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Transferrin Polymorphism versus Growth Rate in Lambs, Polish Long-wool Sheep

I. Frequency of genes and genotypes of transferrin in flock of Polish Long-wool Sheep

Summary

The subject of this study was to identify the transferrin polymorphism and analyse changes in the frequency of alleles and genotypes of the transferrin in a flock of Polish long-wool sheep on the grounds of lambs from the four subsequent years. The flock comprised 3419 lambs, either sex, from the four subsequent lambings, (i - 920 off, ii - 849 off, iii - 914 and iv - 736 off accordingly). The separation of transferrin variants was done using starch gel electrophoresis. The significant increase in the frequency of BD transferrin phenotype was observed, CC and CE phenotypes, however, decreased and the frequency of Tf BD phenotype gradually dropped. The flock selected for quality traits exhibited a significant growth in the frequency of Tf⁰ allele in subsequent years of the study, whereas the frequency of Tf² allele decreased. The frequency of remaining alleles varied in certain years of the study and no distinguished changes were found. The homozygosity of the polymorphic transferrin system increased in the third year of the study but then dropped its value in the fourth year below the value noted in the first year. Regularities of changes in the frequency of phenotypes and alleles of transferrin in the flock concerned could mark changes in the breeding - selecting scheme.

Key words: Polish long - wool sheep, transferrin polymorphism

Zusammenfassung

Titel der Arbeit: Transferrinpolymorphismus und Wachstum von Lämmern der Rasse Polnisches Langwollschaf. 1. Mitt.: Transferrinvarianten beim Polnischen Langwollschaf

Der Transferrinpolymorphismus wurde untersucht und die Analyse der Frequenzveränderung der Transferrinallele und -genotypen in einer Schafherde der Rasse Polnisches Langwollschaf in vier aufeinanderfolgenden Jahrgängen (I bis IV) durchgeführt. Das Untersuchungsmaterial bestand aus 3419 Lämmern beiderlei Geschlechts und aus 4 Ablammungen (I – 920, II - 849, III - 914, IV - 736 Stück). Das zu untersuchende Blut wurde von den Lämmern im Alter von 5-6 Monaten entnommen. Eine deutliche Zunahme der Frequenz der Transferrin BD bei der gleichzeitig kontinuierlichen Abnahme der Frequenz der Phänotypen CC und CE sowie bei einer langsameren ständigen Abnahme der Frequenz des Phänotypus Tf BC wurde nachgewiesen. In der nach Nutzeigenschaften selektierten Herde stieg in den folgenden Jahren die Frequenz des Allels Tf^D signifikant. Die Frequenz des Allels Tf^C nahm dagegen ab. Die Frequenz der übrigen Allele schwankte in den einzelnen Jahren, ohne deutliche Veränderungen. Die Homozygotie des polymorphen Transferrinsystems stieg im dritten Untersuchungsjahr an, und sank dagegen im vierten Untersuchungsjahr auf einen Wert, der niedriger als im ersten Untersuchungsjahr war. Die festgestellten Regelmäßigkeiten in der Frequenzveränderung der Transferrinphänotypen und -allele in der untersuchten Population Polnischer Langwollschafe könnnen ein Anzeiger der Veränderungen bei der Durchführung des Zucht- und Selektionsprogramms sein.

Schlüsselwörter: Polnisches Langwollschaf, Polymorphismus, Transferrin

Introduction

The structural variability of the proteins which exhibit polymorphism due to genetic conditions is determined by a pair or series of multiple alleles. Transferrin (Tf) is one of such proteins. The locus of transferrin is found on the first chromosome in sheep and cattle (ECHARD et al., 1994) whereas on the thirteenth in pigs (CHOWDHARY et al., 1993; GUERIN et al., 1993; EDFORS-LILJA et al., 1995). The first to study transferrin polymorphism of the sheep blood serum was ASHTON, (1958; 1958a) who found 14 phenotypes controlled by five alleles (TfA, TfB, TfC, TfD, TfE) in sheep of English breeds. Today twelve transferrin alleles are identified according to decreasing electrophoretic mobility in the starch gel: Tfl, Tfl, TfG, Tfl, TfB, TfK, TfC, TfM, TfD, TfQ, TfE and TfP, (ARCHIBALD and WEBSTER, 1986; ERHARDT, 1986; KURYŁ, 1992; RYCHLIK et al., 1996). The transferrin polymorphism was used to identify inter-breed differences and made it possible to establish genetic differentiation within breeds, kinds and generations of animals due to a great variety of frequencies of certain genes and genotypes of transferrin, (BOJCZUK et al., 1988; BALOV and ALEKSEJEVA, 1989; GLAZKO, 1990; KENJI TSUNODA et al., 1990; RODERO et al., 1990; SHIQAN WANG and FOOTE, 1990; UKBAJEV et al., 1990; NGUYEN et al., 1992; TATE et al., 1992; BOJCZUK and BOJCZUK, 1994; KMIEĆ, 1995). The aim of this study was to analyse changes in the frequency of genes and genotypes of transferrin in a flock of the Polish long-wool sheep on the grounds of four subsequent lambings.

Materials and Methods

This study was carried out for four subsequent years in a pedigree flock of the Polish long-wool sheep kept at The State Animal Breeding Station at Bobrowniki. The study flock comprised 3419 lambs, either sex, from the four subsequent lambings - 61,5% twins - (i - 920 out of 1160 born, ii - 849 out of 1128 born, iii - 914 out of 1181 born, iv - 736 out of 1061 born accordingly). There were 34 rams responsible for the first and second lambings, 35 for the third and 36 for the fourth respectively. The lambs concerned formed half-sibling groups of comparable size. The serum was separated from the blood taken to a dry test-tube from the zygomatic vein of lambs at the age of 5-6 months. Transferrin phenotypes were determined by horizontal electrophoresis in starch gel, (SMITHIES, 1955), with the use of combined buffers, (KRISTJANSSON, 1962; KHATTAB et al., 1963). Genotypes of the animals in study were identified on the grounds of the direct interpretation of phenotypes, i.e. results of electroforetic determination, and the codomining of alleles affecting the transferrin polymorphism was taken into consideration. The frequency and number of expected transferrin phenotypes were calculated according to the mathematical formula of Hardy-Weinberg law, the significance verified by χ^2 test, (ŻUK, 1989).

Results and Disussion

Fifteen transferrin phenotypes controlled by the following alleles TfA, TfB, TfC, TfD

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and Tf^E (out of 12 to be possibly found using the method applied) were found in the flock of long-wool sheep. The most frequent phenotypes throughout the whole population in the entire study period were Tf BC (19,43-24,24%), Tf BD (14,78-24,59%), Tf CD (12,69-17,80%) and Tf BB (12,25-14,13%). The least frequent, however, were the following transferrin phenotypes: AA, AE and EE (frequency below 1%), see Table 1. The flock exhibited numerous significant differences (P \leq 0,05 and P ≤ 0,01) of phenotype frequencies between certain years of the study as far as AD, AE, BC, BD, BE, CC, CD, CE, DD and DE phenotypes were concerned. The statistically significant decrease (P

0,01) of the frequency of BC transferrin phenotypes (from 24,24 down to 19,43%), of Tf CC (from 11,52 down to 7,61%) and of Tf CE (from 2,61 down to 0,54 %) was observed along with the steady increase of the frequency of Tf BD (from 14,78 up to 24,59%) - see Table 1. The analysis of the transferrin phenotype frequency in lamb rams showed the statistically significant differences (P \leq 0,05 and $P \le 0,01$) between certain years of the study as far as BC, BD, BE, CE and DD phenotypes were concerned. In that group of animals the steady decrease of the frequency of Tf AD, Tf BC and Tf CE phenotypes was observed along with the steady increase of the frequency of Tf BD - see Table 1. The statistically significant differences (P \leq 0,05 and P \leq 0,01) were also found in the frequency of transferring phenotypes in lamb ewes in the subsequent years of the study. The differences were observed for AB, AD, BD, BE, CC, CD, CE, DD and DE phenotypes - see Table 1. In that group of animals the steady increase of the Tf BD frequency (from 14,90 up to 25,98%) was observed along with the decrease of Tf CC frequency (from 12,42 down to 5,39%), the differences being statistically confirmed, ($P \le 0,01$).

The population of the first three years of lambs did not exhibit any upset of the genetic equilibrium between observed and calculated sizes of phenotype groups. In the fourth year, however, the statistically significant differences ($P \le 0,01$) were found between the observed and expected quantities of transferrin phenotypes - see Table 2. The similar upset of the genetic equilibrium was also observed in flocks of Sudanese (OSMAN, 1967) and Macedonian (EFREMOV and VASKOV, 1968) sheep respectively which was explained by selecting the flock for quality traits (OSMAN, 1967) or crossing to result in the increase of the number of phenotype groups, (EFREMOV and VASKOV, 1968).

The flock in study exhibited statistically significant differences ($P \le 0.05$ and ($P \le 0.01$) in the frequency of Tf^A , Tf^C , Tf^D and Tf^E alleles between certain years of the study - see Table 3. The Tf^A was more frequent ($P \le 0.01$) in the first, second and third years of the study than in the fourth year. The Tf^C was less and less frequent over subsequent years and the statistically significant differences ($P \le 0.05$ and $P \le 0.01$) were observed between the first year and the third and fourth years and between the second year and the third and fourth years of the study. The significant increase of the frequency was observed for the Tf^D (20,27 up to 27,79%) and statistically significant differences ($P \le 0.01$) were found between the first year and the third and fourth years and between the second year and the third and fourth years of the study - see Table 3. The analysis of the frequency of transferrin alleles in lamb rams showed statistically

Table 1 Frequency of transferrin phenotypes in animal groups in years of study (Frequenz der Transferrinphänotypen nach Tiergruppen und Jahren)

	7	n=736	00,00	3,13	2,58	1,09	0,00	14.13	19,43*	24 50ªBC	2210	7,512	1,01	17,80	0,54 ^B	5,30	1 401	1,43	00'0
	Ħ	n = 914	99'0	3,83	3,50	2,62	0.55	13.57	22 53	20.13	O SEABC	6,0	5,85	12,69°	1,20	7,77EAB	O SCEN	0,55	000
Total	п	n =849	0,12	4,24	3,77	2,59	0.24	12.25	22 704	17 00AC	17,00	2,/1-	11,19	13,78	2,47bB	4 473		1,18	0,12
	Ι	n = 920	0,11	3,80	3,70	2.93A	0.22	13 50	ANC NC	24,24	14,78	3,15	11,52	13,37^	2.61**	3 704	2000	2,06	0,22
ā	VI	n = 408	0.00	1.96*	2.70	1 224	000	12,72	17,00	20,54	22,982	2,45	5,3980	18,87bc	0.49D	515	2,0	1,72	00,00
	日	n=467	0.64	4.50°	4.50	3.214	0.43	14.56	14,70	24,03	19,49	0,000	7,49	11.99°	0 64°	7.30E	07,1	0,64°	000
Lamb ewes	п	n = 445	0.22	3,60	737	000	2,70	12,01	16,11	24,94	16,41	2,70€	11,24 ^c	13.26°	2 47eD	, c, c, b	20,0	1,12	0,22
		n = 483	000	4 55	1,0	2,17	1,11	17,0	13,87	22,77	14,90	3,11 ^D	12,42Bb	14.08	1 24	1,4,4	2,11	2.28°	0,21
	2	п=328	8	4.57	2,0	4,4	76,0	00,0	14,63	18,29	22,87 ^E	2,13	10,37	16.46	20,00	1000	5,49	1.22	00,00
	目	n = 447	230	0,0	0,10	2,40	10,2	19,0	12,53	20,36°	20,81 ^D	1,12*	12.30	12.47	27,01	1,19	8,78	0.45	0,00
Lamb rams	п	n=404	6	90,0	0,6,4	4,20	2,48	0,25	12,62	22,52	17,82	2.72	11 14	14.26	14,00	2,48	3,22	1 24	000
	1	n = 437		0,23	2,97	3,20	2,75	0,23	13,27	25,86bB	14.64DE	3.20	10.53	0,00	12,39	4,12~	4,35c	1 82	0,23
Dhenothne	Tf			¥.	AB	AC	AD	AE	BB	BC	BD	BE	1 6	3 (3	3	DD	מע	出出

Frequency in lines designated with the same letter differ significantly. Small letters denote significance at $P \le 0.05$; capitals at $P \le 0.01$.

Observed (obs.) and expected (exp.) number of transferrin phenotypes of differences in animal groups in study (Beobachteter und erwarteter Bestand der Transferrinphänotypen nach Tiergruppen und Jahren) Table 2

	33.	П obs/exp.	E									
		obs/exp.	=	≥	I	п	目	2	I	п	E	2
			obs/exp.	obs/exp.	Obs./ exp.	obs/exp.	obs/exp.	obs/exp.	Obs/exp.	obs/exp.	obs/exp.	obs/exp.
		0/ 1.4	3/ 1.0	0/ 0 5	0/ 17	1, 1,	1					
		20/17.6	14/152	15/10.0	22/ 21 2	2,1 1,2	5/ 2,3	0/ 0,4	1/ 2,7	1/ 2,6	6/ 3,2	0/ 0.8
		17/15.8	11/13.5	8/76	201 /00	16/ 16/	21/ 25,3	8/ 9,4	35/36,6	36/ 34,0	35/ 40,1	23/19,4
		10/10.2	9/114	3/ 68	15/ 110	5,01 /01	481 /17	11/6,4	34/ 33,5	32/31,1	32/32,0	19/13.9
		1/ 1.6	3/ 0.9	0,0%	17, 71	12 10,3	15/ 16,2	5/ 7,0	27/ 20,3	22/ 20,5	24/ 27,8	8/13.9
		51/54.2	56/55 5	48/48 8	1, 2,1	0,1 /1	2/ 0,6	9'0 /0	2/ 4,2	2/ 3,2	5/ 1,5	0/ 1.1
		91/97 4	01/08.7	60/740	0,40 1/01	25/ 26,8	68/ 70,5	56/62,4	125/123,1	104/111,0	124/125,7	104/111.1
BD 64/		72/62,6	93/83 9	75/663	72/ 71 6	32/ 21 .	115/103,0	83/84,8	223/225,3	202/203,2	206/202,1	143/158,9
		11/ 9.9	5/ 63	7/50	0,17 27	1,1/1/2	91/ 90,6	106/92,7	136/136,4	145/133,8	184/174,7	181/158,9
		45/43.8	55/43 8	3472 1	0,21 /07	1,11 /21	0/ 3,1	10/ 7,4	29/ 28,5	23/ 21,0	5/ 9.6	17/ 12.4
		58/563	60/74 5	54/50 2	0,40	20, 49,2	35/ 37,6	22/28,9	106/103,1	95/ 93,0	90/ 81.2	56/ 56.8
		10/89	8/56	2/200	7,00 /00	7,00 /40	26/ 66,1	77/63,0	123/124,9	117/122,5	116/140,4	131/113.6
		13/18 1	27/217	18/73.5	1,11	11/ 10,3	3/ 2,3	2/5,1	24/ 26,1	21/ 19,2	11/ 7,8	4/ 80
		5/ 57	2/48	4/24	7,17	25/ 22,2	34/ 29,1	21/34,4	34/ 37,8	38/ 40,3	71/ 60,7	39/ 56.8
		0/08	2 6	100	17/ /1	6,9	3/ 2,0	7/ 5,5	19/ 15.5	10/ 12.6	5/ 67	11/ 20
		C.0 10	7,0 /0	0,0	1/ 0,6	1/ 0,5	0′0 /0	0/ 0,2	2/ 1,7	1/ 1,0	0/ 0,2	0,0
го п.S.	S.	п.S.	п.S.	n.s.	п.s.	n.s.	n.s.		n.s.	D.S.		

- significance of differences at P ≤ 0,05; •• - significance of differences at P ≤ 0,01; n.s. - non-significant

significant differences for Tf^A , Tf^D , Tf^E alleles between certain years of the study ($P \le 0.05$ and $P \le 0.01$). In that group of animals the steady decrease of the frequency of Tf^C and Tf^E was observed along with the increase of the Tf^D frequency - see Table 3. The statistically significant differences ($P \le 0.05$ and $P \le 0.01$) were also found in the frequency of Tf^A , Tf^C , Tf^D and Tf^E in lamb ewes in the years of the study - see Table 3. That group of animals exhibited the steady increase of the frequency of Tf^D (from 20,29 up to 29,05%) along with the decrease of the frequency of Tf^C (from 33,54 down to 26,59%), the differences being statistically confirmed at $P \le 0.01$. Frequencies of other alleles varied in subsequent years of the study, the significant differences ($P \le 0.05$ and $P \le 0.01$) concerned Tf^A and Tf^E alleles.

Table 3
Frequency of transferrine alleles in animal groups in years of study (Frequenz der Transferrinallele nach

gruppen und Jah Group	Year	Tf ^A	Tf ^B	Tf ^c	Tf ^o	Tf ^{ti}
		4.01	36,61	33,41	20,25 ^{EF}	4,92 ^{DE}
	1	4,81	36,63	32,92	21,17aG	3,34
Ram lambs	11	5,94	35,24	31,32	26,62 ^{EG}	2,01 ^D
	Ш	4,81		29,27	26,22aF	1,98 ^E
	IV	3,96	38,57	29,21	20,22	
	1	6,01 ^D	36,54	33,54 ^{DE}	20,29 ^{HI}	3,62 ^F
Ewe lambs	II	5,17°	35,73	33,26 ^{bF}	22,36 ^J	3,48 ^G
Ewe lamos	III	6,96 ^E	38,86	28,37 ^{bD}	24,95 ^{bH}	0,86 ^{FGI}
	IV	2,94 ^{aDE}	39,09	26,59 ^{EF}	29,05 ^{blJ}	2,33 ^H
				30-90/48940		4 2 4AB
	I	5,43 [^]	36,58	33,48 ^{AB}	20,27 ^{AB}	4,24 ^{AB}
Total	H	5,54 ^B	36,16	33,10 ^{aC}	21,79 ^{CD}	3,41ªC
a court	III	5,91 ^c	37,09	29,81ªA	25,77 ^{AC}	1,42 ^{AC}
	IV	3,39 ^{ABC}	38,86	27,79 ^{BC}	27,79 ^{BD}	2,17 ^{aB}

Frequency in columns designated with the same letter differ significantly. Small letters denote significance at $P \le 0.05$; capitals at $P \le 0.01$.

Table 4 shows frequencies of homozygotes and heterozygotes as far as transferrin genotypes are concerned. Homozygotous genotypes contributed from 27,04 to 31,84% types of transferrin in the study flock. Statistically significant differences ($P \le 0,05$) were noted for homozygotous genotypes of transferrin between the third and fourth years of lambs. Having analysed the ceratin sex within the flock in terms of the frequency of hetero- and homozygotous transferrin genotypes the statistically significant differences ($P \le 0,05$) were found between the second and third years of lamb rams, between the third and fourth years of lamb ewes and between the lamb rams and lamb ewes in the fourth year of the study. - see Table 5. Heterozygotes in the flock of the long - wool sheep amounted to 70%, variations, however, were observed in the certain groups, i.e. from 66,22% (lamb rams, third year) to 75,74% (lamb ewes, fourth year). Domestic flocks exhibited higher share of heterozygotes and the regularity was confirmed by a number of researchers, (LIPECKA, 1984; KMIEĆ, 1986; PORĘBSKA et al., 1990). Discrepancies between quantities of homozygotes and heterozygotes in the population of the study force researchers to keep on studying the

Table 4
Frequency of homo- and heterozygous transferrin phenotypes in animal groups in study (Frequenz der homound heterozygoten Transferrinphänotypen nach Tiergruppen und Jahren)

Group	Year	Number	Hor	nozygous	Hete	rozygous
		of animals	n	Frequency [%]	n	Frequency [%]
	I	437	125	28,60	312	71,40
Ram lambs	II	404	109	26,98b	295	73,02
	Ш	447	151	33,78 ^b	296	66,22
	IV	328	100	30,49°	228	69,51
	I	483	143	29,61	340	70,39
Ewe lambs	II	445	130	29,21	315	70,79
	III	467	140	29,98°	327	70,02
	IV	408	99	24,26 ^{ce}	309	75,74
	I	920	268	29,13	652	70,87
Total	II	849	239	28,15	610	71,85
	III	914	291	31,84*	623	68,16
	IV	736	199	27,04*	537	72,96

Frequency in columns designated with the same letter differ significantly. Small lettes denote significance at $P \le 0.05$; capitals at $P \le 0.01$.

Table 5
Observed and expected numbers of homozygous/heterozygous ref. to transferrin phenotype in groups and years of study (Beobachteter und erwarteter Bestand der Homo- und Heterozygotie hinsichtlich des Transferrinsphänotypus nach Tiergruppen und Jahren)

Group	Year	Total obs. / exp.	Homozygous obs. / exp.	Heterozygous obs. / exp.	Significance of differences
	I	437 / 437,0	125 / 127,3	312 / 309,7	n.s.
Ram lambs	II	404 / 404,0	109 / 118,0	295 / 286,0	n.s.
	Ш	447 / 447,0	151 / 132,2	296 / 314,8	*
	IV	328 / 328,0	100 / 100,1	228 / 227,9	n.s.
	Î	483 / 483,0	143 / 141,1	340 / 341,9	n.s.
Ewe lambs	II	445 / 445,0	130 / 130,0	315 / 315,0	n.s.
	Ш	467 / 467,0	140 / 139,5	327 / 327,5	n.s.
	IV	408 / 408,0	99 / 126,2	309 / 281,8	**
	I	920 / 920,0	268 / 268,4	652 / 651,6	n.s.
Total	II	849 / 849,0	239 / 247,9	610 / 601,1	n.s.
T-T-6772)	Ш	914 / 914,0	291 / 271,0	623 / 643,0	n.s.
	IV	736 / 736,0	199 / 226,0	537 / 510,0	*

^{* -} significance of differences at $P \le 0.05$; ** - significance of differences at $P \le 0.01$; n.s. - non-significant.

problem. They may be caused by a lower survival rate exhibited by homozygotous embryos, (ASHTON, 1959), selection in the flock (COOPER, 1966; EFREMOV and VASKOV, 1968) or prevailance of a certain type in accordance with breeding conditions, (RASMUSEN and TUCKER, 1973). The analysis of the population for quantities of homo- and heterozygous genotypes of transferrin showed differences between theoretical and observed values in the forth year of the study ($P \le 0.05$) as well as in lamb rams ($P \le 0.05$), third year of the study and in lamb ewes ($P \le 0.01$), fourth year of the study. It seems that the upset of the genetic equilibrium in the flock is caused by breding - selecting activities in the parent flock.

Conclusions

Results obtained may show that there were significant changes in the frequency of transferrin phenotypes in the flock of the Polish long - wool sheep, high wool yield flock. The significant increase in the frequency of BD transferrin phenotype was observed along with the steady drop of frequencies of CC and CE accordingly and the slighlty slower steady decrease in the frequency of the Tf BC. The significant increase in the frequency of Tf^D allele was observed in the subsequent four years of the study in the flock selected for quality traits whereas the Tf^C frequency dropped in the same time. The frequency of other alleles varied in certian years of the study and significant changes were not found. The homozygosity of the polymorphic system of transferrin increased in the third year of the study, and then dropped in the fourth year to reach the lower level than in the first year.

Regularities found in changes in the frequencies of phenotypes nad transferrin alleles in the study flock of the long-wool sheep may be markers of changes that associate the breeding - selecting schedule. The regular growth in the frequency of BD transferrin phenotype along with the drop in the Tf CC and rather slow but steady decrease in the frequency of Tf BC and Tf CE were found.

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