambs of equal age and their F_1 and F_2 crosses with the Bergschaf and Weisses Alpenschaf rams. Lambs were fed either in the extensive or semi-intensive system. The group of lambs reared extensively stayed on pasture throughout, in specially prepared rotational paddocks. Lambs from the second feeding group were grazed on pasture (regardless of weather conditions) and returned at night to the fold, where they received ground grain (0.25

kg/day/animal). Animals of both groups had access to water and salt licks with minerals.
At 200 days of age, lambs were slaughtered and their carcasses cut using the method of NAWARA et al. (1963).
Commercial classes of carcass muscling, developed as part of the EUROP grading system (Verordnung Deutsches Ministerium, 1993), were determined. Percentage of valuable cuts (best end of neck, saddle, leg and shoulder) was determined in right half-carcass. Detailed dissection of the leg was performed. *Musculus longissimus dorsi* (*Mld*) area was determined using ECHOSCAN 2 software.
Mld muscle was analysed for dry matter (PN-ISO 1442:2000), crude protein (PN–75/A-04018, 1975), fat (PN–ISO 1444:2000), pH of meat after 24 h cooling (PN-A-82058 1977), and water holding capacity (centrifugal drip) using the Grau and Hamm method (TYSZKIEWICZ, 1969). Sensory characteristics of meat (*Musculus semimembranosus*) were evaluated on a 5-point scale (BARYŁKO-PIKIELNA, 1975).
Higher fatty acids were analysed using gas chromatography (VARIAN 3400) after previously extracting lipids according to FOLCH et al. (1957) and converting free fatty acids into methyl esters.
The results were analysed statistically using multivariate analysis of variance, with genetic group and feeding system as differentiating factors (VOLK, 1973). *Post-hoc* analysis was performed using the Tukey's honestly significant difference (HSD) test. (OSTASIEWICZ, 2001).

Results

Throughout rearing, the lowest daily weight gains were obtained by PMS lambs (141 g), resulting in a preslaughter weight of 31.58 kg. Compared to the other genetic groups, differences in the values of these traits were highly significant. Semi-intensively fattened lambs had more rapid gains (by 44 g per day) and achieved body weights that were 16.5 kg higher than those obtained by extensively reared lambs ($P \leq 0.01$) (Tab. 1).
The lowest dressing percentage was obtained by PMS lambs (38.24%), which differed highly significantly from the other groups of crossbreds by 2.61 percentage units.
The poor meatiness of PMS lambs is confirmed by the percentage of valuable cuts in half-carcass (54.42%), compared to 56.52% in the group of BF × PMS and 60.15% in the group of WAS × (WAS × PMS) crossbreds ($P \leq 0.01$). The difference between these groups of crossbreds was statistically significant. At the same level of significance, a difference of 2.12 percentage units was also found between feeding groups. Similar differences were also found among animal groups in loin eye area. All the crossbred groups had the highest content of muscle tissue (71.25-72.56%) compared to only 65.04% in the PMS group ($P \leq 0.01$). In addition, legs of MPS lambs were characterized by the highest content of fatty tissue (9.63%) ($P \leq 0.01$). The content of muscle tissue was significantly differentiated, and the content of fatty tissue highly significantly differentiated, by the type of feeding. These values were 71.95 and 5.79% in the extensive group, and 68.66 and 8.96% in the semi-intensive group.
In the EUROP classification, best scores were given to the carcasses of crossbreds, especially WAS × (WAS × PMS) (60% in classes E to R) and poorest scores to the carcasses of PMS lambs (50% in classes O and P). The other 50% of carcasses were outside class P (Tab. 1).

Table 1
Daily gains, slaughter results and EUROP carcass classification

Trait		Genetic group					Feeding group	
		pog	BF x pog F_1	BF x (BF x pog) F_2	WAS x Pog F_1	WAS x (WAS x pog) F_2	exten-sive	semi-intensive
	n	10	10	10	10	10	25	25
Daily gain (g)	$\overline{X}$	141^{A}	165^{B}	170^{B}	170^{B}	173^{B}	142^{**}	186^{**}
	SD	10.00	6.25	0.75	13.75	17.00	11.90	11.40
Pre-slaughter body weight (kg)	$\overline{X}$	31.58^{A}	36.12^{Bc}	37.76^{Bc}	37.15^{Bc}	38.73^{Bd}	32.38^{**}	48.88^{**}
	SD	4.03	4.02	3.89	4.10	4.17	4..3	5.2
Dressing percentage	$\overline{X}$	38.4^{A}	41.8^{Bc}	42.5^{Bc}	42.9^{Bc}	43.3^{Bd}	41.1	42.2
	SD	1.4	1.8	2.3	1.1	2.7	1.1	2.0
Carcass grade (number/%)	E					1 / 10		1 / 4
	U		1 / 10	3 / 30	1 / 10	1 / 10	1 / 4	5 / 20
	R		1 / 10	3 / 30	2 / 20	4 / 40	3 / 12	7 / 28
	O	1 / 10	3 / 30	2 / 20	4 / 40	2 / 20	5 / 20	7 / 28
	P	4 / 40	3 / 30	2 / 20	3 / 30	2 / 20	11 / 44	3 / 12
Out of class		5 / 50	2 / 20				5 / 20	2 / 8
Valuable cuts (%)	$\overline{X}$	54.2^{A}	56.2^{Bc}	57.9^{Bc}	56.2^{Bc}	60.5^{Bd}	56.4^{*}	58.6^{*}
	SD	1.2	2.6	1.3	1.2	1.9	1.4	1.4
Loin eye area (cm^2)	$\overline{X}$	6.4^{A}	11.0^{B}	12.2^{B}	11.6^{B}	12.0^{B}	10.7	11.5
	SD	0.9	0.0	0.6	1.3	1.4	0.8	0.1
Leg tissue composition (%) meat	$\overline{X}$	65.4^{A}	71.8^{B}	70.3^{B}	71.5^{B}	72.6^{B}	71.5^{*}	68.6^{*}
	SD	2.0	2.4	4.3	2.5	3.5	2.8	3.4
fat	$\overline{X}$	9.3^{A}	6.4^{B}	7.3^{B}	7.1^{B}	6.6^{B}	5.9^{**}	8.6^{**}
	SD	1.4	2.7	1.3	1.4	1.9	1.8	1.8
bones	$\overline{X}$	25.3^{A}	22.8^{B}	22.4^{B}	21.4^{B}	20.8^{B}	22.6	22.8
	SD	1.4	2.7	1.6	1.6	1.4	1.5	1.1

Notes: PMS – Polish Mountain Sheep, BF – Bergschaf, WAS – Weisses Alpenschaf
A. B – values with different letters differ highly significantly, P≤0.01**
a. b – values with different letters differ significantly, P≤0.05*

No significant differences were found between genotype groups in the basic chemical composition of meat and in the content of dry matter and crude protein, although significant differences were found in the content of fat (P≤0.01). The highest level of crude fat was found in PMS lambs (3.40%), followed by 2.48-2.78% in the crossbreds. The highest centrifugal drip was found in the group of BF × PMS crossbreds (27.30%) and the lowest in the WAS × (WAS × PMS) and BF × (BF × PMS) groups – 23.46% and 24.50%, respectively (P≤0.01). The drip loss of the meat was highly significantly differentiated by feeding. It was 28.08% in the extensive group and only 23.68% in the semi-intensive group (P≤0.01). There were also differences in pH values between these groups (5.62 and 5.93, respectively; P≤0.01). The feeding system did not significantly affect the chemical composition of meat, despite differences in crude fat content were found (2.54-3,04%). The meat of the genetic and feeding groups studied did not show significant differences in terms of sensory traits, while the high scores for these parameters point to the high eating quality of the meat (Tab. 2).

Table 2
Chemical composition, physical and sensory traits of lamb

Trait		Genetic group					Feeding group	
		pog	BF x pog F_1	BF x (BF x pog) F_2	WAS x pog F_1	WAS x (WAS x pog) F_2	exten-sive	semi-intensive
	n	10	10	10	10	10	25	25
Dry matter (%)	$\bar{X}$	23.0	23.5	23.0	23.2	22.0	23.9	23.7
	SD	1.1	0.7	0.3	1.7	0.7	0.4	0.0
Crude protein (%)	$\bar{X}$	19.2	19.8	19.8	20.4	19.0	19.4	20.6
	SD	0.2	0.6	1.2	0.5	0.6	0.7	0.0
Crude fat (%)	$\bar{X}$	3.0^{a}	2.9^{b}	2.1^{b}	2.8^{b}	2.8^{b}	2.4	3.4
	SD	0.5	0.4	0.1	0.5	0.8	0.1	0.2
Water holding capacity (%)	$\bar{X}$	25.2^{AB}	27.0^{A}	24.0^{B}	26.3^{AB}	23.6^{B}	28.8^{**}	23.8^{**}
	SD	2.4	2.5	1.81	3.24	2.01	2.45	2.69
pH	$\bar{X}$	5.77	5.78	5.80	5.77	5.77	5.62	5.93
	SD	0.14	0.13	0.09	0.19	0.06	0.13	0.11
Aroma (pts)	$\bar{X}$	4.29	4.51	4.43	4.63	4.63	4.30	4.66
	SD	0.20	0.18	0.22	0.21	0.19	0.20	0.19
Tenderness (pts)	$\bar{X}$	4.35	4.50	4.47	4.49	4.51	4.42	4.51
	SD	0.35	0.29	0.24	0.35	0.28	0.26	0.33
Juiciness (pts)	$\bar{X}$	4.29	4.38	4.47	4.40	4.48	4.35	4.45
	SD	0,34	0,28	0,30	0,40	0,31	0,34	0,31
Palatability (pts)	$\bar{X}$	4.33	4.46	4.50	4.55	4.60	4.48	4.49
	SD	0.28	0.27	0.36	0.41	0.28	0.31	0.32

For explanations see Table 1

Table 3 gives the composition (profile) of fatty acids. The fatty acid profile was considerably differentiated by the crossbreeding scheme used. Significant differences were found in the total level of particular groups of acids: saturated ($P \leq 0.01$) and unsaturated ($P \leq 0.05$), including polyunsaturated ($P \leq 0.01$ and $P \leq 0.05$), which resulted from differences in the level of the C10:0, C12:0, C14:0, C16:1, C18:0, C18:1, C18:2 and C20:4 acids. The meat fat of PMS lambs was significantly different from that of the crossbreds in the higher level of C18:2 (10.87%), C20:4 (1.86%) and PUFA acids (14.44%). Of the animal genetic groups studied, the meat of WAS × (WAS × PMS) was characterized by a significantly higher level of unsaturated ($P \leq 0.05$), monounsaturated and polyunsaturated (also PUFA n-3) acids, and lower level of saturated acids ($P \leq 0.01$). Significant differences in CLA content were not found in feeding and genetic group.

Compared to semi-intensive feeding, extensive feeding significantly decreased ($P \leq 0.05$) the level of C16:1 and C18:1 acids and the total level of unsaturated acids, and thus increased the level of saturated acids, as determined mostly by the higher proportion of the C18:0 acid. In the group fed in the semi-intensive system, there was a lower level of n-3 polyunsaturated fatty acids.

Discussion

The lowest weight gains obtained in the PMS group conform with the results of other authors (CIURUŚ and DROŻDŻ, 1995). Dressing percentage depends on many factors, in particular the breed and utility type of animals. The available literature provides inconsistent information on the dressing percentage of PMS lambs. In the

Table 3
Fatty acid profile of the *musculus longissimus dorsi* of lambs

Trait		Genetic group					Feeding group	
		PMS	BF × PMS F_1	BF × (BF × PMS) F_2	WAS × PMS F_1	WAS × (WAS × PMS) F_2	extensive	semi-intensive
	N	10	10	10	10	10	25	25
C10:0	$\overline{X}$	1.15^{ab}	0.16^{a}	0.14^{ab}	0.14^{b}	0.16^{ab}	0.15	0.15
	SD	0.05	0.04	0.03	0.04	0.05	0.05	0.04
C14:0	$\overline{X}$	3.05^{ab}	3.25^{a}	2.99^{b}	3.06^{ab}	3.17^{ab}	3.25^{*}	2.96^{*}
	SD	0.52	0.43	0.42	0.36	0.44	0.41	0.39
C16:0	$\overline{X}$	22.59	22.16	21.74	21.87	21.85	22.00	22.09
	SD	1.82	2.04	1.56	1.93	1.76	1.85	2.00
C16:1	$\overline{X}$	0.92^{ab}	0.89^{ab}	0.84^{ab}	0.78^{a}	0.94^{b}	0.82^{*}	0.93^{*}
	SD	0.07	0.09	0.10	0.08	0.07	0.06	0.09
C18:0	$\overline{X}$	23.91^{A}	22.57^{AB}	23.47^{A}	24.55^{A}	20.11^{B}	23.76^{*}	22.08^{*}
	SD	2.01	1.77	1.85	1.65	1.91	1.88	2.12
C18:1	$\overline{X}$	32.03^{AB}	31.93^{AB}	30.44^{A}	28.45^{A}	34.17^{B}	30.14^{**}	32.67^{**}
	SD	3.22	3.21	2.95	2.88	3.15	2.91	3.09
C18:2	$\overline{X}$	10.87^{Aa}	11.79^{AB}	12.66^{Ab}	13.32^{B}	12.60^{Ab}	12.39	12.10
	SD	2.11	2.15	1.95	2.05	2.21	2.09	2.11
γ C18:3	$\overline{X}$	0.267^{ab}	0.261^{ab}	0.293^{ab}	0.314^{a}	0.235^{b}	0.293^{*}	0.255^{*}
	SD	0.08	0.07	0.06	0.09	0.04	0.07	0.08
C18:3	$\overline{X}$	1.74	1.87	1.98	2.05	1.79	1.90	1.87
	SD	0.12	0.11	0.10	0.13	0.11	0.12	0.10
C20:4	$\overline{X}$	1.86^{a}	2.26^{ab}	2.60^{ab}	2.70^{b}	2.42^{ab}	2.44	2.30
	SD	0.18	0.15	0.17	0.16	0.18	0.16	0.19
SFA	$\overline{X}$	50.14^{A}	48.66^{AB}	48.42^{AB}	50.02^{A}	45.72^{B}	49.64^{*}	47.68^{*}
	SD	3.35	3.25	3.48	3.51	3.29	3.45	3.67
UFA	$\overline{X}$	49.86^{a}	51.34^{ab}	51.23^{ab}	49.98^{a}	54.28^{b}	50.36^{*}	52.32^{*}
	SD	3.51	3.59	3.67	3.65	4.21	3.91	3.55
MUFA	$\overline{X}$	32.94^{AB}	31.04^{AB}	31.28^{AB}	29.23^{A}	35.11^{B}	31.15	32.69
	SD	2.88	3.21	3.09	3.11	3.55	3.40	3.71
PUFA	$\overline{X}$	14.44^{A}	17.95^{Ba}	19.44^{B}	20.75^{Bb}	19.17^{B}	18.26	18.64
	SD	1.86	1.94	1.75	1.92	2.01	1.85	1.91
PUFA-3	$\overline{X}$	2.74^{a}	3.04^{ab}	3.31^{ab}	3.34^{b}	3.25^{ab}	3.31^{*}	2.96^{*}
	SD	0.80	0.91	0.94	1.00	0.88	0.94	0.89
CLA	$\overline{X}$	1.18	1.13	1.08	1.07	1.12	1.13	1.09
	SD	0.94	0.90	0.91	0.89	0.93	0.90	0.97

For explanations see Table 1, PUFA n-3: C18:3, C20:5 (EPA), C22:6 (DHA)

studies of other authors (CIURUŚ and DROŻDŻ, 1988), dressing percentage was 37.8% and it was similar to our own findings. In a different study (CIURUŚ and DROŻDŻ, 1995), this parameter ranged from 41.7 to 42.0%..

Fat is that component of meat which shows the greatest dependence on genetic and environmental factors (KĘDZIOR, 2005). This has also been also confirmed by the present study, although studies investigating the effect of breed and crossbreeding on the chemical composition of meat also pointed to stable (KĘDZIOR, 1991) and differing content of protein (ROBORZYŃSKI et al., 2000).

The results of studies indicate that the composition of fatty acids depends on genetic and nutritional factors. A review of literature (KĘDZIOR, 2004) shows that genetic factors, similar to the present study, result in significant differences in the level of

C12:0, C18:0, C18:1 and C18:2, as well as C16:0, C18:3, SFA, MUFA and PUFA acids. It is concluded from the present and other studies (ENDER et al., 2001; FISHER et al., 2000; GRUSZECKI et al., 2001; NÜRNBERG K. et al., 1966) that compared to the medium intensive and semi-intensive feeding systems, the pasture management of lambs results in a lower concentration of the C18:1 acid and a lower total level of unsaturated acids, and thus a higher level of saturated acids, while having an effect on the higher level of essential n-3 PUFA and their favourable ratio in relation to n-6 acids.

Conclusions

It was shown that the crossbreeding of the Polish Mountain Sheep with the Bergschaf and Weisses Alpenschaf alpine rams has a positive effect on both live (higher daily gains) and slaughter traits (higher dressing percentage, leg tissue composition, loin eye area).

Lambs fed semi-intensively dominated in terms of live and slaughter traits, while their extensive grazing increased the muscle tissue content of leg and decreased leg fatness.

The low scores of PMS ram carcasses on the EUROP scale in the higher weight standards prevent the use of this breed for the production of heavy lambs weighing between 35 and 45 kg. The carcasses of crossbreds were within the upper range of the classification and thus are suitable for marketing and exporting to EU countries.

The crossbreeding scheme largely resulted in differences in water holding capacity of meat, the content of intramuscular fat and the profile of fatty acids. Compared to semi-intensive feeding, extensive feeding had a significant effect on higher drip loss, lower level of unsaturated acids and higher level of n-3 polyunsaturated acids, and higher level of saturated acids. The meat of the genetic and feeding groups studied received high scores for sensory characteristics, being indicative of its high eating quality.

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Serum and saliva antibody isotypes against *Haemonchus contortus* in Żelaźnieńska and Wrzosówka sheep naturally infected with gastrointestinal nematodes over 2004 and 2005 grazing seasons

Abstract

Gastrointestinal nematodes from family *Trichostrongylidae* are probably the most important parasites of small ruminants world-wide, causing significant morbidity and loss of production. Also in Poland these parasites cause important health problems of sheep. The immune response of two breeds of sheep – Wrzosówka (Polish Heath Sheep) and Żelaźnieńska (Polish Lowland Sheep) to somatic antigens of gastrointestinal nematode *Haemonchus contortus* were compared. Female sheep of both breeds were kept in the same conditions. Faecal probes were collected four times during the grazing season, and then examined using flotation and sedimentation methods. Serum and saliva samples were collected at the beginning and the end of grazing season. The levels of both serum and saliva IgA, IgG and IgM antibodies reacting with antigens of *H. contortus* were estimated using ELISA method. The Wrzosówka Sheep have shown lower prevalence of infection and lower number of nematode eggs in faeces than the Żelaźnieńska Sheep. Negative correlations between the level of all isotypes of serum and saliva antibodies and prevalence of invasion (also with a number of eggs in faeces) have been found in both breeds of sheep.

Key Words: sheep, gastrointestinal nematodes, antibody isotypes

Introduction

Nematodes from superfamily *Trichostrongylidea* (including families *Trichostrongylidae*, *Haemonchidae* and *Cooperidae*) invade predominantly ruminants like cattle and sheep, and some species can parasitise in horses or rodents. The most common (and most important) in Poland are: *Trichostrongylus sp., Nematodirus sp., Haemonchus contortus* and *Teladorsagia circumcincta*.

Gastrointestinal nematodes are probably the most important parasites of small ruminants world-wide, causing significant morbidity and loss of production. Also in Poland these parasites can be the reason of serious veterinary problems. They can be treated by anthelmintic chemotherapy, however treatment is costly and drug resistance has evolved in all major gastrointestinal nematode species (ROOS, 1997). Because of this and the growing concentration of pesticides in the environment and in food, it is becoming very important to develop other ways of controlling nematode infections in sheep. One option is selective breeding for a reduction in faecal egg counts following natural infection (BISSET et al., 1996). Another idea is to identify the genetic basis for naturally occurring resistance of some breed of sheep against nematode infections. It is known that some breeds of sheep like Red Massai Sheep in East Africa or Polish Longwool (BAKER, 1995; BOUIX et al., 1998) are more resistant than others. NOWOSAD et al. (2003) and GORSKI et al. (2004) found significant differences in prevalence of some parasitic infections among various sheep breeds in Poland. Some of these breeds are typical Polish, rather not raised in other countries. Wrzosówka (Polish Heath Sheep) is a primitive, small fur breed of north short-tailed sheep groups. Sheep of this breed are very well adapted to the difficult environmental conditions,

resistant to diseases and unseasonal. There were many studies udertaken on this old breed, concerning its immune response to infections of gastrointestinal nematodes (MOSKWA, 1999, 2000; MOSKWA et al., 2002). The second breed – Żelaźnieńska sheep (Polish Lowland Sheep) was developed from the Polish Merino crossed with the Leicester Longwool and Łowicz sheep.
The most important aim of our studies was to compare humoral immune response of there two breeds of sheep (kept in the same conditions) to *Haemonchus contortus* somatic antigens. We also observed the changes in level of serum and saliva antibodies in both breeds during the grazing season.

Materials and Methods

The study was carried out on the Polish Wrzosówka sheep and the Żelaźnieńska sheep maintained on a sheep farm in Żelazna (central Poland) and run by the Department of Sheep and Goat Breeding, Faculty of Animal Sciences, Warsaw Agricultural University. All animals were over 2-year-old ewes. In 2004 the flock of about 70 Żelaźnieńska sheep was investigated, and 22 to 27 specimens of this breed in 2005. The Wrzosówka sheep (from 30 to 40 ewes) were examinated only in 2005. The sheep from this farm were treated with levamisol before and after (Żelaźnieńska sheep), or only after (Wrzosówka sheep) the grazing season.
Faecal samples were collected from the rectum of all ewes four times in the year – at the beginning (before treatment with anthelmintic) of grazing season (April or May), in June, August and at the end of season (September or October). The prevalence of gastrointestinal nematodes invasion was estimated using saturated NaCl flotation method. Faecal egg counts per gram of faeces (EPG) were made from a one-g sample of faeces, using the modified flotation method described previously (DOLIGALSKA et al., 1997).
Blood samples were collected by jugular venepuncture using 9ml evacuated tubes (SARSTEDT Monovette, EDTA KE/9ml). Two times in the year (before and after grazing season) Saliva samples were collected from mouth of sheep by sponges also two times yearly. Serum and saliva samples were stored at -20°C before used. Somatic antigens of adult (both sexes) *Haemonchus contortus* stages in concentration 5 μg/ml were used in ELISA tests. IgA, IgM and IgG isotypes of serum and saliva antibodies were detected using HRP-labelled anti-sheep antibodies (ROCKLAND for IgM and IgG, and EIVAI BOIS Laboratories for IgA) , and the absorbance was determined using MRX plate reader (Dynatech Laboratories) with the 450 nm filters.

Results

The prevalence of infection with gastrointestinal nematodes was increasing during the grazing season in both breeds. In the Żelaźnieńska sheep it was growing from 29.4% to 97.1% in 2004 and from 92% to 100% in 2005. The prevalence was lower in the Wrzosówka sheep and it was increasing from 57.5% to 93.8%. Also the egg number per gram of faeces connected with the prevalence was significantly lower in the Wrzosówka sheep than in Żelaźnieńska. This parameter for the Żelaźnieńska sheep increased from 0.86 to 18.14 in 2004 and from 7.55 to 84.86 in 2005. In Wrzosówka the number of eggs per gram of faeces increased from 1.26 to 23.75 (Fig. 1).

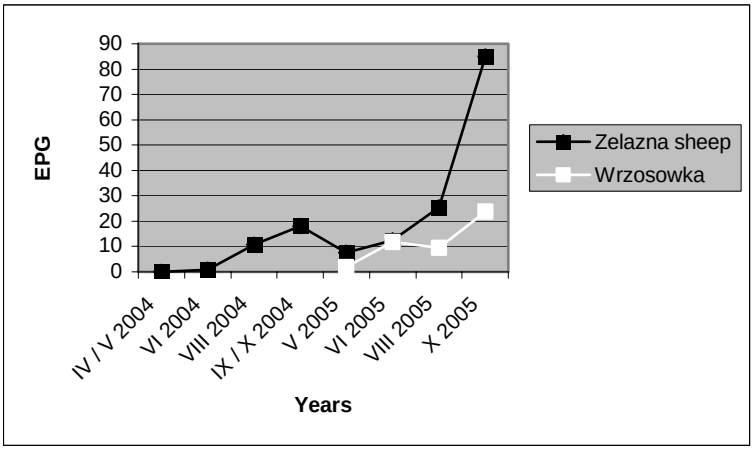

Fig. 1: Changes in number of gastrointestinal nematode eggs in 1 gram of faeces of Zelaznienska and Wrzosówka Sheep. EPG = Eggs Per Gram.

Both examinated breeds developed the humoral immune response to somatic nematode antigens. Negative correlation between the level of all antibody isotypes (in serum and saliva) and the faecal egg count (Table 1) was observed.

Table1
Correlation between the level of serum and saliva antibodies and the faecal egg count.

2004							
Żelaźnieńska sheep				Wrzosówka sheep			
Antibodies (Optical Density)			Eggs per gram of faeces	Antibodies (Optical Density)			Eggs per gram of faeces
IgA	Serum	0.105 -0.05	0.89 - 18.14	IgA	Serum		
	Saliva	0.2 - 0.1			Saliva		
IgG	Serum	1.17 - 1.09		IgG	Serum		
	Saliva	0.3 - 0.2			Saliva		
IgM	Serum	1.05 - 0.7		IgM	Serum		
	Saliva	0.04 - 0.07			Saliva		
2005							
Żelaźnieńska sheep				Wrzosówka sheep			
Antibodies (Optical Density)			Eggs per gram of faeces	Antibodies (Optical Density)			Eggs per gram of faeces
IgA	Serum	0.05 - 0.003	7.55 - 84.86	IgA	Serum	0.06 - 0.01	1.96 - 23.75
	Saliva				Saliva	0.16 - 0.0	
IgG	Serum	1.4 - 0.5		IgG	Serum	0.42 - 0.41	
	Saliva				Saliva	0.21 -0.17	
IgM	Serum	0.7 - 0.4		IgM	Serum	1.1 - 0.4	
	Saliva				Saliva	0.04 -0.16	

Also, both breeds showed the decreasing level of the serum and saliva of all antibody isotypes during the season The only exception was IgM in saliva which increased from Optical Density 0.04 to 0.07 or 0.16 in both breeds. In all the remaining cases the Wrzosówka sheep have shown the lower level of antibodies than Żelaźnieńska ones. ELISA results are shown in Figs. 2, 3, 4 and 5.

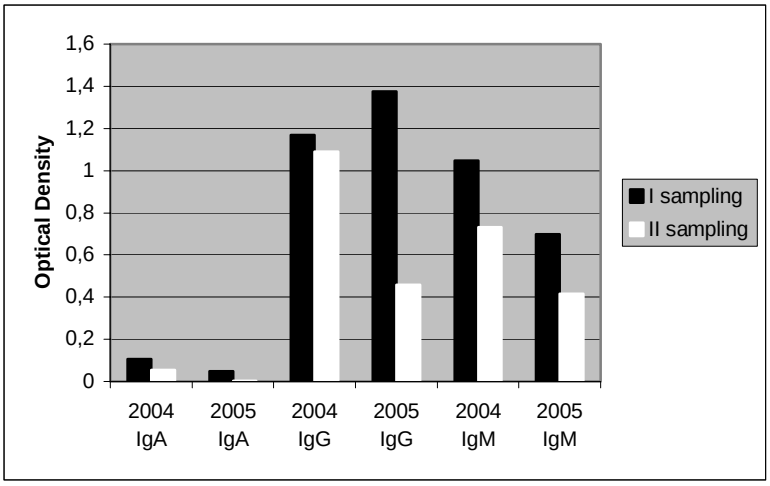

Fig. 2: Serum antibodies of Zelaznienska Sheep reacting with *Haemonchus contortus* somatic antigens. OD = Optical Density. Sampling I – before, Sample II – after grazing season

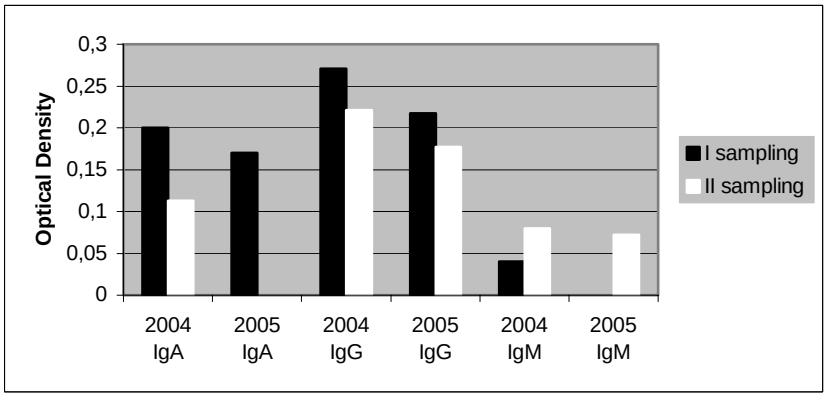

Fig. 3: Saliva antibodies of Zelaznienska Sheep reacting with *Haemonchus contortus* somatic antigens. OD = Optical Density. Sampling I – before, Sample II – after grazing season

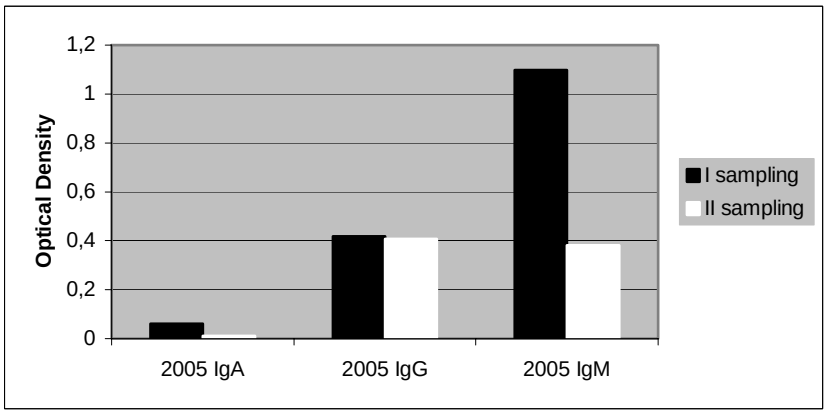

Fig. 4: Serum antibodies of Wrzosowka Sheep reacting with *Haemonchus contortus* somatic antigens. OD = Optical Density. Sampling I – before, Sample II – after grazing season

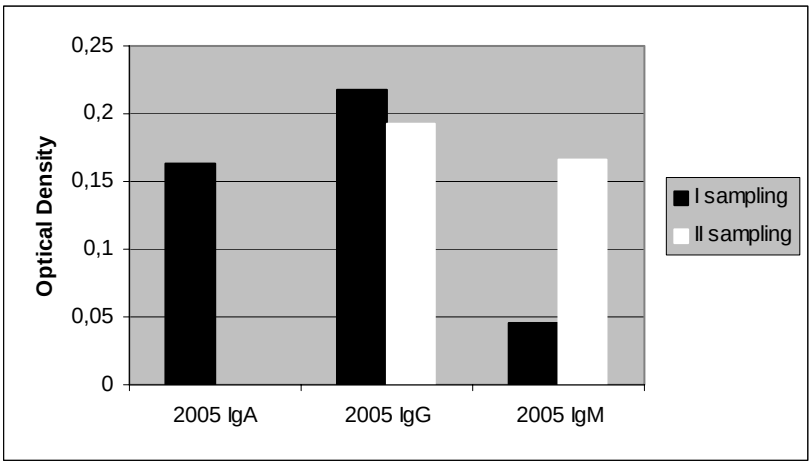

Fig. 5: Saliva antibodies of Wrzosowka Sheep reacting with *Haemonchus contortus* somatic antigens. OD = Optical Density. Sampling I – before, Sample II – after grazing season

Discussion

The faecal egg count increased during the grazing season and the highest was at the beginning of autumn in both breeds. Similar phenomenon was observed in 1997 and 1998 in the Wrzosówka flock maintained at the same farm in Żelazna in Central Poland. Otherwise in 1996 the number of eggs per gram of faeces was higher in the middle of the season (MOSKWA, 1999). The author of these studies has found a considerable variation in faecal egg counts in different years (however, in general the numbers of eggs per gram of faeces were higher than found in our studies), so we cannot treat our results as invariable phenomenon.

We found negative correlation between the level of antibodies and faecal egg counts. It is well known phenomenon typical for gastrointestinal nematode infections (BAKER et al., 1996; DOUCH et al., 1996; MOSKWA, 1999). Sheep grazing on the pasture are exposed to natural gastrointestinal nematode infections and acquire resistance slowly, during the grazing season. The state of immunity can be assessed by measuring the number of nematode eggs passed by infected sheep. A reduction of the number of nematode eggs in faeces is associated with the development of acquired immunity against nematode infection. Because serum antibody level may be negatively correlated with faecal egg counts, the measurement of specific antibody production in infected animals may provide another useful selection criterion and be used to complement faecal egg counts (MOSKWA et al., 2002).

In both the Żelaźnieńska and Wrzosówka sheep the IgM level in saliva increased during the season, whereas the level of other isotypes decreased. Probably the reason of increasing of saliva IgM level was summer infection with *Haemonchus contortus* infective larvae not many days before the second sampling.

The level of serum and saliva antibodies was somewhat higher in the Wrzosówka than Żelaźnienska sheep, but similar. Because of lower prevalence and lower number of nematode eggs in faeces of Wrzosówka, proportionaly the humoral response of this breed is stronger and probably more effective. Some studies have already established the importance of host genotype on resistance of host to parasite infection (STEAR et al., 1995). Some primitive, old breeds of sheep are known to be more resistant to gastrointestinal nematode infections. It was confirmed in such breeds as the African

Red Maasai and Djallonke sheep (BAKER, 1995), or Scottish Soay sheep (COLTMAN et al., 2001). In the last case the authors have shown that resistance to gastrointestinal nematode infections of naturally parasitized animals is associated with the microsatellite polymorphism in gamma interferon gene.
Summarising, it can be noticed that it is possible to certify that Wrzosówka, primitive breed of sheep is more resistant to gastrointestinal nematode infections than the highly selected Żelaźnieńska sheep, however further studies on that problem should be undertaken in more seasons.

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Factors affecting the longissimus dorsi muscle depth and backfat thickness measured by ultrasound technique in lambs

Abstract

The study was conducted to estimate the effect of systematic factors on characteristics of meatiness and fattiness scanned by ultrasound in live lamb. The second aim of the study was to compare two statistical models (with and without regression on lamb liveweight) for analysis of variance in muscle depth and backfat thickness. Ultrasonic measurements collected in field conditions during the years 1999-2005 were available for 6270 purebred Suffolk (SF), 4191 Charollais (CH), 1320 Texel (T) and 2093 Romney (RM) lambs. Lambs were scanned at the age of 80-120 days by 5MHz linear probe behind the last rib vertebra after wool combing. The averages for muscle depth were 25.54, 23.56, 24.04 and 24.29 mm and for backfat thickness with skin 3.22, 2.83, 2.88 and 3.69 mm for SF, CH, T and RM lambs. According to Akaike criterion the statistical model with regression on lamb liveweight better fits the analysed data both for muscle depth and backfat thickness. Including the lamb liveweight effect into model equation caused decrease of influence of dams age and type of birth effects on the variability of both examined traits. On the contrary, effect of sex increased. After adjustment with the same liveweight the female lambs had significantly higher muscle depth and backfat thickness compared to male lambs. This fact is probably caused by different body proportions of males and females. Rams have their body mass concentrated rather in the chest and rack area than in the scanned part of the body.

Key Words: lamb, ultrasound, fat thickness, *longissimus dorsi* muscle

Introduction

The change in production orientation from wool to lamb meat production has occurred in the Czech Republic during the last 15 years. These changes evoked the necessity to aim breeding work at fertility, lamb growth intensity and carcass quality traits. Consumers demand excellently conformed carcasses with optimal fattiness. The potential of real-time ultrasonography for *in vivo* predicting of meatiness and fattiness in sheep has been well reported by many authors (KEMPSTER et al., 1982; RINGDORFER, 1995; GRUSZECKI and SZYMANOWSKI, 1996; STANFORD et al., 1998; MILERSKI, 2001; PUNTILA et al., 2002; JUNKUSZEW and RINGDORFER, 2005). Ultrasound measurements were also implemented into breeding programmes for meat sheep in several countries (SIMM and DINGWALL, 1989; CROSTON and OWEN, 1992; QUANZ, 1995). In 1999 the programme of ultrasound measurements in population of terminal sire breeds Suffolk, Charollais, Texel and Oxford Down was started in the Czech Republic and three years later also Romney was included into the programme. Analysis of effects of systematic factors on lamb growth in the conditions of the Czech Republic was done by KUCHTÍK and DOBEŠ (2006). The goal of this work was to estimate the effect of systematic factors on characteristics of meatiness and fattiness scanned by ultrasound in live lamb and to compare usefulness of two statistical models, with and without regression according to lamb liveweight.

Material and Methods

Ultrasonic measurements of eye muscle depth and backfat thickness were carried out in field conditions during the years 1999-2005. Totally 6270 Suffolk (SF), 4191

Charollais (CH), 1320 Texel (T) and 2093 Romney (RM) male and female lambs were measured in 192 flocks. The age range of scanned lambs was from 80 to120 days. Before the ultrasonic scanning all lambs were weighed. Echocamera Aloka SSD210SX equipped with 5MHz probe UST 5813-5 was used for the majority of the measurements. Since 2001 digital ultrasonic machine Sonovet2000 with 4-7 MHz linear probe LV4-7AD was also used in part of involved flocks. Animals were measured between the last rib and the first lumbar vertebra on the left side of their back after the wool had been combed and coupling agent used to allow good acoustic contact. Muscle depth was measured in the deepest place upright to the body surface. Fat depth was measured over the middle of the eye muscle and included also skin thickness. The measurements were carried out directly on the monitor of the ultrasonic machine after the picture freezing.

Variance of the examined traits was analysed by the use of procedure MIXED from the statistical package SAS (SAS INSTITUTE, 2000). The following statistical models were used:

Model 1:

$$y_{ijklm} = FY_i + AD_j + SEX_k + TB_l + b1 \times ag_{ijklm} + e_{ijklm}$$

Model 2:

$$y_{ijklm} = FY_i + AD_j + SEX_k + TB_l + b1 \times ag_{ijklm} + b2 \times lw_{ijklm} + b3 \times lw_{ijklm}^2 + e_{ijklm}$$

Where:

y_{ijklm} – measurement
FY_i – combined random effect of flock and year
AD_j – fixed effect of dam age - 4 levels
SEX_k – fixed effect of sex - 2 levels
TB_l – fixed effect of type of birth – 3 levels
b1, b2, b3 – fixed regression coefficients
ag_{ijklm} – age of lamb at the time of ultrasonic measurements in days
lw_{ijklm} – liveweight of lamb at the time of ultrasonic measurements in kg
e_{ijklm} - random residual

The analysis was carried out separately for animals of different breeds.

Results and Discussion

The overall descriptive statistics taken from the set of examined animals are displayed in Table 1. Direct comparisons between breeds for examined traits cannot be made because breeds were confounded with flocks.

The highest average liveweight 32.88 kg at the average age 100.8 days was detected in the Romney lambs. On the other hand this breed was characterized also by the highest average backfat thickness 3.7 mm. The differences between average backfat thickness of Romney lambs and the arithmetical mean for the same trait measured on lambs of the other examined breeds were quite marked, and ranged from 0.5 to 0.9 mm. The highest average muscle depth 25.5 mm was found in Suffolk. Charollais and Texel lambs were leaner than the lambs of the other two examined breeds. The lowest average backfat thickness was found in Charollais, but Texel lambs had the best ratio between muscle depth and backfat thickness. ŚLÓSARZ (2004) found in the Whiteheaded Mutton sheep average ultrasonically measured muscle depth 25.4 mm and backfat thickness (without skin) 1.6 mm at the average age 100.9 days and the aveage liveweight 29.6 kg.

Table 1
Descriptive statistics for ultrasound data

	Breed							
	Suffolk n=6270		Charollais n=4191		Texel n=1320		Romney n=2093	
	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.
Age	102.17	9.32	100.99	9.78	101.80	9.45	100.77	9.36
Liveweight	32.52	7.01	28.84	6.86	28.70	6.91	32.88	7.16
Muscle depth	25.54	4.35	23.56	4.27	24.04	4.54	24.29	3.96
Backfat thickness	3.22	0.95	2.83	0.76	2.88	0.92	3.69	1.01

In Tables 2 and 3, the results of analysis of variance for the examined traits according to two different statistical models are presented. As the comparison of Akaike criteria shows, the variant of model with the regression according to lamb liveweight fits the data better in the case of both examined traits and all included breeds. By including this regression into statistical model equation the changes in significance of other factors occured. While in Model 1 the effect of type of birth explained the highest part of variability of both traits measured by ultrasound, after including the liveweight into Model 2 the significance of factors of type of birth and age of dam decreased rapidly and in many cases turned off not to be statistically significant ($P<0.05$). On the other hand the significance of the sex factor was much higher in Model 2 compared to Model 1. The type of birth and the age of dam seems to influence the muscle depth and backfat thickness mainly by means of body weight, less by the carcass composition. On the other hand differences between males and females seem to be caused by different body proportions. OLESEN and HUSABØ (1994) used a model without the liveweight effect for analysis od variance of ram lamb muscle depth and backfat thickness. They found that birth-rearing type, age of lambs and flock were significant sources of variation for both ultrasonic traits. In addition, the age of dam showed a significant effect on muscle depth.

In Tables 4-7 the LSMs for different levels of fixed effects on muscle depth and backfat thickness according to both used statistical models are presented. When data was adjusted according to Model 2, the differences between LSMs for different type of birth were small and in the majority of cases non-significant, contrary to Model 1. Also differences between levels of dam age were rather small under Model 2. Lambs of more than 7-years dams had slightly less depth of muscle in the Charollais, Texel and Romney breeds. This fact could be caused by genetic progress in the lambs muscularity. After adjustment on the same liveweight according to Model 2 the female lambs had significantly higher muscle depth and backfat thickness compared to male lambs. This fact is probably caused by different body proportions of males and females. Rams have a stronger skeleton and have their body mass concentrated rather in the chest and rack area than in the scanned back area. Without including the liveweight into the model equation this fact was overlapped by a higher weight the rams. This is in agreement with the finding of JOHNSON et al. (2005), that muscle to bone ratio of ram lambs was lower than of females.

Table 2
Analysis of variance of muscle depth

		Suffolk				Charollais				Texel				Romney			
		Model 1		Model 2		Model 1		Model 2		Model 1		Model 2		Model 1		Model 2	
Akaike citeria		33445		28899		22409		19076		7286		6140		10798		9415	
d.f.		6004		6002		3995		3993		1249		1247		2044		2042	
	d.f.	F-value		F-value		F-value		F-value		F-value		F-value		F-value		F-value	
Dam age	3	42.5	***	1.5	n.s.	37.2	***	2.3	n.s.	21.2	***	3.6	*	24.3	***	1.6	n.s.
Sex	1	0.3	n.s.	337.6	***	20.1	***	110.2	***	0.6	n.s.	85.5	***	3.5	n.s.	119	***
Litter size	2	309.7	***	3.5	*	221.9	***	5.7	**	64.1	***	0.9	n.s.	110.3	***	0.6	n.s.
Lamb age	1	259.1	***	20.8	***	68.9	***	41.9	***	29.5	***	7.6	**	39	***	4.5	*
Liveweight (LW)	1			712.1	***			861.2	***			244.3	***			255.0	***
LW^2	1			123.2	***			243.2	***			46.3	***			55.3	***

* P<0.05; ** P<0.01; P<0.001

Table 3
Analysis of variance of backfat thickness

		Suffolk				Charollais				Texel				Romney			
		Model 1		Model 2		Model 1		Model 2		Model 1		Model 2		Model 1		Model 2	
Akaike citeria		14162		11835		7833		6638		3082		2574		5022		4153	
d.f.		6004		6002		3995		3993		1249		1247		2044		2042	
	d.f.	F-value		F-value		F-value		F-value		F-value		F-value		F-value		F-value	
Dam age	3	22.27	***	2.1	n.s.	20.11	***	1.47	n	7.61	***	2.9	*	10	***	3.26	*
Sex	1	20.88	***	347.7	***	0.79	n	112.4	***	0.79		37.9	***	5.58	*	175	***
Litter size	2	230.9	***	4.13	*	149.9	***	10.6	***	38.1	***	0.4	n.s.	101.71	***	0.15	n.s.
Lamb age	1	190.5	***	2.46	n.s.	89.7	***	0.3	n.s.	24.2	***	0.86	n.s.	46.11	***	0.08	n.s.
Liveweight (LW)	1			100.34	***			90	***			22.4	***			62.9	***
LW^2	1			0.18	n.s.			3.6	n.s.			0.37	n.s.			1.93	n.s.

* P<0.05; ** P<0.01; P<0.001

Table 4
Effect of systematic class factors on muscle depth – Model 1

Breed/ Rasse	Suffolk		Charollais		Texel		Romney	
	LSM	s.e.	LSM	s.e.	LSM	s.e.	LSM	s.e.
Age of dam								
1 year	23.6	0.3 a	21.7	0.4 a	21.2	0.6 a	21.4	0.5 a
2 years	25.2	0.2 b	23.6	0.3 b	24.0	0.4 b	23.5	0.4 b
3-7 years	26.0	0.2 c	24.3	0.2 c	24.4	0.4 b	24.2	0.4 c
>7 years	25.2	0.3 b	23.2	0.3 b	22.5	0.6 c	23.2	0.4 b
Sex								
male	25.0	0.2	23.5	0.3 a	22.9	0.4	23.1	0.4
female	25.0	0.2	23.0	0.3 b	23.1	0.4	22.9	0.4
Litter size								
1	27.0	0.3 a	25.3	0.3 a	25.3	0.4 a	24.8	0.4 a
2	24.7	0.2 b	22.8	0.3 b	22.9	0.4 b	22.7	0.4 b
3 and more	23.2	0.3 c	21.6	0.3 c	20.9	0.6 c	21.4	0.5 c

LSMs rows different superscripts differ at the significancy level P<0.05 (for Tables 5, 6, 7 also)

Table 5
Effect of systematic class factors on muscle depth – Model 2

Breed	Suffolk		Charollais		Texel		Romney	
	LSM	s.e.	LSM	s.e.	LSM	s.e.	LSM	s.e.
Age of dam								
1 year	25.4	0.2 a	23.4	0.2	24.1	0.4 a	24.5	0.4 a
2 years	25.7	0.1	23.7	0.2 a	24.3	0.3 a	24.3	0.3
3-7 years	25.7	0.1 b	23.7	0.1 a	24.1	0.3 a	24.3	0.3
>7 years	25.6	0.2	23.4	0.2 b	23.3	0.4 b	23.9	0.4 b
Sex								
male	25.0	0.1 a	23.2	0.2 a	23.3	0.3 a	23.7	0.3 a
female	26.2	0.1 b	24.0	0.2 b	24.6	0.3 b	24.8	0.3 b
Litter size								
1	25.7	0.1	23.8	0.2 a	24.0	0.3	24.2	0.3
2	25.7	0.1 a	23.5	0.2 b	23.8	0.3	24.3	0.3
3 and more	25.4	0.2 b	23.4	0.2 b	24.0	0.4	24.3	0.4

Table 6
Effect of systematic class factors on backfat thickness – Model 1

Breed	Suffolk		Charollais		Texel		Romney	
	LSM	s.e.	LSM	s.e.	LSM	s.e.	LSM	s.e.
Age of dam								
1 year	2.96	0.07 a	2.61	0.06 a	2.48	0.12 a	3.09	0.12 a
2 years	3.24	0.06 b	2.87	0.05 b	2.84	0.10 b	3.40	0.10 b
3-7 years	3.39	0.05 c	2.94	0.04 c	2.84	0.09 b	3.51	0.10 c
>7 years	3.16	0.07 b	2.79	0.05 b	2.61	0.12 a	3.34	0.12 b
Sex								
male	3.17	0.06 a	2.79	0.05	2.67	0.10	3.29	0.10 a
female	3.25	0.06 b	2.81	0.05	2.71	0.10	3.38	0.10 b
Litter size								
1	3.58	0.06 a	3.09	0.05 a	3.58	0.06 a	3.09	0.05 a
2	3.13	0.06 b	2.73	0.05 b	3.13	0.06 b	2.73	0.05 b
3 and more	2.91	0.06 c	2.58	0.05 c	2.91	0.06 c	2.58	0.05 c

Table 7
Effect of systematic factors on backfatt thickness – Model 2

Breed	Suffolk			Charollais			Texel			Romney		
	LSM	s.e.		LSM	s.e.		LSM	s.e.		LSM	s.e.	
Age of dam												
1 year	3.24	0.06	a	2.82	0.05		2.94	0.10	a	3.75	0.09	a
2 years	3.32	0.04		2.87	0.04		2.87	0.08	a	3.58	0.08	b
3-7 years	3.35	0.04	b	2.86	0.03		2.79	0.08	b	3.56	0.07	b
>7 years	3.29	0.06		2.80	0.04		2.72	0.10	b	3.51	0.09	b
Sex												
male	3.15	0.04	A	2.75	0.04	a	2.72	0.08	a	3.40	0.08	a
female	3.45	0.04	B	2.93	0.04	b	2.93	0.08	b	3.80	0.08	b
Litter size												
1	3.35	0.04	a	2.90	0.04	a	2.83	0.08		3.61	0.07	
2	3.28	0.04	b	2.81	0.04	b	2.80	0.08		3.59	0.07	
3 and more	3.27	0.05	b	2.80	0.04	b	2.85	0.11		3.60	0.09	

Both examined models – with and without liveweight adjustment could be used in practice, but with different interpretation. While Model 1 provides information rather about muscle and fat tissue growth intensity which are higly correlated with the overall growth intensity, Model 2 provides more information about carcass composition and ratio between tissues. Both models could be combined in selection indexes with appropriate weight coefficients.

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Genetic potential and reproductive performance of Whitebacks – Polish native breed of cows

Abstract

The investigations covered all the cows included in the genetic resources conservation programme and in the registers of the Whiteback breed. The animals are predominantly maintained in small farms in the eastern part of Poland. The genetic variation of these cows was characterized on the grounds of the studies of 11 loci of the DNA microsatellites. In the 11 analysed loci, total 93 alleles were localized, thus mean 8.3 alleles appear in one locus. The alleles from the locus ETH10 of 227bp size, TGLA122 of 170bp, 172bp and 174bp size and ETH3 of 109bp and 115 bp size have not been registered in the world data base so far. Therefore, they may be considered as the typical of the Whiteback cattle. A heterozygosity degree fixed in the analysed cow population proved high – 0.7689,while a polymorphic information content (PIC) ranged from 0.4820 up to 0.8433.The probability of exclusion (PE) for this population amounted to complete 100%. The evaluated cow population was characterized with a mean milk efficiency at 4331.48 kg level with a content of fat – 4.21%,protein – 3.32%,lactose – 4.68% and dry mass – 12.64%.An average rump height of the assessed cows appeared to be 126cm,the body weight – 590kg ,these estimates prove that it represents the "old European" type of dairy -meat cattle.

Key Words: cattle, genetic resources conservation, local breeds, Whitebacks

Introduction

In the early XXth century in Europe, around 230 cattle breeds were recorded, yet 70 pedigrees became extinct, 53 are in danger of it and nearly 30 populations have been included in to the programme of the conservative breeding. In Poland, the Whitebacks is the breed which seemed extinet in the 70's while today it is being restituted (LITWIŃCZUK et al., 2003a). The market conditions in the second half of XXth century were not propitious to maintain biodiversity. More and more widely, the high - performance animal genotypes were used and adapted for the intensive production (REKLEWSKI, 2005). As a consequence, a relatively substantial affinity of the high production breeds was observed manifesting itself with the negative effects of the inbred depression. The studies conducted in the 90's of the last century on the American population of the Holstein-Frisian cattle demonstrated some signs of profitableness decrease in the milk production owing to the growth of inbred in this population, that reached 2.60% level (WIGGANS et al., 1995). In the recent years, some revaluation in the European breeding has been reported so that the local breeds could have a new importance and their utilization can be well-founded economically. Food overproduction and as a result, vast area of land out of crop, is conducive to the extensive animal production based on the local breeds that may positively affect the landscape diversity. Besides, nowadays a demand for some local specific products is increasing, can be aiming at tasting something new of original dietetic values.

Many indications are given of the fact that the only roan breed maintained in Poland for many years has been dappled coloured Whitebacks along with their variety defined as lowland cattle (żuławki cattle). Owing to the region they occur, that is between the Vistula and Bug rivers, this cattle was termed nadwiślanskie, nadbużanskie etc. The lowland cattle (żuławki cattle) became completely extinct in the first half of XXth century, while the Whitebacks were assumed died out in the 70's of last century. In the

late 90's of XXth century, The University of Agriculture in Lublin took up the efforts to restitute the Whitebacks cattle. At that time just single animals of this breed type were encountered in the eastern part of Poland (the region of the Biebrza, Bug, Narew rivers) (LITWINCZUK et al., 2003a; LITWIŃCZUK et al., 2003b; LITWIŃCZUK et al., 2004). The project realized from the year 2000 enabled to restitute some population of the cattle, so in 2003 the Ministry of Agriculture and Rural Development acknowledged it the Polish pedigree, that resulted in the breeding registers opening. These registers have been run at the University of Agriculture in Lublin. From 2003.09.11. this breed has been covered by the project of the genetic resources conservation on the grounds of the programme elaborated by LITWIŃCZUK (2002).

Photo: Whitebacks black cow. Owner Department of Cattle Breeding. University of Agriculture in Lublin. (Photo W. Chabuz)

The main objective of the work was to determine a level of genetic variation and distance in the restituted population of the Whitebacks.

Material and Methods

The investigations covered 86 cows included into the project of genetic resources conservation and recorded in the breeding registers of the Whitebacks pedigree. The animals are mainly kept in small farms in the eastern part of Poland, mostly in the Bug and Biebrza rivers region. The cow nutrition is based on the feeds produced in the farms. During the winter period the animals feed hay, haysilage and silage from maize, whereas in summer the cows graze the pasture. The evaluation of the reproduction performance was performed on the grounds of the information supplied by milk usefulness inspection of 66 cows. Then their milk efficiency was determined after 305

milk days considering the content of fat, protein, lactose and dry mass. Besides, cow caliber and body weight were fixed.

The genetic variation was characterized on the basis of the studies of microsatellite 11 loci of the DNA. The biological material was constituted by the peripheral blood sampled to the disposable test tubes with EDTA as an anticoagulant, then cooled down to 4^0C temperature. Prior to investigations, it was stored frozen (-20°C). The blood examination proceeded in three stages: I – isolation of nuclear DNA, II – PCR- chain reaction of polymerase, III – electrophoretic separation. The polymerase chain reaction was conducted in a thermocycler Gene Amp PCR System 9600, while the electrophoretic separation in the automatic sequenator DNA ABI PRISM 377, at 51°C temperature, at 3000 V voltage,60 mA strength, 200 W power and laser power of 40 mV. The results were analysed with the GeneScan programme. The genetic variation was evaluated by the following statistical indicators:

- microsatellite alleles frequency, estimated with GENEPOP programme;
- heterozygosity observed /Ho/ for each locus as heterozygotic genotypes content;
- heterozygosity expected /He/,termed gene diversity computed after the formula given by OTT (1992) with DISPAN programme;
- polymorphic information content (PIC) for microsatellite loci according to the formula presented by BOSTEIN et al., (1980) aided with DISPAN programme;
- probability of exclusion PE for all the analysed loci was computed after the formula supplied by FREDHOLM et al.,(1996), - DISPAN programme.

A genetic distance between the restituted population of the Whitebacks cattle and black-white cattle maintained in this region was calculated according to the NEI method (1978).

Results

A Whitebacks cattle population which has been restituted currently meets the colour standards characteristic of this pedigree depicted by professor MOCZARSKI (1970).The animals hold a white strip on the back, narrow at the neck and widening towards the rump (Photo).Most of the population have black sides (81.7%) and only few animals (12.9%) show the red colour. The restituting cow population belongs to the mean calibrated cattle, height at withers – 126.6 cm, body length– 156.3 cm, chest depth – 68.5 cm, circumference of fore cannon – 18.3 cm and body weight – 579.7 kg, while calf body weight at birth – 36.5 kg (Tab. 1). Cow milk performance (estimated after 66 lactations) averaged from 4776.3 kg of milk in I lactation up to 5454.4 kg at IX lactation (Tab. 2). The mean cow performance for whole assessed population was 4331.5 kg milk with a content of fat – 4.21%, protein – 3.32%, lactose – 4.68% and dry mass – 12.64%.

The analysis of the genetic variation of the restituted population was conducted on the basis of microsatellite DNA studies. In the estimated group of animals, in 11 loci DNA there were identified total 95 alleles. Alleles number in a single locus ranged from 4 (BM1824) up to 12 (TGLA227, TGLA53, TGLA 122), mean 8.3 alleles fell to one locus. Among 93 identified alleles, only 20.4% exhibited low frequency (0,02).

Table 1
Characteristics of habit and calibre of the restituted Whitebacks cows population in Poland

Specification	$\overline{x}$	SD
Height at withers /cm/	126.58	4.39
Height at sacrum /cm/	127.99	4.30
Oblique length of body /cm/	156.33	8.42
Width of chest /cm/	48.59	6.86
Depth of chest /cm/	68.51	4.41
Circumference of chest /cm/	192.49	10.62
Width of hip /cm/	54.76	3.74
Length of round /cm/	52.67	3.42
Width of pin bones /cm/	23.78	6.92
Circumference of fore cannon /cm/	18.35	1.05
Body weight of cow /kg/	579.73	81.35
Calf body weight at birth /kg/	36.48	3.06

Heterozygosity observed (Ho)and expected (He)were calculated for all the loci (Tab. 4).The estimated value Ho for the Whiteback population oscillated from 0.6627 for the locus ETH3 up to 0.8604 for the TGLA122.High values of this indicator also appeared in the locus TGLA53 (08539) and BM1824 (0.8089). The mean heterozygosity observed value was 0.740.The highest heterozygosity expected (He) was recorded for the locus TGLA 122 (0.8686),whereas the lowest for the SPS115 (0.5111).The mean heterozygosity level for whole the population was 0.7686.

Table 2
The first results on restituted whitebacks cows performance (at each lactation)

Lac-tation	Number of lac-tation	Milk		Fat				Protein			
		kg		kg		%		kg		%	
		$\overline{x}$	SD	$\overline{x}$	SD	$\overline{x}$	SD	$\overline{x}$	SD	$\overline{x}$	SD
1	9	3776.33	705.78	150.22	30.81	3.99	0.48	119.44	24.00	3.16	0.12
2	6	4070.33	1315.01	185.00	71.43	4.50	0.66	141.17	48.73	3.45	0.28
3	9	4665.44	1037.05	195.33	62.35	4.18	0.83	153.67	32.17	3.31	0.23
4	7	4424.57	756.11	188.57	46.94	4.24	0.59	140.43	25.11	3.18	0.18
5	13	4308.38	943.74	185.08	66.26	4.22	0.83	142.85	34.47	3.35	0.29
6	7	4149.14	1295.33	173.71	70.84	4.12	0.57	139.29	43.06	3.37	0.35
7	5	3694.40	531.38	158.60	50.56	4.23	0.80	125.20	30.16	3.36	0.34
8	5	4742.20	1192.94	205.00	64.42	4.28	0.53	158.20	36.53	3.36	0.30
9	5	5454.40	919.61	240.00	47.13	4.38	0.22	183.40	26.35	3.38	0.17
Total	66	4331.48	1032.47	184.55	59.40	4.21	0.64	143.24	35.68	3.32	0.26

The highest polymorphic information content (PIC) and the greatest probability of exclusion (PE) was noted for the locus TGLA122 (PIC 0.8433, PE 0.7176), while the lowest values for these indicators were found in the locus SPS115 (PIC 0.4820, PE 0.3158). The high polymorphism indices were also obtained in the locus TGLA53 (0.8298) and BM2113 (0.8037). For the other microsatellites loci of the DNA, the values were contained in the interval 0.6868 up to 0.7909.

Table 3
Allele frequency in 11 microsatellite loci in Whitebacks

Locus	Allele (bp)	Częstość (q)	Locus	Allele (bp)	Frequency (q)
BM 1824	179	0.29	TGLA 53	153	0.22
	181	0.15		157	0.18
	183	0.26		159	0.19
	189	0.30		161	0.16
BM 2113	126	0.11		163	0.05
	128	0.14		165	0.04
	132	0.04		167	0.04
	134	0.28		169	0.06
	136	0.16		171	0.01
	138	0.11		175	0.01
	140	0.16		179	0.02
ETH 3	109	0.03		181	0.02
	115	0.02	TGLA 122	140	0.06
	117	0.38		142	0.15
	119	0.1		144	0.17
	121	0.24		148	0.02
	125	0.13		150	0.12
	127	0.09		152	0.22
	129	0.01		154	0.04
ETH 10	213	0.01		162	0.06
	215	0.1		164	0.13
	217	0.04		170	0.01
	219	0.26		172	0.01
	221	0.37		174	0.01
	223	0.09	TGLA 126	115	0.01
	225	0.06		117	0.06
	227	0.07		119	0.52
ETH 225	141	0.10		121	0.22
	145	0.06		123	0.02
	147	0.10		125	0.06
	149	0.28		127	0.11
	151	0.39	TGLA 227	79	0.02
	153	0.06		81	0.03
	155	0.01		83	0.31
INRA 23	199	0.02		85	0.11
	201	0.11		87	0.03
	207	0.18		89	0.06
	209	0.12		91	0.16
	211	0.20		93	0.03
	213	0.07		95	0.06
	215	0.28		99	0.13
	217	0.02		101	0.05
SPS 115	246	0.68		105	0.01
	248	0.02			
	250	0.07			
	252	0.06			
	254	0.12			
	256	0.02			
	258	0.03			

Table 4
Level of heterozygosity observed (Ho) and expected (He). polymorphism information content (PIC) and probability of exclusion (PE) in whitebacks

Locus	H_o	H_e	PIC	PE
BM 1824	0.8089	0.7446	0.6868	0.4855
BM 2113	0.7441	0.8353	0.8037	0.6553
ETH 3	0.6627	0.7684	0.7276	0.5550
ETH 10	0.7441	0.7914	0.7555	0.5953
ETH 225	0.7701	0.7514	0.7059	0.5274
INRA 23	0.7727	0.8250	0.7909	0.6363
SPS 115	0.5057	0.5111	0.4820	0.3158
TGLA 53	0.8539	0.8573	0.8198	0.6969
TGLA 122	0.8604	0.8686	0.8433	0.7176
TGLA 126	0.6781	0.7359	0.6912	0.5101
TGLA 227	0.8089	0.8475	0.8215	0.6893

Discussion

The results presented in Table 1 indicate that the present Whitebacks are characterized with a substantially greater caliber as compared to the information provided by other authors – Professors ROSTAFIŃSKI (1920), KONOPIŃSKI (1926) and PAJĄK (1958), (from 122.5 up to 123.5 cm height in rump). Alike, body weight (579.7kg) appears to be far higher than that mentioned by the authors above (359.5 – 457.5 kg). The increased caliber of the evaluated cow population has also improved the cow milk efficiency. The current average cow performance in lactation is by nearly 1000 kg higher than mentioned by PAJĄK (1958) in the 50's of the last century and by 1600 kg compared to the 30's of XXth century. According to the data (from the Circles of Cowshed Inspection) concerning the years 1935-36 the mean milk performance of the Whitebacks kept in the Polesie region was 2650 kg milk with a fat content 3.89%. Similar values were given by SACHS (1935), (2765 kg milk of 3.8% fat) for the cows from the Brest District. The present performance of cow productivity in the restituted population proves that it has been still the cattle that was not improved with Holstein-Frisians, thus hopeful to conserve the gene pool of this native breed.

The aptness of this thesis has been confirmed by the cytogenetic examinations. In 3 out of 11 analysed loci, the alleles were identified ,whose size expressed in bp is not found within the ranges given in the world literature (BISHOP et al., 1994; BREZINSKY et al., 1993a, BREZINSKY et al.,1993b; GEORGES et al., 1992; MOMMENS et al., 1994; SOLINAS et al., 1993). These are the following alleles: 227 bp in the locus ETH10, 170bp,172bp and 174bp in the TGLA122 and 109bp and 115bp in the ETH3.So these alleles can be assumed characteristic of the Whitebacks pedigree as it was also by the studies run by GRZYBOWSKI et al. (2003). A high number of alleles in a locus proves its high polymorphism. Thus, the most polymorphic appeared to be the loci TGLA227, TGLA122 (12 alleles each), while the least BM1824 (4 alleles). The analyzed group of the Whitebacks demonstrated a considerably higher mean number of alleles in a single locus (8,3) as against the bw cattle (5,3 alleles) studied by ŻURKOWSKI et al. (2004).A polymorphic content was closely connected with a heterozygosity index. The heterozygosity observed calculated for the analyzed population, (heterozygotic genotypes content in a locus)as well as heterozygosity expected He (called the gene diversity) indicate a relatively high genetic variation in a animal stock. It is confirmed by a great number and high

frequency of alleles. The locus TGLA122 makes a good example where 12 alleles were identified and the computed heterozygosity observed (Ho) had 0,8604 value.
An indicator characterizing the genetic structure of the studied animal population is probability of exclusion (PE). It depends on the number of alleles in a locus and the equal distribution of each allele occurrence frequency. In the case of the analysed Whitebacks, the highest value of probability of exclusion (determined on the grounds of a single locus) was recorded for TGLA122 (0,7176). Despite the distinct differences in the PE values computed for each locus (from 31% in SPS115 up to 71% in TGLA122), this value (i.e. PE) estimated on the basis of all 11 analyzed microsatellite loci DNA was complete 100%. In the mentioned above studies (ŻÓRKOWSKI et al., 2004) concerning the Whitebacks, the highest level of probability of exclusion was also stated in the locus TGLA53 (68%) and combined treatment of all the loci gave 100%, too.
Analysing the differences in the gene frequency at each locus in the animals born in the years 1989-1999 and after 2000, it was stated to be higher in the case of 43 alleles, lowered in 38 ones and persisted at the same level in 12. It is worthy of notice that all 6 alleles typical of the the Whiteback cattle were also recorded in the animals born after the year 2000. In the case of 3 alleles,that is 115 bp in locus ETH 3 .227 bp in ETH 10 and 174 bp in TGLA 122, their frequency decreased by 0.01,for one (172 bp in TGLA 122) remained at the same level and for two 115 bp in ETH and 170 bp bp in TGLA 122 even slightly grew (by 0.02 and 0.03).
The realized studies prove that the Whitebacks cattle population restituted currently represents the “old European” type of meat-dairy cattle. The performed analysis on the genetic variation on the basis of polymorphism of microsatellite DNA sequency has confirmed explicitly the distinct separateness of this population from the black-white cattle maintained in this region at present.

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Effect of maintenance system on the reproduction of sheep as well as the level of some morphological and biochemical blood indicators

Abstract

The studies were conducted on thirty ewes of two genotypes. For each genotype the ewes were divided into two groups: stabling and pasture. The objective of the investigation was to determine the influence of maintenance system on chosen morphological and biochemical blood indicators as well as the parameters of reproduction traits of sheep. In the first year of the studies the ewes of both groups obtained similar value concerning the size of litter. In the second year a higher value of this indicator was noted for the ewes housed indoors. In the third year of the observation the size of litter in both studied groups was high, ranging from 2 to 2.11. The biochemical analysis revealed a higher content of Hb and Ht in the blood of the ewes kept on the pasture compared with the stabled ones. Also the activity of the ALAT and AspAT enzymes in blood serum in each of the three phases, i.e. in the mating season, the fifth month of pregnancy and the second month of lactation was higher for the ewes kept on pastures compared with the stabled ones. In the pasture group, a higher activity of LDH at pregnancy and lactation and the received differences were statistically significant comparing with the indoor ones. The content of triglycerides cholesterol in blood serum of pasture ewes at all studied periods was lower than in the blood serum of stabled ewes. Moreover, in both studied groups the content of triglycerides and cholesterol was heightened at pregnancy comparing with the mating season and lactation. The content of protein for the studied ewes at mating and pregnancy was at a similar level, the increase in the concentration of this parameter was irrespective of the maintenance system at lactation.

Key Words: sheep, maintenance system, blood, laparoscopy, reproduction

Introduction

In order to increase of sheep effectiveness and environment protection as well as to meet the expectations of the consumers, the pasture system of keeping sheep is used (LIPECKA et al., 2002; NIŻNIKOWSKI, 2003). However, the climatic and environmental conditions of Poland enforce the necessity of using farm facilities for the most of the year and keeping sheep indoors (KIEĆ and MUSZYŃSKA-WARSIEWICZ, 1999). Nowadays, a great interest is observed in keeping the sheep in the vegetation period, as well as beyond it, exclusively outdoors (ANTCZAK et al., 2002; KLEWIEC et al., 2002; PATKOWSKI et al., 2003). The maintenance of sheep in summer in continuous pasture does not negatively affect the condition and reproduction of sheep (PATKOWSKI et al., 2003).

The inter dependence between productive traits of animals and some indicators of their blood has been studied for quite a long time. The level of physiological indicators in the blood of the animals may help evaluate the demand for nutrients and the evaluation of the regularity of feeding. The information on shaping the level of the blood indicators is scarce, however, and not fully recognized. The available data contain the values for sheep not considering their physiological state (WINNICKA, 1997), however, foreign standards are not always useful in our conditions due to different environment as well as diet (GŰNDOĞAN and SERTESER, 2005).

The objective of the studies was to determine the effect of various maintenance systems on chosen morphological and biochemical blood indicators as well as the parameters of the traits of sheep reproduction.

Material and Methods

The studies were conducted in 2001 – 2004 at the Experimental Station Bezek. The observation covered the ewes of two genotypes: RB – (25% Polish lowland sheep, 25% prolific race, 50% berrichon du cher race) – 16 specimens and RS – (25% Polish lowland, 25% prolific race, 50% Suffolk race) – 14 specimens.

The experiment was set out on young 8-month-old animals. For each genotype the ewes were divided into two groups: stabled and pasture. Since September 2001, the pasture group was kept on the meadow only, having the access to a canopied shed, whereas the indoor group in the sheep shed with no access to the sheep-run. Feeding RS and RB ewes in both maintenance systems was accordant with the demand of individual physiological phase. At winter feeding, the sheep of both groups were given corn silage, meadow hay, pulses straw and a little supplement of concentrate. At summer feeding, the sheep of the studied groups received the same basic feeds; only for the indoor group corn silage was supplemented with green forage from field cultivation. However, the pasture group, when having access to the meadow, at times was also fed with green forage from field cultivation. In the studied groups mating was performed by the group mating system at the following dates: from 15th November 2001 to 3rd January 2002 – the first mating period, from 17th September to 30th October 2002 – the second mating period and from 16th September to 2003 – the third mating period. In the mating period, the level of ovulation rate was evaluated by means of laparoscope techniques on the basis of the number of *corpus luteus* on both ovaries (PATKOWSKI, 2001). The investigations were executed after three weeks from beginning of mating. During the period of lambing (about 1 month), the ewes of the pasture group were moved to the sheep shed, then they came back to the pasture with their lambs. Every year the fertility as well as the size of litter was evaluated. For biochemical analyses, the blood was sampled from mothers at then after three weeks from beginning of mating, in the fifth month of pregnancy and in the half second month of lactation in each year of studies. The blood was always sampled before the morning pasture. In hyparinised blood haematocrit (Ht) was determined by the microhaematocrit method, the content of hemoglobin (Hb) – by the calorimetric Driabikin method (PINKIEWICZ et al., 1971, BOMSKI, 1989) while in blood serum the activity of alanine aminotransferase (ALAT), aspartico aminotransferase (AspAT) and lactate dehydrogenase (LDH) – by the kinetic method, using Cormay monotests. Moreover, in blood serum the level of protein, cholesterol and triglicerydes was described by the colorimetric method, using Cormay monotests.

The obtained results were statistically determined by the method of *two-factor* variance analysis (SAS, 2004).

Results

The conducted studies revealed that each year the fertility of the ewes of the studied genotypes maintained in the pasture system stayed at a similar level. In the first year of the studies the fertility of the mothers of RB and RS genotypes in the pasture system was 87.5% and 85.7%, respectively (Tab.1). However, in two subsequent years of the studies this reproduction trait in both genotypes reached the value of 100%. The same fertility in each of the three years of studies was observed in the ewes of RS genotype maintained in the indoor system. The ewes of RB genotype in the same system in the

first and second year had a slightly lower fertility (87.5%) than in the third year of the studies (100%, Tab. 1).

Table 1
Reproduction rate of ewes in relation to maitenance system and genotype

Traits/ year of investi-gation	Pasture						Stabling					
	RB		RS		Total		RB		RS		Total	
	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
Fertility (%)												
1st year	87.5	35.4	85.7	35.4	86.7	35.2	87.5	35.4	100.0	0.0	93.3	25.8
2nd year	100.0	0.0	100.0	0.0	100.0	0.0	87.5	35.4	100.0	0.0	93.8	25.0
3rd year	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0
Ovulation rate (No.)												
1st year	1.75	0.71	1.86	0.69	1.80	0.67	1.75	0.71	1.57	0.53	1.67	0.62
2nd year	2.14	0.38	2.00	0.53	2.07	0.46	2.25	0.71	2.37	0.52	2.31	0.60
3rd year	2.20	0.42	2.54	0.66	2.39	0.58	2.22	0.44	2.33	0.50	2.28	0.46
Litter size (No..)												
1st year	1.57	0.53	1.50	0.52	1.54	0.52	1.57	0.53	1.43	0.53	1.50	0..52
2nd year	2.00	0.00	1.63	0.74	1.79	0.58	2.43	0.53	1.75	0.46	2.07	0.59
3rd year	1.80	0.42	2.15	0.69	2.00	0.60	2.00	0.50	2.11	0.60	2.06	0.54

The pasture system positively affected the level of ovulation of the ewes of both studied genotypes. In this system it was observed that the older the animals (subsequent years of studies), the higher the level of ovulation of sheep and in the case of RB genotype it ranged from 1.75 to 2.20, whereas for RS genotype - from 1.86 to 2.54 (Tab. 1).

The size of litter of RB genotype ewes of the pasture group was slightly lower (the value from 1.57 to 2.00) than the indoor group (1.57 to 2.43) (Tab. 1). In the ewes of RS genotype it ranged from 1.50 to 2.15 in the pasture group and from 1.43 to 2.11 in the stabled one. Comparing the size of the litter in relation to the maintenance system of ewes, it was found out that in the first and third year of studies it was at the same level. Only in the second year of the studies, the decrease in this parameter (1.79) in the case of the ewes of the pasture group was noted, comparing the ewes of the indoor group (2.07, Tab. 1).

As the result of the conducted studies, a different level of Hb in blood of the studied ewes (Tab. 2) was found out. The value of this indicator ranged from 12.5% to 23.6g%. The highest content of haemoglobin in the blood of the ewes of the pasture group occurred in the fifth month of pregnancy (22.1g %), however, the lowest at lactation (14.7g %). A parallel concentration of haemoglobin in the fifth month of pregnancy differed statistically significantly depending on the system of maintenance.

Table 2
Haemoglobin concentration and haematocrit in blood of ewes in relation to maintenance system and genotype

Traits	Pasture						Stabling					
	RB		RS		Total		RB		RS		Total	
	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
Concentration of haemoglobin (g%)												
mating period	19.5	4.6	19.6	4.9	19.5	4.7	18.7	4.9	20.0	4.3	19.4	4.6
5th month of pregnancy	20.2	5.1	23.6	7.7	22.1^{x}	6.8	19.7	6.7	19.9	7.8	19.8^{x}	7.2
lactation	15.1	4.9	14.4	4.6	14.7	4.7	12.5	2.9	13.6	2.9	13.0	2.9
Haematocrit (%)												
mating period	35.2	3.2	35.8	4.4	35.5	3.9	35.9	3.5	35.9	2.0	35.9	2.8
5th month of pregnancy	39.1	2.8	39.9	3.0	39.5$^{x}_{x}$	2.9	36.4	3.2	36.1	2.7	36.2$^{x}_{x}$	3.0
lactation	35.8	3.2	35.8	3.5	35.8	3.3	35.0	4.8	34.4	3.0	34.7	4.0

^{x} - statistically significant differences between maintenance systems at $P < 0.05$
^{xx} - statistically significant differences between maintenance systems at $P < 0.01$(also for Tables 3 and 4)

Table 3
Activity of some enzymes in blood serum of ewes in relation to maintenance system and genotype

Traits	Pasture						Stabling					
	RB		RS		Total		RB		RS		Total	
	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
Alanine aminotransferase (U/l)												
mating period	16.8	3.6	15.9	3.6	16.3	3.6	16.2	2.9	15.4	4.2	15.8	3.6
5th month of pregnancy	14.2	3.4	15.5	3.6	14.9	3.5	15.4	3.4	14.5	3.2	14.9	3.3
lactation	16.4	2.5	15.4	3.7	15.8	3.3	15.0	2.8	13.9	4.4	14.5	3.6
Aspartico aminotransferase(U/l)												
mating period	89.9	14.5	90.2	21.5	90.1	18.5	82.0	19.1	91.8	15.3	87.1	17.7
5th month of pregnancy	90.3	19.0	86.8	13.5	88.4	16.1	81.9	10.8	80.9	18.1	81.4	14.9
lactation	100.4	13.4	95.2	12.1	97.4	12.7	97.8	14.5	101.4	18.7	99.5	16.5
Lactate dehydrogenase (U/l)												
mating period	570.9	105.1	610.2	90.2	592.8	97.9	607.1	75.4	607.6	76.7	607.4	75.2
5th month of pregnancy	852.3	211.6	915.7	250.4	887.3^{xx}	233.6	782.1	151.4	837.8	161.8	810.5^{xx}	157.7
lactation	787.6	153.5	824.4	173.1	809.3^{x}	164.3	737.9	168.5	738.5	133.4	738.2^{x}	150.9

Table 4
Concentration of triglicerydes, protein and cholesterol in blood serum of ewes depending on maintenance system and genotype

Traits	Pasture						Stabling					
	RB		RS		Total		RB		RS		Total	
	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
Triglicerydes (mg/dl)												
mating period	14.2	4.1	14.0	4.5	14.1^{x}	4.3	16.4	5.2	18.2	7.0	17.3^{x}	6.2
5th month of pregnancy	24.9	5.6	23.1	5.7	23.9	5.7	24.3	5.9	23.9	6.4	24.1	6.1
lactation	17.4	3.9	18.8	5.4	18.3	4.8	21.4	4.4	19.5	4.5	20.5	4.5
Protein (g/dl)												
mating period	6.7	0.8	7.1	0.7	6.9	0.8	6.9	0.8	7.0	0.7	7.0	0.7
5th month of pregnancy	7.0	0.7	7.0	0.7	7.0	0.7	6.6	0.6	6.8	0.5	6.7	0.6
lactation	7.5	0.6	7.3	0.5	7.4	0.5	7.0	0.6	7.6	1.1	7.3	0.9
Cholesterol (mg/dl)												
mating period	64.7	5.7	64.8	16.1	64.7^{x}	12.5	67.4	15.1	83.4	27.1	75.8^{x}	23.4
5th month of pregnancy	80.2	11.4	80.8	15.0	80.6	13.4	81.8	19.8	89.2	20.7	85.6	20.4
lactation	75.7	15.5	77.2	18.5	76.6	17.2	80.0	16.7	84.8	19.3	82.3	17.9

The highest level of haematocrite was noted in the blood of both studied groups of sheep in the fifth month of pregnancy (939.5% in the pasture group and 36.2% in the indoor group). The difference proved to be statistically highly significant. A slightly lower level of this indicator was noted at lactation (35.8% and 34.7%, respectively). A similar value was obtained at mating period (35.5% and 35.9%, respectively) (Tab. 2).
The activity of ALAT and AspAT enzymes in blood serum in all three studied periods was higher in the ewes maintained indoors (Table 3). Only in the 5th month of pregnancy, for the ewes of the indoor group, a slightly higher activity of AspAT (99.5U/1) was noted comparing with the ewes of the pasture group (97.4U/1). This difference was not statistically significant, however (Tab.3). The lowest activity of AspAT enzyme was determined for both studied groups of ewes in the 5th month of pregnancy (in pasture group – 88.4U/l, in stabled group – 81.4U/l), and the highest at lactation (97.4U/l and 99.5U/l, respectively).
A slightly higher activity of LDH enzyme was found out in the blood serum of RS ewes both in the pasture and indoor group than in the ewes of RB genotype of both groups. The highest activity of this enzyme was observed in the fifth month of pregnancy for the ewes of the pasture and indoor groups (887.3U/1 and 810.5U/l, respectively). The difference was statistically highly significant. At lactation, a slight decrease in the activity of LDH in blood serum of the ewes kept on the pasture (809.3U/l) and indoors (738.2U/1) was noted, however, between the systems of maintenance the difference was still statistically significant. The highest activity of this

enzyme was seen at the mating period. For the ewes of the pasture group it was 592.8U/l, and for the ewes maintained indoors 607.4U/l (Tab. 3).
The concentration of triglicerydes in blood serum of the ewes, mean for both genotypes, ranged from 14.1mg/dl to 23.9mg/dl in the pasture group and from 17.3mg/dl to 24.1mg/dl in the indoor group. Statistically significant differences were shown for the concentration of triglicerydes in blood serum of the ewes at the period of mating between the pasture group (14.1mg/dl) and indoor 917.3mg/dl). In the 5th month of pregnancy the increase in the content of triglicerydes in blood serum was noted, both for the ewes kept on the pasture and indoors (23.9mg/dl and 24.1mg/dl). However, at the period of lactation in both systems of maintenance, there occurred the decrease in the concentration of this parameter in blood serum, and it was 18.3mg/dl and 20.5mg/dl, respectively (Tab. 4).
The highest content of cholesterol was found out for the ewes maintained in both systems in the 5th month of pregnancy (pasture – 80.6mg/dl, indoors – 85mg/dl). However, at lactation there occurred the decrease in the concentration of cholesterol to the level of 76.6mg/dl and 82.3mg/dl, respectively. A statistically significant difference in the content of cholesterol was observed between the ewes kept in two different systems at the mating period. The ewes maintained on the pasture had the concentration of 64.7mg/dl and indoors -75.8mg/dl (Tab. 4).
The concentration of protein in blood serum of the ewes of the studied genotypes maintained by the pasture system ranged from 6.7g/dl to 7.5g/dl, and the indoor system from 6.6g/dl to g/dl. The highest content of this parameter was noted at lactation (Tab. 4).

Discussion

The results of the reproduction traits showed that the system of maintenance did not significantly differentiate the obtained values of fertility, ovulation rate and the size of litter. They therefore confirm earlier self-studies (PATKOWSKI et al., 2003) as well as the reports of ANTCZAK et al. (2002) and KLEWIEC et al. (2002). It should be also noted that the level of reproduction traits, apart from the system of maintenance, also depended on the year of studies and the genotype. LIPECKA et al. (2002) and NIŻNIKOWSKI (2003) stated that the maintenance of sheep in extensive breeding is possible and it does not significantly affect fertility or prolificacy. The analysis of the reproduction traits allows to state that RB and RS genotypes may be well maintained both on pasture and indoors.
The results of haematological tests show the growth of Hb and Ht concentration in the 5th month of pregnancy both in the pasture and indoor group. In the case of the ewes kept on the pasture this increase was higher and the obtained differences were statistically relevant. At lactation, irrespective of the genotype and the maintenance system, the decrease in Hb concentration in the blood of the studied ewes was observed. A similar declining tendency of Hb content in the blood of sows at lactation was observed by CZECH et al. (2000). The genotype did not differentiate Ht concentration in the blood of the studied ewes. KRASUCKI and GRELA (1997) also paid attention to the variability of Ht content in the blood of sows depending on the physiological period.
In the blood serum of the ewes of the pasture group in all studied periods, the content of triglicerydes and cholesterol was lower than in the blood serum of indoor ewes. In

both studied groups the content of triglicerydes and cholesterol was heightened at pregnancy comparing with the mating period and lactation. At the mating season the difference in the level of triglicerydes and cholesterol between the mothers of the studied groups depending on the maintenance system appeared to be statistically significant. According to CZECH et al. (2000), a significant decrease of lipid substances in the post-parturition animals is connected with higher use of lipid, protein and sugar resources of the organism. However, the content of total protein stayed at a similar level in the studied ewes irrespective of the period of investigation. A similar concentration of total protein was noted for sheep by GŰNDOĞAN and SERTESER (2005). The activity of LDH in the 5th month of pregnancy was markedly heightened in both studied groups. In the 5th month of pregnancy the difference in the level of LDH between the mothers of the studied groups depending on the maintenance system appeared to be statistically significant. According to GANONG (1994), such a state is typical for this physiological period.

The level of all the studied blood indicators was characterized by a high variability. The system of sheep maintenance influenced the activity of ALAT, AspAT and LDH enzymes in blood serum. It also affected the concentration of triglicerydes and cholesterol. High individual variability of the studied indicators reveals that their level depends on environmental factors (maintenance system), but also on the physiological state of an animal. Irrespective of the maintenance system of sheep, the level of chosen morphological and biochemical blood indicators stayed within referential values for this species (WINNICKA, 1997).

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Evaluation of factors affecting the repeatability of ultrasound measurements of the musculus longissimus in lambs

Abstract

The study was designed to evaluate the repeatability of measurements on ultrasound images of the *musculus longissimus dorsi* (MLD) cross-section taken by two independent teams of operators using ultrasound equipment with different parameters of probe working frequency. A total of 60 ewes of two different synthetic lines (BCP and SCP) and Polish Lowland Sheep (20 animals per group) were investigated. All the animals were of similar age and their body weight ranged from 35 to 38 kg. The MLD and the layer of overlying external fat were measured. A probe was placed at the last thoracic rib. The equipment used was ALOKA SSD-900 with a UST-5818-5 5 MHz transducer and Echoson ts 1000 exd ALBIT with an LA – 3.5 3.5 MHz transducer. Each team performed the measurements using Multiscan ver. 14.02 software. MLD height ranged from 2.47 to 2.68 cm, MLD width from 5.32 to 5.67 cm, with muscle area of 10.10-10.20 cm^2 and thickness of fat of 0.196-0.258 cm. The correlations between the measurements taken by the two teams within images from particular probes were positive, very high and statistically significant ($P \leq 0.01$). The greatest similarity of measurements (A-3.5 MHz) was obtained for the height of MLD ($r_{xy} = 0.973$), muscle measurements (0.872-0.984) and fat measurements ($r_{xy} = 0.524$) (B-5 MHz device). Results of measurements taken by different teams of operators (Z1 and Z2) using different devices (A-3.5 MHz and B-5MHz) were compared. For thickness of skin and fat, r_{xy} was 0.139 and 0.340, respectively, while the correlation coefficients for MLD cross-section area ranged from 0.581 to 0.730. Collection and measurement of ultrasound images requires great skill and high qualifications, and particular attention should be given to the measurement of subcutaneous fat. In view of the literature data, the present results indicate that 5 MHz ultrasound probes should be recommended for wide use.

Key Words: lamb, *musculus longissimus*, loin, ultrasonography, probe

Introduction

Ultrasound technique and computer tomography are increasingly used for live evaluation of muscling in sheep (KNAPIK, 1994; KNAPIK et al., 2001; ŚLÓSARZ, 2004). The musculus longissimus in the lumbar part and the overlying layer of external fat are monitored. The collected images are used to measure the thickness and area of visible tissues, and the results provide data for selection indices (JUNKUSZEW and RINGDORFER, 2005). Because of the need to compare the results of evaluation between flocks, it is necessary to eliminate measurement differences resulting from the equipment or measurement technique used (ŚLÓSARZ et al., 1995; ŚLÓSARZ, 2004). The aim of the study was to evaluate the repeatability of ultrasound measurements of the ovine musculus longissimus taken by two independent teams of operators using ultrasound equipment with different parameters of operating frequency.

Material and Methods

The study was carried out at the Experimental Station of Small Ruminants in Bezek, belonging to the Agricultural University in Lublin, Poland. A total of 60 ewes of two different synthetic lines (BCP and SCP) and the Polish Lowland Sheep (20 animals per group) were investigated. All the animals were of similar age and their body weight ranged from 35 to 38 kg. The *musculus longissimus dorsi* (MLD) behind the last thoracic vertebra was monitored. After pulling the wool apart, an ultrasound probe was

applied transversely to MLL, and ultrasound gel was used to ensure good contact. Ultrasound measurements were performed using an ALOKA SSD-900 device with a UST-5818-5 transducer (B-5MHz). This type of transducer uses the electronic linear scanning method with a scanning width of 61 mm and frequency of 5 MHz. The image obtained is of good sharpness, with clearly visible boundaries of particular tissue layers. Using a 5 MHz transducer to monitor the muscling and fatness of lambs, in which the width of the muscle examined is greater than the scanning width of the transducer, it is necessary to obtain two partial images and put them together as a single complete image. The second ultrasound device was Echoson ts 1000 exd ALBIT (A-3.5 MHz) with an LA – 3.5 3.5 MHz linear probe and operating width of 100 mm. Both ultrasound devices generated 256 greyness level images. These devices were used by two independent teams of operators (Z1 and Z2) with previous experience in ultrasound measurements. The *musculus longissimus dorsi* (MLD) cross-section images were stored in computer memory and each team carried out the measurements using Multiscan ver. 14.02 software. The thickness of fat and skin over MLD and the depth, width and area of this muscle were determined. The measurements were made twice and the arithmetic mean of both measurements was the final result. The results obtained were used to calculate Pearson simple correlations between the analogous measurements performed with images of muscle cross-section obtained using two different devices (A-3.5 MHz and B-5MHz) and between the measurements made by two independent teams of operators.

Table 1
Measurements of the *musculus longissimus dorsi* (MLD) images recorded by two teams of operators using A-3.5 MHz and B-5 MHZ devices

Item	Team	Probe			
		A-3.5 MHz		B-5 MHz	
		X	SD	X	SD
Muscle height (cm)	Z1	2.60	0.30	2.47	0.27
	Z2	2.68	0.32	2.50	0.30
Muscle width (cm)	Z1	55.80	0.45	56.65	0.51
	Z2	53.19	0.73	55.36	0.70
Thickness of fat (cm)	Z1	0.26	0.07	0.21	0.09
	Z2	0.25	0.10	0.20	0.07
Muscle area (cm^2)	Z1	10.20	1.73	10.11	1.51
	Z2	10.15	2.28	10.10	1.90

** P≤0.01

Table 2
Correlations between measurements performed by two independent research teams on muscle cross-section images carried out using a 3.5-MHz probe device

Team Z2 / Team Z1	Muscle height	Muscle width	Thickness of fat	Muscle area
Muscle height	0.973**			
Muscle width		0.752**		
Thickness of fat			0.866**	
Muscle area				0.850**

** P≤0.01

Results

The results of MLD cross-section measurements are given in Table 1. MLD thickness ranged from 2.47 to 2.68 cm, MLD width from 5.32 to 5.67 cm, MLD area from 10.10-10.20 cm^2 and fat layer thickness from 0.196 to 0.258 cm. The standard

deviation values were invariably low, which indicates that the analysed material was uniform. Table 2 gives the correlation coefficients between measurements done on MLL cross-section images obtained using a 3.5 MHz linear probe by two measuring teams (Z1 and Z2). The coefficients were positive, very high and statistically significant ($P \leq 0.01$). The best convergence of results ($r_{xy} = 0.973$) was obtained for MLD depth. Similar relationships were found while analysing the measurements on images obtained using am A-5 MHz probe (Tab. 3). In this case the coefficients of correlation for measurements of muscle cross-section images ranged from 0.872 to 0.984, with lower convergence of results obtained for fat thickness measurements ($r_{xy} = 0.524$). Lower coefficients of correlation were observed when comparing the measurements taken on images obtained using different devices (A-3.5 MHz and B-5 MHz) and different operator teams (Tab. 4).

Table 3
Correlations between measurements performed by two independent teams on muscle cross-section images carried out using a 5 MHz probe device

Team Z2 / Team Z1	Muscle height	Muscle width	Thickness of fat	Muscle area
Muscle height	0.984**			
Muscle width		0.872**		
Thickness of fat			0.524**	
Muscle area				0.887**

** $P \leq 0.01$

Table 4
Correlations between measurements performed by two independent teams on muscle cross-section images carried out using 3.5 MHz and 5 MHz probe devices

Team Z2, 3.5 MHz probe / Team Z1,5 MHz probe	Muscle height	Muscle width	Thickness of fat	Muscle area
Muscle height	0.587**			
Muscle width		0.655**		
Thickness of fat			0.139**	
Muscle area				0.730**

** $P \leq 0.01$

For skin and fat thickness r_{xy} was 0.139 and 0.340, respectively, while the coefficients of correlation for the measurements of MLD cross-section ranged from 0.581 to 0.730.

Table 5
Correlations between measurements performed by two independent teams on muscle cross-section images carried out using 3.5 MHz and 5 MHz probe devices

Team Z2, 5 MHz probe / Team Z1, 3.5 MHz probe	Muscle height	Muscle width	Thickness of fat	Muscle area
Muscle height	0.581**			
Muscle width		0.611**		
Thickness of fat			0.340**	
Muscle area				0.706**

** $P \leq 0.01$

Discussion

The results obtained are part of studies aimed to increase the accuracy and repeatability of ultrasound measurements. JUNKUSZEW and RINGDORFER (2005) reported that the coefficients of correlation between the depth of MLD and the muscle tissue content of carcass are 0.692 for ultrasound measurements done on MLD cross-

section images and 0.824 for results based on measurements obtained using computer tomography. WAGENPFEIL et al. (1996) obtained high coefficients of correlation between muscling of the dorsal part evaluated in points and MLD depth measured using the ultrasound image (r_{xy} = 0.61). The same authors showed a high conformity between the area of the above muscle measured planimetrically post-mortem and its area measured using the ultrasound image (r_{xy} = 0.52). The high correlation between MLD depth and carcass muscle content is currently used to construct selection indices.
In animal ultrasonography it is therefore necessary to make measurement methods uniform to achieve the highest possible repeatability of the results. At present, muscle cross-section image measurements are monitored using several types of linear probes with an operating frequency of 3.5, 5, 7.5, 8 and 10 MHz (GRUSZECKI and SZYMANOWSKI, 1996; RINGDORFER, 1995; ŚLÓSARZ, 2004). The present findings showed that regardless of the working frequency, the measurement results were similar (Tab. 1). Slight differences could be due to the accuracy of the ultrasound image obtained, which is much more detailed for a 5-MHz probe (ŚLÓSARZ ,2004). This was reflected in the analysis of repeatability of measurements, especially when the image of skin and fat over loin eye was analysed (Tab. 2 and 3). For a 5 MHz probe, the repeatability of measurements was lower (0.524) compared to the results obtained using a 3.5 MHz probe. In the other cases analysed, the repeatability of ultrasound measurements using different transducer types did not show significant differences between the results obtained, and the results of 0.973-0.974 for MLD depth or 0.850-0.887 for MLD area are considered satisfactory. Comparison of the results obtained with the results of correlation between particular measurements and the adipose and muscle tissue content in sheep shows that the measurements were more accurate for a 5 MHz probe. These were r_{xy}= 0.692 for muscle height and r_{xy}= 0.740 for fat and skin thickness (JUNKUSZEW and RINGDORFER, 2005). Slightly lower values of these correlations were obtained by ŚLÓSARZ (2004). When using a 3.5 MHz probe, the above correlations were lower at r_{xy}= 0.3482 and r_{xy}= 0.5142 (SZYMANOWSKI, 1998). Comparison of the ultrasound measurements performed by two teams of operators (Z1 and Z2) with ultrasound images of MLD cross-sections obtained using A-3.5 MHZ and B-5 MHZ devices is given in Tables 4 and 5. The repeatability of measurements was only satisfactory for MLD area (0.706-0.730). The other MLD measurements taken on the MLD cross-section image were characterized by fairly low repeatability (0.139-0.655). This may result from differences in the ultrasound images obtained, especially in the visibility of boundaries between tissue layers, which are much clearer for 5-MHz probes. It may be possible to eliminate these differences by making the probe frequencies uniform, as evidenced by the results obtained for repeatability of measurements presented in Tables 2 and 3. These data are evidence that both measuring teams interpreted the images in much the same way. An alternative method is to develop appropriate conversion factors so as to make the measurements more comparable.
Live measurements taken on ultrasound images of MLD cross-section using A-3.5 and B-5 MHz devices have shown that the results obtained are highly convergent. The collection and measurement of the ultrasound images of muscle cross-sections required great skills and high qualifications, and a special care should be taken while measuring the subcutaneous fat layer. In view of the literature data, the present results indicate that 5-MHz ultrasound probes should be recommended for wide use.

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A phylogenetic comparison of wild sheep *(Ovis musimon)* and domestic sheep *(Ovis aries)*represented by BCP synthetic line using mitochondrial cytochrome b gene sequence analysis

Abstract

Based on mitochondrial DNA (mtDNA) cytochrome b (cyt b) gene was partially sequenced and analysed in order to investigate genetic diversity between domestic sheep (Ovis aries) synthetic line BCP n=16 and wild sheep mouflon (Ovis musimon) n=24 from the Sowie Mountains in Poland. All 18 haplotypes were observed which formed two distinct clades in *O. aries* BCP synthetic line and three clades in mouflon. 14 single nucleotide polymorphic sites (SNPs), were found in domestic sheep, while 22 SNPs in mouflon. Average value of haplotype distance in *O.aries* was 0.037, while distance among mouflon haplotypes was 0.041. The distribution of haplotypes of wild and domestic sheep suggested intercontinental dispersal genus *Ovis*. Our data supports the hypothesis that some modern domestic sheep like synthetic line BCP and European mouflon from the Sowie Mountains in Poland derive from a common ancestor.

Key Words: *Ovis*; mitochondrial DNA, sequence identity

Introduction

Domestic sheep have played an important role in human history, but the origins of the modern domestic sheep *(Ovis aries)* are not well understood. Molecular genetics has proven highly informative for investigating the relationship between animal populations as well as for documenting the levels of genetic variation resident within breeds(MEADOWS et al, 2005). Wild sheep of the genus *Ovis* are Holarctic, with a distribution of extant species and subspecies that span a few continents. For example Euroasian and American wild sheep represent very distinct evolutionary entities (BUNCH et al., 2006). On the other hand some authors state the Siberian snow sheep *(Ovis nivicola)* was classified in the subgenus *Pachyceros* (GEIST, 1971; VALDEZ, 1982). Today *nivicola* is commonly referred to as the snow sheep and is anatomically very similar to the Alaskan Dall sheep *(O. dalli dalli)*. The Siberian snow sheep *(O. nivicola)* inhibit the most northen range of the Euroasian wild sheep which comprises an expanse of mountain ranges in northern Russia (BUNCH et al., 2005). For the domestic sheep *(Ovis aries)* a large number of wild and possibly ancestral species and subspecies exist. Basing on morphological data several Euroasian wild sheep of the highly polymorphic genus *Ovis* have been suggested as ancestors of domestic sheep (HIENDLEDER et al., 2001; HIENDLEDER et al., 2002). According to ZEUNER (1963) the urial *(O. vignei)* was first domesticated in the Aralo-Caspian basin and these domestic forms subsequently spread throughout the Middle East and into Europe. Another line of domesticated sheep derived from mouflon *(O. musimon* or *O. orientalis)* stock that was brought to Europe and mixed with the urial derivatives, but also argali (*O.ammon*) alleles have been introduced repeatedly into these lines (HIENDLEDER, 1998). The major finding within domestic sheep is a biphyletic pattern where mitochondrial haplotypes from two distinct clades A and B (WOOD and PHUA, 1996). Clade A has been found in two breeds from central Asia. Clade B has been observed in a range of breeds from Europe and includes sequences derived from

European mouflon *(O. musimon)* (HIENDLER et al., 2002). To estimate genetic parameters between specimens or even between populations using sequence diversity is a method which does not require a lot of samples and gives exactness and reliable results (MEADOWS et al. 2005, HIENDLEDER et al.2002, BUNCH et al.2006, MATSUNAGA et al.1998) The aim of this study was to investigate genetic diversity in wild sheep mouflon *(O. musimon)* from the Sowie Mountains and selected domestic sheep *(O. aries)* of the lineage BCP.

Materials and Methods

The samples from soft tissue (muscle) were collected from the Sowie Mountains wild population of mouflon (*Ovis musimon*) in Poland, n=24 and soft tissue (muscle) samples from synthetic line of domestic sheep BCP n=16. BCP synthetic line of domestic sheep contain (Polish Lowland Sheep 37,5%, Charolaise 25%, Berrichone du Cher 25%, Romanov or Olkuska –12,5%) Genomic DNA was extracted from soft tissues using QIAamp DNA Mini Kit following the manufacturers instructions. The PCR reaction was amplyfing only a part of the cytochrome b sequence (without tRNA-Glu fragment). Primers Cyt b1 (5' –cca tcc aac atc tca gca tga tga aa- 3' and Cyt b 2 (5' –gcc cct cag aat gat att tgt cct ca -3') were used to amplify a 359 bp fragment (307 bp without primers). PCR conditions: after initial denaturation at 94 ^{o}C for 1 min, 94 ^{o}C for 5 s, 55 ^{o}C for 30 s, 35 cycles, anieling temperature at 58^{o}C and the last elongation at 72^{o}C was prolonged to 3 minutes (BRODMAN et al., 2001). PCR products were purified using QIA-quick PCR purification kit according to the producer manual. The purified PCR products were sequenced on a DNA sequencer (ABI Prism 377, Perkin-Elmer). The sequences were analysed in Chromas software programme and subjected to a BLAST search (www.ncbi.nlm. Nih.gov/blast/blast.cg). DNA sequences from the EMBL database were used to compare cytochrome b gene sequences of mouflon and domestic sheep. For that purpose 307 bp of 1140 bp of total cytochrome b genes were compared to each other. A rapid method was developed to distinguish the differences into mtDNA lineages. It is a method using single nucleotide polymorphism (SNP) genotyping sequences. We were working on DnaSP 4.0 software freeware programme (ROZAS et al., 2003) and estimated the diversity of nucleotide sequence (Pi), number of monomorfic (invariable) sites (Msites), number of polymorphic (variable) SNPs (Psites), number of haplotypes (Hn), the haplotype diversity (Hd) and the variance of haplotype diversity (VHd).

Nucleotide diversity is defined as the average number of nucleotide differences per site between any two DNA sequences.

From this distance data, an mtDNA phylogenetic (neighbour-joining) tree was constructed and parsimony analysis was performed using the PHILIP version 3.5 (FOLSTEIN, 1993) with the GeneDoc and Gene-Bee programme. We used an outgroup *O. mongolian1* partially nucleotide sequence GenBank accession no. AY879584 (MEADOWS et al., 2005) and *O. musimon* partially nucleotide sequence GenBank accession no. D84203 (www.ncbi.nlm. Nih.gov/blast/blast.cg).

Results and Discussion

A total of 18 mtDNA haplotypes could be distinguished among the 40 wild and domestic sheep analysed (Table 1). In the domestic sheep 14 mutations were found and in mouflon 22. Sequences differences between *O. aries* haplotypes ranged from

0.009 to 0.070, where average value was x = 0.037. While distances among mouflon haplotypes ranged from 0.009 to 0.065, the average value was x = 0.041 (Table 2). Both the distance analysis and the parsimony analysis suggested that the investigated domestic sheep BCP synthetic line derive from two different maternal sources. The neighbour-joining tree (Fig. 1) constructed with the data described above reveals two major branches BCP synthetic line domestic sheep haplotypes.

Table 1
Summary statistics from mtDNA sequence for mouflon and domestic sheep

Species	*Ovis musimon*	*Ovis aries*
n	12	6
Msites*	285	293
Psites	22	14
Hn	12	6
Hd	0.88	0.87
VHd	0.00116 (SD=0.034)	0.00926 (SD=0.096)
Pi	7.23	6.29

* Msites is the number of monomorfic (invariable) sites; Psites is the number of polymorphic (variable) SNPs; Hn is the number haplotypes observed; Hd is haplotype diversity; VHd is the variance of haplotype diversity with standard deviation (SD), Pi is the nucleotide diversity

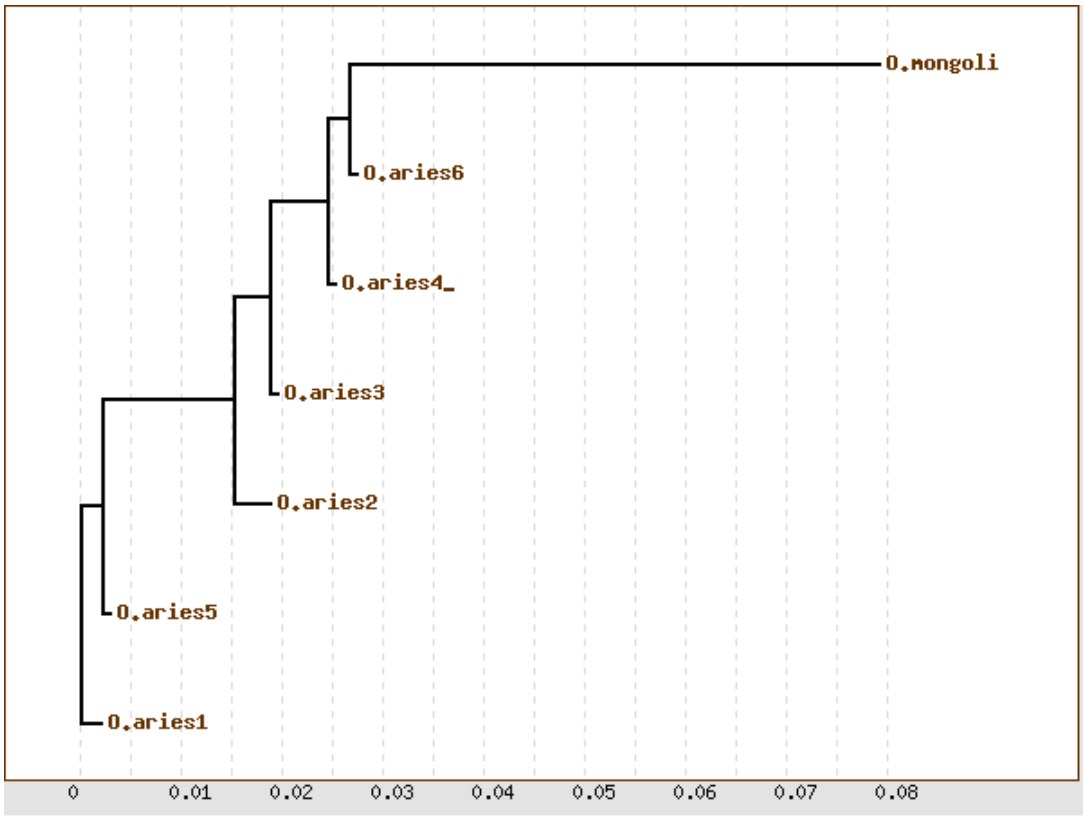

Fig. 1: Unrooted neighbour-joining phylogenetic tree of mtDNA haplotypes investigated domestic sheep (*O.mongolian* is an outgroup)

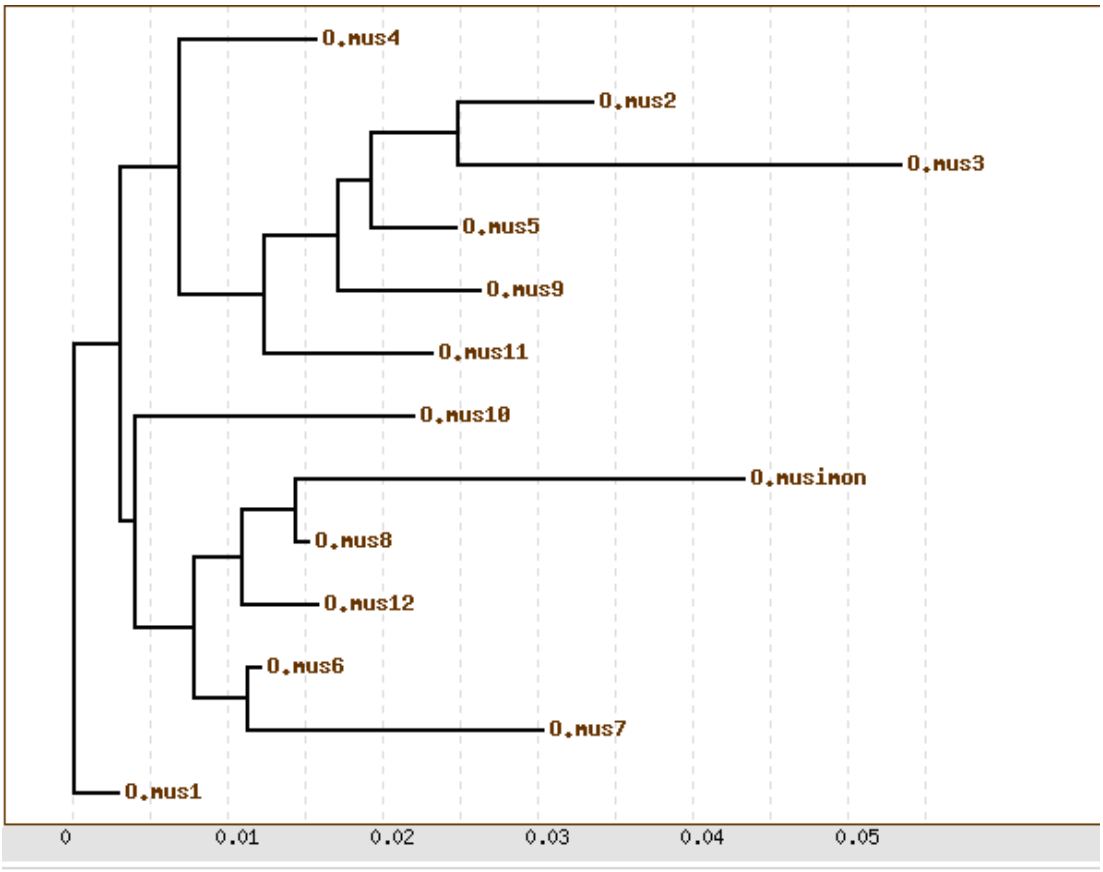

Fig. 2: Unrooted neighbour-joining phylogenetic tree of mtDNA haplotypes investigated mouflon (*O.musimon* is an outgroup)

From the different branches that contain mouflon mtDNAs, three distinct lineages are apparent. One, the *O.mus*1 lineage and the other contains *O.mus*7,6,8,10,12 and closely related outgroup *O.musimon* from Asia. The last branch contains sequences *O.mus* 2,3,4,5,9,11. (Fig. 2). Our study demonstrates threephiletic origin of BCP synthetic line of domestic sheep and the average value of genetic distance shows positive effect of breeding work. Because the mouflon population from the Sowie Mountains is introduced to this population of this region of Poland, it is necessary to continue the molecular investigation.

The most-phylogeny of genus *Ovis* was studied supported by morphological characters (GEIST, 1971; VADEZ, 1982) and mitochondrial DNA molecular methods (BUNCH et al, 2006; HIENDLEDER et al., 1998, 2002) demonstrated that transitions were included in the analysis of mitochondrial genes, a greater number of characters are provided, making possible a better estimate of Ovidae relationships, at the lower hierarchical levels. The cyt-b gene sequence is favourable for examination of the relationship between closely related species, subspecies or subpopulations.

Genetic distance between mtDNA haplotypes of domestic sheep and mouflon show only minor variation and suggested that investigated cytochrome 307 bp region is well conserved in the mouflon *O. musimon* from the Sowie Mountains and BCP synthetic line domestic sheep. This likely results in the small number of sequence differences that distinguish most haplotypes. The results of Meadows and all show high levels of gene flow between European wild and domestic breeds of sheep for example the

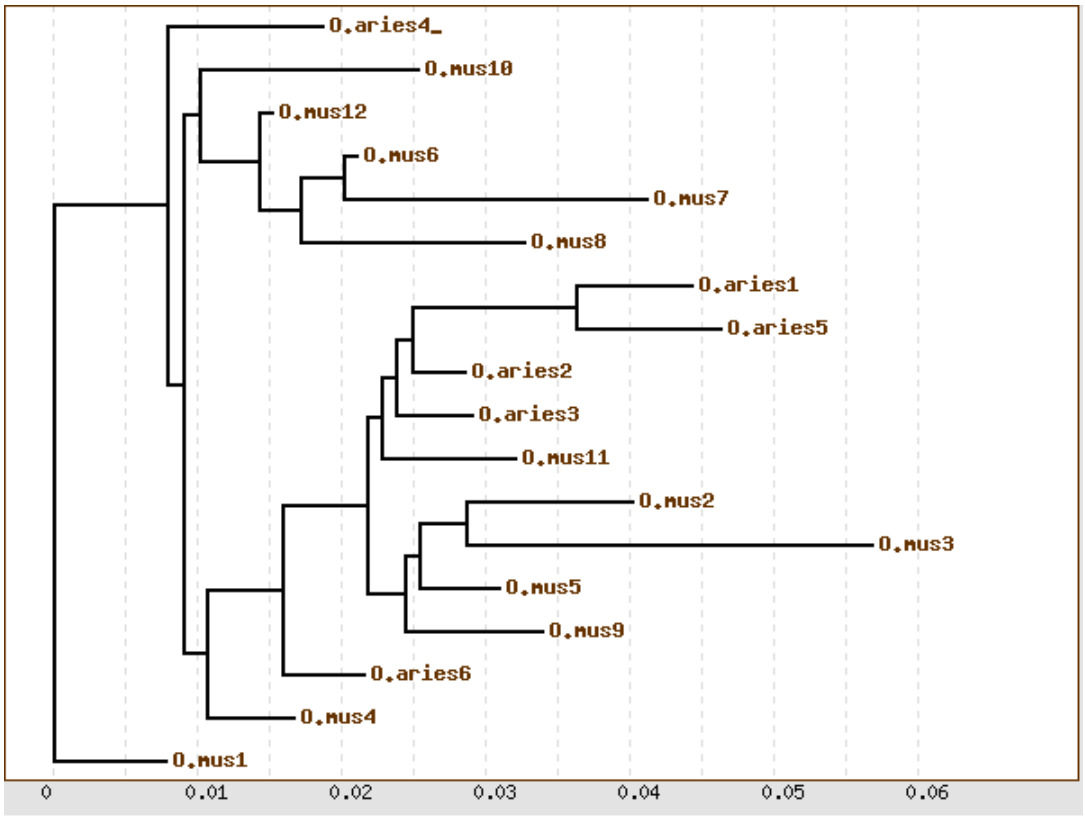

Fig. 3a: Phylogenetic tree of mtDNA haplotypes investigated wild and domestic sheep: unrooted neighbour-joining tree with mouflon (*O.musimon* 1-12) and BCP synthetic line domestic sheep (*O.aries* 1-6)

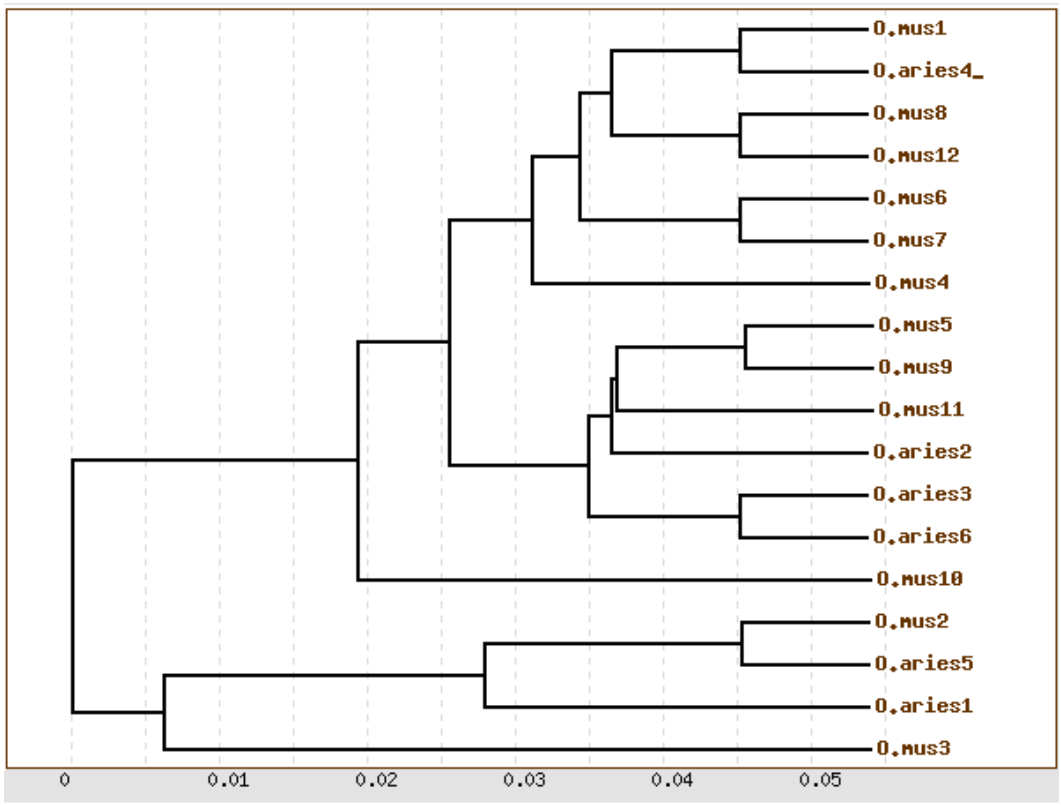

Fig. 3b: Phylogenetic tree of mtDNA haplotypes investigated wild and domestic sheep: unrooted tree derived from the cluster algorithm with Dayhoff matrix analysis of the same data

fragment of neighbour-joining tree shows four mouflons contained mtDNA sequences that clustered in one branch origin with the Tyrolean stone sheep, Oxford down sheep and Forest sheep (B haplotypes or clade B), (MEADOWS et al., 2005). Clade B haplotypes observed in a range of domestic sheep breeds from Europe and even New Zealand includes sequences derived from the European mouflon *(Ovis musimon),* (HIENDLEDER et al., 2002). In domestic animals bi- or multiphyletic origins are not uncommon. The results in this paper (Fig. 3) and the conclusions of Hiendleder strongly suggest that mouflon is a possible progenitor of one clade domestic sheep haplotypes. The position of mouflon haplotypes in our phylogenetic trees tends to support these hypothesis.

Table 2
Sequence divergence between *O. aries* and *O. musimon* mtDNA haplotype

Haplotype no.	Ovis ssp.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1	O. musimon 1																	
2	O. musimon 2	0.044																
3	O. musimon 3	0.044	0.035															
4	O. musimon 4	0.022	0.039	0.048														
5	O. musimon 5	0.030	0.022	0.030	0.017													
6	O. musimon 6	0.017	0.044	0.052	0.022	0.030												
7	O. musimon 7	0.026	0.052	0.061	0.022	0.030	0.009											
8	O. musimon 8	0.017	0.052	0.052	0.030	0.039	0.009	0.017										
9	O. musimon 9	0.030	0.030	0.030	0.026	0.009	0.030	0.030	0.030									
10	O. musimon 10	0.030	0.057	0.065	0.035	0.035	0.030	0.030	0.030	0.035								
11	O. musimon 11	0.022	0.030	0.048	0.026	0.017	0.030	0.030	0.030	0.017	0.035							
12	O. musimon 12	0.017	0.044	0.044	0.022	0.030	0.017	0.026	0.009	0.030	0.039	0.030						
13	O. aries 1	0.052	0.026	0.061	0.057	0.048	0.061	0.070	0.061	0.057	0.070	0.039	0.052					
14	O. aries 2	0.022	0.030	0.039	0.035	0.017	0.030	0.039	0.030	0.017	0.035	0.017	0.030	0.039				
15	O. aries 3	0.017	0.026	0.044	0.030	0.022	0.026	0.035	0.026	0.022	0.039	0.013	0.026	0.035	0.013			
16	O. aries 4	0.009	0.035	0.044	0.022	0.022	0.017	0.026	0.017	0.022	0.022	0.022	0.017	0.052	0.013	0.017		
17	O. aries 5	0.044	0.009	0.035	0.039	0.022	0.044	0.052	0.052	0.030	0.057	0.030	0.044	0.026	0.022	0.026	0.035	
18	O. aries 6	0.017	0.035	0.044	0.030	0.030	0.026	0.035	0.017	0.022	0.039	0.022	0.017	0.044	0.022	0.009	0.017	0.035

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Arch. Tierz., Dummerstorf **49** (2006) Special Issue, 317-324

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The level of some bioactive components in the fat fraction of sheep's milk and cheese

Abstract

The study was aimed at determining the level of bioactive components in the fat fraction of sheep's milk and five types of sheep's milk cheese (curd, soft, brine, scalded-smoked and semi-hard cheese). The applied sheep's milk processing technologies resulted in significant differences in the fat and cholesterol content of cheeses, with no statistically significant effect on the fatty acid profile of fat in particular types of cheese.

The retention of milk lipid components in cheese was found to vary. Cholesterol retention was generally low (11-19%), with non-significant differences between the types of cheese. Retention of fat and fatty acids was similar for particular types of cheese but clearly differed among them. By far the lowest retention of fat and fatty acids (31% on average) was found in scalded-smoked cheese (oscypek), followed by soft (bryndza) and semi-hard cheese (66 and 73%, respectively), and curd (bundz) and brine (feta-type) cheese (81 and 80%, respectively). The present study allows a preliminary conclusion that in terms of the retention of lipid components and the health-promoting quality of sheep's cheeses, the least favourable results were obtained when milk was processed into scalded-smoked cheese (oscypek), whereas the results for other types of cheese are considered satisfactory and uniform.

Key Words: sheep, milk and cheese, fat, cholesterol, fatty acid

Introduction

Food products used in human nutrition should meet the nutritional requirements while containing nutrients that benefit the body. Among the basic nutrients, it is worth noting the amount of fats and particularly the dietetic value of fats, which is determined mainly by the fatty acid profile. In addition to meat products, the main sources of animal fats are milk and milk products. Preliminary findings indicate that the fat content and fatty acid profile of ruminant milk depend on a number of genetic, physiological and environmental factors, especially nutrition (BORYS and PISULEWSKI, 2001; HAENLEIN, 1995; PATKOWSKA-SOKOLA et al., 2005; REQUENA et al., 1999; TRABALZA-MARINUCCI et al., 1993).

Sheep's milk processing into cheese and its dietetic quality have been the focus of attention of many research centres in countries with a tradition for milk utilization of sheep and goats (HAENLEIN, 1995). In Poland, especially in the lowland areas, relatively few studies on the milk utilization of sheep and milk processing into cheese have been conducted over the last ten years (GUT et al., 1999; PAKULSKI et al., 2002). No research has been done in Poland on the efficiency of processing sheep's milk into different types of cheese and comparison of their dietetic and health properties.

The present study aimed at determining the lipid (fat, fatty acid and cholesterol) profile of sheep's milk and 5 types of sheep's milk cheeses, as well as the retention of these milk components in cheeses depending on cheese-making technology.

Materials and Methods

Sheep's milk obtained from milk ewes of the prolific and dairy line of the Koluda sheep [37.5% East Friesian + 37.5% prolific breed (Finn or Romanov) + 25% local genera-purpose breed (Polish Merino, Lowland or Longwool Kamieniecka)] was used

as the initial material. We studied the lipid profile of bulk milk and 5 types of cheese made from that milk: curd cheese (“bundz” - Bu), soft cheese (“bryndza owcza” - Br), brine cheese (“feta” type - Ft), scalded-smoked cheese (“Koluda oscypek” - Os) and maturing semi-hard cheese (“Koluda cheese” - Pt).

All types of the cheese were made from heat-treated (pasteurized) milk and produced in accordance with the Experimental Station Koluda Wielka standards. During the production of soft, brine and maturing cheeses, pasteurized milk was acidified with appropriate bacteria cultures prior to rennet addition. Milk for the production of all types of cheese was curdled with rennet for 40-60 min at 32-36°C.

The initial stage of making curd, soft and brine cheeses was similar. The milk clot was cut into fine grains and the resulting cheese mass was used to form cheese blocks, which were then pressed, sprinkled with salt and cooled. After 24 h, the curd cheese was ready for eating. Curd cheese blocks intended for soft cheese were left at room temperature for 4-6 days and ground with a 2-2.5% addition of salt. The ground cheese mass was stored in closed containers for 10-14 days until the cheese was ripe. During the brine cheese production, cubes of 200-300 g were formed from curd cheese blocks, placed in airtight containers and immersed in salty, pasteurized whey to ripen for 4-6 weeks.

The scalded-smoked cheese was produced from a cheese clot broken into grains the size of beans, which were portioned and scalded several times with water at 70-75°C. Cheese mass was then manually squeezed to remove excess whey. After the cheeses were formed, they were cooled in cold water and wet salted for 24 h in brine. Finally, the cheeses were “cold” smoked with a smoke from fruit trees.

The maturing semi-hard cheese was produced from cheese mass formed into blocks and subjected to “self-pressing”. The formed blocks were salted for 24 h in a brine solution, after which the cheese matured for 4-5 weeks at 10-12°C.

The experiment was carried out from July to mid-October. During this period, sheep were kept indoors and fed with forages available from field crops and concentrate mixtures supplemented with hay or straw.

After weaning lambs at 2 months of age, ewes were milked mechanically twice a day. Each type of cheese was made in 4 experimental batches at 3- to 4-week intervals starting from the 3rd month of lactation.

Chemical analyses were performed using representative samples of pasteurized bulk milk and cheese. Cheeses were sampled for analysis when ready for consumption.

The fat content of milk and cheese was determined using the method of Soxhlet according to standard PN-73/A-82111.

Specialist analysis of the fatty acid’s profile (including CLA) and cholesterol was carried out in the fat extracted from the milk and cheeses. Fat extraction from the analysed products was performed in accordance with standard procedures provided by FOLCH et al. (1957). The composition of fatty acids was determined using the method of KRAMER et al. (1997) as modified by the Meat and Fat Research Institute in Warsaw (BORYS et al., 1999). The content of fatty acids was determined using gas chromatography (Hewlett Packard model 6890 with a flame-ionization detector and Rtx 2330 column, 105 m × 0.25 mm × 20 μm).

The cholesterol content was determined in accordance with procedures provided by THOMPSON and MEROLA (1993) using a Hewlett Packard gas chromatograph 6890 equipped with a flame-ionization detector and column HP-1 (25 m × 0.20 mm × 0.11

μm). The working condition of the apparatus: feeder - 310°C, column - 250°C (4 min.) - 5°/min. - 300°C (5 min.), detector - 310°C, carrier gas - helium (100 kPa), devisor - 25:1. Cholestane was used in the analysis as an inner standard, added before the extraction. Before the samples were analysed, the retention time of cholestane and cholesterol was established by means of chromatographic analysis of the model solution of those compounds after derivation.
The retention of the analysed milk components in cheese was calculated as a percentage ratio between their absolute content in cheese and in the milk used to produce the cheese.
The results were analysed statistically in a one-way design using the ANOVA procedure of the Statistica 6.0 PL packet (STATISTICA, 2002). Significant differences between the types of cheese were estimated using Duncan's multiple range test.

Results

The average fat and cholesterol content of bulk milk processed into different types of cheese and the fatty acid content of bulk milk fat did not differ significantly (Table 1). However, differences between mean numerical values for a number of components analysed were considerable. Batches of milk used for processing into different types of cheese differed by a maximum of 13.3% for fat content (milk used to produce Pt and Os cheeses) and by a maximum of 35.5% for cholesterol content (Ft and Br milk). In terms of the fatty acid content of fat, differences between particular batches of milk were quite large in some cases. Marked differences were found for the content of short-chain SFA (C10:0-C10:0) between Ft and Pt milk (11.8%) and for the content of linolenic acid C18:3, CLA and Ω3 PUFA, which were 41.8, 20.7 and 30.9% higher in Pt than in Os milk, respectively (Table 1). Milk processed into scalded-smoked cheese (Os) was characterized by a 23.9% higher (less beneficial) Ω6:Ω3 PUFA ratio in fat than Ft milk.
The sheep's milk processing technologies resulted in significant differences in the fat and cholesterol content of the cheeses obtained (Table 2). The highest fat content was characteristic of Pt cheese, followed by Bu, Br and Ft (21.4% lower on average), and by far the lowest fat content was found in Os cheese (57.8% lower than in Pt cheese and an average of 46.3% lower than in Bu, Br and Ft cheeses, which were similar in this respect). Bu and Ft cheeses contained significantly less cholesterol than Br, Os and Pt cheeses, and the differences of 27.5% on average were statistically significant at $P<0.05$.
There were no statistically significant differences in the content and proportions of fatty acids in the fat of sheep's milk cheeses compared (Table 2). However, as for milk, marked differences in the fatty acid profile were observed between the fats of cheeses compared. The composition of fatty acids in Bu, Br and Ft cheeses and their health quality parameters that are calculated on this basis, were generally similar and intermediate in relation to the extreme Os and Pt cheeses. Considerable differences (46% at the most) between these cheeses concerned the content of most fatty acids and fatty acid groups as well as health quality parameters (Table 2). The lack of significant differences could result from the relatively small number of observations (4 processing batches), with large variation in the analysed parameters of the lipid profile.

Table 1
Fat and cholesterol content of milk and the fatty acid content of milk fat in the milk processed into different types of cheese

Components	Milk processed into different types of cheese:					SEM
	Bu n = 4	Br n = 4	Ft n = 4	Os n = 4	Pt n = 4	
Fat; g/100 g	6.23	6.34	5.80	5.62	6.37	0.153
Cholesterol; mg/100 g	10.2	9.4	12.7	11.2	12.1	0.621
Fatty acids (g/100 g):						
SFA	68.55	68.15	70.07	69.85	68.72	0.381
- incl.: C 4:0	2.30	2.22	2.42	2.27	2.42	0.061
C 6:0	2.02	1.95	2.17	2.10	2.10	0.036
C 8:0	2.15	2.02	2.27	2.22	2.00	0.044
C 10:0	7.57	7.02	7.80	8.02	6.60	0.234
Σ C 4:0 - C 10:0	14.05	13.22	14.67	14.62	13.12	0.259
C 12:0	4.92	4.47	4.82	5.25	3.92	0.231
C 14:0	12.05	11.65	11.82	12.30	11.05	0.282
C 15:0	1.85	1.92	1.75	1.82	1.87	0.037
C 16:0	27.20	27.87	28.62	28.07	29.50	0.414
C 17:0	1.70	1.75	1.65	1.62	1.75	0.051
C 18:0	6.37	6.85	6.32	5.75	7.07	0.301
UFA	31.17	31.56	29.61	29.80	31.04	0.400
MUFA	26.47	26.60	24.82	25.20	26.07	0.380
- incl.: C 14:1	0.60	0.57	0.52	0.57	0.52	0.017
C 16:1	1.72	1.65	1.67	1.85	1.62	0.071
C 18:1T	3.57	3.50	2.90	3.70	2.82	0.236
C 18:1	19.42	19.80	18.65	17.95	19.95	0.453
PUFA	4.69	4.96	4.79	4.60	4.99	0.101
- incl.: C 18:2	2.52	2.65	2.50	2.60	2.50	0.053
C 18:3	0.70	0.80	0.82	0.67	0.95	0.068
CLA	0.94	0.93	0.89	0.82	0.99	0.042
Ω PUFA6	2.75	2.87	2.72	2.80	2.72	0.057
Ω3 PUFA	1.00	1.15	1.17	0.97	1.27	0.071
UFA:SFA	0.456	0.464	0.423	0.427	0.452	0.008
PUFA:SFA	0.069	0.073	0.068	0.066	0.073	0.002
DFA:OFA	0.609	0.631	0.564	0.555	0.619	0.017
Ω6:Ω3 PUFA	3.082	2.857	2.443	3.210	2.195	0.242

Bu – curd cheese, Br – soft cheese, Ft – brine cheese, Os – scalded-smoked cheese, Pt – maturing cheese; SEM – standard error of the arithmetic mean
SFA - Σ: C4:0, C6:0, C8:0, C10:0, C12:0, C14:0, C15:0, C16:0, C17:0, C18:0, C20:0 and C22:0
MUFA - Σ: C10:1, C12:1, C14:1, C15:1, C16:1, C17:1, C18:1 and C20:1
PUFA - Σ: C18:2, C18:3, C20:4, C20:5, C22:5 and C22:6; UFA = MUFA + PUFA,
Ω6 PUFA - Σ: C18:2 and C20:4; PUFA Ω3 - Σ: C18:3, C20:5, C22:5 and C22:6;
DFA = MUFA + C18:0; OFA = SFA - C18:0

There was also a tendency towards poorer PUFA:SFA ratio in Os cheese compared to other cheeses (11.0% lower on average) and differences in the Ω6:Ω3 PUFA ratio between different types of cheese, which were favourably the lowest for Pt, followed by Ft (30.8% higher), and similar for the other cheeses (Bu, Br and Os – 22.8% greater than for Ft and 60.5% greater than for Pt on average).

The retention of milk cholesterol in cheese was relatively low (11-19%) and varied considerably according to the type of cheese (Table 3). Higher retention for this undesirable component was found for Bu and Br than for Ft, Os and Pt cheeses, but the differences averaging 5.8 percentage units were not significant.

The level of fat and fatty acid retention in particular types of cheese was highly similar but markedly different depending on the type of cheese (Table 3) By far the lowest retention of fat and all fatty acids analysed was characteristic of the scalded-smoked cheese (Os), and all the differences (from 35.3 percentage units in relation to Br cheese

to 50.5 percentage units in relation to Bu cheese on average) were highly significant. The highest retention of fat and fatty acids was obtained for curd cheese (Bu) and brine cheese (Ft) (80.5%), followed by semi-hard and soft cheese (7.7 and 14.5 percentage units lower, respectively; NS).

Table 2
Fat and cholesterol content of cheese and the fatty acid content of cheese fat

Components	Type of cheese					SEM
	Bu n = 4	Br n = 4	Ft n = 4	Os n = 4	Pt n = 4	
Fat; g/100 g	18.77Da	20.14Bc	19.71Cb	10.49ABCD	24.86Aabc	1.214
Cholesterol; mg/100 g	56.5acd	80.6ab	57.5bef	77.8cf	76.9dr	3.392
Fatty acids (g/100 g)						
SFA	69.22	68.50	70.17	70.57	68.47	0.388
- incl.: C 4:0	2.15	2.35	2.20	2.30	2.35	0.070
C 6:0	2.10	2.05	2.07	2.17	2.17	0.050
C 8:0	2.17	2.10	2.25	2.35	2.15	0.041
C 10:0	7.60	7.27	8.00	8.55	6.87	0.223
Σ C4:0-C10:0	14.02	13.77	14.52	15.35	13.55	0.240
C 12:0	4.85	4.65	5.17	5.67	3.97	0.252
C 14:0	12.12	11.77	11.97	12.65	10.90	0.308
C 15:0	1.80	1.90	1.92	1.85	1.82	0.036
C 16:0	28.27	27.77	28.92	28.07	29.10	0.353
C 17:0	1.62	1.65	1.62	1.55	1.77	0.046
C 18:0	6.12	6.57	5.62	5.05	6.95	0.310
UFA	30.57	31.07	29.42	28.99	31.12	0.391
MUFA	25.65	26.02	24.42	24.37	26.02	0.361
- incl.: C 14:1	0.60	0.57	0.57	0.60	0.50	0.021
C 16:1	1.65	1.67	1.72	1.92	1.60	0.079
C 18:1T	3.37	3.40	3.20	3.60	2.67	0.239
C 18:1	18.90	19.27	17.72	17.17	20.17	0.500
PUFA	4.92	5.05	5.00	4.62	5.10	0.099
- incl.: C 18:2	2.55	2.70	2.72	2.62	2.57	0.057
C 18:3	0.80	0.72	0.77	0.60	0.87	0.074
CLA	1.07	1.05	0.92	0.92	1.02	0.046
Ω6 PUFA	2.77	2.92	2.95	2.82	2.80	0.060
Ω3 PUFA	1.07	1.07	1.12	0.87	1.27	0.082
UFA:SFA	0.442	0.455	0.420	0.411	0.455	0.008
PUFA:SFA	0.071	0.074	0.071	0.065	0.075	0.002
DFA:OFA	3.091	3.072	3.188	3.179	3.130	0.048
Ω6:Ω3 PUFA	3.471	3.703	2.920	3.582	2.233	0.419

For explanations see Table 1.

It is worth noting that among single fatty acids, the highest retention of all types of cheese was found for CLA, while in the analysed groups of acids in all types of cheese except Pt, the lowest retention was characteristic of Ω3 PUFA acids (Table 3).

Discussion

The fat content of milk, which was used to make the analysed cheeses, fell within reference ranges for sheep's milk (KUNACHOWICZ et al., 2005; PRANDINI et al., 2001) and was not different from the values found in other studies (BORYS, 2004) conducted in comparable conditions (breed, stage of lactation in sheep, type of feed).
The fatty acid profile of sheep's milk, found in the present study, differed to a certain degree from the results reported by other authors (BORYS, 2004; MARQUES and BELO, 2001), possibly due to differences in feeding, breeds of sheep and stage of

lactation. Generally, however, the content of SFA, MUFA and PUFA (including Ω6 and Ω3 PUFA) and their mutual proportions fell within ranges reported by these authors for sheep's milk. The CLA content of milk was similar as in other Polish breeds of sheep: Polish Mountain and Longwool (CIURYK et al., 2001; PATKOWSKA-SOKOLA et al., 2005), higher than that obtained by BORYS (2004) during winter-summer feeding (0.72 g/100 g fat) and higher than the reference values for sheep's raw milk (0.64 g/100 g fat) (PRANDINI et al., 2001).

Table 3
Retention of fat and cholesterol in the cheeses from milk and retention of major fatty acids in the cheese fat from milk fat (%)

Components	Type of cheese					SEM
	Bu n = 4	Br n = 4	Ft n = 4	Os n = 4	Pt n = 4	
Fat	81.1A	66.0D	80.5B	30.6ABCD	73.1C	5.044
Cholesterol	16.3	18.8	11.4	11.5	12.5	1.371
Fatty acids:						
C 10:0	81.7B	68.7D	83.7A	33.2ABCD	76.4C	5.152
C 14:0	81.6A	66.5D	80.5B	31.7ABCD	72.1C	4.964
C 16:0	83.2A	65.6D	82.9B	30.8ABCD	72.2C	5.219
C 18:0	78.3A	63.3D	73.4B	27.2ABCD	71.7C	4.902
C 18:1T	76.7A	64.6D	72.9B	29.8ABCD	69.0C	4.674
C 18:1	79.3A	65.0D	77.8B	29.5ABCD	74.0C	4.953
C 18:2	82.0A	66.8D	81.9B	31.1ABCD	75.3C	5.118
C 18:3	82.1A	58.5D	80.3B	28.1ABCD	68.4C	5.413
CLA	86.0A	75.2D	84.7B	35.3ABCD	75.9C	5.495
Σ C4:0-C10:0	81.2B	69.2D	82.5A	32.7ABCD	76.0C	5.157
SFA	82.1A	66.3D	81.1B	31.2ABCD	72.9C	5.079
UFA	79.4A	65.3D	78.7B	30.1ABCD	73.2C	4.928
MUFA	78.5B	64.9D	78.9A	29.9ABCD	72.9C	4.910
PUFA	83.0A	67.3D	78.1B	30.9ABCD	74.7C	5.021
Ω6 PUFA	81.8B	66.8D	83.0A	31.1ABCD	76.3C	5.183
Ω3 PUFA	75.9A	60.3D	68.0C	27.0ABCD	73.4B	5.153
DFA	79.2A	64.9D	77.3B	29.6ABCD	72.9C	4.892

For explanations see Table 1.

Worth noting is the relatively low level of cholesterol in the analysed milk, which was twice as low as that found by BORYS (2004) in the milk of crossbred sheep of similar breeds under comparable rearing and feeding conditions. Compared to the reference values for sheep's curd cheese and different semi-hard cheeses of pecorino type, the SFA content of cheese fat, found in the present study, was slightly lower, the MUFA content was similar and the PUFA content was higher (PRANDINI et al., 2001). In other Italian studies (TRABALZA-MARINUCCI et al., 1993), the fat of pecorino-type cheese had a similar proportion of MUFA (23.7-25.5%) and a much lower proportion of PUFA (2.7%). In maturing sheep's cheeses, REQUENA et al. (1999) obtained a lower content of SFA (56-62%) and an accordingly higher proportion of UFA than in the Pt cheese obtained in our study.

Comparable studies on the level of CLA in sheep's curd cheese were performed by BORYS (2004), who obtained a lower level of this desirable health-promoting component (0.62 g/100 g fat). The reference values given for the CLA content of sheep's cheeses (PRANDINI et al., 2001) were lower than those obtained in our study for sheep's curd cheeses and higher than those obtained for semi-hard cheeses of the pecorino type (0.75 and 1.50 g/100 g fat, respectively).

The cholesterol content of curd cheese (Bu) was favourably lower than in the same

cheese in the study of BORYS (2004) (74 mg/100 g). It should be noted that the level of cholesterol found in both sheep's milk and sheep's milk cheeses, was generally lower than tabular values given for cow's milk and cow's milk cheeses (KUNACHOWICZ et al., 2005) – 14 mg for milk and 71-99 mg/100 g cheese for different types of cheese.

The lowest and generally very low indicators of fat and fatty acid retention in the scalded-smoked cheese (Os) are attributed to the effect of production technology. Repeated scalding of cheese mass with hot water (70-72°C) caused much greater losses in fat components compared to the other types of cheese. The lack of comparable studies in the available literature prevents the discussion of results obtained for the retention of fat and fatty acids in the types of cheese studied. An additional difficulty is that sheep's cheeses are usually produced using craft methods based on a variety of traditional recipes and technologies.

Overall, the sheep's milk used to produce the 5 types of cheese was characterized by uniform content of the analysed lipid components (fat, fatty acids and cholesterol) that was favourable in terms of health quality.The applied milk processing technologies resulted in significant differences in the fat and cholesterol content of sheep's cheeses, with no statistical effect on the fatty acid profile of fat in particular types of cheese.

The level of retention of the analysed milk lipid components in cheese was differentiated by the milk processing technology used. Cholesterol retention was generally low (11-19%), and differences between the types of cheese were not significant. Retention of fat and fatty acids was similar within particular types of cheese and markedly different across different types of cheese. By far the lowest retention of these components (31% on average) was found for scalded-smoked cheese (oscypek), followed by soft (bryndza) and semi-hard cheese (66 and 73%, respectively), and the highest for curd (bundz) and brine cheese (feta) (81 and 80%, respectively).

The results of the present study allow a preliminary conclusion that from the viewpoint of the retention of lipid components and health-promoting quality, the least favorauble results were obtained when sheep's milk was processed into scalded-smoked cheese. In the case of the other cheeses, the results in this respect are considered satisfactory and generally uniform. Further study using broader material is required to draw more far-reaching conclusions.

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Different level of crude protein and energy – protein ratio in adult quail diets

Abstract

The experiment allowed us to evaluate the influence of low-protein diet feeding on the performance, health, and egg quality of Pharaoh Japanese quails. The control-group quails were fed on layers complete mash diet containing 21% crude protein, with an energy-protein ratio 0.56:1. The treatment groups II and III were fed on the diet containing 19% crude protein and having different energy-protein ratios, respectively 0.61:1 and 0.56:1. Laying performance achieved in group III did not differ significantly from that of the control quails, whereas that of group II was significantly lower compared to the other groups. The diet with reduced crude protein and increased energy-protein ratio (group II) resulted in statistically highly significant decrease in body weight as compared with the control and group III. Group II quails consumed significantly ($P<0.01$) the most feed to produce 1 egg as well as 1 kg of eggs. Low-protein diets did not affect most of the quality parameters of eggs. No influence of the diets could be observed in relation to dry matter content or crude protein level in either yolk or white of egg.

Key Words: low-protein diets, quails, performance indices, egg quality

Introduction

The experiment is a part of a complex research project focused on application of low-protein diets in quail feeding. Changes in the life style of people in many countries as well as their growing and diversifying purchase power cause that the consumers start to demand less common poultry products of ostriches, emus, or quails. However, on the other hand, many consumers expect the producers to offer lower prices for food products, which can be achieved by reducing the cost of production. One of the ways leading to reduced cost of feeds is to reduce their protein content. The breeds and varieties of quails farmed presently are characterized by quite a wide range of dietary requirements. The poultry is also able to adapt to lowered supply of nitrogen in the diet through their better management of its compounds. Another significant, ecological aspect of low-protein diet feeding is that the reduced amount of nitrogen is discharged into the environment with manure (SZCZUREK and PISULEWSKI, 1996). JAMROZ et al. (1984) as well as LECLERCQ and TESSERAND (1993) have not observed any negative influence of moderately reduced level of crude protein in the diet, on the performance or slaughter value of chicken broilers. Similar results for quails were reported by DASZKIEWICZ et al. (1998). ZELENKA et al. (1984), who applied feeding with varied crude protein level, did not demonstrate significant differences in the breast muscle content or carcass fat content in quails. Conversely, limited feeding, as opposed to *ad libitum* feeding, resulted in differences in the composition of body tissues and in slightly delayed laying.

The aim of this study was to investigate the influence of diets with crude protein level reduced to 19% and with two different energy-protein ratios on the laying performance, feed intake, and egg quality of Pharaoh quails.

Material and Methods

The experiment was carried out on 120 adult Pharaoh quails (during the period between 6 and 25th weeks of age), of which three feeding groups were formed with five subgroups, eight birds each. The experiment was performed in the facilities of the Department of Poultry and Ornamental Bird Breeding, the Agricultural University of Szczecin, Poland. The quails were self reared and were raised under optimal microclimate conditions. The diet consisted of feed mashes of the nutritional value corresponding to the recommendations of NUTRIENT REQUIREMENTS OF POULTRY (1996). In the 6th week of age, the birds were weighed individually and were distributed to the three groups so as each group comprised birds of similar body weight. The quails were kept in cages throughout the experiment in the room at 20-22°C and under light regime subject to natural changes, but with light available for not less than 17 hours a day.

The diet of group I (control) was a mash containing 21% crude protein and 11.7 MJ of metabolic energy (according to NUTRIENT REQUIREMENTS OF POULTRY, 1996), whereas in groups II and III, respectively, 19% crude protein and 11.7 MJ of metabolic energy and 17% crude protein and 10.6 MJ of metabolic energy. Experimental mashes (applied in groups II and III) differed also in the energy-protein ratio. In group II it was 1:0.61, while in group III 1:0.56, thus it was just like that in the control (I). The chemical composition of the diets has been presented in Table 1.

Table 1
Chemical composition of quail feed mixes (%)

Item	Groups		
	I	II	III
Matabolizable energy MJ/kg	11.70	11.70	10.63
Protein crude	21.02	19.05	19.01
Energy to protein ratio	1:0.56	1:0.61	1:0.56
Fibre crude	3.73	3.59	4.02
Calcium	2.51	2.49	2.50
Available phosphorus	0.55	0.55	0.55
Na	0.16	0.16	0.16
Lysine	1.21	1.21	1.20
Methionine + cystine	0.79	0.79	0.79

All the birds were fed *ad libitum*, and the feed refusals were weighed once a week. The quails also had unlimited provision of water.

The experiment tooks 19th weeks, during which laying performance, egg weight, deaths rate, and feed consumption were recorded. In the final stage of the experiment, the layers were individually weighed to record their final body weights, and egg quality evaluation was carried out. For this purpose, 25 eggs from each group were collected. The evaluated quality traits of eggs involved: specific gravity, which was determined in sodium chloride solutions of density ranging from 1.058 g/cm^3 to 1.082 g/cm ^{3} (increasing gradually by 0.004 unit), egg shape index, and index of white and yolk, as well as pH of white and yolk using a Sentron 3001 pH-meter. Dry matter content and crude protein were assayed in both yolk and white through proximate

analysis.
From each group, 11 quails were slaughtered and examined post-mortem in order to determine the influence of feeding the diet poorer in protein on the development of selected internal organs. Liver, pancreas, caecum, and small intestine were dissected and their proportion in the body weight was calculated. The data were analysed statistically (using the Statistica package). Significance of differences between groups was tested using one-way ANOVA with the Duncan test (STATISTICA Software).

Results

In the 25th week of age, the highest body weights were attained by the females of group III (210.0 g), and these did not differ significantly from the control group quails (208.4 g). Significantly lower body weights were recorded in group II, as compared with the other groups (187.9 g), in which the birds received the mixture containing 19% protein and with extended energy-protein ratio (Table 2).
The quails of group II consumed daily the most feed, 29.1 g. Group II quails used the most feed to produce an egg (42.8g), highly significantly higher than in the remaining groups. Similar was the feed consumption per 1 kg of eggs. In the group of quails that received the mixture containing 19% protein and with extended energy-protein ratio it was higher by about 16% and 12% in relation to, respectively, group I and III (Table 2).

Table 2
Body weight, feed conversion

Item		Groups		
		I	II	III
Body weight of female in 6 week	g	206.6 ± 13.7	207.2 ± 14.2	206.5 ± 16.0
Body weight of female in 25 week	g	208.4 A ± 21.0	187.9 B ± 16.0	210.0 A ± 19.6
Feed intake	g/quail/day	28.0 ± 1.12	29.1 ± 1.67	28.3 ± 1.30
Feed conversion	g/egg	36.9 A ± 4.28	42.8 B ± 9.14	38.2 A ± 6.05
	kg/kg eggs	3.19 A ± 0.43	3.72 B ± 0.80	3.32 A ± 0.58

a,b - differences significant at P≤0.05; A,B - differences significant at P≤0.01 (also for Tables 3-6).

Table 3
Laying usefulness indicators

Item	Groups		
	I	II	III
Egg production to 25 week of life: number/quail	98.1 A ± 10.0	87.9 B ± 12.7	96.6 A ± 12.7
%	80.1 A ± 8.2	71.7 B ± 10.3	80.4 A ± 8.4
Average number of eggs in week (number/quail)	5.60 A ± 0.57	5.02 B ± 0.73	5.63 A ± 0.59
Average egg weight in whole research period (g)	11.2 ± 0.16	11.2 ± 0.14	11.3 ± 0.19
Egg shape index	1.30 ± 0.8	1.29 ± 0.09	1.26 ± 0.11

The highest mean egg production per layer during the studied period was obtained from the birds of the control group, 98.1 eggs, only by some 2 eggs more than in group III, where the diet contained reduced protein level and with unchanged energy-protein

ratio. These two groups exhibited increased laying rates, statistically highly significantly ($p < 0.01$) higher compared to group II. Similar values were recorded for the mean weekly number of eggs per layer (Table 3).
All three groups produced eggs of similar weight over the entire period of the experiment; an average egg weight was 11.2 g, with no significant differences.
Yolk proportion was the highest in group III, 31.2%, whilst the lowest in group I, 29.3%, with significant differences only between groups I and III, both in the proportion of white and yolk in egg mass (Table 4).

Table 4
Morphological composition of quail eggs

Item		Groups		
		I	II	III
Egg weight	g	11.2 ± 0.83	11.4 ± 1.18	11.4 ± 0.57
White weight	g	6.86 ± 0.51	6.82 ± 0.62	6.74 ± 0.44
	%	61.2 A ± 1.97	59.8 A,B ± 1.49	59.1 B ± 1.36
Yolk weight	g	3.29 ± 0.38	3.54 ± 0.52	3.55 ± 0.22
	%	29.3 a ± 2.02	30.9 b ± 1.87	31.2 b ± 1.43
White to yolk ratio		2.08 : 1	1.92 : 1	1.89 : 1

Table 5
Qualitative traits of quail eggs

Item		Groups		
		I	II	III
Specific gravity	g/cm^3	1.068 ± 0.004	1.066 ± 0.007	1.069 ± 0.005
White height	mm	5.26 ± 0.89	5.74 ± 0.51	5.14 ± 0.65
White shape index		0.130 ± 0.008	0.129 ± 0.009	0.126 ± 0.011
Yolk shape index		0.45 ± 0.084	0.41 ± 0.044	0.41 ± 0.047
White				
pH		9.17 A ± 0.08	9.14 A ± 0.12	8.80 B ± 0.10
Dry matter	%	12.7 ± 0.60	12.6 ± 0.57	12.7 ± 0.74
White content	%	10.4 ± 0.61	10.2 ± 0.42	10.3 ± 0.53
Yolk				
pH		6.48 A ± 0.16	6.60 A ± 0.23	6.08 B ± 0.11
Dry matter	%	53.4 ± 1.11	53.1 ± 0.71	53.2 ± 0.93
White content	%	15.6 ± 0.49	15.9 ± 0.41	16.0 ± 0.35

Eggs obtained from the layers fed on diets differing in protein did not differ significantly in relation to a majority of the studied traits. The level of white, which is an index of its density, was the highest in group II eggs (5.74 mm), while the white index was similar in all groups. Yolk index, on the other hand, was the same in eggs of both treatment groups (0.41), and slightly higher in the control group (0.45), the differences, however, being non-significant (Table 5). However, statistically

significant differences in pH of an egg yolk and white were stated. The reduced level of the proteins in mixture maintaining however the energetic and protein relation as in the control mixture, caused the depreciation of pH of an egg yolk and white. Reduced protein level in the diet with unchanged energy-protein level, as in the control, resulted in highly significant reduction of white and yolk pH. On the other hand, it did not affect the level of dry matter and crude protein in the yolk and white of egg.

Table 6
Participation of some internal organs in relation to body weight

Item		Groups		
		I	II	III
Body weight	g	202.9 A ± 11.80	182.0 B ± 7.72	205.5 A ± 15.93
Liver	%	3.01 ± 0.40	3.40 ± 0.59	3.08 ± 0.34
Pancreas	%	1.25 a ± 0.20	1.52 b ± 0.24	1.30 a ± 0.16
Small intestine	%	1.34 A ± 0.23	1.77 B ± 0.28	1.66 B ± 0.16
Caecum	%	0.71a ± 0.11	0.85 a,b ± 0.28	1.02 b ± 0.24

No significant differences were found in the liver percentage between the groups, which ranged between 3.01% (group I), and 3.40% (group II). The percentage of pancreas in the body weight was the highest in group II birds (1.52%). In relation to the remaining groups, these differences were statistically significant, $p < 0.05$. Higher proportion of small intestine was found in the treatment groups II and III, respectively, 1.77% and 1.66%. The values attained in these groups did not differ significantly from each other, whereas differed highly significantly from the control, in which the proportion of small intestine was lowest (1.34%). Caeca proportion in the body weight was also higher in the treatment groups, with significant ($p < 0.05$) differences recorded between groups III and I (Table 6.

Discussion

The performance recorded in our experiment implies that the experimental diet with extended energy-protein ratio had a negative influence on the body weight of birds and that there are no contraindications as to feeding a mixture with reduced protein level, however, with unchanged energy-protein ratio as in a standard mash.

In our previous experiment it was found that the protein level in the diet had no influence on the body weight of the quails and their mortality. Reduced level of protein to 17% resulted, however, in a significant drop in laying performance, lower egg weights, and poor egg quality. Feed consumption per 1 kg of eggs was also the lowest if the diet containing 17% crude protein was applied (TARASEWICZ et al., 2006). Other authors, who applied protein restrictive feeding, found that the energy-protein ratio and amino-acids level had a stronger influence on performance than the protein level (ALAO and BALNAVE, 1985; COON et al., 1981).

The results of our studies correspond to those by ŚWIERCZEWSKA et al. (2000) on chicken broilers. In the quail experiment carried out by ZELENKA et al. (1984), who applied feed mixtures with varied protein level during raising, no significant influence of the diets on the breast muscles content was found. GEBHARDT-HENRICH and MARKS (1995) observed lower body weight in quail fed on restrictive feeding; in two

weeks after completion of the limited feeding, however, the body weight returned to normal.

Our experiment has not revealed increased mortality of layers, and the recorded deaths were still due to mechanical injuries (wedging a wing in the cage mesh). No influence on the mortality of quails has also been presented by SEHU et al. (2005).

De FREITAS et al. (2005) prove that quails regulate feed intake depending on the level of energy in the ration. These authors observed better feed use in the diet containing less energy. Conversely, SEHU et al. (2005) recorded significantly higher consumption and use of feed in groups of lower energy value.

According to DE FREITAS et al. (2005), high laying rate can be achieved on diets containing 18% crude protein and 2585 kcal metabolic energy per kg. On the other hand, SEHU et al. (2005) confirm the lack of significant influence of diets containing varied level of crude protein and energy on this performance parameter and egg quality. They noted, however, a significant influence of feed mixture on the egg weight. In the groups where quails were fed more energetic feeds, significantly higher egg weights were achieved. This did not find confirmation in the studies of DE FREITAS et al. (2005). These authors found a trend of the egg weight to increase under growing dietary protein level and a trend to diminish the weight of egg when the energy content increased. ABOUL-ELA et al. (1992) have found an increase in the egg weight with an increasing level of protein in the diet, with a significant increase up to 18% in the light strains and to 21% in the heavy strains.

In the analysed experiment, the quail layers were treated as both production fowl and as an experimental model of hen layers.

It is possible to feed adult quails on mixtures with protein and energy levels reduced in relation to the standard feed mixtures of the nutritional value recommended by NUTRIENT REQUIREMENTS OF POULTRY (1996) – without a negative influence on most performance parameters (feed intake, laying rate, egg weight, egg quality). It has been demonstrated that not the protein level, but rather the energy-to-protein ratio is the chief factor of quail performance.

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Comparison of the reproduction traits and body weight at birth of the Polish White Improved goat to its crossbreds with boer goat bucks

Abstract

The research was carried out on 211 dams and 379 kids of the Polish White Improved goats and their crossbreds with the boer goat. The animals were organized in 5 experimental groups, totally. Reproduction indicators and body weight at birth were examined due to genotype, litter size and sex of animals. The upgrading of the Polish Improved White goat with the boer bucks has no influence on decreasing of body weight at birth, litter size or rearing indicators as well as the survivability of kids, in exception of the higher litter size (e.g. quadruplets).

Key Words: goats, birth type, sex, reproduction, body weight at birth

Introduction

The recent increase of the goat breeding has been affected by the increasing interest in goat milk production (BOSEK et al., 2003; NIŻNIKOWSKI et al., 1996). Furthermore, the studies on reproduction traits (BŁASZCZYK et al. 2002) and fleshiness of goats appear more and more often in scientific journals (BIDWELL-PORĘBSKA et al. 1995; GRUSZECKI et al., 1997; KALINOWSKA et al., 1997ab). Few studies concerning meat goats, the boer goat mainly, focus on meat performance (BIDWELL-PORĘBSKA, et al. 1995; STANISZ and GUT, 2003). Also only a few reproduction studies on goats are available (NIŻNIKOWSKI et al., 2003). The increasing interest in the boer goats might be caused by the recent development of agroturistic activities as well as some possibilities of usage of this breed in landscape management and in the agro-environmental programmes. Due to these aims the breeding work focuses on creating the meat type of the Polish White Improved goat by upgrading it with the boer goat. The pursuits of increasing the boer goat genes in genotype arouse the interest in the examination of fleshiness traits. Therefore, this study presents the results of scientific work taken on chosen reproduction parameters and body weight at birth in the Polish White Improved goats upgraded with the boer breed.

Material and Methods

The experiment was carried out in 1992-2005 at the Sheep and Goats' Experimental Farm in Żelazna (Warsaw Agricultural University property). The upgrading of the Polish White Improved goat with the boer bucks took place each year of the experiment to increase the contribution of genotype of this breed. Dams used in crossing were not older than 10 years. Due to the experimental plan the 6-months-old goats of 0, 50, 75 and 87.5% of boer goat genotype were crossed first time with pure boer bucks and therfore we obtained offspring of 50, 75, 87.5 and 93.75% of boer breed. The mating season was carried out in autumn months (October, November, December) in every experimental year using a grouping service. Also data concerned fertility and sterility of dams, annual litter size (prolificacy calculated on singles, twins and quadruplets) and survivability of kids till 7^{th} day of age as well as rearing

indicators and body weight at birth in both male and female kids were collected. The offspring was born after mating of dams with 5 boer bucks.

Data concerning reproduction traits were calculated due to the linear model given by PETERSSON and DANELL (1985), adjusted to the least squares method (HARVEY, 1987). The results were transformed further into least square means (LSM) and standard errors of means (SE) and compiled in tables. In case of dams' fertility and litter size, the model concerned the following variation sources: dam's genotype, year and order of kidding. Due to survivability and rearing indicators as well as body weight at birth of kids, the factors of kid's genotype, year and order of kidding, birth type, sex and two bifactorial interactions (genotype*sex, birth type*sex) were considered. Accordingly to genotype, birth type and sex, the analysis of differences between groups in factors were calculated with Duncan test (RUSZCZYC, 1981).

Results and Discussion

The effect of the examined factors on chosen reproduction traits and body weight of kids at birth are shown in Table 1. The fertility indicator was highly affected (P≤0.01) by genotype, whereas the prolificacy indicator – by the significant influence of mother's age (P≤0.05). The survivability of kids was affected only by the birth type, whereas rearing indicator was affected by both genotype (P≤0.05) and year of kids' birth (P≤0.01). The other factors and interactions had in significant influence on the examined traits. The body weight at birth was influenced by almost every source of variability (both, on highly-significant and significant levels) despite the interaction of genotype x sex.

The obtained standard errors of means (SE) of the examined traits were highly varied probably due to the effects of environmental influence during 13 years of the experiment. Due to this fact the results were assumed within the examined factors and interactions as well as the last square means were used to present results. It allowed the interpretation of the results purified from sources of variability considered in the model of the experiment.

Table 1
The effects of chosen factors and interactions on reproduction traits and body weight at birth in goats

Traits	Effect of:					Interactions:		N	$\bar{x}$	SD
	genotype	year	no of kidding (dam's age)	litter size	sex	Genotype x sex	litter size x sex			
Fertility (heads)	XX	NS	NS					211	0.99	0.07
Prolificacy (heads)	NS	NS	X					210	1.81	0.72
Kids' survivability till 7th day of age (heads)	NS	NS	NS	XX	NS	NS	NS	379	0.95	0.23
Rearing indicator (heads)	X	XX	NS	NS	NS	NS	NS	379	0.84	0.36
Body weight of kids at birth (kg)	XX	XX	XX	XX	X	NS	X	349	3.47	0.84

X - P ≤ 0.05; XX P ≤ 0.01; NS – non-significant

The effect of genotype was presented in Table 2. Fertility indicators in the group of goats with 87.5% of boer genotype content were statistically higher (P≤0.01) than in other groups. Rearing indicators showed statistically higher values (P≤0.01) in case of Polish White Improved goats in comparison to other experimental groups. The level of

this indicator appeared to be definitely higher in all experimental groups of boer goat content.

Table 2
The effect of upgrading Polish White Improved goat with boer bucks on reproduction traits and body weight at birth

Traits		Content of boer genotype (%)				
		0 (A)	50 (B)	75 (C)	87,5 (D)	93,75 (E)
Fertility (heads)	n	140	35	20	16	-
	LSM	0.82	0.82	0.78	0.97	
	SE	0.19	0.19	0.19	0.19	
		D	D	D	ABC	
Prolificacy (heads)	n	140	35	19	16	-
	LSM	1.82	1.85	1.86	1.83	
	SE	0.40	0.40	0.53	0.74	
Kids' survivability till 7th day of age (heads)	n	201	59	64	32	23
	LSM	0.85	1.00	1.00	1.00	1.00
	SE	0.04	0.06	0.07	0.08	0.1
Rearing indicator (heads)	n	201	59	64	32	23
	LSM	0.71	1.00	1.00	1.00	1.00
	SE	0.06	0.09	0.11	0.13	0.16
		Bcde	A	a	a	A
Body weight of kids at birth (kg)	n	181	56	60	31	21
	LSM	3.46	4.07	4.36	4.15	3.76
	SE	0.12	0.18	0.21	0.25	0.32
		BCd	A	Ae	a	C

a, ...,e - $P \leq 0,05$; A, ...,E - $P \leq 0,01$; LSM – least square mean; SE – standard error

All groups of crossbreds did not differ statistically between each other. The positive effect of crossing with boer goat on rearing indicators of kids in case of lack of statistically significant differences in litter size among all groups was evidenced. That may lead to a statement, that the upgrading of Polish White Improved goat with boer goat does not cause decreasing of litter size in the crossbreds and allows parallel improvement of rearing indicators. Concerning meat production in goats the results are very important. Similar patterns of differences were mentioned in the research of NIŻNIKOWSKI et al. (2003) carried out on a lower number of crossbreds' groups.

The genotype of kid also affected the increase of body weight at birth together with the increase of boer genotype content. Only kids with 93.75% of boer genotype content gained statistically less body weight ($P \leq 0.05$) than the other kids at the same age from group of 75% of boer genotype content. Similar tendencies were observed by STANISZ & GUT (2003) in the study on meat performance of kids.

Litter size (Tab. 3) affected the survivability indicators of goats. The lowest values were observed in the quadruplets in comparison to the others. Similar tendencies were also observed in rearing indicator of kids despite the lack of a significant influence of litter size as it was also reported in the study of NIŻNIKOWSKI et al. (2003).

Accordingly to the predictions, the single born kids had the highest body weight at birth. The lowest body weights of kids were observed in triplets, whereas twins and quadruplets gained similarly intermediate ($P \leq 0.01$) body weights between singles and triplets.

Table 3
Effect of litter size of kids on rearing indicators and body weight at birth

Traits		Litter size			
		1 (A)	2 (B)	3 (C)	4 (D)
Kids' survivability till 7th day of age (heads)	n	74	211	81	13
	LSM	1	1	1	0,88
	SE	0.06	0.05	0.05	0.1
		D	D	D	ABC
Rearing indicator (heads)	n	74	211	81	13
	LSM	1	1	0.97	0.86
	SE	0.09	0.08	0.08	0.15
Body weight of kids at birth (kg)	n	66	203	70	10
	LSM	4.42	3.99	3.49	3.93
	SE	0.18	0.16	0.17	0.32
		BCD	AC	ABD	AC

A, ...,E - $P \leq 0.01$; LSM – least square mean; SE – standard error

The estimation of the influence of kid's sex (Tab. 4) on survivability and rearing as well as body weight at birth reported the dominance of male kids over female kids only in rearing parameter.

The obtained results are in opposition to the results reported in the study of NIŻNIKOWSKI et al. (2003), where the dominance of female kids over male kids was observed in both survivability and rearing indicators. These observations show the necessity of further research in the field of meat goats or their crossings, because in milk goats these tendencies might be slightly different.

Table 4
Effect of sex of kids in liter on rearing indicators and body weight at birth

Traits		Sex	
		Male kids	Female kids
Kids' survivability till 7th day of age (heads)	n	206	173
	LSM	1.00	0.99
	SE	0.06	0.05
Rearing indicator (heads)	n	206	173
	LSM	1.00	0.99
	SE	0.09	0.08
Body weight of kids at birth (kg)	n	194	155
	LSM	4.15 x	3.76
	SE	0.18	0.16

x - $P \leq 0.05$; LSM – least square mean; SE – standard error

Generally summarizing, definitely wider range of influence of the examined factors on body weight at birth was reported, than on reproduction parameters. Body weight at birth was higher due to an increasing content of boer goat genotype. Also the effect of litter size on body weight at birth was the highest in singles and this effect was declining within increasing litter size to three kids. Similarly, the male kids dominated over female kids in body weight at birth.

The positive effect of upgrading on dams' fertility and rearing of kids was observed. The fertility was the highest in the group of goats with 87.5% of boer genotype, but the rearing indicator was the lowest in Polish White Improved goats. Survivability was the lowest in case of the quadruplets. Moreover, no effect of kids' sex on survivability and rearing indicator was reported.

Generally, the results showed that upgrading white improved goat with boer bucks does not cause the decrease of body weight at birth, litter size as well as rearing indicators and survivability of kids, despite the largest litters for instance quadruplets.

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Semen characters in reproductive AI boars depending on polymorphism in steroid 21-hydroxylase gene

Abstract

The steroid 21-hydroxylase gene (*CYP21*) located on chromosome 7 in pigs in SLA region class III in the DNA segment between regions SLA class I and SLA class II is regarded as a "gene candidate" of reproductive characters. The purpose of the study was to define the frequency of mutation in the selected *CYP21* gene segments in boars as well as to determine the correlation between the marked genetic variants: *CYP21*/*Nci*I and *CYP21*/*Hae*III, and qualitative and quantitative characters of the semen under study. The study included 214 boars from which semen was taken to inseminate sows. The *CYP21* polymorphism was determined by means of the PCR–RFLP method. The product of 247 bp was digested with *Nci*I enzyme identifying two alleles of the *CYP21* gene. The frequency of A was 0.1659, whereas that of B was 0.8341. In the boar herd under study three genotypes occurrence was stated, namely: *CYP21*/NciI with frequency of 0.0561 – AA, 0.2196 - AB and 0.7243 for BB. The received product of *CYP21* gene – 509 bp was digested with *Hae*III enzyme and two alleles of the *CYP21* were identified. The frequency of A allele was 0.1145, whereas that of B was 0.8855. In the herd under study the occurrence of the following three genotypes was stated: *CYP21*/*Hae*III and its frequency – 0.0047 - AA, 0.2196 - AB and 0.7757 for BB. An analysis of the relation between the *CYP21*/NciI and *CYP21*/*Hae*III and the boars' under study semen characters showed that there are some possibilities to improve boars utility reproductive characters taking the examined polymorphisms into consideration.

Key Words: boars, steroid 21-hydroxylase gene (CYP21), semen characters

Introduction

The utility reproductive characters are of basic importance for the profitability of pigs production. Using sows potential fertility (the number of ovulatory egg cells) depends on the quality of boars used for reproductive purposes. They should be characterized by the best qualitative and quantitative semen parameters, properly developed reproductive organs and high sexual activity. Studies conducted over the past few years by various research centres suggest some possibilities of using polymorphism of some genes to improve the characters related to reproduction of pigs. One of the most important enzymatic complexes participating in the synthesis of adrenal steroids is the steroid 21-hydroxylase (KUPCZYK et al., 1996). It takes part in the synthesis of mineralocorticoids and glucocorticoids. It converts substrates of 17–hydroxyprogesterone and progesterone into 11–deoxycorticosterone (SIMPSON, 1979, 2000), thus leading to the synthesis of cortisol and aldosterone (GEFFROTIN et al., 1990). The Steroid 21-hydroxylase gene (*CYP21*) located on pig chromosome 7 (GEFFROTIN et al., 1987) in region SLA class III (GEFFROTIN et al., 1990) in the DNA segment between regions SLA class I and SLA class II (GEFFROTIN et. al., 1991) was regarded as a "gene candidate" of reproductive characters.

The purpose of this study was to define the frequency of mutation in the *CYP21* gene recognized by restriction enzymes – *Nci*I and *Hae*III in artificifial insemination (AI) boars and the quantitative and qualities characters of semen under study.

Materials and Methods

The study was carried out on 214 boars (Table 1) used for reproductive purposes and kept at AI stations. All the boars were kept in the same environmental conditions and used exclusively for inseminating purposes.

Table 1
Number of boars in breed groups

No.	Breed	Number of boars
1	Polish Landrace	54
2	Duroc x Pietrain	49
3	Polish Large White	39
4	Hampshire x Pietrain	28
5	PIC	19
6	Pietrain	15
7	Polish Synthetic Line	10
Total		214

The data collected on ejaculates of 214 boars within 1997-2002 concerned such semen characters as: ejaculate volume, sperm concentration, percentage of sperm alive, number of alive sperms per ejaculate and number of insemination doses. To analyse the relations dependence, data on 8001 ejaculates was used, taken from boars aged 221-585 days in order to eliminate the influence of the age on the characters under study. The period of semen collection was divided into two seasons: season I – 1 April to 30 September (spring–summer season), season II – 1 October to 30 March (autumn–winter season), which apart from the year, breed and father's influence were taken into consideration while determining variability. The DNA for the study was isolated from the whole peripheral blood sampled into vacuum test tubes containing the K_3EDTA as an anticoagulant. The isolation was carried out by means of the Master Pure^{TM} Genomic DNA Purification Kit of Epicenter Technologies. Such an isolation procedure produced DNA of 75-85 μg/ml concentration and over 85% purity.

Genotypes of the steroid 21-hydroxylase gene (*CYP21*) were determined by the PCR-RFLP method. The detection of mutation located on intron 7 of CYP21 was conducted by means of specific starter sequences: forward primer 5'–CTCCCCTAATTGGCACAAAG–3' and reverse primer 5'–ATTGCTGAGGTGCTGCGT–3', as well as the appropriate thermal profile: 1/94°C/5 min, 2/94°C/40s, 3/58°C/40s, 4/72°C/40s, 5/72°C/7 min. The reaction was conducted in 35 cycles – stages from 2 to 4 for PCR reaction which resulted in receiving a DNA fragment of 247 bp. The amplified fragment was digested with 5 units of restriction enzyme *Nci*I which recognizes CC↓(C/G)GG sequence at 37°C for the period of 4-5 hours. However, the detection of mutation located on intron 5 of *CYP21* gene was conducted with the application of specific starter sequences: forward primer 5' – GAC CCA GGA GTT CTG TGA GG – 3' and reverse primer 5' – CTC TCT GCC CCA GTT CTT CC – 3', as well as the appropriate thermal profil: 1/94°C/5 min., 2/94°C/30s, 3/60°C/50s, 4/72°C/50s, 5/72°C/5 min. The reaction was conducted in 35

cycles – stages from 2 to 4 for PCR reaction. This resulted in receiving a DNA fragment of 509 bp which was digested with 5 units of *Hae*III endonuclease that recognizes CG↓CC sequence at 37°C for 3-4 hours. The restriction fragments of the DNA were separated by electrophoresis in 2-2,5% agarose gels with the addition of ethidium bromide in buffer 1xTBE. Afterwards the gels were visualized and analysed in UV rays and recorded according to Vilber Lourmat system.
The statistical analysis of the relation was carried out by means of the SAS System (General Linear Model Procedure – the SAS System) according to the following model:

$$Y_{ijklm} = \mu + M_i + O_j + R_k + Z_l + Y_m + S_n + (ZSR)_{lmn} + W_S + G/H_t + e_{ijklmnstw}$$

where: Y_{ijklm} – observation; μ - mean for the herd; M_i – effect of *i*th mother; O_j – effect of *j*th father; R_k – constant effect of *k*th boar bred (j = 1, 2, ... , 7); Z_l – effect of *l*th insemination station (l = 1, 2); Y_m - effect of *m*th year (m = 1, 2, ... , 6); S_n – effect of *n*th season (n = 1, 2); $(ZSR)_{lmn}$ – interaction effect of appropriate model effects; W_s – effect of *s*th boar's age; G/H_t – effect of tth gene's genotype (*CYP21*/*Nci*I i *CYP21*/*Hae*III); $e_{ijklmnstw}$ – error.
The results were presented in tables with mean and standard deviations and number of ejaculates under study. The differences significance, verified by means of Duncan's test, was marked.

Results and Discussion

In order to determine *CYP21* genotypes the product of 247 bp was digested with *Nci*I enzyme recognizing CC↓(C/G)GG sequence on introne 7 of *CYP21* and thus different sequences of bands in agarose gel were obtained. These sequences enabled the identification of two A and B alleles conditioning the occurrence of three genotypes: AA – bands of size: 205 and 42 bp; AB – bands of size: 205, 150,55 and 42 bp and BB – bands of size: 150,55 and 42 bp compared to a mass formula of DNA pUC19/*Msp*I. Frequencies of the identified genotypes and *CYP21/Nci*I alleles in the boar herd under study are presented in Table 2. The frequency of A allele was 0,166 whereas the frequency of B was 0,834. Similar frequency of *CYP21/Nci*I proved KNOLL et al., 1998 – 0,25 for A allele and 0.75 for B allele in the pigs of Large White breed and respectively in the pigs of Landrace, however, it was significantly different in pigs of Duroc breed (respectively: 0.0 and 1.0) and in the pigs of Pietrain breed (respectively: 0.04 and 0.96). As it results from the data in Table 2 the frequency of particular *CYP21*/*Nci*I alleles varies from breed to breed. The frequency of A allele ranged from 0.359 in Polish Large White boars to 0.033 in Pietrain boars. The steroid 21-hydroxylase AA genotype exhibited the frequency of 0.0561 in the herd under study. The highest frequency was in Polish Large White boars - 0.1282 whereas in Pietrain and PIC boars this genotype was not found at all. The heterozygotic AB genotype exhibited the frequency of 0.2196 and it was the highest in Polish Landrace boars, however, it was the lowest in Pietrain boars -0.0667. The homozygotic BB genotype exhibited the highest frequency - 0.7234 in the tested herd. The highest frequency of this genotype was found in boars of Pietrain breed - 0.9333 whereas the lowest – in boars of Polish Large White - 0.4103. In the herd of reproductive boars under study statistically significant differences were stated. They existed between the observed

numbers and the theoretically calculated ones according to Hardy and Weinberg's rule for *CYP21*/*Nci*I – ($Chi^2 = 741.89$; $P \leq 0.00001$) genotypic groups.

Table 2
The frequency of *CYP21*/*Nci*I and *CYP21*/*Hae*III genotypes and alleles of boars under study

Breed	Polymorphism	Genotype *CYP21*			*CYP21* allele	
		AA	AB	BB	A	B
Polish Landrace	*CYP21*/*Nci*I	0.0370	0.2593	0.7037	0.1667	0.8333
	CYP21/*Hae*III	-	0.1852	0.8148	0.0926	0.9074
Duroc x Pietrain	*CYP21*/*Nci*I	0.0612	0.1020	0.8367	0.1122	0.8878
	CYP21/*Hae*III	-	0.1020	0.8680	0.0510	0.9490
Polish Large White	*CYP21*/*Nci*I	0.1282	0.4615	0.4103	0.3590	0.6410
	CYP21/*Hae*III	-	0.2051	0.7949	0.1026	0.8974
Hampshire x Pietrain	*CYP21*/*Nci*I	0.0357	0.1071	0.8571	0.0893	0.9107
	CYP21/*Hae*III	-	0.3571	0.6429	0.1786	0.8214
PIC	*CYP21*/*Nci*I	-	0.2105	0.7895	0.1053	08947
	CYP21/*Hae*III	-	0.2632	0.7368	0.1316	0.8684
Pietrain	*CYP21*/*Nci*I	-	0.0667	0.9333	0.0333	0.9667
	CYP21/*Hae*III	0.0667	0.4667	0.4666	0.3000	0.7000
Polish Synthetic Line	*CYP21*/*Nci*I	0.1000	0.2000	0.7000	0.2000	0.8000
	CYP21/*Hae*III	-	0.2000	0.8000	0.1000	0.9000
Total	*CYP21*/*Nci*I	0.0561	0.2196	0.7243	0.1660	0.8340
	CYP21/*Hae*III	0.0047	0.2196	0.7757	0.1145	0.8855

The specific PCR reaction resulted in the next product of 509 bp that was digested with the *Hae*III restriction enzyme and subjected to electrophoresis. The above process produced different sequences of restriction fragments on the agarose gel which enabled the identification of two A and B alleles conditioning the occurrence of the following three genotypes: AA – bands of 438 and 71 bp; AB – bands of 438, 350, 88 and 71 bp and BB – bands of 350, 88 and 71 bp compared to the DNA pUC19/*Msp*I mass formula. In the tested herd the frequency of the A allele was of 0.1145 whereas that of the B was 0.8855 (Table 2). A little higher frequency of the A allele in the pigs of Large White - 0.32 and Pietrain breed -0.23 proved KNOLL et al., 1998. Similarly, in 2002 KMIEĆ et al., showed higher frequency of the same allele in AI boars - 0.1989, as well as (KMIEĆ & ZIEMAK, 2002) in the herd of sows of Landrace breed – 0.1475.

The A allele exhibited the highest frequency in boars of Pietrain breed - 0.3, whereas the lowest frequency was in boars coming from crosses of Duroc and Pietrain breeds (D x P) – 0.0510. In the tested herd of boars of different breeds the steroid 21-hydroxylase genotype AA was found only in the group of boars coming from Pietrain breed and its frequency was only 0.0667. However, the AB genotype was found with the frequency of 0.2196 and that of the BB was 0.7757. The frequency of AB

according to breed group in the tested herd of boars ranged from 0.1020 (Duroc x Pietrain) to 0.4667 (Pietrain) , and that of the BB genotype from 0.4666 (Pietrain) to 0.8680 (Duroc x Pietrain) – see Table 2. The AB genotype was found with a fairly high frequency in boars coming from crosses of Hampshire and Pietrain (0.3571) and in boars from PIC (0.2632). However, the BB genotype was identified with a fairly high frequency in boars from crosses of Duroc and Pietrain (0.8367) as well as in boars of Polish Landrace breed (0.8148) – see Table 2. In earlier studies of pigs coming from Polish Landrace, Large White and Pietrain, as well as Duroc and Hampshire the AA genotype was not found (KMIEĆ et al., 2002; KMIEĆ & ZIEMAK, 2002). The disturbance of genetic balance between the observed numbers and the theoretically calculated ones according to Hardy and Weinberg's formula for *CYP21*/*Hae*III – ($Chi^2 = 1.47$; $P \leq 0.47829$) genotypic groups was not stated in the tested herd of AI boars.

Table 3
Values of tested semen characters in reference to *CYP21*/*Nci*I genotype

Character		*CYP21*/*Nci*I genotype			Total
		AA	AB	BB	
Number of ejaculate		466	1722	5813	8001
Ejaculate volume (cm^3)	Mean	226.0^{A}	213.4^{AB}	$223,2^{B}$	221.3
	SD	92.4	80.5	102.6	78.6
Sperm concentration (mln/cm^3)	Mean	599.0^{A}	606.3^{AB}	598.6^{B}	600.3
	SD	113.9	127.8	124.3	124.4
Sperm alive percentage	Mean	71.8^{AB}	72.1^{B}	72.8^{A}	72.6
	SD	4.3	4.9	5.2	4.8
Number of alive sperms in ejaculate (mld)	Mean	93.5^{A}	90.1^{AB}	93.7^{B}	92.9
	SD	36.8	33.1	31.1	32.0
Number of insemination doses	LSM	25.5^{AC}	23.5^{AB}	24.7^{BC}	24.5
	SD	9.4	8.9	8.4	8.6

Means in rows marked with the same letter differ significantly at $P \leq 0.05$.

Relations between the *CYP21*/*Nci*I and *CYP21*/*Hae*III polymorphism and such characters of the semen as: ejaculate volume, sperm cells concentration, percentage of sperm alive per ejaculate and number of insemination doses in ejaculate were subjected to an analysis. The average ejaculate volume of the boars under study was 221.3 cm^3. While analysing the relations between *CYP21*/*Nci*I polymorphism, it was proved that ejaculates with the highest volume were obtained from the boars of AA genotype - 226.0 cm^3, whereas ejaculates with the lowest volume - 213.4 cm^3 came from the boars of AB genotypes – see Table 3. The differences in ejaculates volume between boars of different *CYP21*/*Nci*I genotypes were confirmed statistically. The concentration of sperm cells in ejaculates coming from the boars of AB genotype was 606.3 x $10^6/cm^3$ and it was higher than the mean of the tested herd and the mean of the boars with the remaining *CYP21*/*Nci*I genotypes (BB – 598.6 x $10^6/cm^3$ and AA - 599.0 x $10^6/cm^3$). The differences were confirmed statistically – see Table 3.

Considering the interrelations between the *CYP21*/*Nci*I polymorphism and the average percentage of sperm alive in boars semen it ought to be concluded that the percentage of sperm alive in the semen of boars with the BB genotype was significantly higher than that in the semen of boars with AA genotype and also significantly higher in the semen of boars with AB genotype than with AA genotype. The differences obtained from the above study were confirmed statistically – see Table 3. It was also indicated that significantly lower number of sperm alive was found in the semen of boars with the AB genotypes than in ejaculates coming from boars with BB and AA genotypes and the differences obtained were confirmed statistically – see Table 3. On average, 24.5 insemination doses were received from one ejaculate in the tested herd of boars. The highest number of insemination doses was obtained from ejaculates taken from boars with the AA genotype and the fewest – from boars with the AB genotype. The differences were confirmed statistically – see Table 3. Slightly lower number of insemination doses (23.4) per ejaculate was received earlier by KMIEĆ et al., 2002.

Table 4
Values of tested semen characters in reference to *CYP21*/*Hae*III genotype

Character		*CYP21*/*Hae*III genotype			Total
		AA	AB	BB	
Number of ejaculate		48	2060	5893	8001
Ejaculate volume (cm^3)	Mean	186.0^{AB}	223.0^{B}	$218,1^{A}$	221.3
	SD	67.3	78.5	78.2	78.6
Sperm concentration (mln/cm^3)	Mean	600.6	601.5	599.5	600.3
	SD	137.1	126.1	123.8	124.4
Sperm alive percentage	Mean	70.0^{AC}	73.3^{BC}	72.2^{AB}	72.6
	SD	0.0	5.4	4.5	4.8
Number of alive sperms in ejaculate (mld)	Mean	77.8^{AB}	94.5^{B}	91.0^{A}	92.9
	SD	29.5	29.3	28.4	32.0
Number of insemination doses	Mean	20.3^{AB}	24.7^{A}	24.2^{B}	24.5
	SD	9.3	8.6	8.0	8.6

Means in rows marked with the same letter differ significantly at $P \leq 0.05$.

Table 4 presents the average values of the boar semen characters in relation to the *CYP21*/*Hae*III polymorphism. It was indicated that ejaculates taken from boars with the AA genotype were of significantly lower volume compared to those with AB and BB genotypes. However, analysing the relations between *CYP21*/*Hae*III genotypes and sperm cells concentration, its lowest value was found in boars with BB genotype but the differences were not confirmed statistically. However, the lowest percentage of sperm alive and the lowest number of sperm alive in one ejaculate was found in ejaculates taken from boars with AA. The differences were confirmed statistically (Table 4). One ejaculate taken from boars with the AA genotypes produced 20.3 insemination doses on average, whereas the ejaculates coming from boars with the AB and BB genotypes produced significantly more insemination doses (respectively: 24.7 and 24.2). The AB boar ejaculates produced on average 4.4 insemination doses more

than the ones with the AA genotype and 3.9 insemination doses than the ones coming from boar of the BB genotypes. The difference was confirmed statistically (Table 4).

Conclusions

The results obtained from the experiments led up to the following statements and conclusions:

1. In the tested herd of reproductive AI boars two alleles (*CYP21*^{A} and *CYP21*^{B}) of the steroid 21-hydroxylase *CYP21*/*Nci*I and *CYP21*/*Hae*III gene were found whose role is to control three genotypes occurrence. The frequency of *CYP21*^{A} allele was respectively – 0.1659 and 0.1145, whereas that of *CYP21*^{B} allele – 0.8341 and 0.8855. The frequency of the AA genotype was respectively – 0.0561 and 0.0047, that of the AB genotype – 0.2196 whereas that of the BB genotype – 0.7243 and 0.7757.
2. The analysis of *CYP21*/*Nci*I genotypic groups in the herd under study indicated the disturbance of genetic balance between the observed numbers and those theoretically calculated ones according to Hardy and Weinberg's rule. However, such a disturbance was not stated for *CYP21*/*Hae*III genetic groups.
3. Statistically significant relations were found between the *CYP21*/*Hae*III and *CYP21*/*Hae*III genotypes and the tested qualitative and quantitative characters of reproductive boars' semen.
4. No significant influence of the *CYP21*/*Hae*III polymorphism on the sperm cells concentration in ejaculate was stated, whereas such an influence was observed for the *CYP21*/*Nci*I polymorphism – the highest concentration of sperm cells in ejaculates coming from the genotype AB boars.
5. The studies proved the occurrence of the *CYP21*/*Nci*I and *CYP21*/*Hae*III polymorphism in selected sequences of the steroid 21-hydroxylase gene and the results obtained from the analysis of relations suggest a possibility of using the existing polymorphism in the steroid 21-hydroxylase gene to improve some utility reproductive characters of boars.

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Nutritional value of the meat of Pomeranian breed lambs and crossbreeds of Blackheaded and Texel rams

Abstract

Selected indexes of the nutritional value of meat of 100-day-old lambs of the Pomeranian sheep (P) and crossbreeds of Blackheaded (PB) and Texel (PT) rams were studied. Samples of meat were taken from the longest dorsal muscle (*m. longissimus dorsi)* and the chemical composition, physical and chemical properties, water/protein ratio, muscle fibres thickness and the cholesterol, collagen, amino acids in the proteins and of fatty acids contents in the inter muscle fat were determined.

It was found that the meat from PB and PT crossbreeds, as compared to pure breed P lambs, was characterized by a higher content of protein, exogenous amino acids and collagen, lower content of cholesterol, lower water absorption, a higher physiological maturity, expressed as the water/protein ratio, higher diameter of muscle fibres, better UFA/SFA ratio and a higher proportion of PUFA. The DFA/OFA ratio which is the most favourable from the dietetic point of view, was found in the inter muscle fat of the PB lambs, while the least favourable ratio was found in the P lambs. The meat from the PT lambs contained less fat and got lower marks for tenderness, juiciness and flavour intensity. If the nutritional culinary value of meat was taken into account, the best option was that of crossing Pomeranian ewes and Blackheaded rams.

Key Words: crossbreeding, lambs, meat, cholesterol, collagen, amino acids, fatty acids

Introduction

The growing social wealth and diet awareness have resulted in higher requirements concerning food of animal origin – especially meat, which is the prime source of protein for humans. As human work requires less and less effort, the energy needs have decreased, along with an increase in an interest in the biological value of meat – to meet the criteria of functional food and restrict the occurrence of the so-called "diseases of civilisation" (PROST, 1996; HOFFMAN et al., 2003). According to the present state of knowledge, lamb is considered to be the daintiest, most easily digestible meat, with high nutritional value and outstanding pro-health values (BRZOSTOWSKI et al., 2004; JANKUSZEW, 2004). Increasing requirements can be met by the meat obtained from light milky lambs (13-30 kg), which is well muscled, low-fat and yields high-quality meat (PIWCZYŃSKI et al., 2001; BORYS et al., 2003; BRZOSTOWSKI et al., 2005). With the current breed structure in Poland, meat quality can be enhanced by crossing regional breeds and varieties of sheep with meat of mediocre quality with rams of meaty breeds.

The purpose of this study was to determine the chemical composition, physical and chemical as well as sensory properties, the amino acids composition of the proteins and the fatty acids profile of the inter muscle fat in 100-day-old lambs of the Pomeranian sheep and its crossbreeds F_1 with Blackheaded and Texel rams.

Material and Methods

100-day-old lambs – rams – of the Pomeranian sheep (P) and its F_1 crossbreeds with Blackheaded (PB) and Texel (PT) rams were used in the study – 12 animals in each group. Apart from their mothers' milk, the lambs were given balanced doses of hay, fodder concentrate mixture CJ and maize-silage. The feeding level was determined

based on the standards recommended for suckling lambs by the Institute of Animal Husbandry (RYŚ, 1998).
Mean live body weights of lambs were as follows: P - 27.89 kg, PB - 28.64 kg and PT - 28.91 kg. After slaughtering and cooling down the carcasses, samples of meat for analysis were taken from the longest dorsal muscle (*m. longissimus dorsi*). The following were determined: dry matter by drying the samples at 105°C; total protein by KJELDAHL's method; raw fat by SOXHLET'S method; raw ash by burning at 550°C; energetic value with a KL 10 calorimeter with an adiabatic bomb; water absorbability by the GRAU' and HAMM method (1953), the lightness of colour was measured with a "Specol" spectrocolorimeter with a R 045 remission attachment at the wavelength of 560 mm, pH was measured 24 hours after the slaughter with a Radiometr PHM 22 pH-meter. The calculation of the water/protein ratio was based on the water and protein content in the meat.
Amino acids were determined with an automatic AAA-T-339M amino acid analyser manufactured by MIKROTECHNA. Sulphur amino acids (cystine and methionine) were determined as cysteine acid and methionine sulphone. Cholesterol was determined with an EPOLL 20 colorimeter, according to the method developed by KOMPRDA et al. (2000); collagen content was calculated from hydroxyproline content by the method developed by BLOMFIELD and FARRAR (1964).
Fatty acids composition in the inter muscle fat was determined by esterification (PEISKER, 1964), followed by gas chromatography, with the use of a PYE Unicam, seria104 flame ionisation detection chromatograph with a 2.1 long, 4 mm in diameter glass column (ŻEGARSKA et al., 1979).
Muscle fibre thickness measurement was conducted on sections of the longest lumbar muscle (*m. longissimus lumborum*), preserved in neutralised (pH 7.4) 10% formalin and sealed in paraffin blocks. Thus obtained microtome sections were coloured with haematoxylin-eosin. The thickness was measured by a computer analysis of the microscopic image with LUCIA 3.52a computer software.
The thermal processing for the meat sensory analysis was conducted by the method developed by BARYŁKO-PIKIELNA (1975). A five-point scale was used with the following qualities of the cooked meat: tenderness, juiciness, flavour (intensity and desirability) and taste (intensity and desirability).
The results were analysed statistically by the method of analysis of variance in a one-factor orthogonal design, with the STATISTICA 7.1 computer software.

Results

The meaty breeds rams used in the crossbreeds with the Pomeranian female sheep significantly affected some of the meat qualities of the lambs of F_1 generation (Tab. 1). The meat obtained from the lambs of crossbreeds from Blackheaded and Texel rams, as compared to the meat of pure breed lambs, contained more protein ($P \leq 0.05$) and collagen ($P \leq 0.01$), less cholesterol ($P \leq 0.01$), its water absorbability was worse ($P \leq 0.01$) but the muscle fibres were thicker ($P \leq 0,05$). The lowest amounts of inter muscle fat were contained in the meat of PT lambs, the highest calorific value was measured for the meat of PB lambs ($P \leq 0.05$). The calculated water to protein ratio indicates the lowest physiological maturity of the P lambs, and the highest was of the meat of PB ($P \leq 0.05$) and PT lambs.

Table 1
The quality properties of lamb meat

Specification		Groups					
		P		PB		PT	
		$\bar{x}$	v	$\bar{x}$	v	$\bar{x}$	v
Dry matter	(%)	22.43	4.48	23.51	5.03	23.04	2.71
Crude protein	(%)	18.86^{b}	4.74	19.99^{a}	5.86	19.80^{a}	3.01
Fat	(%)	2.02^{a}	34.32	1.93^{a}	26.38	1.63^{b}	25.78
Ash	(%)	1.15	4.10	1.12	4.79	1.11	4.62
pH_{24}		5.48	1.64	5.47	2.47	5.48	2.16
Index W/B Water/Protein		4.11^{a}	5.16	3.83^{b}	7.44	3.89	3.61
Gross energy /100g		107.28^{b}	4.10	113.85^{a}	5.63	106.84^{b}	2.21
Water holding capacity cm^2		7.54^{B}	14.57	8.54^{A}	6.75	9.12^{A}	6.90
Color	(%)	18.67	12.37	18.81	14.17	18.18	9.82
Cholesterol	mg/100g	64.41^{A}	14.80	42.64^{B}	5.75	47.01^{B}	9.25
Collagen	mg/100g	224.35^{C}	6.53	251.30^{B}	8.51	283.1^{A}	12.97
Fibre thicknes *m.longissimus lumborum* µm		23.47^{b}	4.49	24.68	7.89	25.66^{a}	7.89

a. b. - $P \leq 0.05$; A. B. C – $P \leq 0.01$

Table 2
Amino acid content in meat protein obtained from studied lambs (%)

Amino acid	Groups					
	P		PB		PT	
	$\bar{x}$	v	$\bar{x}$	v	$\bar{x}$	v
Threonine	4.59^{b}	4.43	4.78^{a}	0.89	4.69	2.12
Valine	5.20	2.15	5.25	2.43	5.21	2.83
Methionine	2.90^{b}	0.50	3.07^{a}	1.30	2.96	1.42
Isoleucine	4.56	1.07	4.70	1.19	4.58	0.45
Leucine	8.11	1.36	8.16	0.47	8.13	1.78
Phenylalanine	3.60^{B}	2.36	3.87^{A}	0.89	3.92^{A}	0.89
Histidine	3.08^{B}	1.47	3.47^{A}	0.17	3.40^{A}	2.08
Lysine	8.24^{B}	1.29	8.51^{A}	1.05	8.59^{A}	1.26
Tryptophan	1.18^{B}	0.42	1.18^{B}	0.49	1.20^{A}	0.48
Total essential (ESAA)	41.46^{b}	41.43	42.99^{a}	62.72	42.68^{a}	58.04
Aspartic acid	9.37	0.14	9.18	0.86	9.21	2.34
Serine	4.03^{A}	2.37	3.83^{B}	1.25	3.89^{B}	1.11
Glutamic acid	15.42^{b}	0.90	15.63^{a}	0.69	15.46^{b}	2.64
Proline	3.66^{a}	0.46	3.51^{b}	1.36	3.76^{A}	1.15
Cystine	1.19^{A}	0.49	1.13^{B}	1.45	1.07^{C}	1.40
Glycine	4.28	1.03	4.23	1.05	4.21	1.69
Alanine	5.61	1.30	5.49	0.34	5.59	1.02
Tyrosine	3.88^{a}	14.38	3.13^{b}	0.18	3.10^{b}	1.84
Arginine	6.32	0.48	6.19	0.66	6.24	1.05
Total non- essential (NEAA)	53.76	20.54	52.32	21.69	52.53	20.94
ESAA/NEAA	0.77^{b}	11.21	0.82^{a}	15.01	0.81^{a}	10.16

a. b. - $P \leq 0.05$; A. B – $P \leq 0.01$

As a result of the crossing, significant changes took place in the amino acid composition of the lamb meat (Tab. 2). Compared to the P meat, the meat of the PB and PT lambs contained more lysine, phenylalanine and histidine and less serine,

cystine and tyrosine. ($P \leq 0.01$; $P \leq 0.05$). Compared to the meat of P lambs, that of the PB lambs contained more threonine, methionine an glutaminic acid and less proline ($P \leq 0.05$), while the meat of PT lambs had less tryptophan. The differences in the contents of single amino acids in meat between the groups of lambs under study significantly affected the sum of exogenous amino acids, giving the advantage to the crossbreeds. Hence, the exogenous/endogenous amino acids ratio proved more favourable for the protein from PB and PT lambs than for the P lambs ($P \leq 0.05$).

A comparison of the fatty acid profile of the inter muscle fat of the lamb meat (Table 3) shows that a better composition and proportion of saturated and unsaturated fatty acids was found in crossbreeds. Compared to the P lambs, the inter muscle fat of PB and PT lambs had less saturated fatty acids and more saturated ones – mainly polyunsaturated, which means that the ratios UFA:SFA and PUFA:MUFA were more favourable in terms of their health values. In terms of the DFA and OFA contents and proportion, the meat of PB lambs was the most valuable, followed by PT lambs. The PB lambs had the least OFA and the largest amounts of DFA, which made the DFA:OFA ratio the most favourable.

Table 3
Fatty acid composition in intramuscular fat (%)

Fatty acid	Groups					
	P		PB		PT	
	$\bar{x}$	v	$\bar{x}$	v	$\bar{x}$	v
$C_{12:0}$	0.73	25.47	0.75	12.26	0.83	13.99
$C_{14:0}$	6.55^{a}	14.54	6.44	8.55	6.41^{b}	7.37
$C_{15:0}$	0.63^{B}	13.62	0.62^{B}	12.13	0.74^{A}	12.00
$C_{16izo:0}$	0.46	10.95	0.41	19.63	0.40	26.84
$C_{16:0}$	26.54	3.14	25.73	6.27	26.10	4.40
$C_{17:0}$	1.17	5.04	1.25	17.23	1.21	4.29
$C_{18:0}$	13.11^{a}	5.11	13.03^{a}	11.07	12.56^{b}	6.27
$C_{20:0}$	0.13^{b}	10.46	0.15	39.41	0.17^{a}	27.00
Saturated (SFA)	49.32^{a}	3.85	48.38^{b}	3.90	48.42^{b}	2.18
$C_{14:1}$	0.75^{b}	14.80	0.67^{B}	10.61	0.84^{Aa}	8.90
$C_{16:1}$	4.65	4.68	4.57	5.72	4.61	7.24
$C_{17:1}$	0.87^{b}	9.78	0.82^{b}	16.40	0.94	5.55
$C_{18:1}$	36.25	6.09	36.32	3.22	36.24	3.30
$C_{20:1}$	0.31^{b}	11.08	0.35	25.44	0.37^{a}	17.22
Monounsaturated (MUFA)	42.83	5.35	42.73	3.25	43.00	2.09
$C_{18:2}$	6.49^{b}	13.15	7.24^{a}	18.16	7.02^{a}	6.89
$C_{18:3}$	0.35	25.61	0.33	30.82	0.30	15.16
$C_{20:4}$	1.01^{b}	40.63	1.32^{a}	49.97	1.26^{a}	30.08
Poliunsaturated (PUFA)	7.85^{Bb}	3.70	8.89^{A}	3.79	8.58^{a}	2.09
Unsaturated (UFA)	50.68^{b}	3.70	51.62^{a}	3.79	51.58	2.09
UFA:SFA	1.03^{b}	8.03	1.07^{a}	7.65	1.07^{a}	4.28
PUFA : MUFA	0.18^{b}	22.67	0.21^{a}	23.16	0.20	10.03
DFA = UFA + $C_{18:0}$	63.79	2.63	64.65	2.95	64.14	2.72
OFA = SFA – $C_{18:0}$	36.21^{a}	4.73	35.35^{b}	5.44	35.85	4.60
DFA : OFA	1.76^{b}	7.20	1.83^{a}	7.99	1.79	7.37

a. b. - $P \leq 0.05$; A. B – $P \leq 0.01$

Table 4
Sensory evaluation of lamb meat (scores)

Specification	Groups					
	P		PB		PT	
	$\bar{x}$	v	$\bar{x}$	v	$\bar{x}$	v
Aroma intensity	4.67	5.87	4.67	13.96	4.83	12.31
Aroma desirability	4.67	10.06	4.83	8.05	4.92	10.06
Tenderness	4.67[a]	12.76	4.52[a]	12.58	4.13[b]	18.85
Juiciness	4.42[a]	13.74	4.35[a]	14.29	3.92[b]	17.37
Taste intensity	4.50[a]	11.61	4.67[a]	10.55	4.17[b]	15.34
Taste desirability	4.67	12.82	4.83	8.05	5.00	0.00
General evaluation	4.59	8.46	4.65	4.00	4.50	12.67

a. b. - $P \leq 0.05$;

The results of the sensory evaluation of the meat (Table 4) show that its quality is very high regardless of the lamb genotype. The highest average rating was achieved by the meat of PB lambs (4.65 points), while the lowest was for PT lambs (4.50 points). Compared to the meat of PB and P lambs, that of PT lambs got higher marks for juiciness, tenderness and taste intensity ($P \leq 0.05$).

Discussion

Nutritional value of lamb is closely linked to its chemical composition, particularly to the protein content, to the amount and composition of fats and the amount of cholesterol in it. The quality and nutritional value of lamb is determined by many factors, including the animal's genotype. The results of studies conducted by PIENIAK-LENDZION et al. (1998), LIPECKA et al. (2000). PIWCZYŃSKI and MROCZKOWSKI (2000), HOFFMAN et al. (2003), BRZOSTOWSKI et al. (2004), indicate certain genetic conditioning of some chemical and physical properties of lamb meat. The breed of ram used for crossing usually conditioned the raw fat content rather than the protein content in meat. The use of Blackheaded and Texel rams for crossing with the Pomeranian female sheep significantly increased the amount of protein in the meat obtained from their offspring. A higher content of protein in the meat obtained from crossbreeds was also found in the studies conducted by LIPECKA et al. (2000), HOFFMAN et al. (2003) and BRZOSTOWSKI et al. (2005). As in this study, the Texel rams used in the studies conducted by KORZENIOWSKI et al. (1986) and KĘDZIOR (1991) reduced the fat content in the meat of their offspring, while Blackheaded rams did not affect this feature. In the current study, the meat obtained from PT lambs got significantly lower marks for tenderness, juiciness and taste intensity. A higher fat content in the muscle fibres of P and PB lambs may have loosened the muscle tissue and enhanced the taste of the meat (DANKOWSKI and ZIELIŃSKA, 1999).

Apart from the fat content, meat tenderness can be determined by the amount of collagen – the main component of connective tissue – and by the thickness of muscle fibres. The meat containing less collagen and whose muscle fibres are thinner is more tender (OPRZĄDEK and OPRZĄDEK, 2000). A higher amount of collagen and thicker muscle fibres in the PT lambs undoubtedly deteriorated the quality of their meat. Thinner muscle fibres are better digested by proteolytic enzymes and better utilised by the human body (KŁOBUKOWSKI et al., 2003).

Reducing the amount of cholesterol, which is considered to be a hazardous factor in atherosclerosis, is a challenge for animal husbandry (ARSENOS et al., 2000; BORYS and PRZEGALIŃSKA-GORĄCZKOWSKA, 2005). Compared to the meat of other animal species (BAROWICZ and JANIK, 1998; HASIK et al., 1999), the meat of lambs, particularly that obtained from crossbreeds, is considered to be a low cholesterol product (P–64.41; PB–42.64 and PT-47.01 mg/100g). The results indicate that by the appropriate selection of sheep breeds for crossing the level of cholesterol in meat can be controlled. This was confirmed by the studies conducted by ARRSENOS et al. (2000), BRZOSTOWSKI et al. (2004); however, the effect of genotype on cholesterol level was not found in the study by KACZOR et al. (2000) and BORYS and BORYS (2001).

The nutritional value of meat is determined not only by the amount of protein contained in it, but also by its biological value, which is most affected by amino acid composition. Amino acids consumed together with meat are used for synthesising new proteins and take part in the synthesis of many biologically active compounds (HASIK et al., 1999; GAWĘCKI and HRYNIEWIECKI, 2004). Of the 18 amino acids found in meat, the human body can synthesise only some, called endogenous; the others, called exogenous, have to be supplied with food in appropriate proportions. As in the studies conducted by ELGASIM and ALKANHAL (1992), JANDASKA et al. (2003), the biological value of the protein analysed in this study was determined mainly by such amino acids as lysine, histidine and methionine – which are necessary for growth and development – tryptophan, which takes part in reproduction and lactation (HASIK et al., 1999). A better relation of exogenous to endogenous amino acids was found in the protein of PB and PT lambs (0.82 and 0.81, respectively) than in the P lambs (0.77). A similar relation of the two amino acid groups (0.83) was found by ELGASIM and ALKANHAL (1992). Differentiation of the amino acid composition of meat proteins depending on the lamb genotype was also observed by JANDASKA et al. (2003).

The dietetic value of meat can be expressed as the ratio of saturated and unsaturated fatty acids contained in the inter muscle fat. The level of polyunsaturated fatty acids (PUFA) is a particularly significant value as they are an important in the human diet (HASIK et al., 1999). The different levels of fatty acids in various groups of lambs resulted in a better ratio of UFA:SFA and PUFA:MUFA in PB and PT lambs. From a dietetic point of view, the amount level of unsaturated fatty acids (UFA) is particularly important as they prevent the occurrence of hypercholesterolemia, which is considered to be the prime risk factor in coronary disease (HASIK et al., 1999). As was shown in this study, the inter muscle fat in the PB and PT lambs (8.89% and 8.58%, respectively) contained more UFA than that in P lambs (7.85%).

The genotype of lambs positively affected the level and proportions of DFA and OFA. In terms of the amount and proportions of the hypo- (DFA) and hypercholesterolemic (OFA) acids, the most valuable meat was produced by PB lambs, followed by PT and P. The fat from the PB and PT crossbreeds contained more DFA and less OFA and their proportion in it was better (PB-1.83, PT-1.79, P-1.76). DFA acids are believed to reduce the absorption of cholesterol and bile acids from food and to affect the synthesis of lipoproteins (BARTNIKOWSKA, 1993). A significant effect of the lamb genotype on the fatty acid profile in the inter muscle fat was also shown in the studies conducted by KACZOR et al. (2000), BORYS and BORYS (2001), JANKUSZEW and GRUSZECKI (2003). The changes in the proportions of groups of acids of various

level of unsaturation, shown in previous studies and in those conducted by other authors, indicate the possibility of controlling the profile of fatty acids by appropriate selection of breeds for crossing.

Thanks to a high protein content and biological value, low amount of cholesterol, and good profile of fatty acids, the meat of lambs, particularly crossbreeds, can be recommended in dietetic nutrition. Due to the nutritional and culinary value of the meat, the option in which Blackheaded rams are crossed with Pomeranian female sheep seems to be the preferred one.

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Characteristic of Polish heath sheep grazing behaviour on fallow lands during vegetative period, concerning pasturage time and weather conditions

Abstract

The study was carried out under 3 years' observation on fallow lands during vegetative period (from June until October). Parameters of grazing activity (time of grazing, distance and speed of walking) due to weather conditions (average month temperature and rainfall level) were examined. It is concluded that the effect of the month of vegetation period on speed of sheep walks on pasture was remarked. Also the correlation between daily walking distance made by sheep and time of grazing as well as the correlation between grazing intensity and the fall level were observed. No effect of temperature on grazing intensity was found. While the amount of rainfall increased the time of grazing and distances made by sheep became shortened, especially in October, when these two traits were perfectly reported.

Key Words: Polish heath sheep, pasturing, weather conditions, grazing activity

Introduction

The increasing share of fallow lands causes particular changes in the environment that may not be always positive. Moreover, progressing plant succession forces different agricultural practices, which often uncontrolled may cause significant problems with usage of fallow lands.

Due to these facts, the study on adjustment of fallow lands for sheep pasturage purposes has been undertaken. In Polish conditions, this adjustment should consider indigenous breeds of sheep, considering Polish heath sheep especially. Particularly, the estimation of nutritional value of fallow lands' herbage is very important (GROBEREK et al., 2003a, 2004bc). GROBEREK et al. (2004b) indicated full ability of the Polish heath sheep for pasture purposes on fallow lands suggesting optimal animal density due to maintaining animals' condition and ability to lamb production.

The range of pasture usage of sheep on fallow lands may be a result of herbage nutrition value as well as several factors caused by climate in a particular region and grazing activity of sheep (CHRUPEK et al., 2005; GROBEREK et al., 2003a, 2004a). In this connection, the study shows the results of the research led on grazing activity in the Polish heath sheep due to climatic conditions during the vegetative period on a farm located in the north-western Wielkopolska province.

Material and Methods

The research was led on 8-year-fallow land on the 50ha farm situated in the north-western Wielkopolska province. The observations took placed in three successive years. The flock of ewes with offspring (approx. 200 heads) of the Polish heath sheep were pastured on fallow lands. The sheep density was about 0.15 AU/ha. Observations were carried out from June to October during 3 successive days a month.

The aim of the study was to calculate time spent by sheep on pasture (min) and estimation of daily distances made by walking sheep (m). Moreover, the speed of walking was also reported as the result of two previously mentioned factors. Paths of wandering sheep were marked on a map of the farm and then the distances were evaluated due to the map scale. The times of going out on pasture and return to the farm were noted and then the time spent on pasture by sheep was calculated. The observations were done by one observer, who watched animals from 300 m distance using binocular.

The results were calculated with LSM analysis in SPSS software for Windows (2003) due to the following model:

$$y_{ijk} = \mu + a_i + b_j + ab_{ij} + e_{ijk}$$

where: y_{ijk} – of trait value

μ – overall mean

a_i – constant effect of the year (i=1, 2, 3)

b_j – constant effect of the month (j=VI, VII, VIII, IX, X)

ab_{ij} – constant effect of i-year with j-month

e_{ijk} – random residual effect

Due to grazing behaviour traits such as time spent by sheep on pasture, distances covered by sheep and speed of walking, in case of significant influence of month the differences were estimated with Duncans' test (RUSZCZYC, 1981) and shown in tables 1, 2, 3 and 4.

The characterization of climatic conditions was based on 3-year-data obtained from the local Meteorological Station IMGiW in Piła (average monthly temperature (°C) and monthly sum of rainfalls (mm)). The correlation coefficients between climatic conditions indicators and behavioural traits were calculated (RUSZCZYC, 1981). These traits, which correlation coefficients were statistically significant, are shown on figures due to the month of vegetation.

Table 1
Climatic data characteristic based on the information from the Meteorological Station in Piła

Month	I year		II year		III year	
	Average monthly temperature (°C)	Monthly rainfall sum (mm)	Average monthly temperature (°C)	Monthly rainfall sum (mm)	Average monthly temperature (°C)	Monthly rainfall sum (mm)
May	14.0	25	16.3	73	14.8	33
June	14.7	66	17.1	45	18.1	44
July	19.9	56	19.8	32	19.0	64
August	18.9	66	20.6	111	18.8	15
September	12.0	121	13.6	44	13.7	25
October	11.3	20	6.9	115	4.8	38

Results and Discussion

Climatic data of average monthly temperature and average rainfall are presented in Table 1. It may be clearly seen that climatic conditions varied in every month and in every year of the experiment. However, in the 3rd year of the experiment, the lower rainfall level was observed in contrast to previous 2 years. That may lead to a statement that dry conditions of breeding dominated in the 3rd year of the experiment

and, therefore, directly affected fodder yield in this area (GROBEREK et al., 2004b). These specific circumstances affected also the sheep behaviour (Table 2).

Table 2
Effect of examined traits and interaction on grazing behavior parameters in sheep

Traits	Year of research	Month	Interaction: year x month	X	SD
Time of grazing (min)	X	NS	X	376.20	97.18
Distance of walking (m)	XX	NS	XX	5733.85	1727.05
Speed of walking (m/min)	XX	X	X	15.38	3.96

X – P ≤ 0.05; X – P ≤ 0.01; NS – non-significant

All examined traits were influenced by the year of the research, whereas speed of walking was also affected by the month of pasturing. Interaction: year^{x}month had statistically important effect on all traits (P≤0.05 or P≤0.01). These results were compatible to tendencies reported in other experiments, where despite the examined factors (GROBEREK et al., 2004ab) the effect of the other environmental phenomena such as height of insects' flight and suckling by lambs were also reported (CHRUPEK et al., 2005).

Table 3
Effect of vegetation period on grazing behaviour parameters in sheep

Traits		Month				
		June (A)	July (B)	August (C)	September (D)	October (E)
Number of observations		8	9	8	7	7
Time of grazing (min)	LSM	419.61	296.77	398.33	409.05	342.77
	SE	34.98	32.39	34.98	37.40	37.40
Distance of walking (m)	LSM	4873.00	4633.11	6831.50	4920.11	6219.77
	SE	621.81	575.68	621.81	664.74	664.74
Speed of walking (m/min)	LSM	11.78^{bcE}	16.10^{ad}	16.65^{ad}	11.74^{bcE}	18.20^{AD}
	SE	1.42	1.32	1.42	1.52	1.52

A,B,C,D,E - P≤0.01; a,b,c,d,e - P≤0.05

The effect of month of pasturing, especially on the speed of walking, was shown in Table 3. Generally, sheep walked more slowly on pasture in June and September, whereas in October - more speedily. In June and September sheep resided definitely longer on pasture, whereas in July this time was the shortest, although this factor did not have a statistical effect. Moreover, the longest distance was made in October and August, that in case of August it may be explained by worsening of fodder conditions (GROBEREK et al., 2004b) or shortening of the day. Generally the speed of walking increased proportionally due to shortening of the days and worsening of fodder conditions, that was supplemented by longer and faster walking to cover nutritional demands of sheep.

Table 4
Correlation coefficients between examined traits

Items	Time of grazing (min)	Distance of walking (m)	Temperature **(°C)**	Rainfalls (mm)
Time of grazing (min)	**0.535**^{x}	-0.016	0.045	**0.495**^{x}
Distance of walking (m)		**0.820**^{xx}	-0.099	**-0.501**^{x}
Speed of walking (m/min)			-0.160	-0.347
Temperature **(°C)**				-0.112

XX - P≤0.01; X - P≤0.05;

Correlation coefficients between traits of sheep grazing activity and climatic data were presented in Table 4. The positive effect and statistically important (P≤0.05) relation between time and daily distance of walking on pasture were observed. The distribution of these values covering month of vegetative period was also shown on Figure 1. At large, if sheep resided longer on pasture, the distances increased. Similar, although definitely higher, correlation coefficient was observed between the distance of walking and speed of wandering sheep. Figure 2 confirms that the observation shows values accordingly to the month of the vegetation period. It may be ascertained, that the distance made by sheep on pasture depended not only on the length of pasturing, but also on the speed of walking.

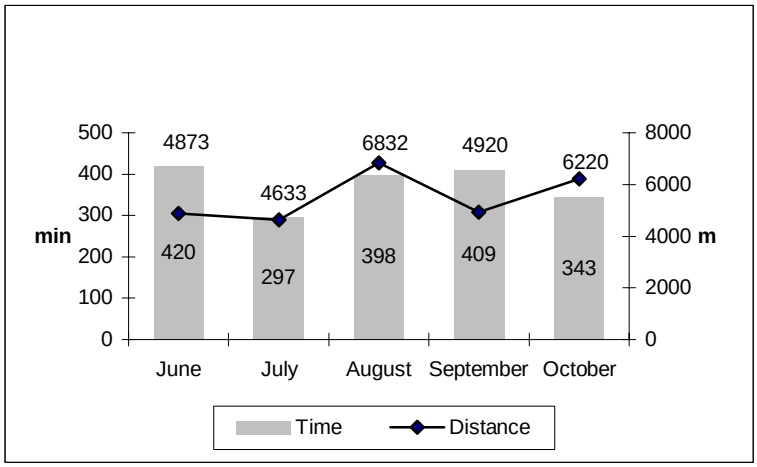

Fig. 1: Changes of time of grazing and distance of walking due to month of vegetation period (P≤ 0.05)

Correlation coefficients between climate and grazing activity of sheep showed negative and significant relationships between rainfalls level and time of grazing as well as distance of walking on pasture. Summarizing, if the rainfall level was higher, the time of grazing and distance of walking were shorter and the other way round.

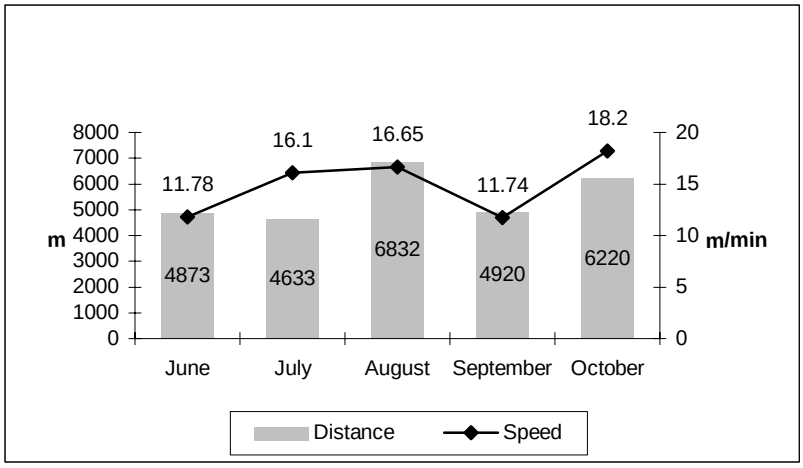

Fig. 2: Changes of distance of walking and speed pf walking due to month of vegetation period ($P \leq 0.01$)

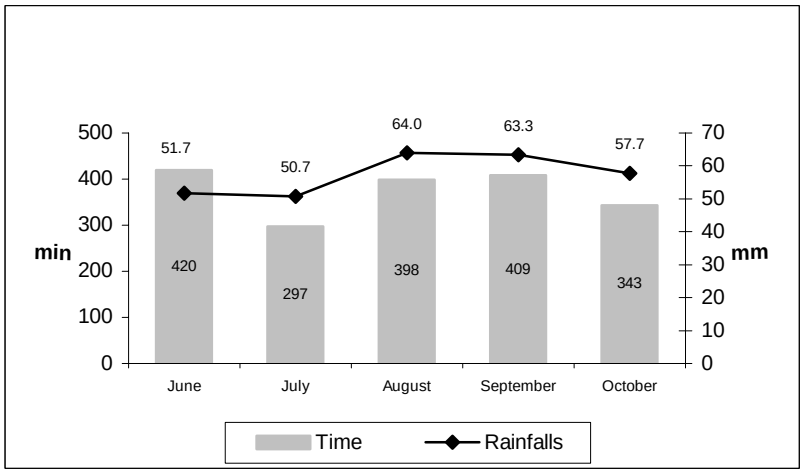

Fig. 3: Changes of time of grazing ($P \leq 0.05$) and rainfall level due to month of vegetation period

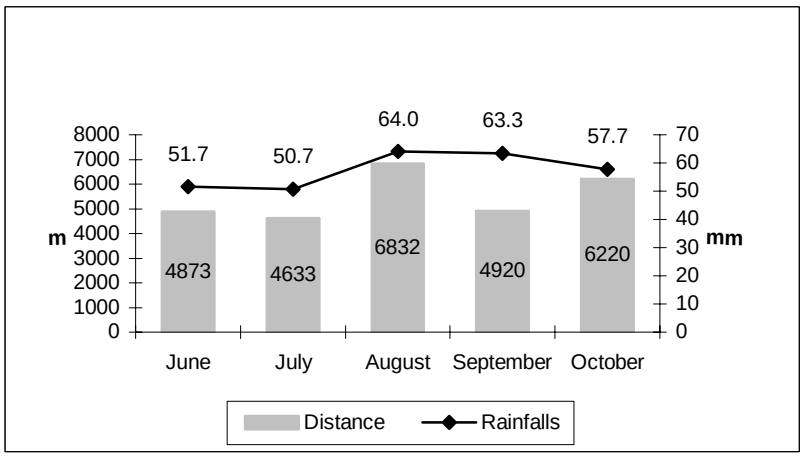

Fig. 4: Changes of distance of walking ($P \leq 0.05$) and rainfall level due to month of vegetation period

These tendencies were also pictured on Figure 3 and 4. Generally, traits of grazing activity were in stronger relationship with the rainfall level than with temperature during the vegetative period. Similar patterns of pasture behaviour were also reported in the study of CHRUPEK et al. (2005) and GROBEREK et al. (2004a).

In this study taken on grazing activity of the Polish heath sheep on fallow lands during the vegetative period it is concluded that:

- the month of the vegetation period affected the speed of walking of sheep on pasture,
- the length of distances of walking sheep during the day depended on the time of grazing on pasture and the speed of walking,
- grazing activity of pasturing sheep depended on the rainfall level and was independent from temperature. If the amount of rainfall increased, the time of grazing and distance of walking were shortened and the other way round, which was perfectly observed in October.

The obtained results provide some information for further strategy of sheep pasturing on fallow lands due to climatic conditions during the vegetative period. The information may be successfully applied in agro-environmental programmes.

Acknowledgements

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The influence of length day on melatonin and prolactin secretion during lactation in asesonal sheep

Abstract

The study demonstrated that during the period of milking, the duration of the daylight had a significant effect on the lactation parameters in ewes lambed in January and June. Marked differences were noted in the levels of milk production in sheep, during the period of shortening daylight, i.e. in autumn, as the sheep lambed in June produced 50% less milk than ewes lambed in January. The dwindling milk production at the time of a shortening photoperiod resulted from the different course of melatonin and prolactin secretion. In the period of shortening daylight, the concentration of melatonin increased, while the secretion of prolactin decreased, thereby contributing to a lower milk synthesis.

Key Words: melatonin, prolactin, lactation sheep

Introduction

The effect of light on the behaviour of animals has been common knowledge for a long time. In most of the animals studied to-date, the biosynthesis of melatonin was shown to follow the rhythm depending on changes in the light conditions. The highest concentrations of this hormone, often dubbed *"the hormone of darkness"* are found at night (REITER, 1991 a, b; ARENDT, 1986).The role of melatonin was mostly associated with its effects on the reproductive system. The most recent studies have proven, however, that melatonin can also modulate the concentrations of prolactin, which is one of the principal hormones responsible for triggering and maintaining lactation in mammals. The hormonal status of these animals (rams), so different from that of lactating females, cannot help in elucidating precisely the relationships that exist in a light-lactation-milk production system (MORGAN et al., 1994; LINCOLN et al., 1995; MISZTAL et al., 1999). This study has therefore been designed to discover the effect of changing the lambing date (changing of day length) on the secretion of melatonin and prolactin, in connection with the milk production levels in an aseasonal sheep race, i.e. the Polish Merino.

Material and Methods

The studies were conducted in the Experimental Station of the Department of Sheep and Goat Breeding, at the Agricultural University in Krakow. The experiments involved 40 ewes from the Polish Merino race, aged 4-5 years and weighing 60 ± 5 kg. The ewes were randomly allocated to two groups, with 20 animals per group. Mating after oestrus synchronization was performed on 5th September for the first group (Group I) of sheep and on 15th January for the second (Group II). In both cases, the Polish Merino rams weighing 85±5 kg were used. The eves of Group I lambed from 20th-25th January while of Group II – from 18th-24th June. From the pre-mating preparation period up to the 4th month of the pregnancy, ewes were fed according to standards, with traditional seasonal fodder (forage pasture, hay and an additional feed

concentrate (NORMS, 1993). From the 5th month of pregnancy to drying off, sheep received a 1.5 kg pelleted diet (7.5 MJ-net energy and 220 g crude protein) and an *ad libitum* hay supplement. Throughout the experiments, these sheep as well as those in the first experiment were kept indoors but allowed to use outside runs during the day. Lambs stayed with their mothers until 56 days of age, after which they were weaned and ewes were used for milking. When the offspring were reared, the milk production of sheep was estimated based on the weight gains of the lambs from 2 to 28 days of age, using a conversion factor of 4.5 l milk per kg weight gain of lamb. During the period of milk use, ewes were milked twice a day using an Alfa-Laval milking machine. Milk yields were recorded individually at 10-day intervals. From day 20 of lactation to drying off, blood was drawn from the sheep every 30 day to monitor the changes in prolactin and melatonin secretion. Blood samples were collected from the time of sunset over a period of 6 h, at 60 min. intervals, through a catheter inserted into the jugular vein 6 h before. After centrifugation in heparinized tubes, plasma was stored at -20°C until hormone assays were performed.

Analytical Techniques

Concentrations of prolactin and melatonin in the blood plasma were measured by radioimmunoassay techniques. The plasma prolactin concentration was assayed by the radioimmunoassay (RIA) double-antibody method, using antiovine-prolactin and antirabbit-gammaglobulin antisera according to WOLINSKA et al. (1977). Melatonin was assayed in unextracted plasma according to the method of FRASER et al. (1983), modified by MISZTAL et al. (1996). The milk performance parameters and plasma prolactin and melatonin concentrations are expressed as means ± SEM. The Mann-Whitney test was used to assay the differences between groups in the parameters of lactations. The effects of the treatments on the hormone concentrations were analyzed by one-way analysis of variation (ANOVA). An a priori level of statistical significance was set at $P<0.05$ for all tests. Post-hoc differences were determined using Scheffe's test.

Results

The studies showed the significant effect of the lambing date on the lactation parameters in ewes during the milking period. The sheep lambed in January (Group I) were milked for an average of 104 days, whereas those lambed in June (Group II) – only for 66 days. In these milking periods, the Group I ewes produced 37.5 ± 9.6 l of milk, whereas those lambed in June – less than 50%, i.e. a mere 15.8 ± 5.1 l. (Fig. 1). The profile of prolactin secretion in the Merino sheep lambed in January showed a higher diversity compared with the changes in melatonin concentrations (Fig. 2). With the lengthening photoperiod, the melatonin level lowered and in May it amounted to a mere 61.0 ± 60.5 pg/ml, whereas in the same period, the prolactin concentrations was at its highest, reaching 298.7 ± 74.0 ng/ml. In July, yet another peak in prolactin levels (217.4 ± 68.3 ng/ml) was noted, again statistically significantly different ($P \leq 0.05$) from other collections, except for the sample collected in June (180.3 ± 69.2 ng/ml). The melatonin concentrations did not show significant differences compared with the previous value (67.3 ± 40.7 pg/ml and 61.0 ± 60.5 pg/ml). However, from July till September, the melatonin secretion was more intense. In September, the concentration

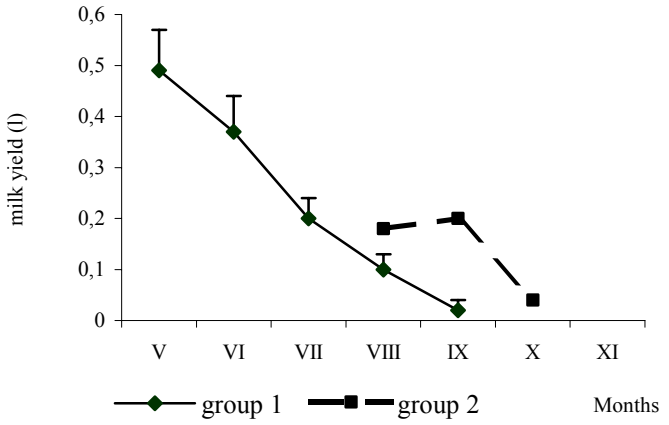

Fig. 1: Mean (± SEM) monthly milk yield of Merino sheep lambed in January (group 1), and June, (group 2). See text for statistical comparisons.

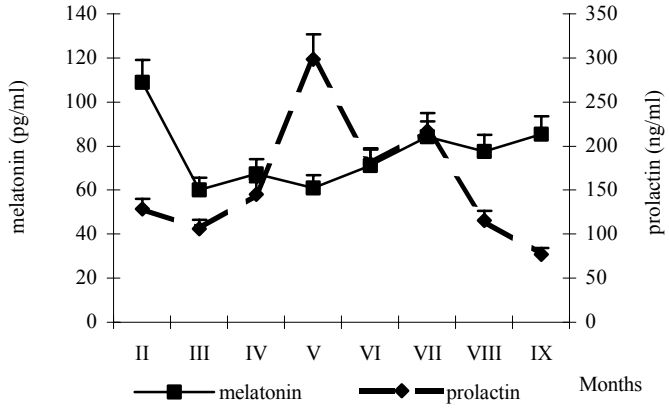

Fig. 2: Mean (± SEM) plasma melatonin and prolactin concentrations in Merino sheep lambed in January. See text for statistical comparisons.

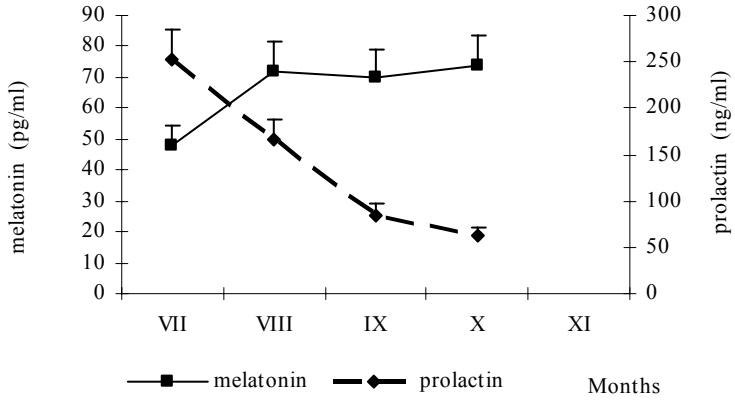

Fig. 3: Mean (± SEM) plasma melatonin and prolactin concentrations in Merino sheep lambed in June. See text for statistical comparisons

of this hormone amounted to 85.3 ± 56.0 pg/ml, whereas the prolactin level decreased to 77.1 ± 57.5 pg/ml, so perhaps also contributing to the decrease in milk production (Fig. 2). The highest daily milk yield in ewes lambed in January was observed in May

where it was 0.49 ± 0.09 l/day on average, while the melatonin was at its lowest value of 61.0 ± 60.5 pg/ml. From June through to September, a decrease in the milk yield occurred from 0.37 ± 0.05 l/day to 0.01 l/day. The concentrations of melatonin over that period showed an upward tendency, from 71.2 ± 50.5 pg/ml in June to 85.3 ± 56.0 pg/ml in September. Over the period of milking of ewes, the melatonin concentrations were stabilised, with an upward tendency at the time of the shortening photoperiod.

The results of the studies, concerning the changes in melatonin and prolactin concentrations in Merino sheep lambed in June, indicated that the highest prolactin concentrations (251.8 ± 62.6 ng/ml) was found in the first sample collection which took place in July, with the statistically significant difference ($P \leq 0.05$) compared with any other sample. The melatonin levels at that time (48.1 ± 38.9 pg/ml) were the lowest (Fig. 3). From July onwards, a systematic decrease in the concentrations of prolactin was observed, with a simultaneous increase in melatonin concentrations. In September, the melatonin level was 73.2 ± 45.0 pg/ml, whereas the prolactin level dropped evidently to a mere 85.09 ± 67.0 ng/ml, which was significantly less ($P \leq 0.05$), compared with the first sample collected in July (251.8 ± 62.6 ng/ml) and the second sample collected in August (165.5 ± 87.4 ng/ml). At the time of the changing photoperiod to the increased duration of the dark phase, the prolactin concentrations decreased: in October it was 62.8 ± 42.5, ng/ml and again the difference was statistically significant ($P \leq 0.05$), compared with the levels in samples collected in July (251.8 ± 62.6 ng/ml) and August (165.5 ng/ml ± 87.4); in this period the concentration of melatonin increased by 3.8 pg/ml compared with the previous concentrations and amounted to 73.8 ± 34.1 pg/ml. The lowest level of prolactin (22.3 ± 16.4 ng/ml) was noted in November, and this value was significantly different ($P \leq 0.05$) from the concentrations found in July (251.8 ± 62.6 ng/ml), August (165.5 ± 87.4 ng/ml) and September (85.09 ± 67.0 ng/ml). The melatonin concentrations in the last collection in November did not differ significantly from that found in October, and was 73.2 ± 45.0 pg/ml. The profile of the melatonin concentrations was stabilized and the differences were statistically insignificant. The highest milk yield in this group of sheep (0.18 ± 0.07 l/day) was found in August, when the prolactin concentrations was 165.5 ± 87.4 ng/ml, and the melatonin concentrations was 72.0 ± 37.3 pg/ml. In the following month, the secretion of melatonin rose to 73.2 ± 45.0 pg/ml and the milk yield dropped to 0.14 ± 0.05 l/day. In October, the melatonin secretion was the most intensive (73.8 ± 34.1 pg/ml) while the daily milk yield dropped to 0.03 ± 0.06 l/day.

Discussion

The differences in lambing dates and the associated lengths of the photoperiod, affected significantly the lactation parameters in sheep during the time of their use for milk production. The sheep which began to lactate in January, could be milked for a longer time compared with ewes lambed in June, that produced 50% less milk compared with ewes lambed in January. The duration of lactation and milk productivity found in the presented study in ewes lambed in January, is comparable with the results obtained by OSIKOWSKI et al. (1999) and WAŻNA et al. (2000). Ewes lambed in January, demonstrated the highest milk productivity in their first months of milking i.e. in May; the concentration of prolactin was also highest in these months whereas the melatonin secretion was at the lowest level. In subsequent months the melatonin levels rose while that of prolactin declined, and the milk production

dropped as well. The ewes which started lactation in June, in their first month of milking, i.e. in August, showed a higher level of prolactin and lower melatonin concentrations than the ewes lambed in January. These differences can be explained by the physiology of the lactation process, as for the sheep of Group I, it was the first month of milking, whilst for the sheep of Group II – the first month of milking. The profile of seasonal melatonin secretion in the ewes of Group I has shown characteristics of an annual rhythm described by MISZTAL et al. (1997), with a systematic increase in secretion levels from June onwards, whereas both prolactin concentration and milk production decreased over the same period. The levels of prolactin and of milk production in ewes lambed in June in the first two months of milking, can be compared to these two parameters in ewes of Group I, i.e. lambed in January, in the two last months of milking. These results indicate that the shortening daylight brought about an increase in melatonin concentration and a decrease in prolactin secretion, in sheep beginning lactation in January and June. The studies by GUT et al., (2001) confirmed the possibility of maintaining milk production in the Merino sheep throughout the year, but the lactation of sheep lambed in May and June lasted for only four months, while in those lambed in January it lasted for ca. ten months. Our own research corroborates these results, as the moving of the lambing date to the spring and summer months in aseasonal sheep exerted a conclusive impact on the lactation parameters during the milking period. Moreover, these results show that the lactation parameters are closely linked with the secretion of melatonin and prolactin. The literature data on the course of lactation in the Merino sheep lambed at various dates is scarce because the aseasonal characteristics of the race seemed to suggest, that the length of the photoperiod should not have a significant effect on milk production. Because of the absence of data on melatonin and prolactin profiles in lactating sheep, the results of this study can be related only to observations made in barren sheep (LINCOLN et al., 1995; MISZTAL et al., 1997). There is an interesting observation of the fact that the melatonin secretion in the aseasonal sheep studied, maintained similar levels from February till October, and that despite this, there was a drop in prolactin concentration and an inhibition of milk synthesis during the period of shortening daylight. This type of relationship can only be explained by the increasing role played by the biochemical signal from melatonin, prolonged in the period of shortening daylight and therefore resulting in decreased secretion of prolactin in response to it (REITER, 1991a; MORGAN et al., 1992; TRICOIRE et al., 2002).

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Prion protein (PrP) gene polymorphisms and breeding for resistance to scrapie in Polish Merino sheep

Abstract

Genetic susceptibility or resistance of sheep to scrapie is associated with polymorphisms particularly in the three codons 136, 154 and 171 of the ovine prion protein (PrP) gene. The aim of the study was to reveal the PrP haplotype and genotype distribution in the Polish Merino breed and to make a forecast of the application of a scrapie resistance breeding programme for this breed. PCR-RFLP analyses were done in order to determine PrP haplotypes based on the polymorphisms in codons 136, 154 and 171. The analysis of 98 Polish Merino sheep revealed dimorphisms only at the two codons 136 (A and V) and 171 (R and Q). 35.7% of the individuals harboured the genotype ARQ/ARQ, and 54.1% carried the genotype ARR/ARQ. The frequency of the ARR/ARR genotype was low (7.1%). A simulation study was done in order to calculate assumed frequencies of the PrP genotypes in the next generations. For the first generation the observed allele frequencies, after excluding ARQ/ARQ rams, were used in the study. For further breeding only ARR/ARR rams were used and PrP genotypes of female progeny were not considered. Employing this strategy in the Polish Merino it is possible to increase the ARR/ARR genotype frequency up to 87% in F_4.

Key Words: resistance to scrapie, prion protein gene, Polish Merino

Introduction

Scrapie is a fatal neurodegenerative disease of sheep and goats that belongs to the group of transmissible spongiform encephalopathies (TSEs). The causative agent of scrapie (called PrP^{sc}) is believed to be a pathological form of host-encoded cellular prion protein PrP^{c}. PrP^{sc} is its conformational isoform which is infectious and resistant to proteolytic enzymes (PRUSINER, 1998).

In the ovine prion protein (PrP) gene, polymorphisms have been observed in various codons, e. g. in codons 112, 127, 136, 137, 138, 141, 143, 151, 154, 171, 176 and 211 (LAPLANCHE et al., 1993; BELT et al., 1995; BOSSERS et al., 1999; ELSEN et al.,1999; TRANULIS et al., 1999; VACCARI et al., 2001). Previous studies showed that the five PrP haplotypes (in the following called "alleles") $A_{136}R_{154}R_{171}$ (ARR), ARQ, AHQ, ARH and VRQ are closely associated with genetic resistance or susceptibility to naturally acquired and experimentally induced scrapie in sheep (MACIULIS et al., 1992; GOLDMANN et al., 1994; BELT et al., 1995; HUNTER et al., 1996). These alleles are based on variations at the three codons 136 (alanine to valine, A/V), 154 (arginine to histidine, R/H) and 171 (glutamine to histidine or arginine, Q/H/R). In general, the VRQ allele, or the ARQ allele in breeds where the VRQ allele is rare, is linked to the highest risk of scrapie. In contrast, the genotype ARR/ARR confers the lowest susceptibility to scrapie (HUNTER et al., 1996; HUNTER et al., 1997; DAWSON et al., 1998). However, it has to be pointed out that these findings relate to classical scrapie and do not apply completely to recently identified atypical scrapie cases (BENESTAD et al., 2003; BUSCHMANN et al., 2004; MOUM et al., 2005). The epidemiological importance of atypical scrapie has not been clarified up to now (MOUM et al., 2005).

There is a possibility that feed additives for cattle are scrapie-infected, therefore after consumption cattle are prone to develop bovine spongiform encephalopathy (WILESMITH and WELLS, 1991). A new variant of Creutzfeld-Jacob disease in humans is highly possible to be epidemiologically linked with BSE, therefore scrapie should be considered also as a potential threat for public health (BROWN, 1997). For this reason, programmes were started in member states of the European Union (regulation 100/2003/EC) aiming at breeding for scrapie resistant sheep by selecting for the ARR allele and simultaneous elimination of the VRQ allele.
Hitherto no case of scrapie has been detected in Poland. However, occurrence of ovine scrapie in neighbouring countries like Germany and Slovakia (MATÚŠKOVÁ et al., 2003; LÜHKEN et al., 2004; GRETZSCHEL et al., 2005) reminded to consider scrapie resistance breeding schemes for the Polish sheep population. The most advantageous approach is to construct breed-specific scrapie resistance breeding schemes based on PrP allele and genotype frequencies. For example for breeds with low frequency of the ARR allele or lack of ARR homozygous rams breeding recommendations should be different than for breeds in which the ARR allele is predominant (DRÖGEMÜLLER et al., 2001a).
The aim of the study was to investigate the occurrence and frequencies of PrP alleles in the Polish Merino breed and to make a forecast of the application of a scrapie resistance breeding programme for this breed.

Materials and Methods

The investigation was carried out with a total of 98 healthy sheep (8 rams, 90 ewes) of four scrapie-unsuspected Polish Merino flocks from the kujawsko-pomorskie region, including all rams (n = 1 – 4) and 25% randomly chosen ewes (n = 15 – 38) of each flock. Blood from jugular vein was collected in ethylenediaminetetraacetic acid (EDTA)-treated plastic tubes. High molecular weight DNA was extracted from the whole blood using MasterPure™ DNA Purification Kit for Blood (Epicentre Technologies) following manufacturer's instructions. To identify 15 PrP genotypes based on polymorphisms at codons 136 (A/V), 154 (R/H) and 171 (Q/H/R) two RFLP analyses using restriction enzymes (New England Biolabs) *Bsp*HI and *Bsp*DI (LÜHKEN et al., 2004) were employed after optimizing modifications. Additionally, the polymorphism at codon 171 of the ovine *PRNP* was determined by a third RFLP analysis using restriction enzyme *Bse*LI (Fermentas). PCR amplification of a 144 bp fragment was performed in 25 µl reaction volume containing approximately 200 ng of genomic DNA, 10 pmol of each primer, forward 5'-GTGTACTACAGACCCGTGGA-3' and reverse 5'-TCGCTCCATTATCTTGATGT-3', 200 µM of each dNTP, 2.0 mM $MgCl_2$ and 1 U *Taq* polymerase (Fermentas) in 1-fold reaction buffer. Temperature profile of the reaction was: denaturation at 94°C for 1.5 min followed by 45 amplification cycles of 15 s at 94°C, 20 s at 56°C, 30 s at 72°C and final extension of 5 min at 72°C. For digestion of the 144 bp fragment with restriction enzyme *Bse*LI samples were incubated for 4 h at 55°C. Electrophoresis was done as described by LÜHKEN et al. (2004). After the digestion with *Bse*LI four fragments can be observed: 81 bp and 20 bp fragments which occur in the presence of arginine at codon 171, a 101 bp fragment which occurs in the presence of other alleles and a 43 bp fragment which serve as a positive control for restriction digestion. In order to verify

results of PCR-RFLP reactions, 15% of the samples were genotyped by direct sequencing of PCR products (LÜHKEN et al., 2004).
In a simulation study estimated allele frequencies were used to calculate expected genotype frequencies in the generation F_1. It was assumed that female progeny was not selected for PrP genotypes and the breeding values for performance traits were randomly distributed over all PrP genotypes. Simulated breeding for ARR-homozygous animals was initiated by the exclusive utilization of ARR/ARR and ARR/ARQ rams. In order to generate F_2, F_3 and F_4 generation only ARR homozygous rams were used. Allele frequencies in the group of F_1 ewes were used to calculate genotype frequencies in the generation F_2. Employing this strategy, expected genotype frequencies in generation F_3 and F_4 were estimated.

Results

The analysis of 98 Polish Merino sheep revealed dimorphisms only at the two codons 136 (A and V) and 171 (R and Q). There was no variation in codon 154, therefore all animals were homozygous for arginine (R) at this position. Moreover, no animal was carrying the histidine (H) encoding allele at codon 171. Consequently, only three of five important PrP alleles were found in the investigated sheep: ARR, ARQ and VRQ. A total of five different genotypes were identified: ARR/ARR, ARR/ARQ, ARQ/ARQ, ARR/VRQ and ARQ/VRQ, including genotypes linked to the highest scrapie susceptibility. PrP allele and genotype frequencies in the Polish Merino are shown in Tables 1 and 2, respectively.

Table1
Frequencies (%) of prion protein (PrP) alleles in the Polish Merino breed in respect of flock and sex

Allele Flock	ARR	ARQ	VRQ
Flock 1	33.3 ♀ 32.1 ♂ 50.0	66.7 ♀ 67.9 ♂ 50.0	0 ♀ 0 ♂ 0
Flock 2	26.2 ♀ 28.9 ♂ 0	73.8 ♀ 71.1 ♂ 100	0 ♀ 0 ♂ 0
Flock 3	50.0 ♀ 50.0 ♂ 50.0	50.0 ♀ 50.0 ♂ 50.0	0 ♀ 0 ♂ 0
Flock 4	32.9 ♀ 31.1 ♂ 50.0	63.4 ♀ 64.9 ♂ 50.0	3.7 ♀ 4.0 ♂ 0
Total	35.2 ♀ 35.0 ♂ 37.5	63.3 ♀ 63.3 ♂ 62.5	1.5 ♀ 1.7 ♂ 0

The ARQ allele was predominant with a mean of 63.3%, ranging from 50.0% to 73.8% between the flocks. The mean frequency of the ARR allele was lower (35.2%), ranging from 26.2% to 50.0%. The VRQ allele was very rare and appeared in only three animals (1.5%), originating from the same flock (Table 1). Furthermore, 35.7% of individuals harboured the genotype ARQ/ARQ and 54.1% of the sheep carried the ARQ allele in combination with the ARR allele (Table 2). The frequency of the ARR/ARR genotype was low (7.1%) (Table 2). Only one ram and six ewes carried this genotype. In the group of rams three genotypes ARR/ARR, ARQ/ARQ and

ARR/ARQ were detected, with percentage frequencies of 12.5, 37.5 and 50.0, respectively (Table 2).

Table 2
Frequencies (%) of prion protein (PrP) genotypes in the Polish Merino breed in respect of flock and sex

Genotype Flock	ARR/ARR	ARR/ARQ	ARQ/ARQ	ARR/VRQ	ARQ/VRQ
Flock 1	0 ♀ 0 ♂ 0	66.7 ♀ 64.3 ♂ 100	33.3 ♀ 35.7 ♂ 0	0 ♀ 0 ♂ 0	0 ♀ 0 ♂ 0
Flock 2	4.8 ♀ 5.3 ♂ 0	42.9 ♀ 47.4 ♂ 0	52.4 ♀ 47.4 ♂ 100	0 ♀ 0 ♂ 0	0 ♀ 0 ♂ 0
Flock 3	14.3 ♀ 15 ♂ 0	71.4 ♀ 70 ♂ 100	14.3 ♀ 15 ♂ 0	0 ♀ 0 ♂ 0	0 ♀ 0 ♂ 0
Flock 4	7.3 ♀ 5.4 ♂ 25.0	46.3 ♀ 45.9 ♂ 50.0	39.0 ♀ 40.5 ♂ 25.0	4.9 ♀ 5.4 ♂ 0	2.4 ♀ 2.7 ♂ 0
Total	7.1 ♀ 6.7 ♂ 12.5	54.1 ♀ 54.4 ♂ 50.0	35.7 ♀ 35.6 ♂ 37.5	2.0 ♀ 2.2 ♂ 0	1.0 ♀ 1.1 ♂ 0

Results of the simulation study are shown in Table 3. The ARR/ARR genotype frequency in generation F_1 was three times higher (21%) compared to the actual population (7.1%). In the next generations, the simulated breeding programme led to an instant increase in the frequency of the ARR allele. Consequently, expected frequencies of the ARR/ARR genotype in the second, third and fourth generation were 47.5%, 73.7% and 86.9%, respectively.

Table 3
Calculated frequencies (%) of prion protein (PrP) genotypes in the next generations in the Polish Merino breed (simulation study)

PrP genotypes	Frequencies (%) of PrP genotypes in generation:			
	F_1	F_2	F_3	F_4
ARR/ARR	21.0	47.5	73.7	86.9
ARR/ARQ	52.0	51.7	25.8	12.9
ARQ/ARQ	25.3	0	0	0
ARQ/VRQ	0.7	0	0	0
ARR/VRQ	1.0	0.8	0.4	0.2

Discussion

The results of this investigation provide data on the PrP gene polymorphisms in codons 136, 154 and 171 in the Polish Merino breed. Regarding field studies in scrapie-affected countries, among the three different PrP alleles identified in the Polish Merino, the ARR allele is linked with the lowest susceptibility to classical scrapie while the remaining two alleles ARQ and VRQ are connected with a high risk of classical scrapie (HUNTER et al., 1996; HUNTER et al., 1997; DAWSON et al., 1998). Similar occurrence but different frequency of alleles in the Polish Merino breed were detected by NIŻNIKOWSKI et al. (2006). In this study a higher frequency of the ARR allele (56.5%) and a lower frequency of the ARQ allele (41.9%) were detected. These deviations in the pattern of PrP genotypes might be caused by a smaller group of tested animals in NIŻNIKOWSKI et al. (2006) study and/or flock effects in our study. Differences in the genotype frequencies may also be a result of the lack of

exchange of rams between flocks. Comparing our data with German Merinoland and Merino Longwool, the ARQ allele occurred also predominantly in these breeds but in much higher frequencies of 85.4% and 76%, respectively (DRÖGEMÜLLER et al., 2001a). In contrast to the Polish Merino the VRQ allele was absent in German Merinoland and Merino Longwool (DRÖGEMÜLLER et al., 2001a). Moreover, the AHQ allele recently described to be linked with high susceptibility to atypical scrapie (BENESTAD et al., 2003; MOUM et al., 2005) was detected in these two German Merino breeds (DRÖGEMÜLLER et al., 2001a, b) but not in the Polish Merino, either in this study nor by NIŻNIKOWSKI et al. (2006). This difference may be a result of different breeding history of the German Merino breeds compared to the Polish Merino.

Based on the determined PrP allele and genotype frequencies in the Polish Merino and taking into consideration the scrapie resistance breeding schemes applied in other European countries, first indications for breeding the Polish Merino sheep with low susceptibility to scrapie can be done. In general, the scrapie resistance breeding schemes focus on the elimination of alleles linked with the high scrapie susceptibility and gradual increasing of the ARR allele frequency, especially in order to obtain ARR homozygous rams. Regarding the fact that ARQ and VRQ alleles are linked with high risk of classical scrapie, a breeding programme should first aim at decreasing the frequency of ARQ and eradicating the VRQ allele in the Polish Merino breed. In order to increase the ARR allele frequency in Polish Merino only ARR homozygous and heterozygous rams, depending on their availability, should be taken in the initial step of the scrapie resistance programme.

To our knowledge this is the first simulation study of applying a scrapie resistance breeding programme in the Polish Merino breed. According to the obtained results and considering the even more promising data from NIŻNIKOWSKI et al. (2006) for the same breed, it should be possible to increase the ARR allele frequency in the Polish Merino rapidly. However, employing the scrapie resistance programme for this breed will be connected with additional costs for breeders, caused by the necessity of genotyping rams and the need of exchange ARQ/ARQ rams for the ARR allele carriers. In addition, the value of PrP genotyped rams may increase or decrease, depending on their genotype. Taking into consideration the high frequency (64.8%) of the glutamine encoding allele at codon 171 that is associated with high risk of scrapie (HUNTER et al.,1996, 1997; DAWSON et al., 1998), the population of the Polish Merino has to be regarded as susceptible to scrapie. Therefore applying a scrapie resistance programme in this breed makes sense, especially regarding the fact that scrapie is endemic in various European countries (e. g. MATÚŠKOVÁ et al., 2003; ACIN et al., 2004; BAYLIS et al., 2004; GRETZSCHEL et al., 2005) and that present regulations of the European Community aim at eradicating ovine scrapie by selecting for the ARR allele.

New breeding schemes that will focus on scrapie resistance should also regard the probability of unfavourable genetic correlation between the PrP alleles and genotypes and production traits. Field studies undertaken in different European countries showed no unfavourable significant associations between PrP alleles and genotypes and meat performance traits in German meat sheep breeds and no significant influence of these alleles and genotypes on a lean Growth Index Score in the British Suffolk sheep (PROKOPOVÁ et al., 2002; DE VRIES et al., 2004a). However, weak associations

between PrP genotypes and some traits, i.e. litter size and 135-day weight in Texel, multiple births in Suffolk or total number of lambs born in some German breeds were found (BRANDSMA et al., 2004; ALEXANDER et al., 2005; DE VRIES et al., 2004b). Taking into consideration that not all breeds and traits have been investigated hitherto and considering differences between breeds, a scrapie resistance breeding programme for the Polish Merino and the other Polish sheep breeds should be managed carefully with simultaneous monitoring of correlations between PrP genotypes and production traits.

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