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Possible new technologies to estimate the muscle fiber number for use in animal production

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Archives of Animal Breeding A Archiv für Tierzucht

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PREFACE

European meat and fish producers are continuously challenged for efficiency of production, animal welfare and meat/fish quality. In meat/fish production muscle growth is the most important trait of the production economy and the muscle growth rate may influence the quality of the meat produced. Muscle fibres are formed during foetal development, and number and hypertrophic growth determines the growth rate of the animal to a large extend. The number of muscle fibre formed during foetal development is directly related to postnatal muscle growth. However, because studies on the number of muscle fibres are tedious and costly it is important that research in this area is complementary instead of duplicating. An initiative was consequently taken to initiate a COST Action entitled "The importance of prenatal events for postnatal muscle growth in relation to the quality of muscle based foods". The main purpose of the Action is to explain genetic and environmental variation in prenatal events (myogenesis and satellite cell behaviour) in an attempt to find new and alternative methods to be used in selection for optimising postnatal growth and meat/fish quality. Moreover the objectives are firstly to pass on the increased knowledge in this area to the scientific community, primary producers, and the derived food industry, and secondly to stimulate research, education, exchange of knowledge, technical experiences, and the mobility among scientists within the participating countries of this Action.

The present Action (COST Action 925) started in 2004, and the first three working group meetings were held in Oporto, Portugal, at the University of Thessaly, Greece and in Viborg, Denmark. Results from these meetings were published in Archives of Animal Breeding, Volume 48 (2005), in Archives of Animal Breeding, Volume 49 (2006) and Archives of Animal Breeding, Volume 50 (2007), respectively.

The fourth working group meeting was held in Viborg, Denmark. The purpose of this meeting was to fulfill the fourth milestone of the Action "*Possible new technologies to estimate the muscle fibre number for use in animal production"* and was carried out in serial sessions covering the two working groups:

- ***WG 1****: Environmental variation in prenatal events in relation to postnatal growth and meat/fish quality*
- ***WG 2****: Genetic variation in prenatal events and its effect on postnatal growth and meat/fish quality*

This Special Issue of Archives of Animal Breeding contains the contributions of the participants to the sessions of the two working groups presented as one invited paper, 16 oral contribution and 20 posters that report on current scientific activities and results. Furthermore, a workshop on the relationship between gene expression and muscle fibre number was held followed by plenary discussion.

We acknowledge and thank all the authors for their contribution to the sessions at the meeting and to this Special Issue. Furthermore, we appreciate COST for making this work possible. Finally, we thank the Editor-in-Chief and the Editorial Board of Archives of Animal Breeding for the opportunity to publish the contributions of this working group meeting under COST Action 925.

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Intrauterine growth retardation in livestock: Implications, mechanisms and solutions

(Intrauterine Wachstumsretardierung beim Nutztier: Implikationen, Mechanismen und Lösungen)

Abstract

Intrauterine growth retardation (IUGR) is a significant problem in livestock production. It adversely affects neonatal survival, postnatal growth performance, efficiency of feed utilization, tissue composition (including protein, fat and minerals), meat quality, long-term health of offspring, and adult onset of disease. Genetic, epigenetic and environmental factors (including nutrition), as well as maternal maturity impact on the size and functional capacity of the placenta, placental vascular growth, uteroplacental blood flows, transfer of nutrients from mother to fetus, the endocrine milieu, as well as embryonic development of myocytes, adipocytes and other cell types. Growing evidence suggests that arginine-derived signaling molecules (nitric oxide and polyamines) play an important role in regulating these key physiological and biochemical processes. Thus, modulating arginine-metabolic pathways can enhance embryonic/fetal survival and growth, and provide a useful approach to prevent and treat IUGR.

Keywords: arginine, fetal growth, livestock, placenta, postnatal growth

Zusammenfassung

Intrauterine Wachstumsretadierung ist ein bedeutendes Problem in der Tierproduktion mit negativen Auswirkungen auf die Lebensfähigkeit Neugeborener, postnatales Wachstum, Futterverwertung, Gewebezusammensetzung (Protein, Fett, Mineralien), Fleischbeschaffenheit und Gesundheit. Genetische, epigenetische und Umweltfaktoren sowie das Sauenalter beeinflussen die Größe und funktionelle Kapazität der Plazenta, ihre Durchblutung und den Transfer von Nährstoffen von der Mutter zum Fetus, das endokrine Milieu sowie die embryonale Entwicklung von Myozyten, Adipozyten und anderen Zellen. Arginin-abgeleitete Signalmoleküle (Stickoxide, Polyamine) spielen eine wichtige Rolle bei der Steuerung diese physiologischen und biochemischen Prozesse. Demnach kann die Modulation der Arginin-Stoffwechselwege die embryonale/fetale Überlebensrate und Wachstum verbessern und somit Ansätze zur Vermeidung und Behandlung intrauterine Wachstumsretadierung liefern.

Schlüsselwörter: Arginin, fetales Wachstum, Viehbestand, Plazenta, postnatales Wachstum

Introduction

Litter size in mammals is a maternal trait affected by complex factors, including ovulation rate, uterine capacity, and embryonic/fetal survival (WU et al., 2006). Interestingly, heritability of litter size is low, which has been estimated to be 0.09-0.11 in pigs (URBAN et al., 1966; LUND et al., 2002) and sheep (RAO and NOTTER, 2000). Modern highly prolific sows ovulate 20 to 30 oocytes, but only deliver 9 to 15 piglets at term because of high prenatal losses (TOWN et al., 2005). The first peak of losses occurs during the peri-implantation period between days 12 and 15 of gestation. Fetal losses seem to be highest around days 35-40 of gestation, followed by mid-gestation losses from days 55 to 75, and finally the period immediately before farrowing. The highest average fetal losses at day 35 occur in large litters (>10 fetuses), suggesting a relationship between the uterine capacity and fetal mortality (TOWN et al., 2005). The greatest restraint on litter size in pigs is placental development and

function in early gestation and uterine capacity at all periods of gestation, rather than simply the number of ovulations or embryos (BAZER et al., 1988). Because heritability for the number of piglets born alive is low (0.06-0.09; HALEY and LEE, 1992; LUND et al., 2002), improvement in litter size through animal breeding has been slow. Indeed, in the U.S., litter size in swine increased at the rate of 0.052 pigs/year between 1980 and 2000 (JOHNSON, 2000).

Among domestic animals, pigs exhibit the most severe naturally occurring IUGR (WU et al., 2006). Before day 35 of gestation, porcine embryos are uniformly distributed within each uterine horn. After this time in gestation, uterine capacity becomes a limiting factor for fetal growth even though fetuses are distributed relatively uniformly (BAZER et al., 1988). At birth, runt piglets may weigh only one-half or even one-third as much as the largest littermates, and key organs involved in nutrient digestion and utilization in runt pigs are disproportionately smaller than those of the larger littermates (WU et al., 2006). Placental insufficiency is a major factor contributing to low birth-weights of piglets. IUGR has permanent negative impacts on neonatal adjustment, preweaning survival, postnatal growth, feed utilization efficiency, lifetime health, tissue composition (including protein, fat and minerals), meat quality, as well as reproductive and athletic performance (WU et al., 2006). Because IUGR remains a significant problem in mammals as indicated for pigs (Table 1), increasing embryonic/ fetal survival, growth, and development is of enormous importance for optimizing livestock production.

Table 1
Litter size and birth weights of pigs

	Piglets born in Spring[1]	Piglets born in Fall[2]
Litter size (number of gilts), n	46	46
Total number of piglets born, n	516	505
Number of piglets born alive, n	484	453
Number of piglets born alive per litter, n	10.52 ± 0.14	9.84 ± 0.12*
Number of piglets born dead per litter, n	0.72 ± 0.03	1.13 ± 0.05*
Average birth weight of all piglets born, kg	1.35 ± 0.03	1.30 ± 0.02
Average birth weight of piglets born alive, kg	1.37 ± 0.03	1.32 ± 0.02
Percentage of piglets born dead, %	6.2	10.3*
Percentage of piglets born alive, %	93.8	89.7*
at various ranges of birth weights		
0.50-0.69 kg	2.6	4.1*
0.70-0.89 kg	6.8	9.2*
0.90-1.09 kg	12.5	14.4*
1.10-1.29 kg	32.8	28.9*
1.30-1.49 kg	27.6	26.9
1.50-1.69 kg	14.7	13.8
1.70-1.89 kg	3.0	2.7

Data are means ± SEM. Gilts (Yorkshire × Landrace dams and Duroc × Hampshire sires) were bred at ~8 months of age and fed daily a 2-kg diet (WU et al., 2005). Data were analyzed by ANOVA or Chi-square for randomized complete block design (MATEO et al., 2007).
*P<0.05 vs piglets born in Spring; [1]Piglets were born between March 22 and April 5 in 2003-2007; [2]Piglets were born between October 25 and November 7 in 2003-2007

Role of placental growth in embryonic/fetal development

The functional capacity of the placenta must be adequate for normal development of the fetus. Immediately after implantation of the conceptus, various genes are expressed to initiate placental formation (VONNAHME and FORD, 2004). Remarkably, the

placenta undergoes rapid formation of new blood vessels (angiogenesis) and marked growth during pregnancy (REYNOLDS and REDMER, 2001). The pig possesses a noninvasive, diffuse type of epitheliochorial placentation, and its placenta grows rapidly between Days 20 and 60 of gestation and its development is maximal by Day 70 of gestation (BAZER et al., 1988). Placental angiogenesis is necessary to increase placental-fetal blood flow and, thus, the supply of nutrients from mother to fetus. Notably, the Meishan pig, which has 3 to 5 more piglets per litter than US or European pig breeds, exhibits more vascularization in the placenta (VONNAHME and FORD, 2004), enabling the Meishan fetus to obtain sufficient nutrients from a relatively small placenta (BAZER et al., 1988). Insufficient placental vascularization may lead to a progressive deterioration in placental function and a decrease in placental transfer of oxygen and nutrients to the fetus (WU et al., 2006). Thus, understanding the mechanisms that regulate placental growth is crucial for improving and controlling the survival, growth and development of fetal pigs.

Role of NO and polyamines in placental growth

Besides insulin-like growth factors and vascular endothelial growth factors, nitric oxide (NO) and polyamines (putrescine, spermidine, and spermine), which are all products of arginine catabolism, also play crucial roles in angiogenesis, embryogenesis, placental trophoblast growth, uteroplacental blood flow, as well as fetal growth and development in the uterus (WU et al., 2006). Consistent with this notion, we recently discovered unusually high concentrations of arginine in porcine allantoic fluid during early gestation. Particularly, arginine plus ornithine account for 50 % and 55 % of the total allantoic fluid α-amino acid nitrogen on Days 40 and 45 of gestation, respectively. Concentrations of arginine and ornithine in allantoic fluids on Day 60 of gestation are lower than on Days 35 to 45 of gestation, but remain the greatest among all amino acids. The arginine family of amino acids is also highly abundant in ovine conceptus (e.g., 10 mM citrulline and 25 mM glutamine at Day 60). These novel and intriguing observations have raised important questions regarding the role of arginine in growth and development of the conceptus. In support of this notion, rates of NO and polyamine synthesis in both porcine and ovine placentae are highest during early gestation when placental growth is most rapid (KWON et al., 2004; WU et al., 2005; WU et al., 2006). Therefore, we proposed that impaired placental growth (including vascular growth) or function, possibly owing to reduced placental synthesis of NO and polyamines may contribute primarily to IUGR in response to both maternal undernutrition and overnutrition (WU et al., 2006).

Myocytes and adipocytes are derived from a common mesenchymal precursor. Therefore, excessive amounts of adipose tissue are developed at the expense of skeletal muscle when embryonic myogenesis is impaired (KABLAR et al., 2003). There are two developing muscle fibers in fetal pigs: primary fibers (formed by the rapid fusion of primary myoblasts between Days 25 and 50 of gestation) and secondary fibers (formed on the surface of primary fiber between approximately Days 50 and 90 of gestation). The numbers of secondary muscle fibers, but not primary muscle fibers, are affected by conditions *in utero* (DWYER et al., 1994). The total number of muscle fibers is fixed at birth and is a major factor affecting growth of skeletal muscle and, therefore, the postnatal growth of animals (NISSEN et al., 2003). The differences in prenatal and postnatal growth rates are related to a lower ratio of secondary to primary muscle

fibers and a smaller size of the fibers (HANDEL and STICKLAND, 1987). Abnormal regulation of intracellular protein turnover, adipogenesis, and mitochondrial biogenesis is likely a major factor responsible for reduced protein deposition in skeletal muscle and increased fat accretion in IUGR fetuses and offspring. In this regard, it is noteworthy that recent results of proteomics studies indicate that the abundance of proteasome is greater in skeletal muscle and liver, whereas levels of eukaryotic translation initiation factor 3 are lower in skeletal muscle of IUGR piglets at birth, compared with piglets with normal birth weights (WANG et al., 2007).

Polyamines are necessary for both proliferation and differentiation of cells and likely mediate growth and development of fetal muscle fibers and adipocytes. In support of this view, we noted that concentrations of arginine, ornithine, and polyamines were reduced by 28 to 35 % in skeletal muscle of IUGR fetal pigs compared to those in average-weight littermates. Similarly, concentrations of arginine, putrescine and spermidine were 26 to 32 % lower in gastrocnemius muscle of IUGR fetal lambs in response to maternal undernutrition. Further, physiological levels of NO inhibit growth of adipocytes (JOBGEN et al., 2006). Because adipose tissue of fetal lambs in underfed ewes has 33 % lower levels of constitutive NO synthase activity, decreased NO availability is expected to facilitate growth of preadipocytes in IUGR lambs. In addition to NO and polyamines, arginine and other functional amino acids (e.g. glutamine, leucine and proline) may also regulate placental growth as well as embryonic/fetal survival, growth and development via the signaling mechanism of mammalian target of rapamycin (mTOR, now known as FKBP12-Rapamycin Complex-Associated Protein 1 or FRAP1; WU et al., 2006).

Mechanisms of fetal programming

Environmental insults during a critical period of fetal development during gestation may have a permanent effect on progeny throughout postnatal life. This phenomenon is termed fetal programming, which may result from alterations in the epigenetic state of the fetal genome and expression of imprinted genes (WU et al., 2006). Epigenetic alterations (stable alterations of gene expression through covalent modifications of DNA and core histones) in early embryos may be carried forward to subsequent developmental stages (WATERLAND and JIRTLE, 2004). Two mechanisms mediating epigenetic effects are DNA methylation (occurring in 5'-positions of cytosine residues within CpG dinucleotides throughout the mammalian genome) and histone modification (acetylation and methylation). Methylation of CpG can regulate gene expression by modulating binding of methyl-sensitive DNA-binding proteins, thereby affecting regional chromatin conformation (OOMMEN et al., 2005). Histone acetylation or methylation can alter positioning of histone-DNA interactions and the affinity of histone binding to DNA, thereby affecting gene expression (WATERLAND and JIRTLE, 2004). Notably, DNA and protein methylation are catalyzed by specific DNA and protein methyltransferases with S-adenosylmethionine as the methyl donor. In addition to methionine, one-carbon unit metabolism, which is also affected by serine, glycine, histidine, choline, and B vitamins (including folate, vitamin B_{12} and vitamin B_6), may provide a molecular mechanism for the impact of maternal nutrition on fetal programming for postnatal growth performance and disease susceptibility (WU et al., 2006).

Improvement of pregnancy outcome via modulation of the arginine-NO pathway

The naturally occurring inability of placentae to supply an adequate amount of nutrients to fetuses in pigs is exacerbated further by the current widespread practice in the swine industry of restricted feeding programs to prevent excessive weight gain of gilts and sows during pregnancy. Although such a feeding regimen can ameliorate farrowing difficulties and appetite reduction during lactation, gilts and sows may not receive sufficient amounts of dietary arginine during early- to late-gestation for supporting optimal embryonic/fetal survival and growth. Remarkably, dietary supplementation with 1.0 % arginine-HCl between Days 30 and 114 of gestation increased concentrations of arginine and ornithine in plasma by 77 % and 53 %, respectively (MATEO et al., 2007). The arginine treatment did not adversely affect body weight or backfat thickness of gilts, but increased the number of live-born piglets by 2 and litter birth-weight by 24 % (MATEO et al., 2007). These findings provide the first evidence for improved pregnancy outcome in pigs through dietary arginine supplementation.

A relative placental deficiency also results in IUGR in litter-bearing sheep (WU et al., 2006). Thus, through an increase in concentrations of cGMP (which mediates the action of NO on blood vessels) and thus uteroplacental blood flow, intramuscular administration of Viagra (3 x 50 mg per day) to ewes receiving 100 % or 50 % of NRC nutrient requirements between Days 28 to 112 of gestation enhanced fetal growth by 20 to 25 % (SATTERFIELD et al., 2007). In addition, intravenous infusion of arginine to underfed ewes (50 % of NRC nutrient requirements) between Days 60 and 147 of pregnancy (27 mg/kg body wt, three times per day) prevented fetal growth restriction (our unpublished data). Furthermore, arginine supplementation to prolific ewes (4-7 fetuses/dam) enhanced fetal development and survival, as well as birth weight (our unpublished data).

An increase in the numbers of live-born pigs and lambs will markedly reduce production costs associated with reproduction and lactation in dams, whereas an increase in the vitality of neonates will increase their survival to weaning (WU et al., 2006). At present, little is known about effects of arginine treatment on conception rate or early embryo survival in any species. Nonetheless, recent findings provide a compelling basis for future studies to define the underlying mechanisms for the beneficial effect of dietary arginine supplementation on embryonic and fetal development. Because long-term oral or intravenous administration of arginine is safe for livestock species (WU et al., 2007), the use of arginine holds great promise in the prevention and treatment of IUGR, as well as improvements of postnatal growth performance and health.

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References

BAZER, F.W.; THATCHER, W.W.; MARTINAT-BOTTE, F.; TERQUI, M.:
Conceptus development in Large White and prolific Chinese Meishan pigs. J. Reprod. Fert. **84** (1988), 37-42

DWYER, C.M.; STICKLAND, N.C.; FLETCHER, J.M.:
The influence of maternal nutrition on muscle fiber number development in the porcine fetus and on subsequent postnatal growth. J. Anim. Sci. **72** (1994), 911-817

HANDEL, S.E.; STICKLAND, N.C.:
The growth and differentiation of porcine skeletal muscle fibre types and the influence of birthweight. J. Anat. **152** (1987), 107-119

HALEY, C.S.; LEE, G.J.:
Genetic factors contributing to variation in litter size in British Large White gilts. Livest. Prod. Sci. **30** (1992), 99-113

JOBGEN, W.S.; FRIED, S.K.; FU, W.J.; MEININGER, C.J.; WU, G.:
Regulatory role for the arginine-nitric oxide pathway in energy-substrate metabolism. J. Nutr. Biochem. **17** (2006), 571-588

JOHNSON, R.:
History of litter size selection. http://www.nsif.com/conferences/2000/johnson.htm

KABLAR, B.; KRASTEL, K.; TAJBAKHSH, S.; RUDNICKI, M.A.:
Myf5 and MyoD activation define independent myogenic compartments during embryonic development. Dev. Biol. **258** (2003), 307-318

KWON, H.; WU, G.; MEININGER, C.J.; BAZER, F.W.; SPENCER, T.E.:
Developmental changes in nitric oxide synthesis in the ovine placenta. Biol. Reprod. **70** (2004), 679-686

LUND, M.S.; PUONTI, M.; RYDHMER, L.; JENSEN, J.:
Relationship between litter size and perinatal and pre-weaning survival in pigs. Anim. Sci. **74** (2002), 217-222

MATEO, R.D.; WU, G.; BAZER, F.W.; PARK, J.C.; SHINZATO, I.; KIM, S.W.:
Dietary L-arginine supplementation enhances the reproductive performance of gilts. J. Nutr. **137** (2007), 652-656

NISSEN, P.M.; DANIELSEN, V.O.; JORGENSEN, P.F.; OKSBJERG, N.:
Increased maternal nutrition of sows has no beneficial effects on muscle fiber number or postnatal growth and has no impact on the meat quality of the offspring. J. Anim. Sci. **81** (2003), 3018-3027

OOMMEN, A.M.; GRIFFIN, J.B.; SARATH, G; ZEMPLENI, J.:
Roles for nutrients in epigenetic events. J. Nutr. Biochem. **16** (2005), 74-77

RAO, S.; NOTTER, D.R.:
Genetic analysis of litter size in Targhee, Suffolk, and Polypay sheep. J. Anim. Sci. **78** (2000), 2113-2120

REYNOLDS, L.P.; REDMER, D.A.:
Angiogenesis in the placenta. Biol. Reprod. **64** (2001), 1033-40

SATTERFIELD, M.C.; BAZER, F.W.; SPENCER, T.E.; WU, G.:
Viagra (Sildenafil citrate) treatment enhanced fetal growth in an ovine model of intrauterine growth retardation. Pediatr. Res. **62** (2007), 381

TOWN, S.C.; PATTERSON, J.L.; PEREIRA, C.Z.; GOURLEY, G.; FOXCROFT, G.R.:
Embryonic and fetal development in a commercial dam-line genotype. Anim. Reprod. Sci. **85** (2005), 301-316

URBAN, W.E. JR.; SHELBY, C.E.; CHAPMAN, A.B.; WHATLEY, J.A. JR.; GARWOOD, V.A.:
Genetic and environmental aspects of litter size in swine. J. Anim. Sci. **25** (1966), 1148-1153

VONNAHME, K.; FORD, S.P.:
Placental vascular endothelial growth factor receptor system mRNA expression in pigs selected for placental efficiency. J. Physiol. **554** (2004), 194-201

WANG, J.; CHEN, L.; LI, D.; YIN, Y.; WANG, X.; LI, P.; DANGOTT, L.J.; HU, W.; WU, G.:
Intrauterine growth restriction affects the proteomes of the small intestine, liver and skeletal muscle in newborn pigs. J. Nutr. **138** (2008), 60-66

WATERLAND, R.A.; JIRTLE, R.L.:
Early nutrition, epigenetic changes at transposons and imprinted genes, and enhanced susceptibility to adult chronic diseases. Nutrition **20** (2004), 63-68

WU, G.; BAZER, F.W.; HU, J.; JOHNSON, G.A.; SPENCER, T.E.:
Polyamine synthesis from proline in the developing porcine placenta. Biol. Reprod. **72** (2005), 842-850

WU, G.; BAZER, F.W.; WALLACE, J.M.; SPENCER, T.E.:
Board-Invited Review: Intrauterine growth retardation: Implications for the animal sciences. J. Anim. Sci. **84** (2006), 2316-2337

WU, G., BAZER, F.W., CUDD, T.A.; JOBGEN, W.S.; KIM, S.W.; LASSALA, A.; LI, P.; MATIS, J.H.; MEININGER, C.J.; SPENCER, T.E.:
Pharmacokinetics and safety of arginine supplementation in animals. J. Nutr. **137** (2007), 1673-1680

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Increasing the temperature at a certain period during the incubation of broiler eggs improves the carcass and breast yields without a negative impact on the meat quality

(Die Erhöhung der Temperatur in einem bestimmten Abschnitt während der Brut von Hähnchen-Eiern verbessert die Schlachtkörper- und Brustausbeute ohne negativen Einfluss auf die Fleischqualität)

Abstract

The aim of the present study was to investigate how a higher temperature at a comparable period during chicken incubation influences the muscle development and the meat quality in the animals post hatch.
Therefore half of fertilized eggs of the broiler genetic Cobb were incubated between ED 7 to 10 at 38.5 ℃ instead of 37.5 ℃ (control group). At the day of hatch (Day 21) the hatch rate was 86.6 % for the normal temperated eggs (NTG) and 82.4 % for the higher temperated group (HTG). The animals were reared until day 35. The broilers (N=30 per sex and incubation group) were then slaughtered and the carcass, breast and leg weights were determined. The meat quality analysis includes the determination of the pH and electrical conductivity 24 h after slaughter and the colour (L*a*b*) 20 min and 24 h after slaughter. Comparing the results between the incubation groups no significant differences in the weight parameters were determined. With regard to the meat quality parameters also no significant differences could be presented. Considering also the gender the HTG cocks had significantly ($P<0.05$) higher carcass, breast, the leg weights were similar. This difference between the incubation groups could not be determined in the hens which had generally lower carcass, breast and leg weights than the cocks. With regard to the meat quality parameters no clear influence of gender and incubation group could be determined. From the presented results it could be concluded that between ED 7 and 10 of the incubation certain steps within the myogenesis are sensitive to a higher temperature. The higher temperature could either have a direct effect on myogenic regulatory systems, or an indirect by a general stimulation of the embryonic metabolism and/or activity. The gender dependence of these differences might be related to temporal differences in the embryonic muscle development of the cocks and hens.

Keywords: broiler, incubation temperature, embryonic myogenesis, meat quality

Zusammenfassung

Das Ziel der Studie war es, zu untersuchen, wie eine höhere Temperatur während eines vergleichbaren Abschnitts der Hähnchen-Brut die Muskelentwicklung und Fleischqualität nach dem Schlupf der Tiere beeinflusst. Dazu wurde die Hälfte befruchteter Eier der Hähnchen-Genetik Cobb zwischen ED 7 bis 10 bei 38,5 ℃ anstatt 37,5 ℃ (Kontrollgruppe) bebrütet. Am Schlupftag (Tag 21) betrug die Schlupfrate 86,6 % für die normal temperierten Eier (NTG) und 82,4 % für die höher temperierte Gruppe (HTG). Die Tiere wurden bis zum Tag 35 gemästet. Dann wurden die Hähnchen (N=30 pro Geschlecht und Brutgruppe) geschlachtet und die Schlachtkörper-, Brust- und Schenkelgewichte bestimmt. Die Analyse der Fleischqualität beinhaltete die Bestimmung von pH-Wert und Leitfähigkeit (LF) 24 h nach der Schlachtung und der Farbe (L*a*b*) 20 min und 24 h nach der Schlachtung. Vergleicht man die Ergebnisse zwischen den Brutgruppen, so konnten keine signifikanten Unterschiede der Gewichtsparameter bestimmt werden. In Bezug auf Fleischqualitätsparameter bestanden ebenfalls keine signifikanten Unterschiede. Berücksichtigt man auch das Geschlecht, hatten die HTG Hähne signifikant ($P<0,05$) höhere Schlachtkörper- und Brustgewichte, die Schenkelgewichte waren gleich. Dieser Unterschied zwischen den Brutgruppen konnte nicht bei den Hennen bestimmt werden, die allgemein niedrigere Schlachtkörper-, Brust- und Schenkelgewichte hatten. In Bezug auf die Fleischqualitätsparameter konnte kein klarer Einfluss von Geschlecht und Brutgruppe bestimmt werden. Aus den dargestellten Ergebnissen lässt sich folgern, dass zwischen ED 7 und 10 der Brut bestimmte Schritte innerhalb der Myogenese empfindlich für höhere Temperaturen sind. Die höhere Temperatur kann entweder einen direkten Einfluß auf myogene Regulationssysteme oder einen indirekten durch allgemeine Anregung des embryonalen Stoffwechsels und/oder der Aktivität haben. Die Geschlechtsabhängigkeit der Unterschiede könnte in Verbindung mit zeitlichen Unterschieden der embryonalen Muskelentwicklung der Hähne und Hennen stehen.

Schlüsselwörter: Hähnchen, Bruttemperatur, embryonale Myogenese, Fleischqualität

Introduction

The investigation of poultry embryos *in ovo* has the advantage that environmental effects could be better controlled because maternal and paternal influences are mainly excluded (PITSILLIDES, 2006). Beside the analysis of avian embryos to generally elucidate important molecular mechanisms of embryonic organogenesis (CHRIST and BRAND-SABERI, 2002; SCAAL and CHRIST, 2004) there are several publications that determined the embryonic and postembryonic body and organ development and the hatchability after varying the incubation conditions or directly manipulating the embryos or embryonic membranes. Considering the incubation conditions the oxygen concentration in the incubation chamber (LEON-VELARDE and MONGE-C, 2004; CHAN and BURGGREN, 2005; MORTOLA and LABBE, 2005), the light colour (ROZENBOIM et al., 2003; HALEVY et al., 2006) or the incubation temperature (CHRISTENSEN et al., 2001; GAULY et al., 2001; von BLUMRODER und TÖNHARDT, 2002; MALTBY et al., 2004; TÖNHARDT et al., 2004; LOURENS et al., 2005; HAMMOND et al., 2007) were varied. In contrast to this, direct manipulations include the *in-ovo*-injection of growth hormones or factors (e.g. IGF), amino acids or neuro-muscular active drugs into the embryos or its embryonic membranes (KOCAMIS et al., 1998; HEYWOOD et al., 2005; MCENTEE et al., 2006; PITSILLIDES 2006).

Interesting are the results by MALTBY et al. (2004) and HAMMOND et al. (2007) who varied the incubation temperature at specific periods during the embryonic myogenesis. MALTBY et al. (2004) presented that a higher temperature between embryonic day (ED) 5 and 8 as well as ED 9 and 12 in turkeys positively influences the muscle fiber and nuclei numbers in the *M. semitendinosus* post-hatch. HAMMOND et al. (2007) could show this effect also in laying hens incubated at higher temperatures between ED 4 and 7. Referring to these investigations the aim of the present study was to investigate how a higher temperature at a comparable period during chicken egg incubation influences the muscle development and the meat quality in the animals post hatch.

Material and Methods

360 eggs of the broiler genetic Cobb 700 were initially incubated at 37.5 °C for 6 days in a commercial incubator. At day 7 the eggs were candled and half of the fertilized eggs (N=160) were transferred to an incubator with identical construction but with a higher incubation temperature of 38.5 °C. After 3 days the higher temperated eggs were transferred back to the 37.5 °C-incubator and incubated there at 37.5 °C until hatch. At the day of hatch (Day 21) the hatchability was determined. At the same day all chicks were sexed.

In an experimental stable the animals (N=60 per sex and incubation group) were reared according to the animal welfare regulations and fed a standard diet *ad libitum* until Day 35. At Day 35 the broilers (N=30 per sex and incubation group) were slaughtered in an experimental abattoir and the carcass, breast and leg weights were determined immediately after dissection.

The meat quality analysis includes the determination of the pH (pH-Star, Matthäus, Germany) and electrical conductivity (LF-Star, Matthäus, Germany) 24 h after slaughter and the colour (L*a*b*) 20 min and 24 h after slaughter (Minolta CR 400, Minolta, Japan). The presented colour values represent the mean of at least four

different measurements.
The statistical analysis of the data was performed with the software package Statistica 7.0 (StatSoft, Hamburg, Germany) following the GLM. The statistical model includes the variables incubation group, gender and the interaction between gender and the incubation group. The P-value was calculated with the Fisher LSD test with a $P<0.05$ being significant.

Results

The hatch rate was 86.6 % for the chicken incubated for the whole period at 37.5 ℃ (normal-temperated group [NT group]) and 82.4 % for the animals incubated at 38.5 ℃ between ED7 and 10 (Higher temperated group [HT group]).
After slaughter and dissection of the broiler at day 35 the carcass, breast and leg weights were comparable between the animals of the NT and HT group ($P>0.05$). The HT chicken had carcass and breast weights of 1631 g and 407 g, respectively, and the NT animals of 1582 g and 385 g (Figure 1).

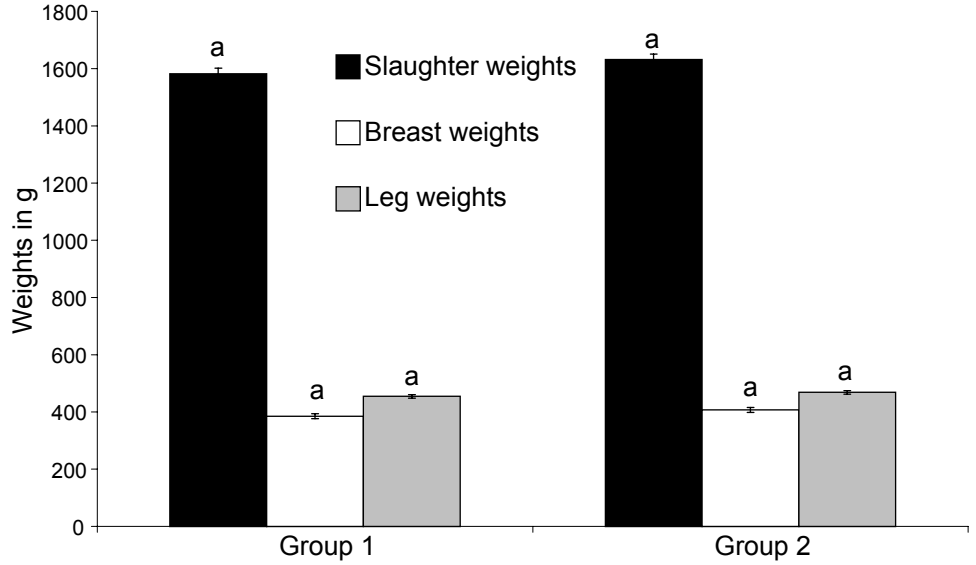

Fig. 1: Mean values (LSM) and standard errors of the slaughter, breast and leg weights of the investigated birds depending on the incubation group (Group 1 = ED 7-10 : 37.5 ℃; Group 2 = ED 7-10 : 38.5 ℃). Considered are 60 birds per incubation group. a – Columns with different letters between the incubation groups differ significantly ($P<0.05$).

Considering also the gender of the broilers the carcass and breast weights of the male animals from the HT group (1758 g; 437 g) were significantly ($P<0.05$) higher in comparison to the NT chicken (1683 g; 401 g). In contrast to this, the slaughter characteristics of the female chicken of both incubation group were comparable ($P>0.05$) but were significantly ($P<0.05$) different from the results of the male animals (Figure 2).
With regard to the quality characteristics of the breast muscles of the slaughtered broilers no significant differences of the pH and electrical conductivity (EC) values and of the colour (L*a*b*) values between the animals of the incubation groups could be found (data not shown). Considering also the gender of the chicken significant differences could only be found in the brightness (L*) values determined 24 h after slaughter in the hens. The NT hens were significantly ($P<0.05$) darker than the female HT broilers. It is interesting to note that the usual increase of the brightness and redness values during storage of the poultry meat was clearer in the hens of both incubation group because the significantly ($P<0.05$) lower L* values in the female breast muscles 20 min after slaughter increased to values comparable with those of the cocks (Table 1).

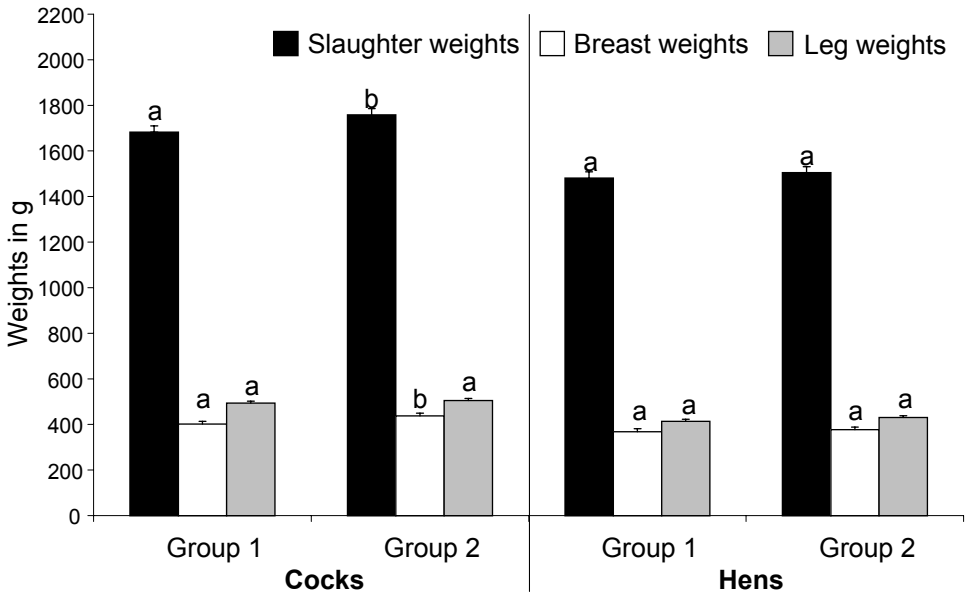

Fig. 2: Mean values (LSM) and standard errors of the slaughter, breast and leg weights of the birds depending on the gender and the incubation group (Group 1 = ED 7-10:37.5 ℃; Group 2 = ED 7-10:38.5 ℃). Considered are 30 birds per gender and incubation group. ab – Columns with different letters between the incubation groups within the same gender differ significantly ($P<0.05$)

Table 1
Mean values (LSM) and standard errors of the pH, electrical conductivity (EC) and colour values (L*a*b*) of the breast muscles depending on the incubation group and the gender of the investigated birds.

	Cocks		**Hens**	
	Group 1 (N=30) (ED 7-10 at 37.5 ℃)	Group 2 (N=30) (ED 7-10 at 38.5 ℃)	Group 1 (N=30) (ED 7-10 at 37.5 ℃)	Group 2 (N=30) (ED 7-10 at 38.5 ℃)
pH_{24h}	6.04 ± 0.03^{a}	6.07 ± 0.03^{a}	6.06 ± 0.03^{a}	6.01 ± 0.03^{a}
EC_{24h} (mS/cm)	4.11 ± 0.15^{a}	4.02 ± 0.15^{a}	4.21 ± 0.15^{a}	4.17 ± 0.15^{a}
Brightness (L*) 20 min	47.83 ± 0.32^{a}	47.82 ± 0.33^{a}	46.14 ± 0.32^{b}	45.93 ± 0.32^{b}
Redness (a*) 20 min	2.70 ± 0.15^{a}	2.60 ± 0.15^{a}	2.65 ± 0.15^{a}	2.67 ± 0.15^{a}
Yellowness (b*) 20 min	2.33 ± 0.18^{a}	2.76 ± 0.18^{ab}	2.97 ± 0.18^{b}	2.74 ± 0.18^{ab}
Brightness (L*) 24 h	49.8 ± 0.45^{ab}	50.04 ± 0.46^{ab}	49.06 ± 0.45^{a}	50.53 ± 0.45^{b}
Redness (a*) 24 h	3.86 ± 0.24^{a}	3.67 ± 0.25^{a}	3.68 ± 0.24^{a}	3.85 ± 0.24^{a}
Yellowness (b*) 24 h	8.69 ± 0.37^{a}	9.13 ± 0.37^{a}	9.08 ± 0.37^{a}	9.24 ± 0.36^{a}

a,b – LSM with different letters within a line group differ significantly ($P<0.05$)

Discussion

The differences in the hatchability are difficult to discuss since the non-hatched chicks have not been investigated. As the ability of a living embryo to hatch depends on several endogenous factors like the energy metabolism, the incidence of malformations or malpositions of the embryo within the egg (ROMANOFF, 1949) further investigations are necessary to clarify the differences between the incubation groups.

It is well known that temperature affects the growth and differentiation of different tissues (HAMMOND et al., 2007). From the presented results – especially in the male broilers – it could be suggested that during ED 7 to 10 certain steps within the embryonic myogenesis had been affected by the higher temperature. This assumption is supported by the result that in chicken during ED 7 to 10 myogenesis is mainly characterized by the formation of secondary myotubes close to the already existing primary myotubes (MILLER and STOCKDALE, 1986). The increased temperature might directly influence molecular steps necessary for the proliferation and differentiation of myoblasts or indirectly affects myogenesis by general stimulation of

the metabolism and the *in ovo* movement of the embryo – as postulated by HAMMOND et al. (2007). A combined direct and indirect effect of the higher temperature on the embryonic muscle development could be also assumed but further investigations are necessary to clarify this hypothesis. The gender dependence of the effect could be related to chronological differences in the muscle development between the male and female embryos – influenced by the sexual hormones. However, data about sex-related differences during myogenesis have not been published up to now and need further investigation.

Considering the quality parameters the pH values determined 24 h after slaughter were in a usual range compared to other investigations (DEBUT et al., 2003; BERRI et al., 2005; BERRI et al., 2007) not indicating a higher incidence of PSE-like meat in the different groups. EC values in chicken meat have not been published but the quite low values in comparison to other farm animal species (e.g. pig) indicate no irregular muscle-to-meat-transition in the meat samples. With regard to the meat colour values the increase in the brightness and redness values in the presented study are in accordance with the results of PETRACCI and FLETCHER (2002). However, to compare the presented data with other studies the colour analysis system has to be considered. In scientific reports that also used the Minolta colorimeter comparable brightness and redness values of the breast muscle had been determined (CAVITT et al., 2004; YOUNG et al., 2004; FANATICO et al., 2005).

In conclusion, an increased temperature between ED 7 and 10 of broiler incubation positively influences the muscle development in the cocks post-hatch without an effect of the meat quality. However, the increased temperature has a negative impact on the hatchability of the chicks.

References

BERRI, C.; LE BIHAN-DUVAL, E.; BAEZA, E.; CHARTRIN, P.; PICGIRARD, L.; JEHL, N.; QUENTIN, M.; PICARD, M.; DUCLOS, M.J.:
Further processing characteristics of breast and leg meat from fast-, medium- and slow-growing commercial chickens. Anim. Res. **54** (2005), 123-134

BERRI, C.; LE BIHAN-DUVAL, E.; DEBUT, M.; SANTE-LHOUTELLIER, V.; BAEZA, E.; GIGAUD, V.; JEGO, Y.; DUCLOS, M.J.:
Consequence of muscle hypertrophy on characteristics of Pectoralis major muscle and breast meat quality of broiler chickens. J. Anim. Sci. **85** (2007), 2005-2011

CAVITT, L. C.; HARGIS, B. M.; OWENS, C. M.:
The use of halothane and succinylcholine to identify broilers prone to developing pale, soft, exudative meat. Poult. Sci. **83** (2004), 1440-1444

CHAN, T.; BURGGREN, W.:
Hypoxic incubation creates differential morphological effects during specific developmental critical windows in the embryo of the chicken (Gallus gallus). Respir. Physiol. Neurobiol. **145** (2005), 251-263

CHRIST, B.; BRAND-SABERI, B.:
Limb muscle development. Int. J. Dev. Biol. **46** (2002), 905-914

CHRISTENSEN, V.L.; MCMURTRY, J.P.; DONALDSON, W.E.; NESTOR, K.E.:
Incubation temperature affects plasma insulin-like growth factors in embryos from selected lines of turkeys. Poult. Sci. **80** (2001), 949-954

DEBUT, M.; BERRI, C.; BAEZA, E.; SELLIER, N.; ARNOULD, C.; GUEMENE, D.; JEHL, N.; BOUTTEN, B.; JEGO, Y.; BEAUMONT, C.; LE BIHAN-DUVAL, E.:
Variation of chicken technological meat quality in relation to genotype and preslaughter stress conditions. Poult. Sci. **82** (2003), 1829-1838

FANATICO, A.C.; CAVITT, L.C.; PILLAI, P.B.; EMMERT, J.L.; OWENS, C.M.:
Evaluation of slower-growing broiler genotypes grown with and without outdoor access: Meat quality. Poult. Sci. **84** (2005), 1785-1790

GAULY, M.; REINER, G.; GROSCHL, M.; DZAPO, V.:
The effects of incubation conditions on the embryonic respiratory and mitochondrial energy metabolism, the thyroid hormone status, and the daily gain in turkeys. Archiv Geflügelk. **65** (2001), 97-105

HALEVY, O.; PIESTUN, Y.; ROZENBOIM, I.; YABLONKA-REUVENI, Z.:
In ovo exposure to monochromatic green light promotes skeletal muscle cell proliferation and affects myofi ber growth in posthatch chicks. Am. J. Physiol. – Regulat. Integr. Comp. Physiol. **290** (2006), R1062-R1070

HAMMOND, C.L.; SIMBI, B.H.; STICKLAND, N.C.:
In ovo temperature manipulation influences embryonic motility and growth of limb tissues in the chick (Gallus gallus). J. Exp. Biol. **210** (2007), 2667-2675

HEYWOOD, J.L.; MCENTEE, G.M.; STICKLAND, N.C.:
In ovo neuromuscular stimulation alters the skeletal muscle phenotype of the chick. J. Muscle Res. Cell. Motil. **26** (2005), 49-56

KOCAMIS, H.; KIRKPATRICK-KELLER, D.C.; KLANDORF, H.; KILLEFER, J.:
In ovo administration of recombinant human insulin-like growth factor-I alters postnatal growth and development of the broiler chicken. Poult. Sci. **77** (1998), 1913-1919

LEON-VELARDE, F.; MONGE-C, C.:
Avian embryos in hypoxic environments. Respir. Physiol. Neurobiol. **141** (2004), 331-343

LOURENS, A.; VAN DEN BRAND, H.; MEIJERHOF, R.; KEMP, B.:
Effect of eggshell temperature during incubation on embryo development, hatchability, and posthatch development. Poult. Sci. **84** (2005), 914-920

MALTBY, V.; SOMAIYA, A.; FRENCH, N.A.; STICKLAND, N.C.:
In ovo temperature manipulation influences post-hatch muscle growth in the turkey. Br. Poult. Sci. **45** (2004), 491-498

MCENTEE, G.M.; SIMBI, B.H.; BAYOL, S.A.; MACHARIA, R.G.; STICKLAND, N.C.:
Neuromuscular stimulation causes muscle phenotype-dependent changes in the expression of the IGFs and their binding proteins in developing slow and fast muscle of chick embryos. Dev. Dyn. **235** (2006), 1777-1784

MILLER, J.B.; STOCKDALE, F.E.:
Developmental Regulation of the Multiple Myogenic Cell Lineages of the Avian Embryo. J. Cell Biol. **103** (1986), 2197-2208

MORTOLA, J.P.; LABBE, K.:
Oxygen consumption of the chicken embryo: interaction between temperature and oxygenation. Respir. Physiol. Neurobiol. **146** (2005), 97-106

PETRACCI, M.; FLETCHER, D.L.:
Broiler skin and meat color changes during storage. Poult. Sci. **81** (2002), 1589-1597

PITSILLIDES, A.A.:
Early effects of embryonic movement: 'a shot out of the dark'. J. Anat. **208** (2006), 417-431

ROMANOFF, A.L.:
Critical periods and causes of death in avian embryonic development. The Auk **66** (1949), 264-270

ROZENBOIM, I.; HUISINGA, R.; HALEVY, O.; EL HALAWANI, M.E.:
Effect of embryonic photostimulation on the posthatch growth of turkey poults. Poult. Sci. **82** (2003), 1181-1187

SCAAL, M.; CHRIST, B.:
Formation and differentiation of the avian dermomyotome. Anat. Embryol. **208** (2004), 411-424

TONHARDT, H.; BRAUCH, N.; VON PLETTENBERG, D.:
Influence of incubation temperature on the noradrenaline concentration in blood plasma and cAMP content in heart muscles cells of chicken embryo. Avian Poult. Biol. Rev. **15** (2004), 235

VON BLUMRODER, D.; TONHARDT, H.:
Influence of long-term changes in incubation temperature on catecholamine levels in plasma of chicken embryos (Gallus gallus f. domestica). Comp. Biochem. Physiol. A-Mol. Integrat. Physiol. **131** (2002), 701-711

YOUNG, J. F.; KARLSSON, A. H.; HENCKEL, P.:
Water-holding capacity in chicken breast muscle is enhanced by pyruvate and reduced by creatine supplements. Poult. Sci. **83** (2004), 400-405

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The influence of maternal feed allowance during mid-pregnancy on prenatal muscle fibre development and postnatal growth and carcass and meat quality at slaughter of the progeny

(Effekte einer Futterzulage während der Trächtigkeit auf die Muskelfaserentwicklung, Wachstum, Schlachtkörper- und Fleischqualität)

A study was conducted in order to assess the effects of an extra feeding during mid-pregnancy on muscle fibre development of the progeny. Two replicates of 103 (replicate 1) and 96 (replicate 2) sows were used. Sows were divided into two experimental groups, control (C) and supplemented (S). Control sows were fed from 2.5 to 3.0 kg/day of a commercial feed and supplemented sows were provided with an extra feed allowance of + 50 % and + 75 % of the same feed for gilts and multiparous sows, respectively, from day 45 to day 85 of gestation. Growth performance throughout the nursery (replicate 1 = 461 and replicate 3 = 497) and growing-finishing periods (replicate 1 = 377 and replicate 3 = 259) was evaluated. Carcass and meat quality traits were studied in the second smallest group of weight (G4, n=90) at slaughter. Muscle fibre characteristics in the *longissimus thoracis* muscle were investigated in pigs from G4 using the histochemical myosinATP-ase reaction (n=70). No clear differences in growth performance were found between treatment groups. Carcass quality at slaughter was neither affected by the experimental treatment. Regarding meat quality traits, S pigs showed a higher ultimate pH (pH_{24h}) in the *semimembranosus* muscle and a lower lightness (L*) in the cross-section of the *longissimus thoracis* muscle compared to the C group of pigs consistently, in both replicates ($P<0.05$). Pigs born from supplemented mothers showed a lower number of muscle fibres with higher mean areas in both cycles ($P<0.15$), accounting for an invariable estimated developmental secondary to primary fibres ratio. In the adult animal, the lower number of muscle fibres in the S group of pigs was reflected in a lower number of type IIB fibres ($P<0.05$), but with larger sizes ($P<0.10$) compared to C pigs.

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The effect of maternal feed intake during the peri-conception period on myogenesis in fetal sheep

(Der Einfluss der Futteraufnahme um den Zeitpunkt der Konzeption auf die Myogenese bei Schafföten)

Observations from studies involving embryo transfers from ewes with contrasting feed intake during the peri-conception period suggest that the environment of the pre-implantation embryo plays a significant role in establishing myogenic potential and, therefore, influences subsequent muscle development (1). The aim of the present study was to investigate whether altering environment of the embryo by altering maternal feed intake during the peri-conception period influences subsequent muscle development on the fetal sheep following natural mating.

The experimental Karayaka breed ewes with a synchronised oestrus were fed roughage and concentrate diet (11.3 MJME/kg dry matter) at either 1.4 × maintenance (H; high) or 0.5 × maintenance (L; low) from 4 to 6 days before until 7 days after mating in individual pen. A total of 11 fetuses were collected on day 90 of gestation and placental weights, fetal body dimensions, fetal femur and tibia lengths, fetal organ and muscle weights were recorded. The numbers, type and size of muscle fibres in *longissimus dorsi* (LD), *semitendinosus* (ST) and *semimembranosus* (SM) muscles were determined.

Maternal feed intake did not influence placental weights, fetal weights, body dimensions, organ developments and muscle weights in fetus. The ratio of secondary to primary fibres (7.6 ± 0.58) in fetal LD muscle samples from low maternal feed intake group was lower than those (9.2 ± 2.75) in high maternal feed intake group (P=0.07). Similarly a lower ratio of secondary to primary fibres in fetal ST muscles but not in SM muscles was observed in low maternal feed intake group (7.14 ± 1.09 vs. 8.93 ± 2.18; P=0.12). A reduced maternal feed intake compared to high feed intake increased the cross sectional area of secondary fibres but not primary fibres in fetal LD (4.51 ± 0.39 vs. 5.74 ± 0.44 μm^2; P=0.10), ST (2.71 ± 0.33 vs. 3.90 ± 0.18 μm^2; P<0.01) and SM (3.20 ± 0.07 vs. 6.15 ± 0.89 μm^2; P=0.06) muscles.

These results indicate that reduced maternal feed intake during the peri-conception period in naturally mated ewes may alter myogenesis without affecting fetal weights and organ developments in sheep.

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References

QUIGLEY, S.P.; KLEEMANN, D.O.; KAKAR, M.A.; OWENS, J.A.; NATTRASS, G.S.; MADDOCKS, S.; WALKER, S.K.:

Myogenesis in sheep altered by maternal feed intake during the peri-conception period. Anim. Reprod. Sci. **87** (2005), 241-251

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Early programming of muscle mosaic hyperplasia in European sea bass

(Frühe Programmierung der Muskelhyperplasie beim Europäischen Wolfsbarsch)

European sea bass (*Dicentrarchus labrax*) in a finfish of major interest for aquaculture. As the development of its hatchery production had gone with an increase of temperature, we characterised the effect of a high and two lower temperatures on *D. labrax* early axial white muscle growth process and analysed the consequences on the subsequent white muscle growth process of *D. labrax* juveniles reared under a same natural seawater temperature.

D. labrax incubated and reared during larval life at constant temperatures of 13 °C, 15 °C or 20 °C were transferred at 1.8 cm total length (TL) under natural seawater temperature and light (Crete, 35 °N). Juveniles were identified by their early temperatures: L13, L15 or L20. Muscle growth process was analysed by quantitative histology on larvae sampled at hatching and on juveniles sampled 30, 100 and 400 days post-transfer and at 5 different equivalent TL to precise the effect of early temperature on the muscle growth process occurring in juveniles with a similar size. We previously showed that, at hatching, larvae incubated at 13 °C had the highest total number of white muscle fibres (TNWF) and a high total number of nuclei (ALAMI-DURANTE et al., 2006) and that, 30 and 100 days after the transfer to natural seawater temperature, the white muscle of L13 juveniles, which included the highest number of small-diameter white muscle fibres, grew more by mosaic hyperplasia than the white muscle of L15 and L20 juveniles (ALAMI-DURANTE et al., 2007). New results indicated that, 400 days post-transfer, the 3 groups of juveniles have again a different muscle growth process: the white muscle of L13 juveniles, which included a significantly higher number of small white fibres (sWF) than that of L15 and L20 juveniles, grew thus more by hyperplasia. These differences in white muscle growth process were not linked to differences in fish size. This was demonstrated by further comparisons of distributions of WFD in juveniles from the 3 batches with similar TL. In 3.2 cm long juveniles, the TNWF of L13 fish was significantly higher than that of L20 ones, that of L15 fish being intermediate, and the number of sWF was significantly higher in L13 fish than in L15 and L20 ones. This later result was yet recorded in 8.6 cm long juveniles, not observed in 14.3 cm and 16.8 cm long ones, but recorded again in 21.6 cm long juveniles. We thus hypothesised and verified that changes in seawater temperature trigger sWF recruitment: this recruitment was high between transfer (April-May) and 3.2 cm TL (May-June) when seawater temperature rose, decreased between July (8.6 cm) and December (16.8 cm) when seawater temperature fell, and increased again in May-June (21.6 cm) when seawater temperature rose again. The periods of high recruitment of new white muscle fibres corresponded to those during which L13 juveniles recruited more new white muscle fibres than other juveniles. L13 juveniles have thus a higher hyperplastic potential, which is expressed during periods of increasing temperature.

This potential of L13 juveniles, which persists at very long term (at least up to 400 days post-transfer), is linked to a higher total number of white muscle fibres and a higher number of nuclei at hatching.
This clearly demonstrates the existence of an early programming of axial white muscle hyperplasia in European sea bass.

Keywords: fish, skeletal muscle, hyperplasia, temperature

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References

ALAMI-DURANTE, H.; ROUEL, M.; KENTOURI, M.:
New insights into temperature-induced white muscle growth plasticity during Dicentrarchus labrax early life: a developmental and allometric study. Mar. Biol. **149** (2006), 1551-1565

ALAMI-DURANTE, H.; OLIVE, N.; ROUEL, M.:
Do European sea bass juveniles coming from different temperatures have the same white muscle growth process? Arch. Tierz. **50** (2007) Special Issue, 25-26

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JOËL BÉRARD[1,2], MICHAEL KREUZER[2] and GUISEPPE BEE[1]

Impact of litter size and birth weight on growth performance, carcass characteristics, meat quality, and post mortem proteolysis in pigs

(Der Einfluss der Wurfgröße und des Geburtsgewichts auf Wachstumsleistung, Schlachtkörper, Fleischqualität und Proteolyse post mortem beim Schwein)

There is a certain evidence that, within litter, low-birth weight (BtW) pigs not only grow slower and have fatter carcasses but also meat quality traits like drip loss or shear force are impaired compared to their high BtW siblings. Because the variability of the body weight (BW) at birth is greater in large compared to small litters, the aim of the present study was to test the hypothesis that effects of BtW on growth performance, carcass characteristics and meat quality differ when pigs originate from small or large litters. In the present study, we also investigated whether BtW of the pig or litter size are related to post mortem proteolysis of various muscle proteins such as titin, nebulin, and integrin, and how the degree of degradation is related to pork quality traits. The 60 Swiss Large White barrows used in this study originated from 20 litters with either less than 10 (S) or more than 14 (L) piglets born per litter. Within each of the S- and L-litters, three barrows were selected per litter at birth: the lightest (L-BtW), the heaviest (H-BtW), and the one with a BtW nearest to the average BtW of the litter (M-BtW). At weaning the barrows were individually penned and they had free access to standard diets until slaughter. At slaughter, the weights of the hot carcass, heart, liver, and kidney were assessed. The pH 24 h post mortem, the drip loss after 48 h, the thawing loss, and the shear force were measured in the *longissimus dorsi* muscle (LD) and in the dark portion of *semitendinosus* (STD). In both muscles collected at 30 min, 24 and 72 h post mortem, proteolysis of intact titin and nebulin as well as of integrin was determined by SDS-PAGE and Western-Blot technique, respectively. The L-BtW (1.2 vs. 1.6 kg) and M-BtW barrows (1.6 vs. 1.9 kg) from L-litters were lighter than from S-litters whereas no differences in BtW were observed in H-BtW barrows (1.9 vs. 2.0 kg) (litter size×BtW interaction; $P<0.01$). Compared to H-BtW and M-BtW barrows, the L-BtW barrows grew slower (0.90 vs. 0.81 kg; $P<0.01$), ingested less feed (2.42 vs. 2.30 kg; $P=0.03$), and were still less efficient (2.71 vs. 2.84 kg feed/kg gain; $P<0.01$). The carcass yields were higher (82 vs. 81 %; $P<0.01$) and livers (1.58 vs. 1. g), and kidneys (0.31 vs. 0.34 kg) were lighter ($P<0.01$) in L-BtW compared to H-BtW barrows. Regardless of the BtW, barrows from S-litters had higher percentages of shoulder (12.4 vs. 12.1 %; $P=0.02$) and lower percentages of omental fat (1.8 vs. 2.1 %; $P=0.06$) than barrows from L-litters. Litter size and BtW had only a small impact on meat quality traits in the LD but the STD was more tender and had a higher 24 h post mortem pH in M-BtW than in H-BtW and L-BtW barrows. Regardless of the litter size, the extent (expressed as the relative abundance of the intact protein band) of titin proteolysis at 24 h post mortem (1.40 vs. 2.57; $P=0.06$) and nebulin proteolysis at 72 h post mortem (0.15 vs. 0.25; $P=0.07$) tended to be greater in the LD of H-BtW than the LD of L-BtW barrows. Even though shear force was not affected by the BtW,

the relative abundance of titin at 24 and 72 h and nebulin at 24 h post mortem was positively correlated ($P \leq 0.09$) with shear force, suggesting that tenderness scores could be impaired by low BtW. At 72 h post mortem, intact integrin of the LD tended to be less degraded (0.17 vs. 0.08; $P=0.08$) in barrows originating from S- than from L-litters. Furthermore, the present results indicated that the relative abundance of the intact integrin band at 24 h post mortem was negatively ($P<0.05$) correlated with the percentage drip and thaw loss. The present results confirm the effect of BtW on growth performance and carcass characteristics. However, the hypothesized impact on meat quality traits could only be partially demonstrated. Although litter size affected average BtW of the L-BtW and M-BtW barrows, its impact on growth performance, carcass characteristics and meat quality was minor. The study confirms the relationships between protein degradation post mortem, and meat quality traits.

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LOUISE B. McNAMARA[1,2], NEIL C. STICKLAND[2], P. BRENDAN LYNCH[1], M. KAREN O'CONNELL[1] and PEADAR G. LAWLOR[1]

The influence of increasing feed intake during gestation on litter size, piglet growth and within litter variation from birth to weaning

(Der Einfluss erhöhter Futteraufnahme während der Gravidität auf die Wurfgröße, das Wachstum und die Variation innerhalb des Wurfs)

Previous research has found that increasing nutrition during mid gestation for sows may increase the ratio of secondary to primary muscle fibres in progeny (DWYER et al, 1994). This improvement is most pronounced in lighter littermates.

The effect of five feed allowances during the gestation of sows (n=238): (1) 30 MJ digestible energy (DE)/day throughout gestation, (2) 60 MJ DE/day from day 25-50 of gestation, (3) 60 MJ DE/day from day 50-80, (4) 60 MJ DE/day from day 25-80 and (5) 45 MJ DE/day from day 80-112, on litter size, piglet growth and within litter variation from birth to weaning were examined. The experiment was a completely randomised design analysed using the general linear models procedure of SAS with effects for treatment, parity grouping and the interaction effect of treatment × parity grouping. A single degree of freedom comparison test was used to contrast treatment 1 with all other treatments.

There was no treatment × parity grouping interaction observed for any variable measured (P>0.05). The main results are presented in table 1 and 2.

Table 1
Effect of feed allowance during gestation on litter size, piglet growth and within litter variation in pig weight

Feed allowance							F	Contrasts			
treatment	1	2	3	4	5	se	test	T1 vs T2	T1 vs T3	T1 vs T4	T1 vs T5
Born alive (No.)	11.46	9.8	10.23	9.4	11.05	0.51		P=0.08		*	
Total born dead (No.)	1.05	1	1.91	1.91	1.22	0.275	*		*	P=0.09	
Birth weight (kg)	1.52	1.54	1.59	1.65	1.49	0.04				P=0.09	
CV birth weight (%)	19	16.4	16.6	17.9	18.9	1.41					
Weaning weight (kg)	7.4	7.9	7.9	7.7	7.6	0.15		P=0.07	*		
CV weaning weight (%)	18.9	18.8	16.7	19.4	17.6	1.16					
ADG birth to weaning (g/d.)	211	228	225	217	217	5		P=0.07	P=0.10		
Sow weight post-farrowing (kg)	257	343	271	286	264	45					
Sow backfat at farrowing (mm)	14.4	17.1	18.1	20.3	16.0	0.69	***	*	***	***	

* P<0.05, ** P<0.01, *** P<0.001

Table 2
Effect of parity group on litter size, piglet growth and within litter variation in pig weight

Parity group	1	2	3	4	5+	se	F test
Born alive (No.)	10.33	10.98	11.19	9.82	9.64	0.510	P=0.10
Total born dead (No.)	1.26	1.13	1.81	1.42	1.47	0.275	
Birth weight (kg)	1.52	1.60	1.55	1.59	1.52	0.040	
CV birth weight (%)	18.0	17.6	17.9	16.8	18.4	1.41	
Weaning weight (kg)	8.0	7.9	7.6	7.6	7.6	0.15	
CV weaning weight (%)	16.4	17.6	20.5	18.2	18.6	1.16	
ADG birth to weaning (g/d.)	229	226	213	214	216	5.0	

The number of pigs born alive was higher on treatment 1 compared with treatment 4 (Table 1; $P<0.05$) and treatment 2 ($P=0.08$). The total number of pigs born dead was nearly one pig higher born dead for both treatments 3 ($P<0.05$) and 4 ($P=0.09$) when contrasted with treatment 1. Birth weight of pigs on treatment 4 was higher than for pigs on treatment 1 ($P=0.09$). Weaning weight was also increased for treatment 3 ($P<0.05$) and treatment 2 ($P=0.07$) compared with treatment 1. A treatment effect was not observed for the coefficient of variation for either birth ($P>0.05$) or weaning weight ($P>0.05$) of pigs. However, there was a difference in average daily gain (ADG) of pigs from birth to weaning where treatment 2 ($P=0.07$) and treatment 3 ($P=0.10$) had higher ADG than treatment 1. Backfat of sows at farrowing was higher on treatments 2 ($P<0.05$), 3 ($P<0.001$) and 4 ($P<0.001$) compared with treatment 1.
There was no parity group effect on total number of piglets born dead ($P>0.05$), mean piglet birth weight ($P>0.05$), CV of birth weight ($P>0.05$), weaning weight ($P>0.05$), CV of weaning weight ($P>0.05$) or growth rate from birth to weaning ($P>0.05$). The total number of piglets born alive tended to increase up to parity 3 and then decrease for subsequent parities ($P=0.10$).
Overall, increasing feed allowance for sows during gestation had little effect on piglet birth weight and was not effective in reducing within litter variation in piglet weight. Increasing feed allowance between days 50-80 of gestation increased the number of piglets born dead per litter. Increased sow backfat at farrowing associated with increased gestation feeding levels may have contributed to sows having difficulty at farrowing and the resultant increase in number of piglets born dead.

Keywords: gestation feeding, born alive, born dead, birth weight, pigs

References

DWYER, C.M.; STICKLAND, N.C.; FLETCHER, J.M.:
The influence of maternal nutrition on muscle fiber number development in the porcine fetus and on subsequent postnatal growth, J. Anim. Sci. **72** (1994), 911-917

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LOUISE B. McNAMARA[1,2], NEIL C. STICKLAND[2], P. BRENDAN LYNCH[1], M. KAREN O'CONNELL[1] and PEADAR G. LAWLOR[1]

Influence of five gestation feeding regimes for sows on lactation feed intake, lactation weight loss, subsequent farrowing rate and litter size

(Der Einfluss von fünf Fütterungsregimes in der Trächtigkeit auf die Laktation, den Gewichtsverlust während der Laktation, die Abferkelrate und die Wurfgröße)

Excessive feed intake during gestation may reduce sow appetite during lactation thus increasing lactation weight loss which has been shown to reduce subsequent reproductive performance (THAKER and BILKEI, 2005). The objective was to determine the influence of five gestation feeding regimes on lactation feed intake, lactation weight change and the subsequent reproductive performance of sows.

Sows (n=238) were assigned to treatments: (1) 30 MJ digestible energy (DE)/day throughout gestation, (2) 60 MJ DE/day from day 25-50, (3) 60 MJ DE/day from day 50-80, (4) 60 MJ DE/day from day 25-80 and (5) 45 MJ DE/day from day 80-110. The experiment was a completely randomised design analysed using the general linear models procedure of SAS with effects for treatment, parity grouping and the interaction effect of treatment × parity grouping. A single degree of freedom comparison test was used to contrast treatment 1 with all other treatments. Duncan multiple range test was used for means separation where parity grouping was compared (Table 2).

No treatment × parity interaction was observed. The main results are presented in table 1 and 2. Lactation energy intake was less for treatment 4 than treatment 1 ($P<0.01$). Lactation weight loss of sows was not affected ($P>0.05$) by gestation feeding. There was a treatment effect for subsequent farrowing rate where treatment 5 (93 %; $P<0.01$) and treatment 3 (84 %; $P=0.09$) sows had higher farrowing rates when contrasted with treatment 1 (66 %) sows. There was no treatment effect associated with subsequent number of piglets born alive ($P>0.05$) and born dead ($P>0.05$).

Mean daily lactation energy intake was higher for parities 2-4 than parity 1 (Table 2; $P<0.05$) and actual lactation weight loss was increased for parity 1, 2 and 3 sows compared with all other sows ($P<0.001$). Parity group 5+ had the lowest subsequent farrowing rate ($P<0.001$) and subsequent born alive ($P<0.01$).

In conclusion, increasing the energy allowance of sows from day 25-80 of gestation reduced feed intake during lactation, however, this did not increase lactation weight loss or reduce subsequent reproductive performance.

Keywords: gestation feeding, lactation, sow

References

THAKER, M.Y.C.; BILKEI, G.:
Lactation weight loss influences subsequent reproductive performance of sows. Anim. Rep. Sci. **88** (2005), 309-318

Table 1
Effect of gestation feeding treatment on lactation feed intake, sow weight loss, litter size and farrowing rate

Gestation feeding treatment	1	2	3	4	5	se	F test	Contrasts			
								T1 vs. T2	T1 vs. T3	T1 vs. T4	T1 vs. T5
Mean lactation energy intake (MJ/day)	76.3	74.8	75.4	65.6	74.5	2.16	*			**	
Total lactation energy intake (MJ)	2143	2097	2117	1793	2068	62.5	*			***	
Actual lactation weight loss (%)	-19.8	-20.6	-18.7	-18.7	-19.8	0.94					
Estimated lactation weight loss (%)	-9.9	-11.7	-8	-9.2	-9.6	1.29					
Subsequent farrowing rate (%)	66	79	84	84	93	6.2	P=0.10		P=0.09		**
Subsequent born alive (No.)	11	10.7	11.1	10.5	11.4	0.53					
Subsequent born dead (No.)	1.77	1.56	1.61	1.48	1.28	0.334					

* P<0.05, ** P<0.01, *** P<0.001

Table 2
Effect of parity group on lactation feed intake, sow weight loss, litter size and farrowing rate

Parity group	1	2	3	4	5 to 8	se	F test
Mean lactation energy intake (MJ/day)	66.7^{b}	76.4^{a}	75.9^{a}	76.4^{a}	72.0^{ab}	2.17	*
Total lactation energy intake (MJ)	1973	2117	2101	2001	2026	62.5	
Actual lactation weight loss (%)	-22.4^{c}	-20.4^{bc}	-20.4^{bc}	-18.3^{ab}	-16.1^{a}	0.94	***
Estimated lactation weight loss (%)	-11.5	-10.0	-9.0	-10.5	-7.4	1.29	
Subsequent farrowing rate (%)	85^{a}	86^{a}	89^{a}	88^{a}	57^{b}	6.2	***
Subsequent born alive (No.)	12.0^{a}	11.8^{a}	11.0^{a}	10.9^{ab}	9.4^{b}	0.53	**
Subsequent born dead (No.)	1.54	1.35	2.21	1.44	1.16	0.334	

a,b,c – within rows, means without a common superscript differ significantly * P<0.05, ** P<0.01, *** P<0.001

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Arch. Tierz., Dummerstorf **51** (2008) Special Issue, 27-28

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Glucose tolerance and muscle characteristics in mice offspring are nutritionally programmed by a maternal high protein diet

(Glucosetoleranz und Muskelcharakterisierung in Mausnachkommen sind durch mütterliche Hochproteindiät diätabhängig programmierbar)

In various studies it has been shown that a low dietary protein intake throughout pregnancy in rodents results in lower birth weights, altered growth characteristics, and age-related loss of glucose tolerance and insulin resistance (METGES and HAMMON, 2005). We have shown that also maternal high protein diet (twice the recommendations) leads to alterations in body composition in young adult rat offspring (DAENZER et al., 2002).

Female mice of 2 strains (selected for high body mass, HBM; and unselected control, CON; n=25 each) were fed isoenergetic HP (40 % protein) or control (C) diets (20 %) from mating to end of lactation (21 day of age). Litters were standardized to 10 pups at birth. Pups were cross-fostered and offspring was tested in 3 combinations of pre- and postnatal (pre-weaning) dietary exposure: HP-C, C-HP, and C-C (n=10 litters each). Male offspring was weaned onto standard chow and continued on this food until tissue sampling. In a subset of animals at age 160 day an oral glucose tolerance test was performed (1 mg glucose/g body mass). Further, we analyzed the influence of prenatal diet (C or HP) on skeletal muscle (*M. rectus femoris*) of these animals at 180 day of age.

The HBM offspring had a better glucose tolerance than the CON offspring irrespective of the diet. Blood glucose peak time was at 15 min in HBM offspring and 30 min in CON offspring, respectively, and maximal blood glucose concentration in HBM was 50 % of that of CON ($P<0.05$). Blood glucose tolerance was better in offspring born to mothers fed C diet during pregnancy and cross-fostered by HP dams independent of the genotype. *M. rectus femoris* was higher in weight in HBM offspring than in CON offspring ($P<0.05$). Offspring of HP mothers in the CON line showed a decreased specific creatine kinase activity that is responsible for storage and supply of energy-rich phosphates for muscle contraction. Offspring of HP mothers in the HBM line exhibited a higher muscle protein concentration compared with control diet offspring.

We conclude tentatively, that programming response due to a maternal high protein diet in mice is genotype-dependent.

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References

METGES, C.C.; HAMMON, H.M.:
Nutritional programming: prenatal nutritional effects on the regulation of growth and metabolism. J. Anim. Feed Sci. **14** Suppl. 1 (2005), 15-30

DAENZER, M.; ORTMANN, S.; KLAUS, S.; METGES, C.C.:
Prenatal high protein exposure decreases energy expenditure and increases adiposity in young rats. J. Nutr. **132** (2002), 142-144

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The influence of maternal nutrition on porcine skeletal muscle fibre phenotype in the offspring

(Der Einfluss der maternalen Ernährung auf die Muskelfasermerkmale der Nachkommen)

Skeletal muscle is a highly dynamic and malleable tissue that is able to adapt to different stimuli that may be placed upon it both during gestation and after birth. Variation in nutrient supply throughout gestation is common, whether in livestock or in the human. It is important to understand the effects of changes in the nutrient supply to the mother during gestation on the subsequent muscle development of the fetus, and hence long-term growth rate and ultimate muscle or meat quality and function. The aim of this study is to determine the effects of increased maternal nutrition (above recommended levels) on the *M. semitendinosus* phenotype of progeny.

Sixty two Large White × Landrace sows were assigned to one of four feeding regimes during gestation; T1 (control group fed recommended level): 30 MJ DE/day throughout gestation, T2: as for T1 but increased to 60 MJ DE/day from 25-50 dg, T3: as for T1 but increased to 60 MJ DE/day from 50-80 dg, T4: as for T1 but increased nutrition to 60 MJ DE/day from 25-80 dg. Light and heavyweight littermate pairs of the same sex were selected at birth and individually fed to slaughter (c.157d). Histochemical and immunohistochemical staining were used collectively to identify the predominantly oxidative region of the *M. semitendinosus* as well as total fibre numbers and proportions of fibre types.

The results imply that increased maternal nutrition alters the skeletal muscle phenotype in the offspring by changing the proportion of fibre types, leading to an increased oxidative capacity. No change in total muscle area or fibre area is observed. The percentage area comprising the deep, more oxidative, portion of the *M. semitendinosus*, oxidative and IIa fibre type proportion, and S : P Ratio are increased in all treatments compared with control. These effects are most significant in T2. The precise molecular mechanism(s) by which these findings occur are being investigated.

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IBRAHIM ALBOKHADAIM and NEIL STICKLAND

Effect of egg incubation temperature on expression of specific genes associated with embryonic development and post-hatch muscle growth in Atlantic salmon (Salmo salar)

(Der Einfluss der Ei-Inkubationstemperatur auf die Expression von Genen der embryonalen Entwicklung und des Muskelwachstums beim Atlantischen Lachs)

Larval muscle development in Atlantic salmon is known to be affected by temperature with consequences for long term growth. The aim of this study was to evaluate the influence of egg incubation temperature on the expression of a number of genes implicated in muscle development.

Salmon eggs were incubated at either 10℃ or 5℃ from fertilization until hatching, after which they were all reared at 5℃. Fish from the two groups were sampled at the eyed stage and at 6 weeks and 21 weeks after first feeding and prepared for molecular studies. Expression of IGF-1, IGF-II, PCNA, myostatin, HSP70, MyoD, and myogenin were analysed using real time reverse transcription polymerase chain reaction (RT-PCR). Muscle cellularity was analysed at the same stages on complete transverse sections taken at the level of the vent.

At the eyed stage, IGF-I expression levels were present in higher amounts (trend) in the 5℃ fish group, and IGF-II expression levels were also higher (trend) in the same group at 6 weeks posthatch. Both PCNA and myostatin expression levels were present in higher amounts in the 5℃ group at the eyed stage. HSP70 expression levels were also higher in the 5℃ group at the eyed stage and at 21 weeks. However, neither MyoD nor myogenin expression levels were affected by temperature at any of the three sampled stages. At the eyed stage and at 6 weeks there was no difference in the number of white muscle fibres between the two temperature groups, but was significantly higher in the 5℃ group at 21 weeks. Nevertheless, at 6 weeks there were more small diameter muscle fibres in white muscle for the fish reared at 5℃ throughout, and larger diameter muscle fibres for the fish initially reared at 10℃. At 21 weeks there were more muscle fibres of smaller diameter in white muscle for the fish reared at 5℃ but no differences in the large diameter fibres.

The trend for high IGF levels in the 5℃ group correlates with the faster growth of this group (ALBOKHADAIM et al., 2007). High levels of PCNA expression also correlate with increase fibre hyperplasia. In this experiment, it is perhaps surprising that MyoD and myogenin expression levels were not also elevated in the 5℃ group. Taken overall this paper demonstrates the significant influence of temperature on gene expression in muscle and its relationship with changes in muscle growth dynamics.

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Foetal programming of postnatal growth and health

(Fötale Programmierung des postnatalen Wachstums und der Gesundheit)

Epidemiological as well as clinical studies have shown that small size at birth increases the risk of developing obesity, type II diabetes, and coronary heart diseases in adulthood. The nutrient supply during foetal development and growth may therefore have an important impact on health, and studies in animal models have also shown that undernutrition during foetal development may decrease postnatal growth – this phenomenon is referred to as foetal programming.

In this study, pigs are used as a model for studying foetal programming. In the first part of the study, 6 Landrace sows were subjected to either a norm diet (3 sows) or a 50% protein reduced but isocaloric diet to the norm fed sows (3 sows) from conception to day 110 in gestation. Just before farrowing the sows were slaughtered and the foetuses were taken out of the uterus. Blood was drained through the umbilical cord, foetuses were weighed and several anatomical measures were made. Organs were weighed, and liver and muscle samples were collected and will be used for RT-PCR and possibly proteome analysis. Sow treatment did not affect these measurements. However, pigs from sow on low protein had significantly less growth of pancreas and heart compared to the rest of the body measurements.

Besides using the nutritional restriction of the sow during gestation as a model for intra-uterine growth restriction (IUGR), the pig is also an important model for this phenomenon as the large variation in size at birth within a litter provides a naturally occurring form of IUGR. This is believed to due to under-nutrition of the foetuses placed in the periphery of the uterus. The birth size was not associated with placement of the foetus in the uterus at day 110 of gestation.

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The influence of sow dietary PUFA source on the fatty acid profile of piglet tissues and blood and on piglet performance

(Der Einfluss der PUFA in der Sauendiät auf das Fettsäureprofil im Gewebe und Blut der Ferkel sowie auf deren Leistung)

The aim of the present study was to examine the effect of sow dietary *n*-3 polyunsaturated fatty acid (PUFA) source on piglet performances and tissue and blood fatty acid composition. Two groups of six sows each were fed different diets from day 45 of pregnancy and during lactation on two commercial farms. On farm I a control diet (palm oil; 25-26 g/kg during pregnancy and lactation) and a fish oil containing diet (20 g/kg) was fed. On farm II the same control diet and a linseed oil containing diet (20 g/kg) was fed. All diets contained equal amounts of linoleic acid. One and three weeks after birth a randomly chosen piglet from each sow was sacrificed to take samples of the *Longissimus dorsi*, liver, subcutaneous fat and blood (erythrocytes and plasma). On both farms, the number of piglets born and weaned were recorded on a larger group (n=64-84) of sows fed the same diets. ROOKE et al. (1998; 2001) performed similar experiments and investigated the fatty acid profile in plasma and various tissues of piglets, but muscle tissue was not investigated. ROOKE et al. (1998) found an increase in the proportion of *n*-3 fatty acids in the order plasma > liver > erythrocytes > spleen > brain > retina by feeding tuna oil compared to soybean oil. Results on piglet performance and the enrichment of piglet tissues in essential *n*-3 fatty acids from feeding of the sows with linseed oil or fish oil will be discussed.

References

ROOKE, J.A.; BLAND, I.M.; EDWARDS, S.A.:
Effect of feeding tuna oil or soyabean oil as supplements to sows in late pregnancy on piglet tissue composition and viability. Brit. J. Nutr. **80** (1998), 273-280

ROOKE, J.A.; SINCLAIR, A.G.; EWEN, M.:
Changes in piglet tissue composition at birth in response to increasing maternal intake of long-chain n-3 polyunsaturated fatty acids are non-linear. Brit. J. Nutr. **86** (2001), 461-470

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Effects of feeding different protein levels to sows during pregnancy on adipose tissue development in the offspring

(Effekte einer Fütterung differenter Proteinniveaus an tragenden Sauen auf die Entwicklung des Fettgewebes der Nachkommen)

Intra-uterine nutritional conditions might affect fetal growth and tissue ontogenesis, and thereby could have a permanent effect on tissue characteristics despite adequate nutrition of the progeny post-natally. This study aimed to determine whether maternal protein diets could be associated with modifications of body composition and adipose tissue traits of the resulting offspring at birth and later age. Sows were fed either control (C, 12 % protein), or isocaloric low-protein (LP, 6 % protein) or high-protein (HP, 30 % protein) diets throughout pregnancy. At day 1 of age, 4 piglets per sow were killed according to their birth weight (one small, two medium, one large, n=85). The remaining piglets were cross-fostered by sows receiving the control diet. After weaning, they were fed commercial normo-protein diets until market age (185 days, n=40). Birth weight was lower on average ($P<0.01$) in LP (1.14 ± 0.03 kg; n=114) and HP (1.25 ± 0.03 kg, n=87) piglets than in C piglets (1.36 ± 0.02 kg, n=136). At day 1 of age, the percentage of subcutaneous fat tended to be higher ($P=0.10$) in LP piglets (11.29 ± 0.27) than in C piglets (10.6 ± 0.27), whereas it did not differ between HP (10.6 ± 0.28) and C animals. Interestingly, the specific activities of the lipogenic enzymes fatty acid synthase and malic enzyme were enhanced by 63 % ($P=0.01$) and 55 % ($P=0.06$), respectively, in subcutaneous fat of LP piglets compared to C piglets. No differences were observed in these traits between HP and C piglets. At market age, no effects of maternal protein diets on the amount and percentage of subcutaneous fat and on back fat thickness were apparent. Likewise, there was no difference in the specific activities of the lipogenic enzymes in subcutaneous fat tissue. In conclusion, preliminary data suggest that maternal protein deficiency could affect body composition and adipose tissue metabolism in the offspring at birth, but does not permanently affect fat metabolism.

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Flesh quality in rainbow trout with high or low fillet yield

(Die Qualität von Fischfleisch von Regenbogenforellen mit hoher und niedriger Filetausbeute)

Increasing fillet yield without any deleterious effect on flesh quality is a major goal for fish farm. The aim of our work was to characterize muscle growth and flesh quality by instrumental and sensorial evaluations) in rainbow trout with different fillet yield. Two groups were sampled, one with a mean low (56 %, LY group) and the other with a mean high (65 %, HY group) raw (non trimmed and non skinned) fillet yield. Both groups presented similar body weight (around 3.6 kg). The trimmed fillet yield was 37 % for the LY group vs. 44 % for the HY group. This higher fillet yield was associated with a higher carcass yield for the HY group (86 % vs. 75 % for the LY group). Loss during the smoking process did not differ between the two groups. Muscle fibre mean diameter was similar for the two groups, so the higher total muscle area of the HY group was associated to a higher number of muscle fibres (343.103 and 419.103 for LY and HY respectively). The area of subcutaneous adipose tissue (measured on one cutlet) was similar for both groups. The HY group presented an higher area of myosepta than the LY group (12.4 % vs. 11.3 %). Concerning flesh quality, fat content was higher for the HY group (9.1 % vs. 8.3 %). The luminosity of the raw flesh was higher for the HY group than the LY one (46.1 vs. 43.5). Mechanical resistance, measured in the caudal part of the fillet, was higher in the HY group (than in the LY group) for the raw flesh, but lower for the cooked flesh. Sensory evaluation did not reveal important difference between the cooked flesh of the two groups. Some differences in appearance were however detected for the smoked fillet : the HY group presented higher area of white stria (criteria for myosepta area) and the flesh colour intensity was lower than in the LY group. In conclusion the higher muscle mass observed in the HY group resulted from muscle fibre hyperplasia, and consequences of this higher muscle mass on flesh quality were rather moderate.

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Growth performance, muscle development, carcass characteristics and meat quality of lambs with varying birth weights

(Wachstum, Muskelentwicklung, Schlachtkörpermerkmale und Fleischbeschaffenheit von Lämmern mit unterschiedlichem Geburtsgewicht)

Intrauterine growth retardation is known to result in low birth weight which may influence postnatal growth, fat deposition, carcass characteristics and meat quality in sheep (1). The present study aimed to investigate the postnatal growth performance, muscle development, carcass characteristics and meat quality of lambs with varying birth weights.

Lambs with high (H; 4.06 ± 0.14 kg; n=7) and low (L; 2.61 ± 0.12 kg; n=8) birth weights at day 110 after birth were fed ad libitum and slaughtered after a feeding period of 55 days. Another group of lambs with low birth weights (LH; 2.68 ± 0.07 kg; n=7) were fed similarly but allowed to reach to the slaughter weight of high birth weight lambs. At slaughter, carcass characteristics, weights of organs, *semitendinosus* (ST), *semimembranosus* (SM) and *gastrocnemius* (GN) muscles were recorded. Samples from muscles including *longissimus dorsi* (LD) were collected for meat quality parameters such as pH at 45 min and 24 h after slaughter, colour at 60 min and 24 h after slaughter, shear force, water holding capacity, dripping loss and cooking loss.

Fat thickness on the loin area was higher in lambs in LH group ($P<0.05$) on the slaughter day. Slaughter weight and carcass weights of lambs in H group were higher than those in L group ($P<0.05$). Carcass yield differed between lambs in different birth weight groups ($P<0.05$). There were no significant differences between birth weight groups in terms of organ weights, pH, water holding capacity and dripping loss. Hunterlab colour measurements of LD and GN muscle samples did not differ between birth weight groups except that b value was higher in LH group which may reflect a higher fat deposition ($P<0.05$). Cooking loss was higher in LD samples of lambs in LH group than L group ($P<0.01$). The weight of ST muscles was higher in the lambs of H and LH groups than those of L group ($P<0.05$).

These results indicate that lamb's birth weight influence posnatal growth performance, carcass characteristics, meat quality. It appears that this effect may be due to higher fat depositions in the carcasses of lambs with low birth weight when they were allowed to reach the body size of lambs with high birth weight.

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References

DE BLASIO, M.J.; GATFORD, K.L.; ROBINSON, J.S.; OWENS, J.A.:
Placental restriction of fetal growth reduces size at birth and alters postnatal growth, feeding activity, and adiposity in the young lamb. Am. J. Physiol. Regul. Integr. Comp. Physiol. **292** (2007), R875-R886

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The influence of fatty acid composition in broodstock nutrition and of temperature during hatching on muscle development and growth in Arctic charr fingerlings

(Einfluss der Fettsäurenzusammensetzung der Diät des Zuchtstocks und der Temperatur beim Schlupf auf die Muskelentwicklung und Wachstum beim Seesaibling)

The Swedish Arctic charr (*Salvelinus alpinus*) breeding program, "Arctic superior", is one example of Arctic charr becoming an important aquaculture species. Arctic charr has been farmed during the latest three decades, resulting in an increased growth obtained by selection, approximately 10 % growth increase/generation. This selection program on Arctic charr started 1984 in Sweden and is an example of a successful selection program leading to enhanced growth and postponed sexual maturation (NILSSON, 1992). Unfortunately, the egg and hatching quality have not been included as selection parameters in the breeding program so far. Therefore, the present work focuses on egg quality from broodstock fed different lipid composition and hatching percentage as well as on impact of temperature on size at hatch and of fingerlings. We have incubated eggs after eying in different temperatures to evaluate if there is an effect on growth of newly hatched fingerlings and size of muscle bunches. That temperature among other traits is of importance for the growth and development of fish fingerlings is reviewed in JOHNSTON (2006). Preliminary results so far show on a difference in size at hatching caused by environmental temperature (2 and 7℃), higher temperature resulting in larger muscle mass of the fingerlings at first feeding.

References

JOHNSTON, I.A.:
Environment and plasticity of myogenesis in teleost fish. Review. J. Exp. Biol. **209** (2006), 2249-2264

NILSSON, J.:
Genetic parameters of growth and sexual maturity in Arctic char (*Salvelinus alpinus*). Aquaculture **106** (1992), 9-19

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Development of a real-time PCR method for determining skeletal muscle fibre type composition in sheep

(Die Entwicklung einer Echtzeit-PCR-Methode zur Bestimmung der Muskelfasertyp zusammensetzung beim Schaf)

Myosin is a major contractile protein in skeletal muscle and its different isoforms reflect differences in the properties of fibres, with the major fibre types being named according to the isoform of myosin heavy chain they contain. Four major adult isoforms of myosin heavy chain (MHC I, IIA, IIX and IIB) have been identified in skeletal muscle from a variety of species (PETTE and STARON, 2000).

We have developed a quantitative PCR method for determining ovine skeletal muscle fibre type composition, based upon the expression of different adult MHC isoform transcripts. Using published partial length ovine cDNA sequences, real-time PCR primers and probes specific for MHC I, IIA and IIX isoforms were generated at the 5'end. To test for cross-reactivity, each MHC isoform cDNA standard curve was spiked with a fixed quantity of each of the other MHC isoform cDNAs. No interference was observed and therefore the primers and probes are specific to their respective MHC isoform. Porcine MHC IIX and IIB cDNA sequences are identical over the region to which the IIX primers and probe were designed and therefore it is likely that they will also detect MHC IIB mRNA, if present. It is not known whether MHC IIB is present in ovine skeletal muscle, but the bovine MHC IIB isoform has only been detected in extraocular muscles (TONIOLO et al., 2005).

The primers and probes were then used to investigate changes in MHC isoform expression with age of sheep. Snap frozen samples of *longissimus dorsi* were obtained from male lambs at 1, 60, 120 and 180 days of age. Total RNA was extracted (Trizol) and cDNA generated using random primers, prior to real-time PCR using the MHC isoform mRNA specific primers and probes. Significant age-related differences in the expression of each isoform relative to total MHC mRNA were observed (ANOVA, $p<0.05$ for each isoform):

The method developed allows the major adult MHC isoform transcripts to be detected independently without interference from each other and can potentially be used to observe changes in muscle fibre type composition at the molecular level.

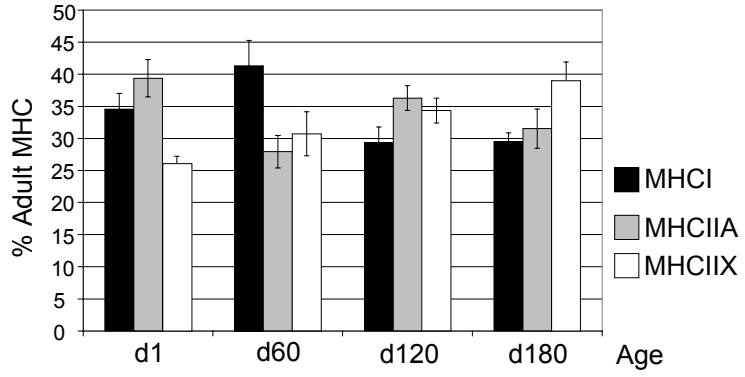

Fig. 1

References

PETTE, D.; STARON, R.S.:
Myosin isoforms, muscle fiber types, and transitions. Microscopy Research and Technique **50** (2000), 500-509

TONIOLO, L.; MACCATROZZO, L.; PATRUNO, M.; CALIARO, F.; MASCARELLO, F.; REGGIANI, C.:
Expression of eight distinct MHC isoforms in bovine striated muscles: evidence for MHC-2B presence only in extraocular muscles. Journal of Experimental Biology **208** (2005), 4243-4253

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Relative abundance of MyHC isoform transcripts and its association with muscularity and meat quality

(Relative Häufigkeit von MyHC Isoform-Transkripten und deren Zusammenhang mit Muskelansatz und Fleischqualität)

Number, size and proportion of muscle fiber types largely affect growth performance and meat quality traits in farm animals due to differences in contractile and biochemical properties, contributing to structural and functional diversity. Muscle fiber types differ in the expression of myosin heavy chain isoforms. We intended to evaluate the application of relative quantification of MyHC isoform transcripts as a new phenotype for further research towards understanding of the significance of muscle type in modulating muscle growth and meat quality. MyHC isoform transcript quantification of animals of a F2 resource population based on Duroc and Pietrain (DUPI) depending on their phenotype in terms of muscle mass and meat quality was conducted using real time PCR and microarray analyses. Abundance of MyHC IIb accounted for more than half of the MyHC transcripts (61.3 %). Real-time PCR quantification of MyHC isoforms I, IIa, IIx, and IIb further revealed that the relative expression of MyHC IIb was higher in pigs with large longissimus muscle. Moreover, transcript quantification by microarray analyses of discordant full-sibs regarding meat quality traits, i.e. *longissimus* pH 1 h p.m. and drip loss, revealed trait-associated expression of MyHC IIb. Frequencies of fibers, determined by ATPase muscle fiber staining, and relative abundance of MyHC isoforms, determined by quantitative RT-PCR, of corresponding pairs of type I, IIa and IIx/IIb were significantly correlated (r=0.71, 0.67, and 0.52, respectively). The study demonstrates that IIb fibers are the most prominent in pigs having large *longissimus* muscle area and implies that IIb is the determining fiber contributing to the differentiation of large and small loineye muscle area in the pig. The relative abundance of MyHC IIb is associated with meat quality traits.

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A method for determining the muscle fibre length

(Eine Methode zur Bestimmung der Länge von Muskelfasern)

Introduction

Muscle fibres are the result of the fusions of thousands of myoblasts to form a long tube. The length of a mammalian muscle fibre is variable, and can be the length of the muscle. They are not, however, usually as long as the muscle, but still the length may even be 340 mm in long muscles (LAWRIE, 1998). Fibres are arranged end-to-end series with their tapering ends overlapping adjacent fibres. About 50 % of their length is of constant diameter, and taper at both ends by 25 % of their length the angle of the taper being about 1 ° (McCORMICK, 1994). There is no data about porcine muscle fibre lengths easily available.

The purpose of this study was to develop a method to estimate the fibre length of a muscle in order to count the total number of fibres in a muscle.

Materials and methods

M. longissimus dorsi muscles from three pigs of about 110 kg live weight were excised on the day following slaughter caudally to 5th *Thoracic vertebra*. Three 1 cm thick slices were cut from both ends at 1/5 of the total length and from the middle, of each excised muscles (Figure 1 A). From each slice five 1×1×1 cm cubes were used for fibre length measurements and five smaller pieces, of about 300 mg, for fibre diameter measurements were cut as shown in Figure 1 B.

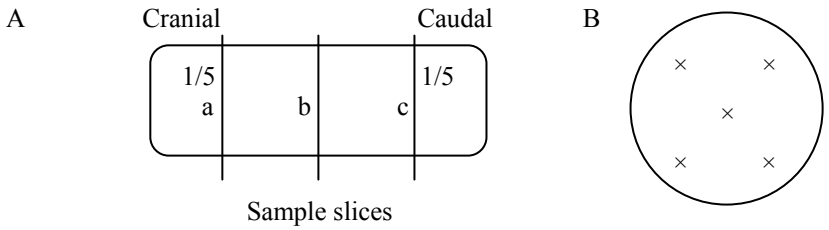

Fig. 1: Sampling from *M. longissimus dorsi* muscle. A – slice locations, B – fiber samples from a slice

Fibre length

Muscle fibres were separated by the HOOPER method (1976; 1981). A sample cube was placed into a 50 ml beaker with 15 ml 4 N HNO_3. Beakers were covered with aluminium foil and kept overnight at room temperature. After the acid treatment the dimensions of the cubes were measured to determine the shrinkage (shortening) caused by the acid treatment. The cubes shrank longitudinally along the fibre axis 10 %. Then each sample cube was inserted into a test tube with 5 ml Ringer-Locke solution. Test tubes were sealed and shaken vigorously by hand and by using test tube shaker, for one minute. Part of the suspension was poured on a slide and left to dry overnight.

The suspension was studied with a microscope (Olympus BH-2, Japan) at 40x magnification, with a commercial Zeiss AxioCam MRc digital camera (Zeiss GmbH, Germany) (Figure 1). Images were analysed with software package (Zeiss KS300 3.0, Germany). Individual fibre fractions were marked manually, on the images at their both ends, and the program then determined the length of the fibre. The results were stored cumulatively. Simultaneously, the number of fractions that visually seemed to be the very end of the tapered end of a fibre, was counted. From each slide sufficient fields were analysed so that the total number of fibre fractions was at least 400. Totally over 20,000 fractions were measured (3 muscles × 3 slices × 5 cubes × more than 400 fractions).
The length of fibres was counted assuming that the tapered ends are equally distributed within the fractions (Equation 1).

L=length of the fibres [mm]

$$L = \frac{\sum F}{(N/2)}$$ F= total length of fractions [mm] (Eq. 1.)

N=number of the tapered ends

Cross sectional area of slices
The slices were photographed, and the cross sectional area was determined using the image analysis program Zeiss KS300.

Number of fibres in a cross section
Due to an error in sample preparation, only two muscles were analysed for fibre numbers. The sample pieces were frozen in liquid nitrogen and kept then in -80℃. Cross sections (12 μm) were cut at -26℃ in a cryostat (REICHERT-JUNG 2800 FRIGOCUT E, Germany). The sections were not stained. From the sections, the counting area of the image and the number of fibres on the counting area were determined. The number of fibres per image was about 100. Two images per sample were analysed, totalling 10 images per slice (30 per muscle, 60 in grand total). The fibre number was counted by dividing the cross sectional area of the slice by the average cross sectional area of fibres.

Results and discussion

The fibres were well separated by the Hooper method. The fractions were clearly seen and marked for the automatic determination in the image. The tapered ends were also easy to identify, but the major obstacle was their small number. It was not possible to use a lower magnification or an automatic determination of the fractions directly from the image, because the first stage of the analysis had to be done manually. On one hand, the tapered ends of the fibres are very thin and difficult to see, but on the other hand, if this done always by the similar way, the fractions of certain size will always be counted similarly. An increase of the fields counted, however, would also much increase the work needed.
The aim of this study was develop a method for fibre length determination. Therefore, the conclusions below are only speculative, especially because the number of tapered ends was so small (varying between 4-14 within the total about 2,000 fractions

counted per slide). In such a small number, a change of one tapered end only has a marked effect on the results.

The fibres seem to be more than 100 mm long (Table 1). They seem to be longer in the middle of the muscle than at the end, which is in agreement with the statement of McCORMICK (1994). In this study we had actually only one end, the caudal (rear) end. The authors did not find any literature data about the length of porcine muscle fibre, and therefore no comparisons were made here. It seems, however, that the lengths obtained with this method are very long, and more analyses are needed for the validation of the method.

The fibre cross sectional areas was similar to that reported previously in Finland (RUUSUNEN and PUOLANNE, 2004) (Table 2). Because the fibres seem to be so long, theoretically there would be no need to have tapered ends at slice c, because it is closer (about 10 cm) to the caudal end of the muscle than the average length of the fibres. (On the contrary, the cranial end of the sample is not at the very end of the muscle, because a part of *M. longissimus dorsi* remained in the forepart of the carcass). Therefore, for reliable results, more slices taken at shorter spacings should be analysed, this would tremendously increase the amount of work needed.

The cross sectional area of fibres is larger in the middle of the muscle, which is a logical consequence of the observation (McCORMICK, 1994) that the fibres are tapered at the end of the muscle. It must be noted, however, that the fibre axis in *M. longissimus dorsi* in not fully parallel with the muscle axis, and therefore the fibres have a slight pennate-type arrangement in the muscle. The number of fibres per muscle cross section is about 1.1–1.3 million, on average 1.2 million. A very rough estimate of the total number of fibres in the whole muscle (length 60-70 cm, weight ca 4.0 kg) is about 4.2 million. Consequently, one fibre, 173 mm long and diameter Ø 40 μm, has a weight of about 1 mg.

Table 1
Muscle fibre lengths of porcine *M. longissimus dorsi* (n=3)

Slice (see Figure 1A)	Muscle fibre length (mm)
a (cranial)	183 (156-298)
b (medial)	218 (186-346)
c (caudal)	137 (112-235)
Grand mean	173

Table 2
Muscle fibre cross sectional area and cross sectional fibre number in porcine *M. longissimus dorsi* (n=2)

Slice (see Figure 1A)	Cross sectional area $\times 10^3$ μm^2	Number of fibres, $\times 10^3$
a (cranial)	5.14	1147
b (medial)	5.17	1153
c (caudal)	4.67	1299
Grand mean	5.00	1198

Conclusions

A rough estimate of the muscle fibre length of porcine muscle length can be made by disintegrating muscular tissue and counting the tapering ends of muscle fibres in relation to the total length of the fibres counted. The method is, however, time consuming and hard to automatize. A fibre type staining could be included to increase the relevant data obtained from samples. The average cross sectional area of the fibres seems to vary within the muscle, being smaller at the ends of the muscle.

References

ABERLE, E.D.; FORREST, J.C.; GERRARD, D.E.; MILLS, E.W.:
Principles of Meat Science. Kendall/Hunt Publishing Company, Iowa (2001)

HOOPER, A.C.B.:
Longitudinal growth of skeletal muscle fibres in lines of mice selected for high and low body weight. Growth **40** (1976), 33-39

HOOPER, A.C.B.:
Length, Diameter and Number of Ageing Skeletal Muscle Fibres. Gerontology **27** (1981), 121-126

McCORMICK, R.J.:
Structure and properties of tissues, in: Muscle Foods – Meat, Poultry and Seafood technology, ed. Kinsman, D.M., Kotula, A.W. and Breidenstein, B.C. Chapman & Hall. New York (1994)

LAWRIE, R.A.:
Lawrie's Meat Science. Woodhead Publishing Ltd. Cambridge (1998)

RUUSUNEN, M.; PUOLANNE, E.:
Histochemical properties of fibre types in muscles of wild and domestic pigs and the effect of growth rate on muscle fibre properties. Meat Science **67** (2004), 533-539

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Can functional near infrared spectroscopy (fNIRS) be used for the estimation of the numbers and types of muscle fibres in sheep?

(Kann funktionelle Nah-Infrarot-Spektroskopie zur Schätzung der Anzahl und Typen von Muskelfasern genutzt werden?)

A novel technique, fNIRS, have succesfully been used for the measurement of oxidative metabolism of muscles in human (1). This study is aimed to investigate whether fNIRS can be used to estimate the numbers and metabolic types of muscle fibres in lambs by measuring oxidative metabolism of *semimembranosus* (SM) muscle in vivo in sheep.

fNIRS is a novel, non-invasive, rapid and inexpensive method that uses light sources and detectors placed on the skin surface to probe the oxygen metabolism of the underlying tissues. A day before slaughter, NIROXCOPE 301, a continuous wave fNIRS device was used to measure the changes in muscle hemodynamic response to electrical stimulation in 120 (n=16) or 165 (n=8) days old lambs. The fNIRS probe was placed on the skin above the muscle and the measurements were acquired during full ischemia with two different stimulation frequencies (40 Hz vs. 150 Hz). NIROXCOPE 301 system provides local concentration changes of deoxyhemoglobin, oxyhemoglobin and oxygen consumption in units of µmol/s. After slaughter, SM muscle was isolated and frozen muscle samples were subjected to ATPase and SDH staining to determine contractile and metabolic types of muscle fibres and the number of fibres in an area.

There were significant positive correlations between the proportion of oxidative muscle fibres in SM muscle and the decrease in oxygen consumption (µmol/s) in response to electrical stimulation on day 120 ($r=0.58$; $P<0.05$) and on day 164 ($r=0.69$; $P=0.06$). Negative correlations were observed for proportions of type I or type IIB muscle fibres and decrease in oxygen consumption ($r=-0.68$; $P<0.05$; $r=-0.79$; $P<0.05$). Additionally there were also negative correlations between the number of muscle fibres per unit area and decrease in oxygen consumption ($r=-0.69$; $P<0.05$).

The preliminary results indicate that fNIRS can be used to study oxygenation status of muscle tissue in sheep and may be a new method to determine metabolic types of muscle fibres in sheep non-invasively. Further studies are required to optimise how fNIRS can reflect the muscle fibre numbers and types in various muscle samples of animals at different age.

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References

YUCETAS, A.; SAYLI, O.; KARAHAN, M.; AKIN, A.:
Measurement of oxidative metabolism of the working human muscles by near infrared spectroscopy. Proc. SPIE. **6081** (2006), 608103-7

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Repeatability of muscle fibre measurements in pig *longissimus* muscle

(Die Wiederholbarkeit von Muskelfasermessungen im *M. longissimus* beim Schwein)

The aim of this study was to determine the repeatability for different muscle fibre characteristics in the pig *longissimus* muscle in order to derive the minimum number of samples per animal needed to obtain reliable estimations. To evaluate the repeatability of the measurements within the animal, intraclass correlation coefficients (ICC, $\hat{\vartheta}$) were obtained by one-way analysis of variance. From each of 23 market weight pigs 5 samples evenly distributed over the *longissimus* cross sectional area at the $12^{th}/13^{th}$ rib level were taken and analyzed for various muscle fibre traits (total number of muscle fibres, mean fibre area, fibre type composition and relative area occupied by each fibre type), using the histochemical myosin ATP-ase reaction. The number of samples required per muscle cross section was found to be different between selected fibre traits. The highest ICCs were obtained for the total number of muscle fibres, and only three samples were necessary in order to obtain reliable estimations ($\hat{\vartheta} \geq 0.8$). However, five or more samples per muscle should be analyzed in order to accurately determine other muscle fibre traits, such as mean fibre area, fibre type composition and the relative area occupied by each fibre type. Type IIA fibres exhibited the highest intra-animal variability for all muscle fibre traits studied. These findings should be taken as a recommendation; their usefulness will depend upon the goal and conditions of future experiments.

References

CERISUELO, A.; SALA, R.; NÜRNBERG, G.; BAUCELLS, M.; REHFELDT, C.:
How many muscle samples are required to obtain reliable estimations of muscle fibre characteristics from pig *longissimus* muscle? Meat Science **76** (2007), 583-587

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DICK F. M. VAN DE WIEL[1] and WEILI ZHANG[2]

Identification of pork quality parameters by proteomics

(Identifizierung von Parametern der Schweinefleischqualität durch Proteomics)

Meat quality is a result of the genetic make up of the animal, its environment and the interaction between the two. Meat quality very much depends on the transition process of skeletal muscle into meat, and is directly related to the properties of the muscle proteins. Whereas genomics is focusing on the influence of genes, the focus of proteomics is on proteins, which is directly related to meat quality parameters of economic interest such as tenderness, taste, colour and waterholding capacity. Furthermore, proteomics allows for prediction of meat quality irrespective of its background, i.e. caused by either genetic or environmental factors. This forms an advantage of proteomics over genomics, because it is estimated that only 15-50 % of meat quality is genetically determined, depending on the quality trait.

After slaughter, pH in muscle tissue decreases rapidly, and together with a fall in temperature and contraction of myofibrils it can cause loss of water and proteins (=exudate or "driploss"), which may vary between 1 and 12 % (w/w).

In our experiment, homogenate of muscle (=proteome) collected immediately after slaughter from 24 pigs with either high or low % driploss (as determined on day 5 after slaughter) has been analysed by 2-dimensional electrophoresis. This is expected to provide information about proteins that are specific for a condition of high or low waterholding capacity, and therefore can be used as a marker in order to predict driploss. By applying this proteomics approach we have identified 3 candidate marker proteins that can predict the water holding capacity of pork, i.e. creatine phospho kinase (CPK), desmin and transcription activator SNF2L1. Based on the concentrations of CPK and desmin, a ratio can be calculated that varies between 2 (low driploss) and 50 (high driploss) (Figure 1).

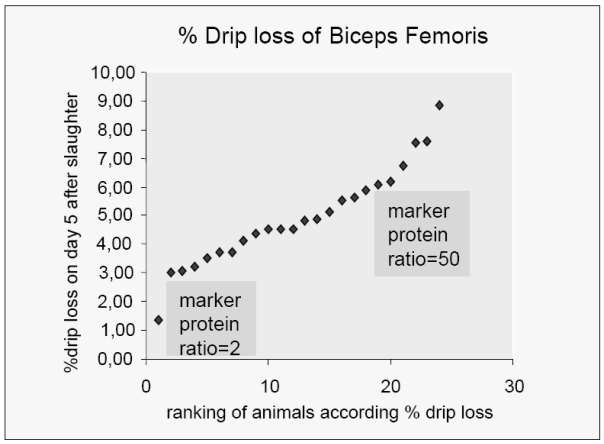

Fig. 1: Ranking of 24 pigs according to increasing driploss in one muscle (*Biceps Femoris*) on day 5 after slaughter, and predictive marker protein ratio's on day 0.

References

VAN DE WIEL, D.F.M.; ZHANG, W.:
Identification of pork quality parameters by proteomics. Meat Science **77** (2007), 46-54

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Surface electromyography as a tool for measuring aspects of meat quality

(Oberflächenelektromyographie zur Messung von Merkmalen der Fleischqualität)

Whilst positive correlations between type I fibres and meat tenderness have been reported (CALKINS et al., 1981), others have found that the strength of the perimysium of muscle is closely related to an increase in the toughness of pork meat during pig growth (FANG et al., 1999). Evoked surface electromyography (SEMG), giving rise to recordings of compound muscle action potentials (CMAP), offers a safe, quick, pain-free, non-invasive and repeatable technique for the assessment of the physiological processes that cause muscles to generate force and produce movement. A total of forty three Large White × Landrace pigs, taken from four litters born at Cockle Park Farm, Morpeth, UK, were used in this experiment. Analysis of SEMG signal parameters performed on *M. longissimus dorsi* using low stimulation frequencies, resulted in a correlation between CMAP parameters and the shear force of muscles post mortem. The exact relation between these parameters and the fiber type composition of porcine muscles still remains to be elucidated, however, the shear force of muscle post mortem can be approximated from the CMAP parameter Area (mV/s) using the equation Shear Force (N)=62.46 - 183.55 × CMAP signal Area (mV/s) ($r=-0.80$, $P=0.01$). Such a correlation could be explained by the fact that a strong SEMG signal with a large Area (mV/s) can be generated by just a few well-coordinated large-cross-sectional area fibers giving rise to relatively little perimysium per unit volume of muscle tissue, although further studies are now needed to prove this association. It is concluded, in the light of these preliminary findings, that this non-invasive method be further evaluated in a study aimed at linking the random variation in fibre type frequency in muscles with post mortem meat quality.

References

CALKINS, C.R.; DUTSON, T.R.; SMITH, G.C.; CARPENTER, Z.L.; DAVIS, G.W.:
Relationship of fiber type composition to marbling and tenderness of bovine muscle. J. Food Sci. **46** (1981), 708-710

FANG, S.H.; NISHIMURA, T.; TAKAHASHI K.:
Relationship between development of intramuscular connective tissue and toughness of pork during growth of pigs. J. Anim. Sci. **77** (1999), 120-130

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Description of in vivo bovine myogenesis using proteomic approach gives new insights for muscle development

(Die Proteomanalyse der In-vivo-Myogenese beim Rind liefert neue Einsichten in die Muskelentwicklung)

Abstract

Bovine myogenesis can be characterized by five key stages during the foetal life. Thus, stages 60 and 110 days post conception (dpc) are witnesses of primary myoblasts and secondary myoblasts proliferation respectively. Stage 180 dpc shows the end of the proliferation step and is the point when the total number of muscle fibres is defined. The last third of bovine foetal life, encompassing 210 dpc and 260 dpc, is mainly characterised by a large metabolic and contractile differentiation step. If histological and biochemical experiments allowed the analysis of bovine myogenesis and allowed to display those stages, many questions remain about the proteomic mechanisms involved in the regulation of the:

i) proliferation of the two myoblasts generations,
ii) cellular transition proliferation/cellular alignment/fusion of the myoblasts in myotubes/ differentiation,
iii) control of the total number of fibre.

A proteomic approach, based on the analysis of the *semitendinosus* muscle from Charolais cattle at key stages, was leaded using a classical two-dimensional electrophoresis and mass spectrometry strategy. We investigate the in vivo proteome of bovine muscle during *semitendinosus* muscle ontogenesis.

Gene Ontology is a modern in silico tool that allows the attribution of a biological function to a single gene or a single protein but it can be used to characterize the main biological function of a particular cluster (composed of several proteins). Analysis of common expression profile of proteins and the use of gene ontology enables us to show some already confirm results and, above all, enables us to find other tracks to investigate the muscular development of large mammals in order to answer our issues.

Keywords: myogenesis, proteomics, proteome dynamics

Zusammenfassung

Die Myogenese ist beim Rind durch fünf Abschnitte in der fötalen Entwicklung gekennzeichnet. In den Stadien 60 und 110 Tage nach der Konzeption (dpc) findet die Proliferation der primären bzw. sekundaren Myoblasten statt. Am Stadium 180 dpc endet die Proliferation und die Anzahl Muskelfasern ist fixiert. In den anschließenden Stadien 210 und 260 dpc findet die Differenzierung hinsichtlich metabolischer und kontraktiler Eigenschaften der Fasern statt. Obwohl histologische und biochemische Experimente Einblicke in die bovine Myogenese erlaubten, bleiben offene Fragen über die proteomischen Mechanismen der Regulation der:

i) Proliferation der beiden Myoblastengenerationen,
ii) Formation der Myotuben und
iii) Kontrolle der Faseranzahl.

Das Proteom des *M. semitendinosus* von Charolais Rindern wurde zu den Schlüsselstadien der Myogenese mittels 2D-Elektrophorese und Massenspektrometrie untersucht. In-silico-Werkzeuge wie ‚Gene Ontology' ermöglichen einzelne Gene und Proteine funktionell zu kategorisieren und auch Cluster von Kompomenten gemeinsamer Funktion zu bilden. Expressionprofilierung und die Anwendung dieser In-silico-Analysen liefern erste Ergebnisse zur obigen Fragen der Muskelentwicklung bei großen Säugetieren.

Schlüsselwörter: Myogenese, Proteom, proteomische Mechanismen

Introduction

Muscle characteristics play a major role in meat quality. As bovine species present mature muscle at birth, most of muscle characteristics (including physiological and metabolic properties) of adult muscle are acquired during foetal life. Myogenic events

of bovine muscles have been described previously using mainly histological and immunological approaches. These studies drawn the conclusion that bovine myogenesis can be described according to the succession of key stages that reflect myogenic events observed for the majority of mammals. Thus, 60 and 110 days post conception (dpc) are characteristic of the proliferation of the primary and secondary myoblasts respectively. At 180 dpc, the total number of fibres is reached. At last 210 and 260 dpc are two main stages of i) contractile and metabolic muscle differentiation and ii) fibres maturation. In our study, these stages were kept in order to perform a proteomic investigation of global proteins modifications occurring during bovine myogenesis. Proteomic approaches are very powerful to give access to the final expression of a whole genome during complex process. Indeed, using two-dimensional gel electrophoresis (2DE) and large data exploration leading to the identification of 246 proteins by mass spectrometry, we described the proteome dynamics of foetal muscle during ontogenesis. The time course analysis of the 110 proteins common to the five key stages allowed the description of the main function and pathway involved in muscle formation thanks to the appropriate use of gene ontology and in silico tools.

Materials and methods

The proteomic investigation performed is described partly in CHAZE et al. (2008) in press. Briefly, four Charolais foetuses for each five stages were collected from cows slaughtered at the INRA experimental abattoir. Samples of the *semitendinosus* (ST) muscle were frozen in liquid nitrogen and stored at -80 °C, until protein extraction, done as previously described (BOULEY et al., 2004). Protein concentration was determined using the 2-DE Quant kit (Amersham, Uppsala, Sweden), an average protein concentration of 6.8 ± 1.5 µg/µl was obtained from bovine foetal ST muscle. Proteins were separated by 2D gel electrophoresis. For the first dimension, iso-electrofocusing (IEF) steps were previously described by BOULEY et al. (2004) with the exception that after a desalting step (50 V, 7 h) proteins were separated under the following conditions, 200 V for 1 h, ramping to 500 V over 1 h, ramping to 1,000 V for 2 h, plateau at 1000 V for 1 h, ramping to 8,000 V over 9 h, plateau at 8,000 V until 73,500 V/h was reached. Second dimension SDS-PAGE was conducted on 12 % polyacrylamide gels in a Protean Plus Dodeca cell system (Bio-Rad, Hercules, USA) at 110 V and 15 mA per gel until the bromophenol blue migration front reached the bottom of all the gels. Two-DE gels were stained with G250 Colloidal Coomassie Blue. Four 2DE were performed for each foetus as technical replicates.

Gels were scanned at 300 dpi with an optical density calibrated ImageScanner (Amersham, Uppsala, Sweden). Image analysis was realised with ImageMaster 2-DE Platinum software (Amersham). Protein spots from the five stages were matched to a single reference gel. The reference gel was a physical gel conducted with an identical amount (in weight) of muscle protein, pooled from all samples analysed.

Data analyses of spots shared by the five stages were carried out using Principal Component Analysis and Hierarchical Clustering Analysis:

- In order to classify proteins presenting the same behaviour during bovine myogenesis, we performed a PCA analysis using the SPAD software.
- In order to group proteins presenting similar expression profiles across the five stages observed, hierarchical clustering analysis was used Hierarchical Clustering Analysis (HCA) was conducted using freely available PermutMatrix v.1.8.5 software (CARAUX et al., 2005).

HCA of the common protein spots was processed according to the Pearson distance. The Ward aggregation procedure was then used to construct the resulting dendrogram, as described previously (MEUNIER et al., 2007).
The coomassie stained spots of interest were identified by MALDI-TOF mass spectrometry. Peptide mass fingerprint (PMF) of spots was determined using a Voyager DE Pro Matrix Assisted Laser Desorption Ionisation Time Of Flight mass spectrometer (Applied Biosystem). Protein identification was done automatically and validations of all proteins were done by hand. Bos taurus NR database was used to identify PMF of trypsin digested proteins.
The Babelomics Fatigo+ tool was used to describe our data from clustering with the control vocabulary of Gene Ontology (AL-SHAHROUR et al., 2005). Groups of protein clusters exhibiting the same expression profile across the five stages of myogenesis were submitted to Fatigo+.

Results

This proteomic study of bovine myogenesis leaded to the detection of 496 protein spots and the identification by mass spectrometry of 246 proteins, mapped on the Figure 1. Among these, one hundred and ten proteins were shared by the five stages covering the whole bovine myogenesis.

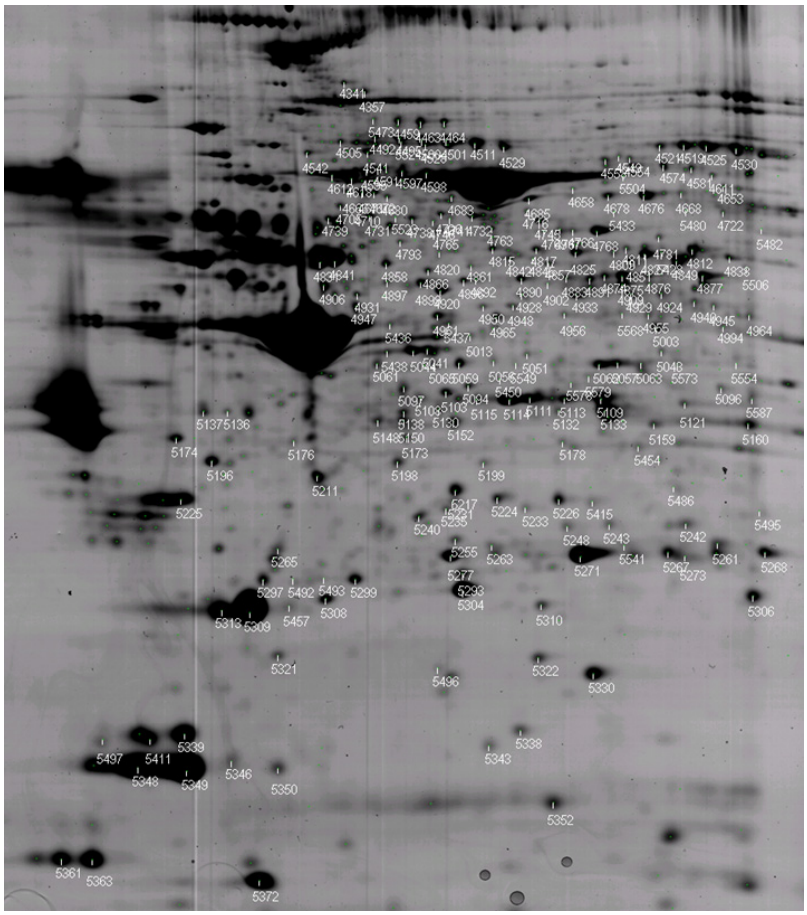

Fig. 1: Cartography of the 246 proteins identified by mass spectrometry (MALDI-TOF) in *semitendinosus* muscle of bovines foetuses during muscle development

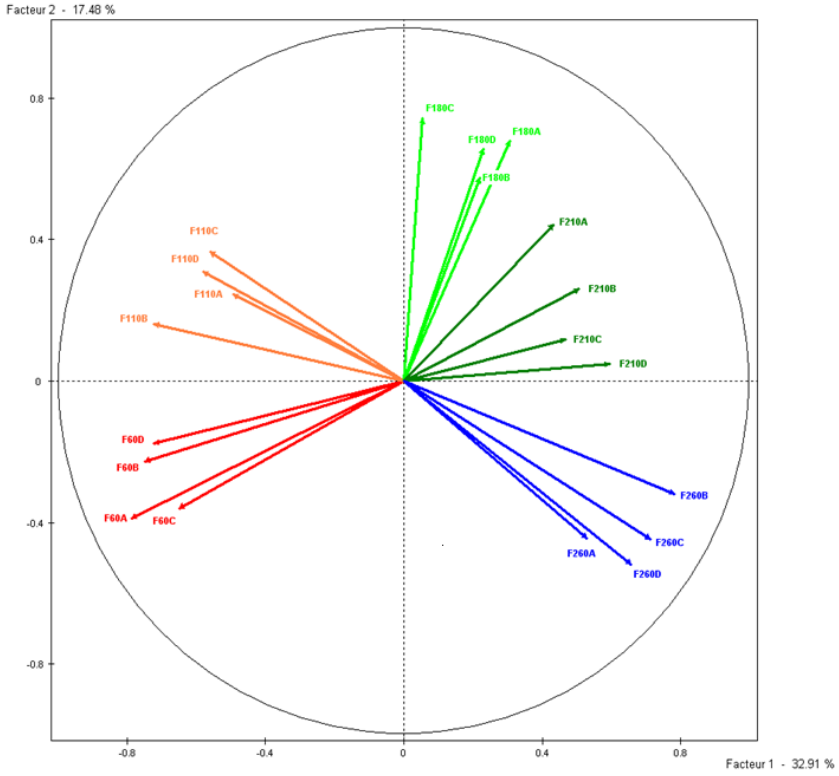

Fig. 2: Principal Component Analysis of the 5 ages with 4 foetuses par stages

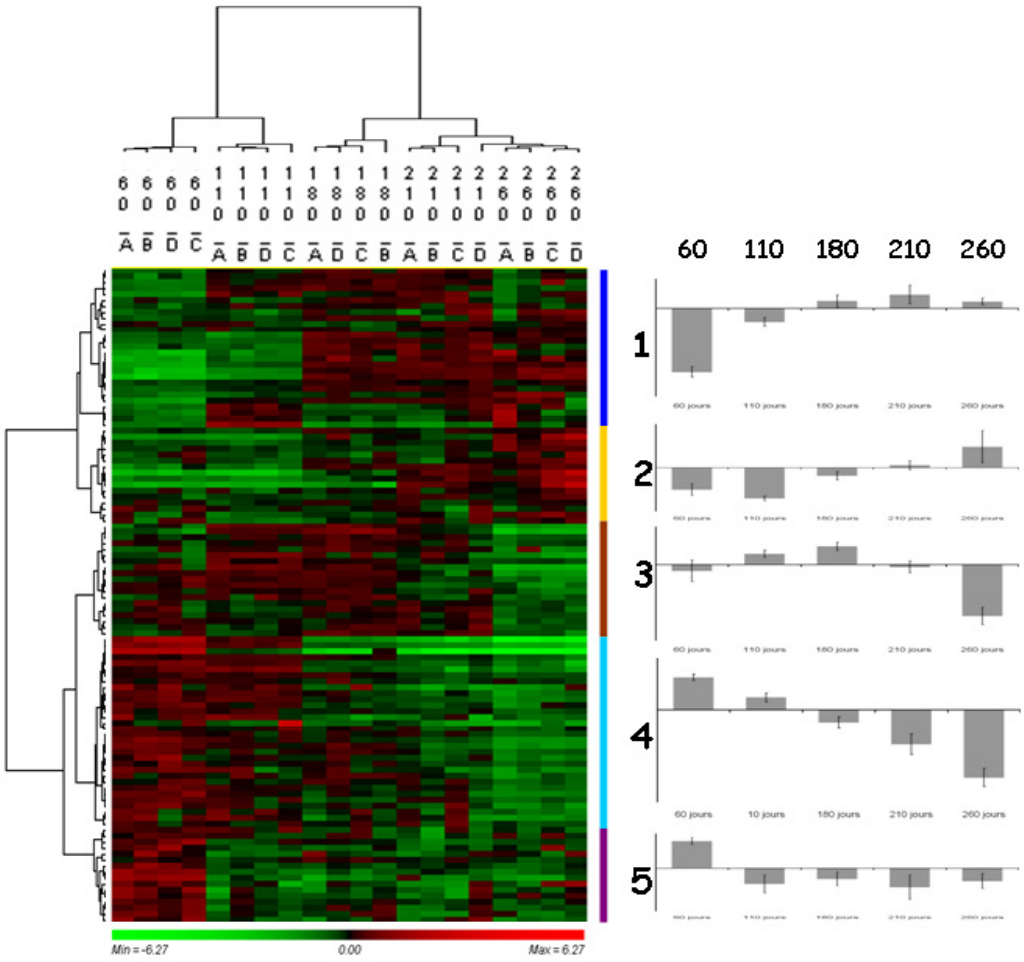

Fig. 3: Hierarchical Clustering Analysis of the 110 proteins common to the 5 stages
1, 2, 3, 4, 5 correspond to the different clusters grouping proteins with a same profile of expression across these stages. In green are proteins with low abundance, in red proteins with high abundance.

Analysis of these 110 proteins during muscle ontogenesis was performed using PCA and HCA analysis. In PCA, gels layout ensured a good gathering of 2D gel electrophoresis replicates since gels belonging to a specific stage were grouped together (Figure 2). Each stage was clearly separated in gels PCA experiments. Stage 60 and 110 dpc set at the opposite of stage 210 and 260 dpc while stage 180 dpc was set in the centre.

Data analysis using hierarchical clustering allowed the gathering of proteins exhibiting the same expression profile across myogenesis (Figure 3). In our study, the 110 proteins common to the five stages were classified in 5 distinct groups. The list of protein belonging to a particular cluster was submitted to the Babelomic's suite in order to characterize each cluster by Gene Ontology controlled vocabulary.

Thus, cluster 1 grouped proteins which abundance increased until 180 dpc and became stable during the last third of gestation. Functional analysis of this cluster indicated an increase of actin filament based process and also an increase of processes linked to cell death.

Cluster 2 consisted of proteins more and more abundant across myogenesis. Proteins grouped in cluster 2 were strongly associated to energy metabolism (cellular carbohydrate metabolic process, energy derivation by oxidation of organic compounds and coenzyme metabolic process). The main location of proteins forming this cluster was cytoplasm for the most part and mitochondria for some proteins.

Cluster 3 showed proteins which abundance increase until 180 dpc and decreased strongly at 260 dpc. GO terms characterising this cluster were linked to tissue organization (cytoskeleton organisation and biogenesis and regulation of striated muscle contraction). Cluster 4 exhibited proteins which abundance decreased across gestation. This cluster was particularly hard to be characterized, nevertheless this decrease in abundance was to be linked to developmental process (cell development) and mRNA splicing (mRNA processing and RNA splicing).

Cluster 5 displayed proteins which abundance was higher at 60 dpc and stable during the rest of myogenesis. This cluster could not be characterized using Gene Ontology vocabulary since it was composed of too little proteins (only 16 proteins) amongst whom five isoforms of albumin and two of transferin were identified.

Discussion

This work represents the first detailed description of protein modifications during foetal myogenesis. It could constitute a reference not only for bovine but also for other species.

The PCA analysis described a good repartition of the different stages in accordance with previous data on bovine myogenesis. Stages 60 and 110 dpc are grouped and opposite to the other stages. The data of PICARD et al. (2002) through the analysis of different myosin heavy chain isoforms (MyHC) at cellular and tissue levels, demonstrated that at these stages the number of primary and secondary myotubes increased. This suggests, a proliferating and fusing activity of myoblasts at these stages which is coherent with the high expression of proteins involved in developmental process and mRNA processing and RNA splicing such as HNRH3 and Apolipoprotein B mRNA editing enzyme, at these stages. On the contrary, stages 210 and 260 dpc, grouped and opposite to 60-110 days, correspond to differentiation process as the abundance of proteins associated to energy metabolism increased during

this period. These are coherent with the previous data indicating an increase of oxidative and glycolytic enzyme activities during the last trimester of bovine gestation associated with contractile differentiation characterised by modifications of MyHC isoforms (GAGNIERE et al., 1997 and 1999).
These data confirm the key stages of 180 days which was previously identified as a stage of the determination of the total number of fibres in charolais foetuses by histochemical analysis (PICARD et al., 2002). We observe numerous modifications of protein abundances and changes in isoforms expression from this stage. Particularly, the expression of proteins involved in tissue organisation falls down from this stage. Interestingly, we observe changes in proteins involved in apoptosis until 180 days. This suggests that the total number of fibres is the result of a balance between proliferation and apoptosis of muscle cells as described in CHAZE et al., 2008 (in press). Among the proteins with an over abundance on 180 days pc, some could constitute good candidates as markers of the total number of fibres. For example, the proteins WARS, PARK7 or CLIC4 whose expression decreased after 180 days pc, could be good markers. Further analyses are in progress to confirm this hypothesis. These types of markers are potentially interesting as the measure of the total number of fibres by histochemical techniques is very complex. Scientists working on myogenesis are looking for this type of markers in order to analyse how to manage the number of fibres which is of crucial importance for meat production.
In conclusion, these proteomics data will be used in the comparison of others types of bovines such as cattle presenting a high muscle development. This will be of importance to explain the differences of muscle development in these different types of animals and to understand the origin of muscle hypertrophy. These data will constitute also, a good basis for studies in comparative biology of myogenesis in different species.

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References

AL-SHAHROUR, F.; MINGUEZ, P.; VAQUERIZAS, J.M.; CONDE L.; DOPAZO J.:
BABELOMICS: a suite of web tools for functional annotation and analysis of groups of genes in high-throughput experiments. Nucleic Acids Res. **33** (suppl_2) (2005), W460-464

BOULEY, J.; CHAMBON, C.; PICARD, B.:
Mapping of bovine skeletal muscle proteins using two-dimensional gel Electrophoresis and mass spectrometry. Proteomics **4** (2004), 1811-1824

CARAUX, G.; PINLOCHE, S.:
PermutMatrix: a graphical environment to arrange gene expression profile in optimal linear order. Bioinformatics **21** (2005), 1280-81

CHAZE, T.; MEUNIER, B.; CHAMBON, C.; JURIE, C.; PICARD, B.:
In vivo proteome dynamics during early bovine myogenesis. Proteomics (2008) [in press]

GAGNIERE, H.; PICARD, B.; JURIE, C.; GEAY, Y.:
Comparative study of metabolic differentiation of foetal muscle in normal and double-muscled cattle. Meat Science **45** (1997), 145-152

GAGNIERE, H.; PICARD, B.; GEAY, Y.:
Contractile differentiation of foetal cattle muscles: intermuscular variability. Repr. Nutr. Dev. **39** (1999), 637-655

MEUNIER, B.; DUMAS, E.; PIEC, I.; BÉCHET, D.; HÉBRAUD, M.; HOCQUETTE, J.F.:
Assessment of Hierarchical Clustering Methodologies for Proteomic Data Mining. Journal of Proteome Research **6** (2007), 358-366

PICARD, B.; LEFAUCHEUR, L.; BERRI, C.; DUCLOS, M.J.:
Muscle fibre ontogenesis in farm animal species. Repr. Nutr. Dev. **42** (2002), 415-431

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Muscle molecular profiles in myostatin-null mice

(Molekulare Profile der Muskulatur von Myostatin-Null-Mäusen)

Myostatin, a member of the TGF-beta superfamily, is an inhibitor of muscle development and of the maintenance of the muscle mass. Invalidation of myostatin is responsible for the development of a hypermuscular phenotype in mice (MCPHERRON et al., 1997) and cattle (GROBET et al., 1997). Myostatin signals through TGF-beta like signal pathway, and also through p38 and akt. Myogenin and p21CKI have been identified as the major physiological targets of myostatin in murine cells. However, data in cattle suggest that there may be additional myostatin downstream events accounting for the development of muscle hypertophy (BOULEY et al., 2005; CASSAR-MALEK et al., 2007).

The main objective of this study was to identifiy molecular targets of myostatin action involved in the regulation of the muscle mass. For this we have examined differences in muscle gene and protein expression between myostatin-null mice (MC PHERRON et al., 1997; n=4) and their control littermates (n=4) by using both transcriptomics (DNA microarray) and proteomics.

As expected, the muscles of 5 week old myostatin-null mice were significantly heavier than those of their control littermates (+48 % to 163 %, $P<0.001$). There was an interaction between the genotype and the muscle type ($P<0.001$) and the *Quadriceps* muscle was chosen for the genomic experiments owing to its weight and marked hypertrophy (+87 %).

Comparison of the protein profiles by two dimensional electrophoresis (4-11 pH gradient, SAM) (MEUNIER et al., 2005) revealed the differential abundance of 28 protein spots (10 up-regulated and 18 down-regulated) and the presence of one spot and absence of one spot in myostatin null mice vs. controls. Mass spectrometry analyses are in progress to identify the candidate spots that may be myostatin targets.

The patterns of muscle gene expression were also compared in the muscles of myostatin-null mice vs their littermates using a microarray (human and murine oligonucleotide sequences) of around 6,000 genes expressed in muscle. SAM analysis revealed the differential expression of 192 up- and 245 down- regulated genes ($FC>1$, $FDR<5$ %). Among them, genes belonging to the Fc epsilon RI signalling pathway, insulin/IGF pathway protein kinase β signalling, carbohydrate metabolism, apoptosis and cell differentiation were up-regulated whereas genes belonging to the canonical Wnt signalling pathway, calcium signalling pathway and cytokine-cytokine receptor interaction were down-regulated. Data mining is in progress to understand the functional relevance of the differential expression of growth factors (e.g. FGF6) and 8 up and 16 down regulated transcription factors that may be key candidates for controlling hypertrophy molecular networks.

The transcriptomic and proteomic data will be further analysed with bioinformatic tools and integrated in order to identify the molecular bases underlying muscle hypertrophy. Thus, this will enable us to identify novel molecular networks that may be myostatin targets underpinning hypertrophy. The expected applications will be undoubtedly in food science for livestock meat production but also in clinical domains concerning the treatment of muscular dystrophy and ageing.

Acknowledgments

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References

MCPHERRON, A.C.; LAWLER, A.M.; LEE, S.-J.:
Regulation of skeletal muscle mass in mice by a new TGF-p superfamily member. Nature **387** (1997), 83-90

GROBET, L.; MARTIN, L.J.R.; PONCELET, D.; PIROTTIN, D.; BROUWERS, B.; RIQUET, J.; SCHOEBERLEIN, A.; DUNNER, S.; MÉNISSIER, F.; MASSABANDA, J.; FRIES, R.; HANSET, R.; GEORGES, M.:
A deletion in the bovine myostatin gene causes the double−muscled phenotype in cattle. Nature Genetics **17** (1997), 71-74

BOULEY, J.; MEUNIER, B.; CHAMBON, CH.; 2, DE SMET, S.; HOCQUETTE, J.-F.; PICARD, B.:
Proteomic analysis of bovine skeletal muscle hypertrophy. Proteomics **5** (2005), 490-500

CASSAR-MALEK, I.; PASSELAIGUE, F.; BERNARD, C.; LÉGER, J.; HOCQUETTE, J.-F.:
Target genes of myostatin loss-of-function in muscles of late bovine fetuses. BMC Genomics **8** (2007), 63

MEUNIER, B.; BOULEY, J.; PIEC, I.; BERNARD, C.; PICARD, B.; HOCQUETTE, J.-F.:
Data analysis methods for detection of differential protein expression in two-dimensional gel electrophoresis. Anal. Biochem. **340** (2005), 226-230

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Muscle markers and regulatory factors in marine fish under different developmental stages and treatments

(Marker für Muskelgewebe und regulative Faktoren beim Seefisch in Abhängigkeit von Behandlung und Entwicklungsstadium)

The research of molecular markers for flesh quality and their relationship with muscle development in fish is still scarce. Myogenic precursors cells have an important role in muscle growth and development, participating in hypertrophic fibre growth, new fibre formation and fibre repair. Many studies are focused to characterize these precursor cells markers (c-met, Pax7, Sox8) and signalling pathways (Akt, MAPK, mTOR) that regulate their function. Also, the Myogenic Regulatory Factors (MRFs) may be useful to determine muscle status in terms of growth and quality in fish.

We studied molecules as c-met, Akt and MAPK in two species of marine fish: Gilthead sea bream (*Sparus aurata*) and sea bass (*Dicentrarchus labrax*) cultured at different dietary conditions. We have also focused our attention on the MyoD2, Myogenin and Myostatin expression in sea bream.

Our studies using western blot in muscle of fish treated with different diets, show that c-met follows the similar pattern than fish weight and size, being the expression higher in those animals with better growth. On the other hand, MAPK and AKT pathways do not show the same tendency and their expression could be more influenced by the post-feeding stage.

By means of Real Time PCR, preliminary studies in primary culture of sea bream myocytes suggest high levels of MyoD2 in early stages of cell development (proliferation stages), while Myostatin seems to be not expressed along the culture. Further studies will be done to elucidate the implication of the different MRFs on muscle formation in sea bream, and to determine the possible effects on IGFs expression.

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Quantitative Real Time PCR of differentially expressed genes in pigs with extreme glycolytic potential values of skeletal muscle

(Quantitative Echtzeit PCR differentiell exprimierter Gene bei Schweinen mit extremem glykolytischen Potenzial der Muskulatur)

Glycolytic Potential (GP) is an important parameter for pork quality as it strongly influences the ultimate pH of meat. In order to identify the genes affecting its variation we compared by microarray analysis the expression of skeletal muscle genes in pigs with divergent values for GP level (RUSSO et al., 2006). Then, among the genes that resulted differentially expressed, a few were considered for validation by quantitative real time PCR (qRT-PCR). Here we report the results obtained comparing the expression profile by qRT-PCR in high and low GP samples of two genes, phospholamban (PLN) and ATP synthase, H+ transporting, mitochondrial F0 complex, subunit C3 (subunit 9) (ATP5G3), that microarray analysis identified as upregulated in muscles with low GP. Total RNA was extracted using Trizol from *longissimus dorsi* muscle of Italian Large White pigs extreme for GP level. The extracted RNA was quantified and integrity assessed by agarose gel. After treatment with DNase the RNA samples were retrotranscribed to cDNA and utilized for qRT-PCR on LightCycler TM system using SYBR Green I. The analyses were carried out in triplicate on individual samples and for the serial cDNA dilutions prepared to construct the standard curve (from 108 to 50 molecules/µl). The results showed that these genes are upregulated in low GP samples ($P<0.05$), confirming the data of microarray. The likely low level of ATP in the skeletal muscle cells of samples of low GP pool could be responsible of the up-regulation for genes involved in generation of precursor metabolites and energy.
These partial results underline the contribution of expression studies to understand the processes influencing the regulation of the GP level in skeletal muscle and to identify genes affecting meat quality.

References

RUSSO, V.; DAVOLI, R.; COLOMBO, V.; SCHIAVINA, S.; STELLA, A.; BUTTAZZONI, L.; FONTANESI, L.: Microarray analysis of gene expression profiling in pigs with extreme glycolitic potential values of skeletal muscles EAAP Proc. 57th Annual Meeting of the EAAP Antalya, Turkey, Sept. 17-20, 2006

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Post mortem proteome degradation profiles of *longissimus* muscle in Yorkshire and Duroc

(Post mortem Proteom-Degradationsprofile vom *M. longissimus dorsi* bei den Rassen Yorkshire und Duroc)

Abstract

Post mortem enzymatic proteolysis changes tough intact muscle tissue into tender meat. The extend of muscle protein proteolysis greatly determines the quality of the meat: inadequate proteolysis produces tough meat, too much proteolysis is associated with increased drip loss which also reduces tenderness and juiciness of the meat. While information on the proteolysis of several specific proteins is available, knowledge regarding the progress of proteolysis on the entire proteome of muscle tissue is limited. Furthermore, breed-specific differences have received little attention. Therefore, this study we investigated breakdown profiles of the *longissimus* proteome of Yorkshire and Duroc pigs. Multiple samples of *longissimus* muscle tissue of five pigs of each breed were collected and drip loss, cooking loss, shear force, as well as proteome profiles, using SELDI-TOF technology, were determined after 1, 2, 3, 7, and 10 days. Drip loss increased with ageing and was slightly higher in Yorkshire than in Duroc *longissimus* while cooking loss was unchanged during ageing and similar in both breeds. Shear force decreased with ageing time. Shear force decreased more in Yorkshire compared to Duroc, but profiles differed between animals. The number of peptides detected with SELDI-TOF was higher in York than in Duroc. It suggests a relationship between ageing-related levels of proteolysis and meat quality traits.

Keywords: proteomics, meat, proteolysis

Zusammenfassung

Enzymatische post mortem Proteolyse überführt intaktes Muskelgewebe in Fleisch. Dabei führt unzulängliche Proteolyse zu zähem Fleisch, zu starke Proteolyse führt zu erhöhtem Tropfsaftverlust, der auch die Weichheit und Saftigkeit des Fleisches verringert. Während Informationen über die Proteolyse einiger spezifischer Proteine vorhanden sind, ist das Wissen über Veränderungen des Proteoms während der Proteolyse begrenzt. Außerdem sind rassenspezifische Unterschiede bisher wenig berücksichtigt worden. Diese Studie zielt darauf ab, die Profile der *M. longissimus* Proteome während der Proteindegradation bei den Rassen Yorkshire und Duroc zu analysieren. Gewebeproben des *M. longissimus* von je fünf Schweinen der beiden Rassen wurden 1, 2, 3, 7 und 10 Tage nach der Schlachtung gewonnen zur Bestimmung von Tropfsaftverlust, Kochverlust und Scherkraft sowie der Darstellung von Proteomprofilen mittels SELDI-TOF. Der Tropfsaftverlust erhöhte sich mit dem Altern und war beim Yorkshire etwas höher als beim Duroc. Beim Kochen blieben die Verluste unverändert während des Alterns und ähnlich in beiden Rassen. Die Scherkraft verringerte sich mit der Zeit. Die Abnahme war beim Yorkshire höher. Die Zahl der Peptiden, die mit SELDI-TOF ermittelt wurden, war beim Yorkshire höher als beim Duroc. Dies deutet auf einen Zusammenhang zwischen alterungsabhängigen Niveaus der Proteolyse und Fleischqualitätsmerkmalen hin.

Schlüsselwörter: Proteomics, Fleisch, Proteolyse

Introduction

Skeletal muscle tissue formation in mammals is a prenatal process (REHFELDT et al., 2004; STICKLAND et al., 2004). After birth the skeletal muscle develops further by length and hypertrophic growth (TE PAS and SOUMILLION, 2001). During life of the animal the muscle determines mobility and stability of the animal. Muscle tissue consists of many cell types including muscle fibers, adipocytes, neurons, endothelial

cells, etc. Muscle mass is determined mainly by muscle fibers. Due to different functional requirements muscles differ in muscle fiber type composition. Muscle fiber types differ in metabolic activity and can be recognized by the expression of muscle fiber type-specific proteins (REGGIANI and MASCARELLO, 2004).
After slaughtering muscle tissue becomes meat. Meat has different characteristics than muscle tissue during life. Post mortem processes called 'ageing of meat' change the alive-muscle characteristics into eatable meat characteristics. A major factor in ageing is the proteolysis of proteins that were required for functioning during life but which induce toughness in meat (KOOHMARAIE 1996; HOPKINS et al., 2002). Due to muscle fiber type differences the proteolysis may differ between muscles. Due to selection proteolysis may also differ between breeds even within a single muscle.
Relatively little knowledge of the changes in the total tissue proteome during ageing (LAMETSCH, 2001, 2002, 2003), and muscle-specific and breed-specific differences have been reported. Therefore, we initiated this study by analyzing the proteome breakdown profiles during ageing in *longissimus* muscle in two pig breeds differing in pork quality parameters.

Materials and Methods

Animals and sampling
Five Yorkshire and five Duroc pigs, raised under similar conditions, were slaughtered at the same day. Growth rate data and backfat data were available for all animals. On the day of slaughter (day 0) longissimus and ham muscle tissue samples were taken from the right carcass side after the carcasses were rapid chilled for 30 min. Samples were taken 1.5 h after slaughter. The longissimus muscle was sampled between vertebrae three and four. Part of the samples were weighed and stored in an open box at 4℃ for drip loss measurements. Starting weights were about 200-300 g each. Part of the samples was snap frozen in liquid nitrogen and stored at -80℃ for future proteomics measurements.
All other samples were taken at day 1 from the left carcass half which was stored intact at 4℃ New samples for drip loss measurements were taken and treated exactly as described above. Other samples were taken from between vertebrae four-five, etc. until vertebrae nine-ten. These samples were stored in a plastic bag at 4℃ and used for determination of cooking loss and shear force as described below.

Meat quality traits
In the slaughterhouse pH at 24 h, conductivity and meat color (Japanese color scale and Minolta) were determined at the day after slaughter. Drip loss was determined by weighing the samples on the day of taking the samples, and reweighing them after drying with a tissue on days 1, 2, 3, 4, 7 and 10. Cooking loss was determined by weighing *longissimus* samples, heating at 70℃ in a sealed plastic bag for 1 h and cooling under running tap water for 1 h at the same days. After weighing, the cooked samples were stored in a sealed plastic bag at -20℃ until shear force determination (HONIKEL, 1998). Each sample was measured six times by taking six sub-samples.

Proteomics profiles
Samples for proteomics were removed from the freezer and the water-soluble fraction of proteins was isolated. Samples were weighed (30-50 mg), placed in 1.5 ml lysis

buffer (10 mM Tris-HCl pH 7.25, 10 mM KCl, 2 % [v/v] Triton X-100, 1 mM PMSF), homogenized on ice using an Ultra-Turrax T25 with dispersion tool S25N-10G (9,000 rpm, 20 s) and incubated on ice for 1 h and vigorously shaken occasionally. Subsequently, the homogenate was cleared by centrifugation (Eppendorf centrifuge, 30 min, 15,000 rpm, 4 ℃ max d), the resulting supernatant was transferred to a new tube and stored at -80 ℃ until further processing. The protein concentration of the cleared supernatant was determined using the Bio-Rad DC assay (Bio-Rad, Veenendaal, The Netherlands).

SELDI-TOF (Biorad) analysis was performed on the Protein Chip System Series 4000 equipment and the data were analyzed using Ciphergen Express Software release 3.0.6. After an initial screening the IMAC30-Cu (IMAC30 EDM buffer kit), CM10 (pH5 buffer) and Q10 (pH 6 buffer) chip array types and binding buffer combinations were chosen. The different ProteinChip® arrays were equilibrated with the respective binding buffers containing 0.1 % Triton. Equal amounts of cleared lysate sample protein was loaded on the arrays together with the respective binding buffers (total volume 250 µl) using a Bioprocessor (Biorad) and incubated for one hour at room temperature with continuous shaking. After removal of the diluted sample extracts, the arrays were subsequently washed with the respective binding buffers followed by a quick rinse with deionised water. After air-drying the arrays at room temperature energy absorbing matrix sinapinic acid (SPA) in 50 % acetonitril and 0.5 % trifluoroacetic acid was added to each spot. Mass analysis of the bound proteins was performed using Protein Chip System Series 4000 (Biorad) in positive operating mode. Mass spectra were collected by the accumulation of lasershots at a laser intensity of 3000 nJ. The highest mass to acquire was set at 150,000 kDa; Focus Mass setting was 50,000 kDa. The instrument was calibrated by using the All-in-one Protein Standard II (Biorad).

The EDM tool of the software was used to create clusters using the settings: S/n-ratio ≥3 and %-age occurrence of peak features=20 %. This means that clusters were created when 1 of 5 of the spectra contained a peak with an s/n ratio of ≥3.

Results

Meat quality traits

Drip loss did not differ between the Yorkshire and Duroc breeds in the *longissimus* muscle, but was significantly different between days of sampling: day of slaughter and day 1 (different carcass sides) as well as over time. After correction for correlated residuals Yorkshire carcasses had 0.15 % more drip loss than Duroc carcasses (not significant). Drip loss increases with day (Figure 1).

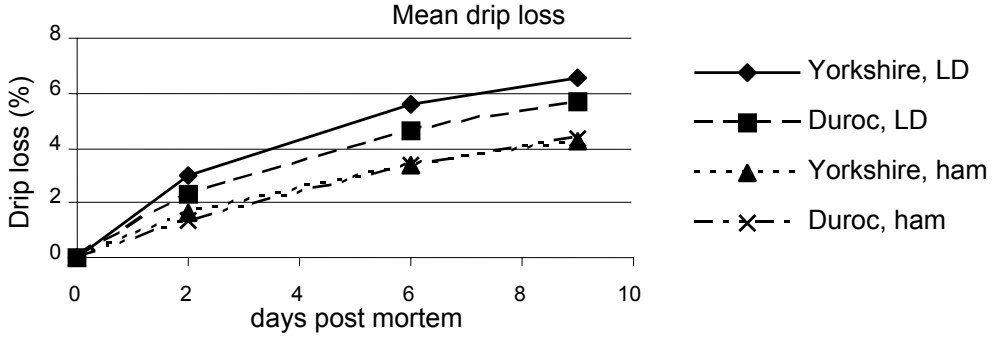

Fig. 1: Mean drip loss of *longissimus* and ham muscles of five Duroc and five Yorkshire carcasses from slaughter to ten days post mortem.

Cooking loss showed similar results as Yorkshire and Duroc breeds did not differ, but both breeds showed a significant day effect. Yorkshire pigs showed on average 0.364 % more cooking loss than Duroc pigs (not significant), but this difference increased over time (Figure 2).

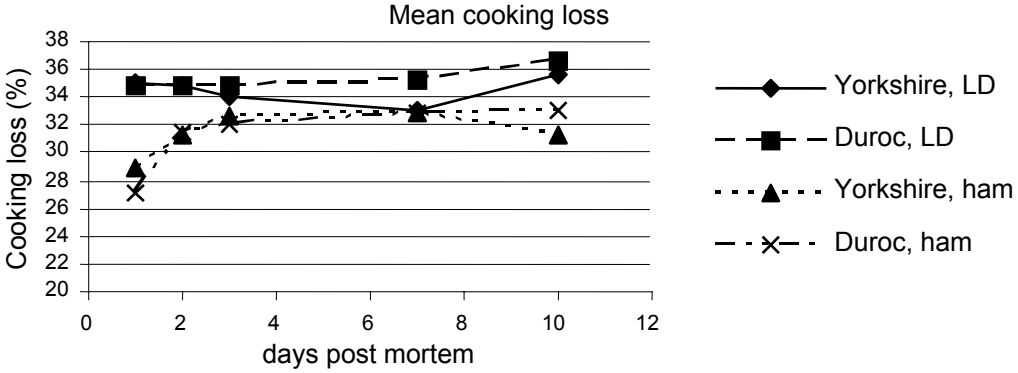

Fig. 2: Mean cooking loss of *longissimus* and ham muscles of five Duroc and five Yorkshire carcasses from slaughter to ten days post mortem

Shear force repeated measurements on a single sample were not significantly different indicating good repeatability of the measurements. Shear force was also not different between Yorkshire and Duroc breeds with Yorkshire pigs being a little less tender than Duroc. Shear force decreased with day in a non-linear pattern (Figure 3).

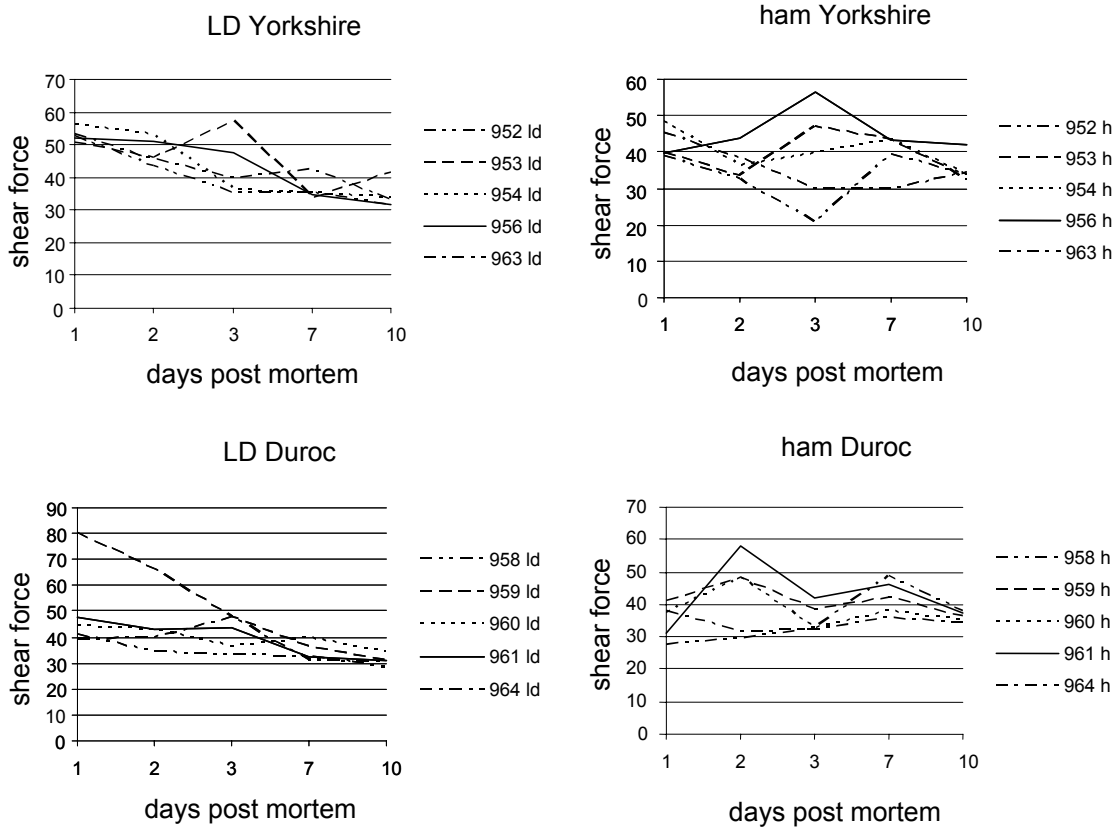

Fig. 3: Shear force measurements (averaged over 6 sub-samples) of *longissimus* and ham muscles of five Duroc and five Yorkshire carcasses from slaughter to ten days post mortem.

Proteomics profiles

Peak height was taken as a measure for expression level. The results show a profile of peaks representing different proteins. On average the profiles of Yorkshire pigs showed more peaks than the profiles of Duroc pigs (data not shown).

Analyzing the results in time suggest four different profiles of protein expressions (Figure 4). In figure 4 representative examples of protein features representative for the four classes of profiles are given.
(1) Profile one suggests that the expression level at day zero is either very low or not existing. After day one the expression increases in time and continues to increase until the end of the experiment on day 10. The increase may either start on day one or on a later day indicating variation within this profile between individual peaks. This profile suggests that the peak is a degradation product. (2) Profile two start similar to profile one but reaches a maximum after which the expression decreases. This profile suggests that the peak is a degradation product that is degraded also. (3) Profile three is the opposite of profile one: starting a high expression the expression decreases. The profile suggests that the peak is an original muscle protein that is degraded. (4) Profile four indicates that the expression of the protein is unchanged over time suggesting that the protein is an original muscle protein that is not degraded.

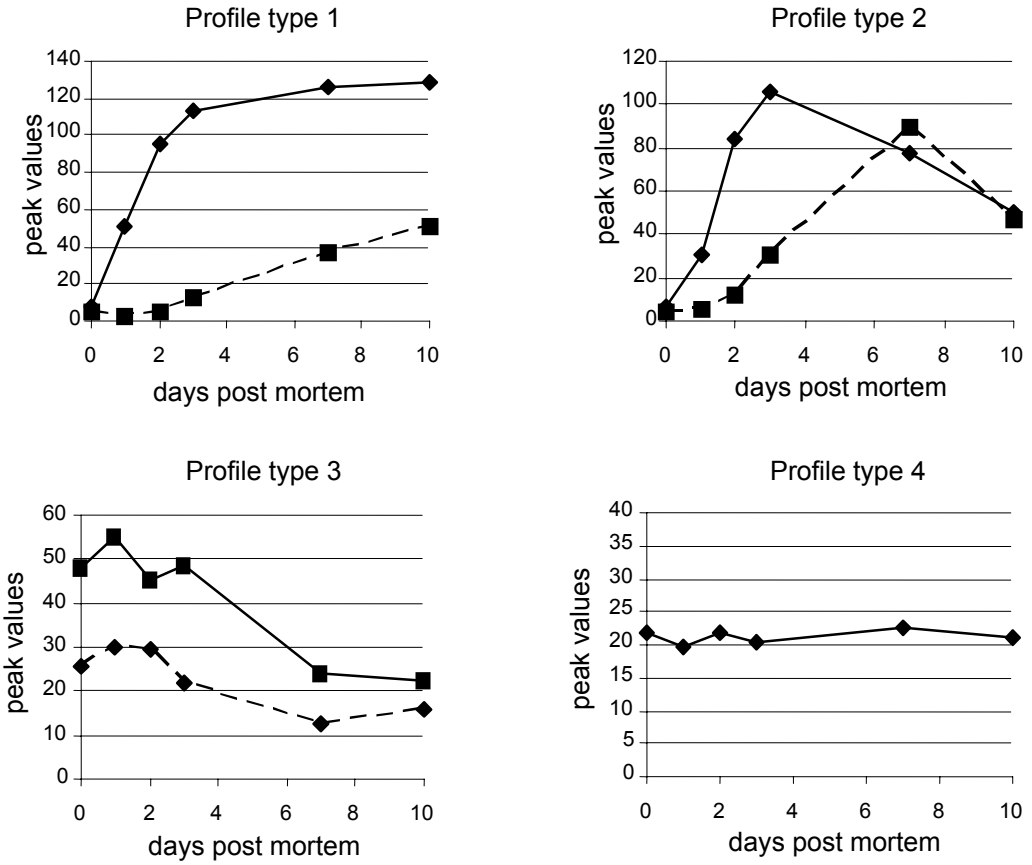

Fig. 4: *Longissimus* muscle proteome degradation profile types. Type 1: Expression at slaughter is low and increases during degradation. The time of the start of the increased expression varies between protein fragments (size). Suggestive for a degradation product. Type 2: Expression at slaughter is low and increases during degradation, but followed by a concomitant decrease. The times of the start of the increased expression and the decrease varies between fragments. Suggestive for a degradation product that itself is degraded further. Type 3: Expression at slaughter is high and decreases during degradation. The time of the start of the decreased expression varies between fragments. Suggestive for a muscle protein that is degraded. Type 4: Peak with a stable expression. Suggestive for a muscle protein that is not degraded during meat aging.

Discussion

Conversion of muscle to meat is regulated by complex interactions of biochemical processes that take place during postmortem storage of the carcass (OUALI, 1992; KOOHMARAIE, 1996). Post mortem proteolysis is one such important process.

Especially the degradation of titin and nebulin, both located in the I-band, is of great interest as electron microscopy of post-mortem meat samples has shown that the major fragmentation of the myofibrils occurs in the I-band area (TAYLOR et al., 1995; BOYER-BERRI et al., 1998; HUFF-LONERGAN, 1995). Several studies have shown troponin T to be degraded postmortem, and this degradation is believed to be closely related to meat tenderness (OUALI, 1992; HO et al., 1994). However, the degradation of the entire proteome is poorly known. Present proteomics techniques enable the study of the proteome.

Our results show that proteolysis continues during the whole period of sampling, i.e. until ten days after slaughtering the animal. While meat quality data showed that differences between animals and breeds are minimal, animals and breeds differed in proteome degradation profiles of the longissimus muscle proteome. This suggests that differences in meat quality between carcasses are related to differences in the degradation of the proteome. Differential rate of degradation or differences in the order of degradation of proteins may affect eating quality traits since pork is mostly partly degraded at the moment of consumption. Most fresh pork is consumed within a week. Characterization of the peptides leading to identification of the proteolytically cleaved muscle proteins may yield more insight in the process of tenderization. Further detailed studies are required with respect to this subject.

From our study it can be concluded that SELDI-TOF is a highly efficient method for the detection of muscle protein degradation products which are generated during the meat tenderization process. Correlation of meat proteome degradation profiles with other meat quality data may lead to the identification of Biomarkers for meat quality traits that can be used in the slaughterhouse. Development of Biomarkers that can be used during breeding or fattening livestock is also envisioned.

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References

BOYER-BERRI, C.; GREASER, M.L.:
Effect of postmortem storage on the Z-line region of titin in bovine muscle. J. Anim. Sci. **76** (1998), 1034-1044

HO, C.Y.; STROMER, M.H.; ROBSON, R.M.:
Identification of the 30 kDa polypeptide in post mortem skeletal muscle as a degradation product of troponin-T. Biochemie **76** (1994), 369-375

HONIKEL, K.O.:
Reference methods for the assessment of physical characteristics of meat. Meat Sci. **49** (1998), 447-457

HOPKINS, D.L.; THOMPSON, J.M.:
Factors contributing to proteolysis and disruption of myofibrillar proteins and the impact on tenderisation in beef and sheep meat. Aust. J. Agric. Res. **53** (2002), 149-166

HUFF-LONERGAN, E.; PARRISH, F.C.; ROBSON, R.M.:
Effect of post mortem aging time, animal age, and sex on degradation of titin and nebulin in bovine longissimus muscle. J. Anim. Sci. **73** (1995), 1064-1073

KOOHMARAIE, M.:
Biochemical factors regulating the toughening and tenderization processes of meat. Meat Sci. **43** (1996), 193-201

LAMETSCH, R.; BENDIXEN, E.:
Proteome analysis applied to meat science: characterizing postmortem changes in porcine muscle. J. Agic. Food Chem. **49** (2001), 4531-4537

LAMETSCH, R.; ROEPSTORFF, P.; BENDIXEN, E.:
Identification of Protein Degradation during Post-mortem Storage of Pig Meat. J. Agric. Food Chem. **50** (2002), 5508-5512

LAMETSCH, R.; KARLSSON, A.; ROSENVOLD, K.; ANDERSEN, H.J. ROEPSTORFF, P.; BENDIXEN, E.:
Postmortem Proteome Changes of Porcine Muscle Related to Tenderness. J. Agric. Food Chem. **51** (2003), 6992-6997

OUALI, A.:
Proteolytic and physicochemical mechanisms involved in meat texture development. Biochimie **74** (1992), 251-265

REGGIANI, C.; MASCARELLO, F.:
Fibre type identification and functional characterization in adult livestock animals. In: Muscle Development of Livestock Animals – Physiology, Genetics, and Meat Quality (Editors, M.F.W. te Pas, M.E. Everts, and H.P. Haagsman). CABI publishers, Wallingford, Oxfordshire, UK, 2004, 39-68

REHFELDT, C.; FIEDLER, I.; STICKLAND, N.C.:
Number and size of muscle fibres in relation to meat production. In: Muscle Development of Livestock Animals – Physiology, Genetics, and Meat Quality (Editors, M.F.W. te Pas, M.E. Everts, and H.P. Haagsman). CABI publishers, Wallingford, Oxfordshire, UK, 2004, 1-38

STICKLAND, N.C.; BAYOL, S.; ASHTON, C.; REHFELDT, C.:
Manipulation of muscle fibre number during prenatal development. In: Muscle Development of Livestock Animals - Physiology, Genetics, and Meat Quality (Editors, M.F.W. te Pas, M.E. Everts, and H.P. Haagsman). CABI publishers, Wallingford, Oxfordshire, UK, 2004, 69-82

TAYLOR, R.G.; GEESINK, G.H.; THOMPSON, V.F.; KOOHMARAIE, M.; GOLL, D.E.:
Is Z-disk degradation responsible for postmortem tenderization? J. Anim. Sci. **73** (1995), 1351-1367

TE PAS, M.F.W.; SOUMILLION, A.:
The use of physiologic and functional genomic information of the regulation of the determination of skeletal muscle mass in livestock breeding strategies to enhance meat production. Current Genomics 2 (2001), 285-304

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Role of STATs in muscle development

(Die Rolle von STAT-Proteinen in der Muskelentwicklung)

This review concerns whether STAT proteins, non-receptor tyrosine kinases with the transcriptional activity are involved in muscle development. In this regard, some reports indicate a negative while others point to positive role of STATs in the regulatory functions of cytokines. Depending on the category of STAT and/or cell type the impact of STATs on signal transduction from cytokine membrane receptors attracts special attention. Findings showing that STATs modulate cross-talk between death and survival pathways led to the idea that dysfunction/hyperfunction of STATs might somehow render muscle cells disincentive/susceptible to proinflammatory cytokines. In view of this, phosphorylation status seems to be crucial to the latter effect of STATs. It is widely known that muscle growth is under control of growth factors (insulin, IGF-s) but also other immunomodulatory cytokines are seriously considered as stimulators/repressors of muscle development and regeneration. This assumption came from microarray analysis of atrophying muscles (STEVENSON et al., 2003). In JAK/STAT (JAK2, STAT5b) signaling pathway differently expressed genes have been shown to regulate hypertrophic responses. It was noticed, that GH/IGFs might act through their cognate receptors by the alternative pathway involving JAKs/STATs (FROST et al., 2002). JAKs/STATs family of tyrosine kinases might affect several signaling pathways either by blocking the intracellular receptor binding domains, or by the transduction of signals from the phosphorylated tyrosine residues. Furthermore, overexpression of the suppresors of cytokine signaling (SOCS), antagonists of JAK/STAT signaling have been frequently reported to retard muscle growth. In contrast, in SOCS deficient mice, muscle growth was accelerated. In the „in vitro“ study carried out on C2C12 muscle cells, the expression of STAT1alpha protein increased during myogenesis and was additionally elevated by insulin (1-10 nM). Apparently, the stimulatory effect of insulin was dose-dependent. Since, proinflammatory cytokines (TNF-alpha, IL-1beta, IFN-gamma) were reported to impair insulin signaling further studies are focused to elucidate the role of JAKs/STATs in the insulin-dependent myogenesis. We hypothesized that cytokine membrane receptors compete for STAT kinase to transduce the intracellular signals. Possible interactions between STATs-targeted proteins are discussed in the aim to clarify the antagonistic effects of growth factors and proinflammatory cytokines. Special concern is put on insulin signaling and possible role of SOCS.

References

STEVENSON, E.J.; GIRESI, P.G.; KONCAREVIC, A.; KANDARIAN, S.C.:
Global analysis of gene expression patterns during disuse atrophy in rat skeletal muscle. J. Physiol. **551** (2003), 33-48

FROST, R.A.; NYSTROM, G.J.; LANG, C.H.:
Regulation of IGF-I mRNA and signal transducers and activators of transcription-3 and -5 (Stat-3 and -5) by GH in C2C12 myoblasts. Endocrinol. **143** (2002), 492-503

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Investigation of the porcine SMAD7 gene as a functional-positional candidate for muscle-related traits

(Untersuchung des porcine SMAD7 Gens als funktionelles positionelles Kandidatengen für Muskelfleischmerkmale)

SMAD7 is a member of the TGF-beta signalling pathway and a negative regulator of myostatin expression. Recently fundamental role for SMAD7 in initiating myogenesis through a SMAD7-MYOD positive feedback loop and abrogation of the myostatin signaling pathway was proposed. Transcriptional profiling of 7 key stages of myogenesis between Pietrain and Duroc breeds (EU-funded project PorDictor – QLK5-2000-01363) using differential display indicated breed-associated differential regulation of the SMAD7 gene. Based on comparative mapping between pig and human SMAD7 maps to interval S0312-SW2166 on porcine chromosome 1 in a major QTL region for carcass composition and meat quality traits identified in the Duroc × Pietrain F_2 (DuPi) population. Taken together there is a strong functional and positional evidence that sequence variation of the SMAD7 gene might affect muscle-related traits possibly via its effects on myogenesis. Comparative sequencing of SMAD7 gene so far revealed four SNP in the fourth exon in 3´ untranslated region. Currently genotyping and functional assays are developed to study effects of the identified sequence variation on function of SMAD7 and consequently muscle-related traits.

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Effects of isoflavones on mRNA expression of IGF1, IGF2, EGF and related growth factor receptors in porcine muscle satellite cell cultures

(Die Einflüsse der Isoflavone auf die mRNA-Expression von IGF1, IGF2, EGF und zugehöriger Rezeptoren in porcinen Satellitenzellkulturen)

Soy that is widely used in human nutrition and in livestock production is a rich source of isoflavones like genistein and daidzein. In skeletal muscle the impact of isoflavones on the growth and metabolism has received very little attention. In addition to the estrogenic or antiestrogenic effects via estrogen receptors, isoflavones are suggested to affect cell growth via inhibition of protein tyrosine kinases (e.g. growth factor receptors). Therefore, the present *in vitro*-study was undertaken to determine whether genistein or daidzein affect mRNA expression of insulin-like growth factor 1 (IGF1) receptor and epidermal growth factor (EGF) receptor and their related growth factors by real-time PCR. For this purpose, porcine proliferating and differentiating skeletal muscle cells were treated over 26 h with 0 (control), 1, 10 or 100 µM genistein or daidzein in serum-free medium (n=3).

Compared with the untreated controls the lower concentrations examined (1 and 10 µM) showed no effects on mRNA expression of the selected genes in proliferating or differentiating skeletal muscle cells. However, high-concentrated isoflavones (100 µM) decreased mRNA expression of IGF1 receptor and growth factors. Genistein at 100 µM caused a decrease on mRNA expression of IGF1, EGF and IGF1 receptor ($P<0.001$, $P<0.001$, and $P<0.01$, respectively) in proliferating cells. In differentiating cells genistein also reduced the mRNA expression of IGF1, EGF and IGF1 receptor genes ($P<0.001$, $P<0.001$, and $P<0.05$, respectively). In contrast, daidzein only affected IGF1 mRNA expression in proliferating cell culture ($P<0.01$), whereas in differentiating cells daidzein reduced transcript expression of IGF1, IGF2, EGF and IGF1 receptor ($P<0.001$, $P<0.01$, $P<0.001$, and $P<0.05$, respectively). The EGF receptor remained unchanged in response to isoflavones.

In summary, the results of the *in vitro*-study suggest that there is no effect of phytoestrogens at concentrations resulting from dietary consumption (1 and 10 µM) on IGF- and EGF-associated gene expression in porcine skeletal muscle tissue. Only high concentrations (100 µM) of isoflavones reduced the mRNA expression of the IGF1 receptor and the examined growth factors, and therefore, may modify their essential autocrine and paracrine actions in the skeletal muscle tissue.

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Divergent selection on "residual feed intake" in pigs: impact on growth performance, muscle compositional traits and meat quality

(Divergente Selektion auf Restfuttermenge beim Schwein: Einfluss auf Wachstumsleistung, Muskelzusammensetzung und Fleischqualität)

In the growing pig, increasing feed efficiency is a way to reduce the production cost. The residual feed intake (RFI) is defined as the difference between the observed daily feed intake (DFI) and the theoretical DFI estimated from maintenance and production requirements (GILBERT et al., 2006). A divergent selection was conducted for 4 generations in Large White males recorded between 35 and 95 kg live weight in order to produce animals with under (RFI-, "efficient animals") or over (RFI+, "luxurious animals") consumption compared to standard requirements. At similar body weight (107.8 ± 8.0 kg), 14 females from each line were slaughtered. Animals from the RFI- line exhibited leaner carcasses with higher muscle content, lower backfat thickness and lower lipid content in the *longissimus* muscle (LM). *Longissimus* muscle fibres were classified as types I, IIA, IIBR (Red) and IIBW (White) as described in LARZUL et al. (1997). The higher muscle content of the RFI- pigs was associated with a hypertrophy of all fibre types and an increase in the percentage of type IIBW fibres in LM. However, the correlative responses of muscle typing and chemical composition to the selection on RFI were not associated with variations in the activities of lactate dehydrogenase (glycolytic metabolism) and citrate synthase (oxidative metabolism), only a slight decrease ($P<0.05$) in β-hydroxy-Acyl-CoA dehydrogenase (lipid β-oxidation) was observed in RFI- pigs. Glycogen content in LM was determined at the level of each myofibre using the periodic acid Schiff staining and computerized image analysis. Glycogen content was higher specifically in type IIBW fibres of RFI- animals (1.43 vs 1.09 %, $P<0.001$). In accordance with their higher glycogen level in the fast glycolytic fibres, the RFI- pigs showed a higher glycolytic potential (162 vs 138 µmol equivalent lactate g-1, $P<0.01$), a lower ultimate pH (5.42 vs 5.58, $P<0.01$), higher drip loss (2.1 vs 1.1 %, $P<0.05$), and meat lightness (L*, 54.1 vs 50.7, $P<0.01$). Altogether, this suggests an impaired meat quality of the RFI- compared with the RFI+ pigs.

Keywords: muscle, muscle fibre, meat quality, selection, residual feed intake, pig

References

GILBERT, H.; BIDANEL, J.P.; GRUAND, J.; CARITEZ, J.C.; BILLON, Y.; GUILLOUET, P.; NOBLET, J.; SELLIER, P.:
Divergent selection on residual feed intake in the growing pig: genetic parameters and responses to selection. J. Rech. Porcines France **38** (2006), 111-117

LARZUL, C.; LEFAUCHEUR, L.; ECOLAN, P.; GOGUE, J.; TALMANT, A.; SELLIER, P.; LE ROY, P.; MONIN, G.:

Phenotypic and genetic parameters for longissimus muscle fiber characteristics in relation to growth, carcass, and meat quality traits in Large White pigs. J. Anim. Sci. **75** (1997), 3126-3137

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Relationship between the expression of development-related genes and properties of porcine hind limb muscles

(Die Beziehung zwischen der Expression von Genen für die Muskelentwicklung und den Eigenschaften der Hüftmuskulatur)

Congenital splay leg in newborn piglets is assumed to be a developmental retardation of skeletal muscle. We have recently investigated the expression of genes with known influence on the development and growth of the skeletal muscle in *M. biceps femoris* of healthy and affected newborn piglets. We identified the gene for MEOX2 (mesenchyme homeobox 2) as being significantly down-regulated in male splay leg piglets compared to healthy males. MEOX2 is involved in the regulation of vertebrate limb myogenesis. The aim of this study was to investigate (1) whether this down-regulation is generally observed in different hind limb muscles and (2) whether there is a relationship between the expression level of the gene and histological and biochemical properties of the respective muscles. To this end, we have isolated total RNA from *Mm. adductores*, *gracilis* and *sartorius* from each three male healthy and splay leg piglets. After reverse transcription of the mRNA-population with random hexamer primers gene specific primers for MEOX2 were used for Real-time-PCR. The individual expression of the gene for 18S rRNA within each muscle was used for standardization of the values. The expression was not different in *Mm. adductores* and *gracilis* but again, a highly significant down-regulation (3-fold) was observed in *M. sartorius* of male splay leg piglets. A first survey on the structure of the respective muscles revealed no significant relationship between histological parameters (e.g. secondary to primary fiber ratio; fiber density) and the observed expression differences. Further investigations are required to explain the observed differences between different muscles and between both sexes within a muscle.

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The analysis of MYF5, MYF6, GHR and IGFR1 expression profile in muscle and liver in growing pigs of different breeds, regarding to their muscle and carcass quality

(Analyse von MYF5, MYF6, GHR und IGFR1 Expressionsprofilen von Muskel und Leber bei Schweinen verschiedener Rassen und ihr Zusammenhang mit Fleischqualität und Schlachtkörpermerkmalen)

Myogenesis is a complex process which starts prenatally and lasts till postnatal period. The variation in the processes involved in myogenesis, indicated by genes expression profile, is essential to understand the differences in breeds, and thereby to use this knowledge in a breeding programme.

In the present study four genes were chosen, basing on the results of our previous association research: MYF5, MYF6, GHR and IGF1. MYF5 and MYF6 belong to a MyoD genes family, coding for transcription factors controlling the processes of myogenesis. MYF5 is responsible for the primary muscle cells migration and proliferation, and satellite cells proliferation in the postnatal process of muscle regeneration. MYF6 is expressed in an early peak together with MYF5 and second time at the later stages of myogenesis, has the high postnatal expression level and acts as a factor of muscle fibres maturation and their differentiated stage maintenance. The factors and hormones of somatotropin axis are essential for body, muscle and adipose tissue development and their expression is proved to be in relation with MyoD genes family expression.

The genes of these two systems are studied as candidate genes for performance traits in pigs. In the present study, the groups of gilts within the breed groups (Polish Large White, Polish Landrace, Pietrain, Duroc and Polish indigenous pig Pulawska) are studied in regard to mRNA level of four described genes expressed in *M. longissimus dorsi*, *M. semimembranosus* and/or in a liver. The samples were taken out of 30, 60, 90, 120, 150, and 210 days old gilts, the RNA was isolated and Real Time PCR analysis was undertaken. It will allow to draw the expression profile of studied genes during a postnatal process of body and muscle development of pigs and to see the possible differences between the breeds differing in carcass quality, maturation time and meat quality.

Presently, in the project the muscle and liver samples were collected and RNA is isolated out of samples of 30, 60, 90 and 120 days old sows. The Real Time analysis was performed for 60 days old animals of different breeds. The observed differences in expression levels of studied genes between the breeds were statistically insignificant. The expression of MYF5 and MYF6 genes were also studied in regard to single nucleotide polymorphisms found in the promoter region in our previous research. In the pigs of TT genotype of MYF6 gene, the significantly higher MYF6 expression then in CC pigs was stated.

References

WYSZYNSKA-KOKO, J.; PIERZCHALA, M.; FLISIKOWSKI, K.; KAMYCZEK, M.; ROZYCKI, M.; KURYL, J.:

Polymorphisms in coding and regulatory regions of the porcine MYF6 and MYOG genes and expression of the MYF6 gene in m. longissimus dorsi versus productive traits in pigs. J. Appl. Genet. **47** (2006), 131-138

WYSZYNSKA-KOKO, J.; KURYL, J.:

Porcine MYF6 gene: sequence, homology analysis, and variation in the promoter region. Anim Biotechnol **15** (2004), 159-173

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Stunning fish

(Betäubung von Fischen)

Plants have long been used by mankind as a source of active compounds for a whole range of activities. One such activity practiced by hunter gatherers and still in use by indigenous tribes of South America is the use of plants as piscicides. Macerated material of plant origin is thrown into rivers, streams or shallow ponds, and then the fish which are stupefied float to the surface and can be collected. Subsequent chemical analysis of plant material has revealed that the active compounds in such plant extracts are rotenoids and saponins. There are some reports in the literature about other plant extracts which may also act as icthyotoxins, one such extract comes from *Eugenia caryophyllata* (clove). The objective of the present study was to establish if saponins and volatile oils from clove could be used as an alternative to current anaesthetics for fish (eg. phenoxyethanol), and to determine their potential mode of action and their impact on meat quality. A method was established for extraction of saponins from the berries of *Sapindus saponaria* (Linné: *Sapindaceae*). In brief, dried berries were milled to a fine grain, defatted and their saponins were solvent extracted and isolated by precipitation with cold acetone. The resulting precipitate was collected by filtration under pressure, washed and the dried precipitate stored at 4℃. In order to test the activity of the saponin extract its capacity to haemolyse red blood cells was assessed. A standard solution was prepared of purified saponin from Quillaja bark (1 mg/ml; Sigma-Aldrich) and its action compared to fractions B and C of the crude saponin isolate (5 ppt) and water extracted saponin (5 ppt). The saponin from Quillaja bark and saponin extract (fractions B and C) caused haemolysis of red blood cells within 45 minutes indicating the isolated material is bioactive; the water extracted saponins had no effect. The saponin standard (0.3 mg/ml) and saponin extract B (0.3 ppt) and C (0.3 ppt) and clove oil (0.6 ppm) were then tested on fish (n=4-6) (platyfish, *Xiphosphorus maculatus*), neither fraction B nor C had any sedative effect as assessed by fin movement and general activity of the fish. In contrast, exposure to saponins from Quillaja bark (0.3 mg/ml and 1 mg/ml) initially caused hyperactivity but within 5 minutes all activity ceased and fish died suddenly. It was not possible to find a dose level at which saponins acted without causing death. In contrast, clove oil (5-10 ppm) had a rapid acting and totally reversible effect and sedated fish within 2 minutes of exposure. Activity as determined by fin movements gave a good index of anaesthetic effect which occurred in 129.4+22.94 (sec) in fish exposed to clove oil. Further dilutions of clove oil (60 ppb) caused an initial period of hyperactivity, which was followed by reduced activity although fish never reached a state of total sedation. The effect of saponins on muscles is currently under investigation. The results of the present study indicate that saponins are not effective as a fish anaesthetic but that clove oil offers a safe and rapid alternative to current anaesthetics.

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Metabolic programming establishes resistance of oxidative-type skeletal muscles to glucocorticoid-induced muscle cachexia in rats

(Metabolische Programmierung führt zur Resistenz oxidativer Skelettmuskulatur gegen Glukokortikoid induzierte Kachexie)

Abstract

The aim of this study was to establish why oxidative type muscles (*soleus* muscle, SM) are resistant to muscle cachexia induced by dexamethasone disodium phosphate overload (2 mg/kg b.w. day^{-1} during 5 consecutive days). DEX treatment was associated with the dramatic drop in total antioxidant status, that was partially corrected by the supplementation with antioxidants. The relationships between the expression of selected proteins (phospho-Ser473-Akt [P-PKB] and Akt [PKB], nucleus encoded subunit IV of mitochondrial cytochrome-c oxidase [NCOIV]) and the activity of selected enzymes (lactate dehydrogenase – LDH, phosphofructokinase – PFK, isocitrate dehydrogenase – ICDH) in SM vs. *gastrocnemius* muscle (GM) were examined in rats additionally treated with antioxidants (sodium ascorbate, ASC, or ascorbic acid phosphate, ASC-P). No changes were observed in the expression of PKB, P-PKB, NCOIV in *soleus* muscle. The activity of LDH was not affected, either. Interestingly, PFK activity in SM was elevated in experimentally treated animals ($P<0.05$) and the highest value was observed after ASC-P ($P<0.01$). In turn, ICDH activity peaked in SM after dexamethasone treatment ($P<0.05$) and dropped during the co-treatment with ascorbate (DEX/ASC). The average expression of NCOIV was significantly higher in GM in contrast to SM, although it decreased considerably after DEX treatment or co-treatment. DEX was also shown to reduce significantly the expression of P-PKB in GM. Anyway, the rise in the activity of PFK suggests that SM started to utilize excess glucose during DEX-induced hyperglycemia. In contrast to SM, the activity of PFK in GM was significantly lower after treatment or co-treatment with antioxidants. Taken together, these results pointed to the metabolic profile of the skeletal muscle that determines the resistance of SM to steroid diabetes-induced muscle cachexia. Antioxidants significantly affect the metabolic profiles of examined muscles.

Keywords: dexamethasone, oxidative-type muscles, muscle cachexia, antioxidant status

Zusammenfassung

Die Studie soll zeigen, warum Muskeln mit überwiegend Muskelfasern vom oxidativen Typ (*M. soleus*, SM) gegenüber Dexamethason-indizierter (DEX 2 mg/kg an 5 aufeinander folgenden Tagen) Kachexie resistent sind. Dexamethasongaben gingen mit einem drastischen Rückgang von Antioxidantien einher, der teilweise ausgeglichen wurde. Die Expression einiger Proteine (phospho-Ser473-Akt, [P-PKB], Akt [PKB], nucleus encoded subunit IV of mitochondrial cytochrome-c oxidase [NCOIV]) und die Enzymaktivität (Laktatdehydrogenase [LDH], Phosphofruktokinase [PFK], Isozitratdehydrogenase [ICDH]) wurde bei Ratten im SM vergleichend zum *M. gastrocnemius* (GM) nach Behandlung mit Antiooxidantien (Askorbinsäure [ASC]) bestimmt. Keine Unterschiede wurden in der Expression von PKB, P-PKB, LDH und NCOIV im SM beobachtet. Die PFK-Aktivität im SM war bei behandelten Tieren erhöht ($P<0,05$) und am höchsten nach ASC-P ($P<0,01$). Die ICDH-Aktivität war am höchsten nach DEX-Behandlung ($P<0,05$) und fiel bei DEX/ASC-Behandlung ab. Die NCOIV-Aktivität war im GM signifikant höher als im SM; sie sank nach DEX oder DEX/ASC-Behandlung. DEX führte auch zur Reduktion der P-PKB Expression im GM. Der Anstieg der Aktivität von PFK zeigt, dass im SM überschüssige Glukose nach DEX-indizierter Hyperglykämie metabolisiert wird. Im Gegensatz dazu war im GM die PFK-Aktivität signifikant erniedrigt nach DEX- oder DEX/ASC-Behandlung. Die Untersuchungen zeigen das metabolische Profil der Skelettmuskeln, das die Resistenz des SM gegen Steriod-Diabetes-induzierte Muskelkachexie bestimmt. Antioxidantien beeinflussen das metabolische Profil signifikant.

Schlüsselwörter: Dexamethason, oxidative TypI Muskelfasern, Kachexie, Antioxidantien

Introduction

The metabolic programming leads to molecular changes which determine the contribution of the particular fibre type to skeletal muscle. Thus, muscle development is related to variety of morphological, biochemical and mechanical criteria. Adult skeletal muscles are composed of multinucleated fibres with metabolic properties which are adapted to the energetic requirements of the tissue, although these properties might be also answerable for specific response to particular stimuli, for example glucocorticoids. These hormones are known to induce muscle cachexia that is manifested by muscle atrophy. The molecular mechanisms of glucocorticoid-dependent muscle cachexia are complex. Most, if not all of the effects are genomic, pointing to indirect action of glucocorticoids on muscle cells (ORZECHOWSKI et al., 2003). By targeting several genes glucocorticoids impair antioxidant capacity leading to oxidative stress (LIN et al., 2004; MANOLI-OREOPOULOS et al., 2004; ORZECHOWSKI et al., 2002). Concomitantly, m-calpain proteolytic system is activated with resultant cell death (ORZECHOWSKI et al., 2003). Glucocorticoids retard muscle growth through the myostatin-dependent mechanism (LANG et al., 2001; MA et al., 2001; MA et al., 2004). However, neither glucocorticoids nor myostatin are causative for disuse muscle atrophy, since both in adrenalectomized and in myostatin knockout mice progress in muscle wasting through leg immobilization is equal or even greater than in wild-type animals (JASPERS and TISCHLER, 1986; MCMAHON et al., 2003). The catabolic effect of proinflammatory cytokines is closely related to muscle cachexia, since TNF-α or IL-1β exert systemic rather than local negative effects (MOLDAWER and COPELAND, 1997). In these circumstances, oxidative-type muscles behave uniquely being extremely resistant to the above-mentioned cachectic factors. The answer to the question whether metabolic programming render muscles less susceptible to atrophy might shed more light on the molecular mechanisms of this phenomenon.

From our preliminary studies (ORZECHOWSKI et al., 2000; 2002) we found that dexamethasone treatment led to muscle cachexia and insulin resistant state in growing rats. In this research we turned into the oxidative-type muscles represented by soleus muscle. Oxidative muscles appeared to be extremely resistant to catabolic action of glucocorticoids and glucocorticoid-induced insulin resistant state. In contrast to glycolytic-oxidative muscles (i.e. *gastrocnemius* muscle), the somatic index (organ wet weight $\times 100$ / body weight, SI in percentage) of *soleus* muscle increased in dexamethasone-treated (DEX) growing rats (ŁOKOCIEJEWSKA et al., 2006). In the present study we used molecular and biochemical analyses to gain insight into the mechanisms of the resistance to dexamethasone-induced muscle cachexia.

Material and methods

Animals

Polish Ministry of Agriculture rules for animal welfare were followed during these experiments. Experimental procedures carried out on animals were approved by the Local Ethic and Animal Welfare Commission of the Warsaw Agricultural University. Wistar male rats (n=120, 12 groups of 10 animals each) at the age of four-weeks were purchased from Institute of Animal Physiology and Animal Feeding, Polish Academy of Sciences (Jabłonna near Warsaw, Poland). They were fed twice a day with standard laboratory rodent chow (WYTWÓRNIA PASZ, ANDRZEJ MORAWSKI, Kcynia,

Poland) containing 13 MJ kg-1 metabolizable energy and 21.2 % w/w crude protein was provided. Any remaining uneaten food was weighed and feed intake was calculated daily. Water was provided ad libitum. Each animal was housed individually in controlled environmental conditions (22 ℃, 75 % humidity, 12 : 12 h light-dark cycle period started 8:00 a.m.). After a 2-week acclimatization period, when the rats were 6 weeks of age (180-200 g), the experimental treatment was begun. Because Dex alters food intake, control animals were pair-fed the average daily amount consumed by the corresponding DEX-treated group. Similarly, the diets of animals from other groups were constituted the average daily amount of food being eaten by DEX-treated animals. Two 5-day experimental periods were investigated. DEX-treated rats received twice a day (at 8.00 a.m. and 4.00 p.m.) the 1 mL of dexamethasone disodium phosphate (Sigma, St. Louis, MO, USA) dissolved in saline (0.85 % w/v NaCl). DEX was loaded by intragastric tube in a daily dose of 2 mg/kg b.w. day-1 during 5 consecutive days. Control animals (CTRL) received 1 mL of saline (vehicle, 0.85 % NaCl). Sodium ascorbate (ASC) and ascorbic acid phosphate (ASC-P) were purchased from Sigma (St. Louis, MO, USA). ASC and ASC-P were given individually or as co-treatment with DEX at dose of 600 mg/kg b.w. day-1 and 785 mg/kg b.w day-1, respectively. Either factor was dissolved in distilled-deionized water (Aqua pro injectione, Polpharma S.A., Poland) and introduced to rats by the intragastric tube in a volume of 1 ml. Time-schedule was the same as for DEX. The whole experiment consisted of six randomized groups of animals (DEX, DEX/ASC, DEX/ASC-P, ASC, ASC-P, CTRL; n=10). After 5 days of treatment animals were anesthetized by intraperitoneal injection of 0.3 ml pentobarbital sodium salt (Pentobarbitalum 26.7 mg/ml; Pentobarbitalum Natrium 133 mg/ml, Morbital, Biowet, Puławy, Poland) and dissected. The entire soleus muscles (SM) and samples of gastrocnemius muscles (GM) were removed, quickly trimmed from connective or visible fat tissue and frozen in liquid nitrogen and stored at -80 ℃ for biochemical or Western blot analysis. Specific growth rates, protein efficiency ratio and somatic indices (organ wet weight ×100 / body weight) were evaluated and the results have been already published (ŁOKOCIEJEWSKA et al., 2006).

Reagents

All reagents for enzymatic analyses were of high purity, and unless otherwise stated they were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Primary goat polyclonal anti-Akt-1 IgG antibody, rabbit anti-p-[Ser473 residue]-Akt-1 IgG antibody, goat polyclonal anti-β-actin IgG antibody, and secondary antibodies donkey antigoat, donkey antimouse, and donkey anti-rabbit were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Primary mouse monoclonal anti-cytochrome-c oxidase subunit IV antibodies were obtained from Molecular Probes (Eugene, Oregon, USA). Sodium dodecyl sulfate (SDS) 10 % (w/v), Sequi-Blot PVDF Membrane 0.2 μm and all reagents for Western blot were also obtained from Bio-Rad Laboratories (Hercules, CA, USA).

Electrophoresis and immunoblotting

Frozen tissues were homogenized in a freshly prepared buffer composed of urea (8.3 M), thiourea (2 M), 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS, 2 %) and dithiotreitol (DTT, 1 %). Insoluble material was removed by

centrifugation at 10,000 g for 30 min at 8℃. Soluble protein concentrations in the supernatant fraction were determined by a protein-dye-binding method (BRADFORD 1976) with a commercial reagent (Bio-Rad Laboratories) using bovine serum albumin (BSA) as standard. Equal amounts of protein (30 μg) isolated from muscles of treated (see previous text) or untreated (CTRL) rats were used for sodium dodecyl sulfate polyacrylamide gel electrophoresis (12 %). After transferring onto PVDF membranes proteins were immunostained by standard methods as previously described (ORZECHOWSKI et al., 2005). Blots were striped and re-probed with β-actin to show that equal amounts of protein were loaded in each line. The enhanced chemiluminescence (ECL) method was used for antibody-antigen complexes detection (Amersham Int. Aylesbury, UK).

Enzyme activities in skeletal muscles

In order to measure the activities of the main enzymes participating in glucose metabolism, frozen muscle samples were homogenized in ice-cold buffer containing sucrose (0.25 M), Tris (0.01 M, pH 8.0), ethylenediaminetetraacetic (EDTA; 0.002 M). After centrifugation at 4 ℃ and 9,000 g for 10 min, the supernatant was collected and kept on ice. Anaerobic metabolism was characterized by phospho-fructokinase (PFK) and lactate dehydrogenase (LDH) activities. PFK catalyzes the transformation reaction of fructose-6-phosphate to fructose-1,6-biphosphate, and LDH is involved in the conversion of pyruvate into lactate. For comparison, the aerobic metabolism was studied by measurements of isocitrate dehydrogenase (ICDH) activity. Assay of PFK activity was performed as previously described (BEUTLER 1971). Activity of LDH was assessed following the disappearance of reduced nicotinamide adenine dinucleotide (NADH) after addition of pyruvate (BERGMEYER and BERNT, 1974). Activity of ICDH was determined by the reduction rate of NADP after addition of isocitrate (BRIAND et al., 1981). All measurements were performed by using the Infinite F200 Tecan Multiplate Reader (Salzburg, Austria) at 340 nm and 30 ℃ for PFK or 28 ℃ for LDH and ICDH. All enzymes activities were expressed as micromoles of substrate per min per gram of tissue wet weight.

Statistical evaluation

Results were statistically evaluated using one way ANOVA and Tukey's comparison multiple range test by GraphPad Prism™ version 4.0 software (GraphPad Software Inc., San Diego, CA, USA). Results are expressed as mean + SEM and a value of $P<0.05$ was determined to be significant, $P<0.01$ as highly significant and $P<0.001$ as very highly significant.

Results

Insulin sensitivity is sustained in oxidative type skeletal muscles upon dexamethasone treatment. It has been well established that glucocorticoid overload impairs growth and results in insulin resistance as evidenced by the occurrence of hyperglycemia and hyperinsulinemia (DARDEVET et al., 1995; DUPONT et al., 1999). In our study, one day before the end of experiment all animals were individually tested for insulin resistance by the use of glucose tolerance test (ŁOKOCIEJEWSKA et al., 2006). Urine glucose test strips confirmed that DEX causes secondary diabetes which was accompanied by oxidative stress (ORZECHOWSKI et al., 2000) evidenced by the

significant drop in blood plasma TAS (data not shown). Diabetic state recessed immediately after DEX withdrawal from the treatment (ORZECHOWSKI et al., 2000; ŁOKOCIEJEWSKA et al., 2006). Previous examination also showed that 5-day administration of DEX led to progressive and significant fall of growth indices in 6-weeks old rats. These changes were caused by both loss of appetite and accelerated whole-body catabolism. Growth retardation induced by DEX was reversible, since the dynamic indices of growth increased steadily during the recovery period. This was not the case when changes in somatic indices were monitored. In contrast to oxidative-glycolytic muscles (*gastrocnemius* muscle, GM), the somatic index of SM increased in dexamethasone-treated (DEX) growing rats. In turn, somatic index of GM increased by co-treatment with sodium ascorbate (ASC) or ascorbic acid phosphate (ASC-P) indicating that antioxidants might directly counteract catabolic action of glucocorticoids or that they indirectly improve GM sensitivity to insulin (ŁOKOCIEJEWSKA et al., 2006). Raise of the somatic index for SM upon DEX treatment confirmed the relative resistance of the oxidative type muscles to catabolic action of glucocorticoids.

The insulin resistant state evoked by DEX is manifested by steroid diabetes and results from several disturbances in insulin signaling system (DUPONT et al., 1999). The above-mentioned observations prompted us to investigate the expression of selected proteins which contribute to the insulin-dependent signal transduction. The total and active (phosphorylated at Ser473 residue) form of Akt were studied. Akt is one among the principal downstream mediators of insulin action, where activation of Akt by its phosphorylation starts after PI3-K catalyzes the production of phosphatidylinositol 3,4-bisphosphate and/or phosphatodylinositol 3,4,5-triphosphate (LAWLOR and ALESSI, 2001). Additionally the expression of cytochrome-c oxidase was evaluated. Previously, we described the expression profile of cytochrome-c oxidase as the reliable indicator of insulin action in muscle cells (PAWLIKOWSKA et al., 2006a). No changes were observed in the expression of Akt, phospho-Ser473-Akt, subunit I (data not shown) and IV of mitochondrial cytochrome-c oxidase in SM (Figure 1). In contrast to oxidative type muscles, dexamethasone induced statistically significant decrease in the expression of subunit IV of COX accompanied by the declined level of both evaluated forms of Akt in GM homogenates (Figure 1). Co-treatments with antioxidants did not influence the levels of selected proteins in SM, but slightly improved the expression of Akt, phospho-Ser473-Akt and COX IV in GM. Results obtained from Western blot analysis strongly indicate, that dexamethasone does not affect insulin sensitivity in soleus muscle, at least at Akt level, although in the oxidative-glycolytic type skeletal muscles it evoked resistance to insulin.

Glucose metabolism and mitochondrial activity are responsible for the resistance of SM to catabolic action of dexamethasone. In order to evaluate the rate of aerobic versus anaerobic metabolism we measured the activity of enzymes which adequately illustrate glucose metabolism in skeletal muscle tissue. In oxidative type muscle (SM) basal activity of ICDH was two-fold higher than in oxidative-glycolytic muscle (GM). In contrast to ICDH, the activity of PFK was almost eight times higher in GM than in SM muscle in untreated rats. Nevertheless, LDH activity did not differ significantly in SM vs. GM of control groups of rats.

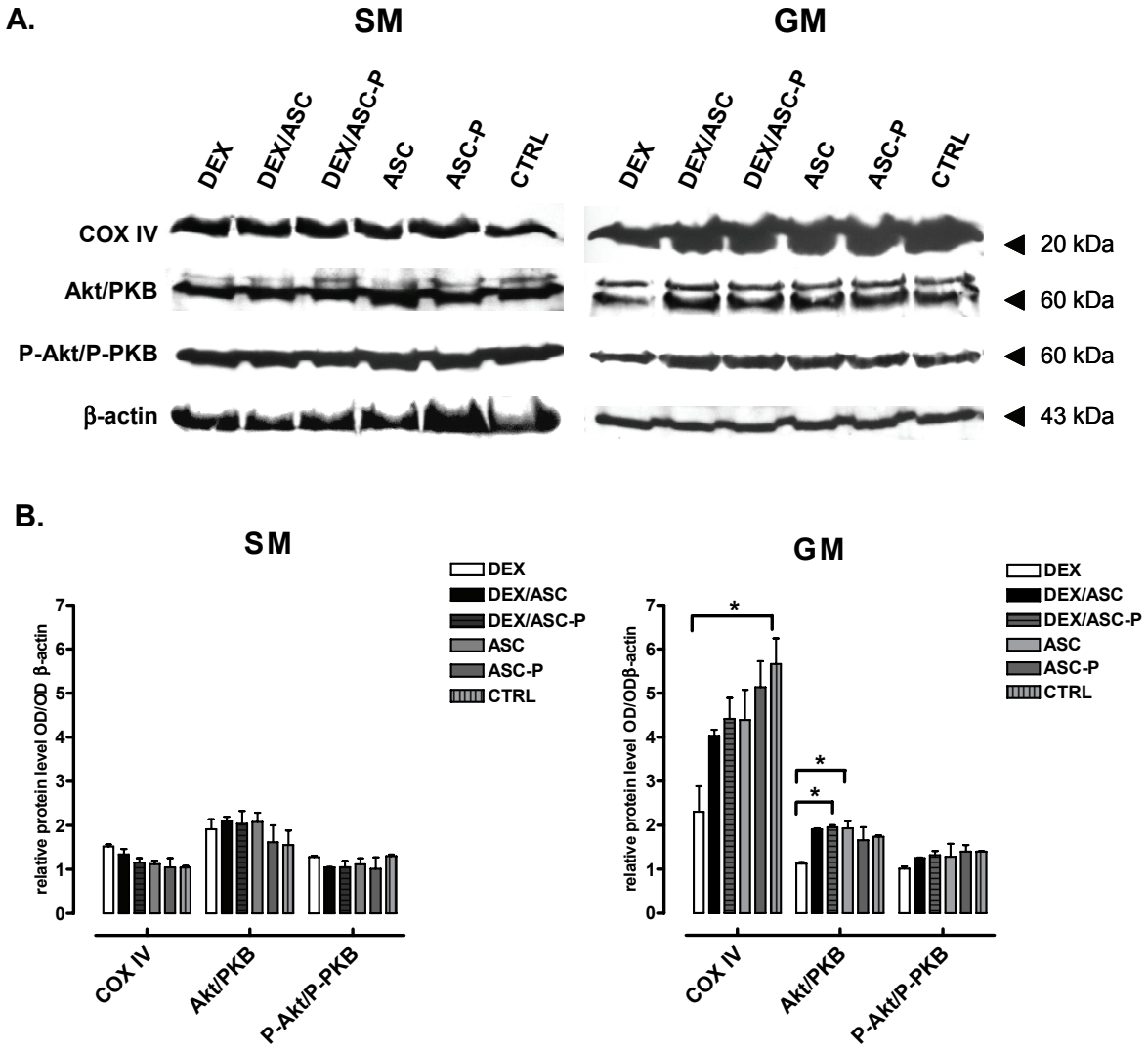

Fig. 1: Western blot analysis of proteins associated with insulin action: subunit IV of cytochrome-c oxidase, Akt/PKB and Ser473-phospho-Akt/PKB. A. From left to right dexamethasone (DEX), dexamethasone with sodium ascorbate (DEX/ASC), dexamethasone with ascorbic acid phosphate (DEX/ASC-P), sodium ascorbate (ASC), ascorbic acid phosphate (ASC-P), control (CTRL). Total protein extracts (30 µg protein per sample) from soleus (SM) or gastrocnemius muscle (GM) of treated (see above) or untreated (CTRL) growing rats were resolved by 12 % SDS/PAGE followed by immunobloting. B. Quantification of protein level. The relative protein level of each evaluated protein was calculated from the relation between the blot optical density (ODxmm2) of every particular protein to that of β-actin. The representative of one from four analyses is shown.

None of the treatments affected the activity of lactate dehydrogenase (LDH) neither in SM nor in GM (Figure 2). In turn, ICDH activity peaked in SM upon dexamethasone treatment ($P<0.05$) and remained below the average control value ($P<0.05$) during co-treatment with ascorbate (DEX/ASC) (Figure 2, panel A). In contrast to SM, ICDH activity in GM remained unchanged ($P>0.05$) irrespective of treatment (Figure 2, panel B). Furthermore, as could be expected, the average level of ICDH activity in GM was lower than in SM, even if the latter was treated with dexamethasone (Figure 2). Interestingly, PFK activity in SM was significantly elevated in some of the experimentally treated rats (DEX, DEX/ASC-P; $P<0.05$) and the highest was observed after ASC-P ($P<0.01$). Astonishingly, the activity of PFK significantly ($P<0.001$) dropped in GM in the experimentally treated rats except the dexamethasone treated group ($P>0.05$; Figure 2, panel B).

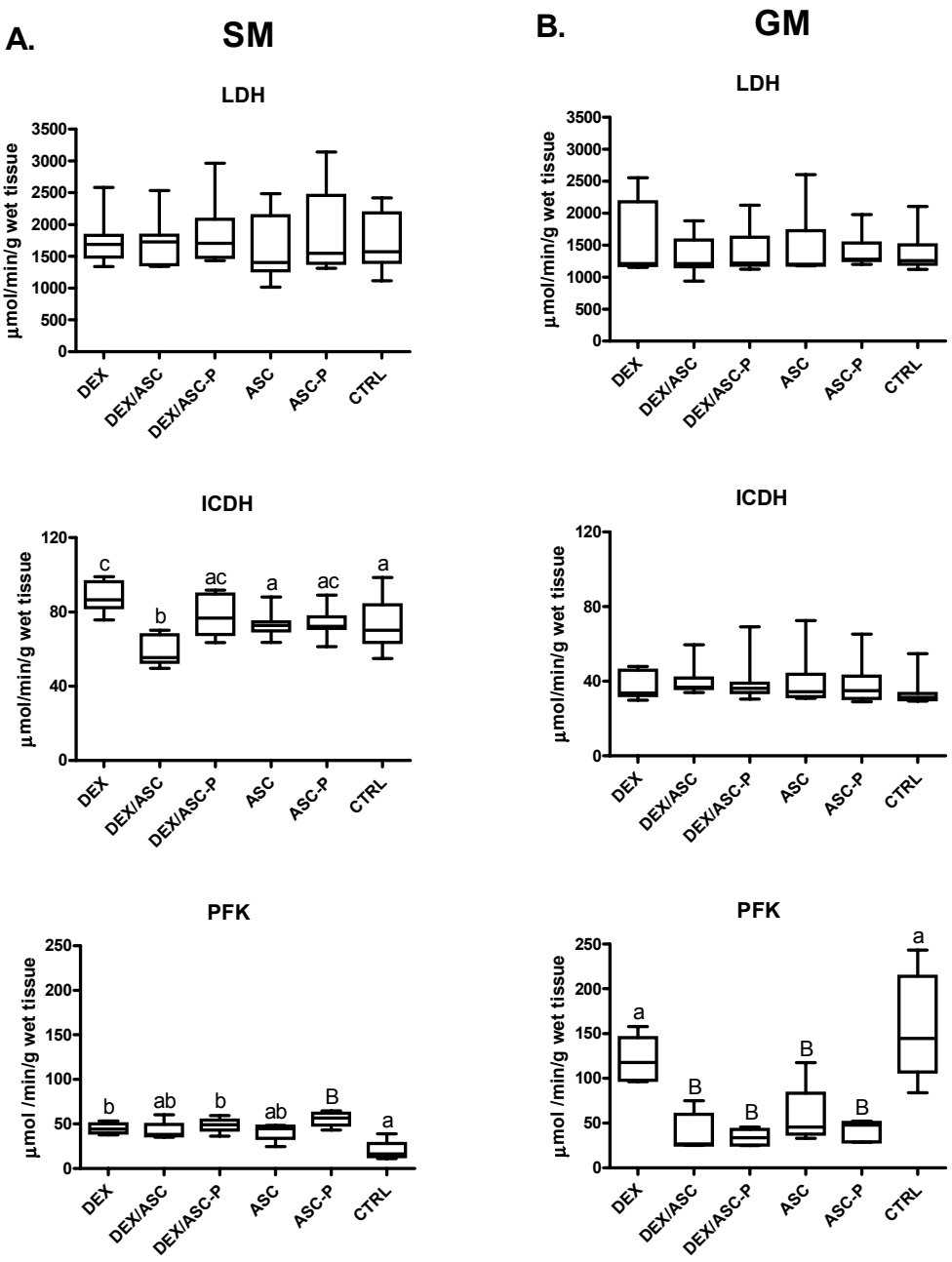

Fig. 2: Graphs representing activities of selected glycolytic and oxidative metabolic enzymes. From top to bottom: lactate dehydrogenase (LDH), isocitrate dehydrogenase (ICDH), phosphofructokinase (PFK) activity of SM (A.) and GM (B.) from treated (DEX, DEX/ASC, DEX/ASC-P, ASC, ASC-P) or untreated (CTRL) growing rats. Frozen muscle samples were homogenized and glycolytic enzyme activity (PFK, LDH) and mitochondrial enzyme activity (ICDH) were determined spectrophotometrically. The specific activity of particular enzyme was expressed as µmol substrate converted into reaction product per min per gram of tissue wet weight. Results were obtained from 6 (for SM) or 4 (for GM) rats of each particular experimental group. Each muscle sample was measured at least in eight replicates. Means that differ significantly ($P<0.05$) from control group are ticked with different lower case letters whereas those that differ very highly significantly ($P<0.001$) are indicated by diverse upper case letters.

Discussion

It has been well established that chronic treatment with glucocorticoids induces side effects featured by insulin resistance and catabolic state of skeletal muscles. Moreover, long-term action of glucocorticoids is also accompanied by the decrease in tissue oxygen consumption and lower rate of oxidative phosphorylation at least in the liver

(ROUSSEL et al., 2004; PANDYA et al., 2004). In our experiments activity of ICDH, LDH and PFK were selected to assess the rate of glucose aerobic vs. anaerobic metabolism in skeletal muscles (SM and GM) which represent distinct metabolic profiles. Since basal activity of LDH in the untreated control rats did not differ significantly in SM vs. GM, we assume that this is not a step-limiting enzyme for aeorobic/anaerobic metabolism of glucose. On the other hand, the differences observed in ICDH and PFK activity in SM vs. GM appeared indicative for metabolic profiles of skeletal muscles. Additionally, differences in the activity of ICDH, PFK but not LDH were observed upon particular treatment. In our experiment, irrespective to type of treatment the LDH activity in SM and GM remained at the level of control group. Surprisingly, PFK activity in SM was elevated in either group treated with DEX. The rise in the activity of the step-limiting enzyme of carbohydrate catabolism (PFK) suggests that soleus muscle started to utilize excess glucose during DEX-induced hyperglycemia but not after ASC- or ASC-P-induced sensitization to insulin.

However, according to DUMAS et al. (2003) no changes in the activity of enzymatic complexes in liver mitochondria were observed upon dexamethasone loading. Since neither the expression of NCOIV nor P-PKB/PKB was affected by DEX in SM we assume that these markers of insulin action imitate relative autonomy of oxidative type muscles to insulin resistance. In contrast to SM, the NCOIV and P-PKB/PKB expression in GM decreased significantly in response to DEX (Figure 1) indicating that oxidative-glycolytic muscles are susceptible to retarded insulin effect. Interestingly, antioxidants ASC or ASC-P entirely prevented GM from the DEX-dependent drop in the expression of COIV and P-PKB/PKB. The latter observation might indicate the link between the oxidative stress and the level of measured proteins and activity of metabolically indicative enzymes. Likewise, antioxidants confined the fall in somatic indices of GM in DEX-treated rats (ŁOKOCIEJEWSKA et al., 2006). Overall, we believe that antioxidants posses limited but considerable anti-cachectic activity. Actually, indirect evidence for that statement could be found in altered activity of enzymes of glucose aerobic/anaerobic metabolism (Figure 2). Regardless of antioxidant used, the activity of PFK dropped in GM but not in SM muscle (Figure 2) advocating for antioxidant-dependent glucose metabolism in oxidative but not oxidative-glycolytic muscles.

It should be taken under consideration that indices of organ wet weight in both SM and liver were increased after 5-day administration of dexamethasone. However, whereas absolute mass of liver was elevated, it remained unchanged in the case of SM (data not shown). Thus, DEX evoked hepatomegaly but did not induce cachexia in oxidative type muscle (SM). We suggest, that the mitochondrial energy expenditure (energy formation) in soleus muscle in DEX-treated rats was increased in response to hyperglycemia. It corresponds to the results of ROUSSEL et al. (2004) who observed similar reaction in the liver. Moreover, we speculate that the effect of glucocorticoids on energy metabolism is not only tissue specific but it also differs between types of muscle fibres. Susceptibility of oxidative-glycolytic muscles to muscle cachexia was reflected by the diminishing magnitude of somatic index of gastrocnemius muscle upon treatment with DEX (ŁOKOCIEJEWSKA et al., 2006) and marked changes in the activity of PFK (Figure 2).

To shed more light on the metabolic effects of glucocorticoids one has to bear in mind that insulin action is markedly impeded in steroid diabetes. Our in vitro experiments

carried out on clonal lines of muscle cells (rat L6 myoblasts, mouse C2C12 satellite cells) have clearly shown that insulin-dependent myogenesis is limited to the cells with active mitochondria. Similarly, the phosphatidylinositol-3-kinase (PI3-K) inhibitor LY 294002 but not MAPK kinase (MEK) inhibitor PD 98059 ceased the morphological changes in mitochondria embedded to energy supply indispensable for insulin pleiotropic effects (PAWLIKOWSKA et al., 2006; PAWLIKOWSKA et al., 2007). More importantly, the retrograde regulation was also evident, since no insulin-dependent effects were observed when either activity of particular respiratory enzyme complexes (OXPHOS) or mitochondrial membrane potential were declined. We did not pursue the insulin effects in fully formed muscle fibres, but it is likely that sensitivity to insulin is altered by the stage of development and more importantly to the type of muscle fibre. In this respect oxidative type muscles seem to be less affected by oxidative stress and insulin, thus being sufficiently efficient to take advantage of transient hyperglycemia that accompany steroid diabetes.
Results obtained from Western blot analysis strongly indicate, that dexamethasone does not affect insulin sensitivity in SM, at least at PKB level, although it induced the resistance to insulin in the oxidative-glycolytic type muscle (GM). Proteomic studies are urgently needed to reveal the molecular mechanism liable to maintain the balance in protein turnover in oxidative-type muscles.

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References

BERGMEYER, H.U.; BERNT, E.:
Lactate dehydrogenase: UV-assay with pyruvate and NADPH. Methods of Enzymatic Analysis, ed. Bergmeyer HU, Academic Press, New York. No 2. (1974), 574-579

BEUTLER, E.:
Phosphofructokinase. In Red Cell Metabolism. A Manual of Biochemical Methods, ed. Beutler E, Grune and Straton, New York, (1971), 42-44

BRIAND, M.; TALMANT, A.; BRIAND, Y.; MONIN, G.; DURAND, R.:
Metabolic types of muscle in sheep: II. Lactate dehydrogenase activity and LDH isoenzyme distribution. Eur. J. Appl. Physiol. Occup. Physiol. **46** (1981), 359-365

DARDEVET, D.; SORNET, C.; TAILLANDIER, D.; SAVARY, I.; ATTAIX, D.; GRIZARD, J.:
Sensitivity and protein turnover response to glucocorticoids are different in skeletal muscle from adult and old rats. J. Clin. Invest. **96** (1995), 2113-2119

DUMAS, J.F.; SIMARD, G.; ROUSSEL, D.; DOUAY, O.; FOUSSARD, F.; MALTHIER, Y.Y.; RITZ, P.:
Mitochondrial energy metabolism in a model of undernutrition induced by dexamethasone. Br. J. Nutr. **90** (2003), 969-977

DUPONT, J.; DEROUET, M.; SIMON, J.; TAOUIS, M.:
Corticosterone alters insulin signaling in chicken muscle and liver at different steps. J. Endocrinol. **162** (1999), 67-76

JASPERS, S. R.; TISCHLER, M. E.:
Role of glucocorticoids in the response of rat leg muscles to reduced activity. Muscle Nerve **9** (1986), 554-561

LANG, C.H.; SILVIS, C.; NYSTROM, G.; FROST, R.A.:
Regulation of myostatin by glucocorticoids after thermal injury. FASEB J. **15** (2001), 1807-1809

LAWLOR, M.A.; ALESSI, D.R.:
PKB/Akt: a key mediator of cell proliferation, survival and insulin responses? J. Cell Sci. **277** (2001), 2903-2910

LIN, H.; DECUYPERE, E.; BUYSE, J.:
Oxidative stress induced by corticosterone administration in broiler chickens (*Gallus gallus domesticus*) 2. Short-term effect. Comp. Biochem. Physiol. B **139** (2004), 745-751

ŁOKOCIEJEWSKA, M.; WAGNER, J.; ZARZYŃSKA, J.; JANK, M.; OSTASZEWSKI, P.; BURDZIŃSKA, A.; SADKOWSKI, T.; OLCZAK, J.; MRÓWCZYŃSKA, A.; ORZECHOWSKI, A.:
Sodium ascorbate (ASC) and ascorbic acid phosphate (ASC-P) differently modulate glucocorticoid-dependent metabolic effects in growing rats. Arch. Tierz. **49** (2006), 41-51

MA, K.; MALLIDIS, C.; ARTAZA, J.; TAYLOR, W.; GONZALEZ-CADAVID, N.; BHASIN, S.:
Characterization of 5'-regulatory region of human myostatin gene: regulation by dexamethasone in vitro. Am. J. Physiol. Endocrinol. Metab. **281** (2001), E1128-E1136

MA, K.; MALLIDIS, C.; BHASIN, S.; MAHABADI, V.; ARTAZA, J.; GONZALEZ-CADAVID, N.; ARIAS, J.; SALEHIAN, B.:
Glucocorticoid-induced skeletal muscle atrophy is associated with upregulation of myostatin gene. Am. J. Physiol. Endocrinol. Metab. **285** (2001), E363-E371

MANOLI-OREOPOULOS, I.; LE, H.; ALESCI, S.; MC FANN, K.K.; SU, Y.A.; KINA, T.; CHROUSOS, G.P.; BLACKMAN, M.R.:
Monoamine-Oxidase-A is a major target gene for glucocorticoids in human skeletal muscle cells. FASEB J. **19** (2005), 1359-1361

MCMAHON, C.D.; POPOVIC, L.; OLDHAM, J.M.; JEANPONG, F.; SMITH, H.K.; KAMBADUR, R.; SHARMA, M.; MAXWELL, L.; BASS, J.J.:
Myostatin-deficient mice lose more skeletal muscle mass than wild-type controlsduring hindlimb suspension. Am. J. Physiol. Endocrinol. Metab. **285** (2003), E82-E87

MOLDAWER, L.L.; COPELAND, E.M. III:
Proinflammatory cytokines, nutritional support, and the cachexia syndrome: interactions and therapeutic options. Cancer **79** (1997), 1828-1839

ORZECHOWSKI, A.; ŁOKOCIEJEWSKA, M.; PAWLIKOWSKA, P.; KRUSZEWSKI, M.:
Preincubation with sodium ascorbate potentiates insulin-dependent PKB/Akt and c-Jun phosphorylation in L6 rat myoblasts challenged with reactive oxygen/nitrogen species. Life Sci. **77** (2005), 496-511

ORZECHOWSKI, A.; OSTASZEWSKI, P.; BRODNICKA, A.; WILCZAK, J.; JANK, M.; BAŁASIŃSKA, B.; GRZELKOWSKA, K.; PŁOSZAJ, T.; OLCZAK, J.; MRÓWCZYŃSKA, A.:
Excess of glucocorticoids impairs whole-body antioxidant status in young rats. Relation to the effect od dexamethasone in soleus muscle and spleen. Horm. Metab. Res. **32** (2000), 174-180

ORZECHOWSKI, A.; JANK, M.; GAJKOWSKA, B.; SADKOWSKI, T.; GODLEWSKI, M.M.; OSTASZEWSKI, P.:
Delineation of signalling pathway leading to antioxidant-dependent inhibition of dexamethasone-mediated muscle cell death. J. Muscle Res. Cell Motility **24** (2003), 33-53

ORZECHOWSKI, A.; GRIZARD, J.; JANK, M.; GAJKOWSKA, B.; ŁOKOCIEJEWSKA, M.; ZARON-TEPEREK, M.; GODLEWSKI, M.M.:
Dexamethasone-mediated regulation of death and differentiation of muscle cells. Is hydrogen peroxide involved in the process? Reprod. Nutr. Dev. **42** (2002), 197-216

PANDYA, J.D.; AGARWAL, N.A.; KATYARE, S.S.:
Effects of dexamethasone treatment on oxidative energy metabolism in rat liver mitochondria during postnatal developmental periods. Drug Chem. Toxicol. **27** (2004), 389-403

PAWLIKOWSKA, P.; GAJKOWSKA, B.; HOCQUETTE, J.-F.; ORZECHOWSKI, A.:
Not only insulin stimulates mitochondriogenesis in muscle cells, but mitochondria are also essential for insulin-mediated myogenesis. Cell Proliferation **39** (2006), 127-145

PAWLIKOWSKA, P.; GAJKOWSKA, B.; ORZECHOWSKI, A.:
Mitofusin 2 (Mfn2) – a key player in insulin-dependent myogenesis in vitro. Cell & Tissue Res. **327** (2007), 571-581

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