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 extent. 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 working group meetings were held the 4th and 5th of October at Laboratory of Nutrition, Growth and Quality of Fish. Centre of Marine and Environmental Research – CIMAR; Rua dos Bragas, 177; 4050 – 123 PORTO, Portugal.

The purpose of the meeting was to fulfil the 1st milestone "Presentation of National work/Defining prenatal events important for postnatal growth performance and meat quality" and was carried out in a joint session and in two working groups:

1. ***WG 1****: Environmental variation in prenatal events in relation to postnatal growth and meat/fish quality*
2. ***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 four papers introducing and reviewing the subject, which were given at a joint session, as well as 26 papers detailing actual research activities, that were given at the working group sessions.

We acknowledge and thank all the authors for their contribution to the session at the meeting and to this special issue. Furthermore, we appreciate COST for making this work possible by funding the Action, this meeting, and the publication of the contributions to the meeting.

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Arch. Tierz., Dummerstorf **48** (2005) Special Issue, 04-10

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Prenatal influences on skeletal muscle development in mammals, birds and fish

Abstract

Muscle development can be influenced by a number of environmental factors and in a range of species. Nutritionally disadvantaged fetuses tend to develop fewer secondary myofibres in their muscles whereas primary fibres are generally unaffected. However, prenatal nutritional restriction can lead to impaired postnatal growth without an effect on muscle fibre number. Connective tissue content in muscle is also influenced by prenatal nutrition. A 1^0C increase in incubation temperature in turkeys for a short period early in incubation leads to prolonged myoblast proliferation and delayed differentiation results in more muscle fibres and muscle nuclei posthatch. Lower incubation temperatures have a similar impact in fish muscle. Stimulating increased movement (through neuromuscular stimulation) in chick embryo also results in chicks at hatch with more muscle fibres and increased aerobic capacity in their muscles. Taken together, these results demonstrate that prenatal environmental factors can influence the balance between proliferation and differentiation within specific cell lineages. This in turn can affect muscle fibre number determination as well as the connective tissue and satellite cell content of muscles.

Key Words: skeletal muscle, prenatal, myogenic factors

Zusammenfassung

Titel der Arbeit: **Pränatale Einflüsse auf die Entwicklung der Skelettmuskulatur bei Säugern, Vögeln und Fischen**

Bei verschiedensten Spezies lassen sich unterschiedliche Einflüsse aus der Umwelt auf die Skelettmuskelentwicklung zeigen. Unter ungünstigen Ernährungsbedingungen bilden sich bei Feten weniger sekundäre Muskelfasern aus, während die Entwicklung primärer Fasern nicht beeinflusst wird. Pränatale Futterrestriktion kann zu verminderter postnataler Wachstumsleistung führen ohne dass ein Effekt auf die Muskelfaseranzahl nachzuweisen ist. Auch das Bindegewebe der Muskulatur wird durch die pränatale Ernährung beeinflusst. Eine kurzzeitige Erhöhung der Temperatur um 1 °C während der frühen Brutphase führt bei Puten zu einer verlängerten Proliferation und verzögerten Differenzierung der Myoblasten und so zu einer erhöhten Anzahl an Muskelfasern und Muskelzellkernen nach dem Schlupf. Bei Fischen hat die Senkung der Temperatur vor dem Schlupf einen ähnlichen Effekt. Stimulation der Muskelkontraktion (durch neuromuskuläre Stimulation) bei Hühnerembryonen führt zu einer erhöhten Anzahl an Muskelfasern und erhöhter aerober Kapazität der Muskulatur beim Schlupf. Diese Ergebnisse belegen, dass pränatal einwirkende Umweltfaktoren auf die Balance zwischen Proliferation und Differenzierung bei bestimmten Zelllinien wirken. Dies beeinflusst die Determination der Anzahl an Muskelfasern, die Ausbildung des Bindegewebes und die Bildung von Satellitenzellen im Skelettmuskel.

Schlüsselwörter: Skelettmuskel, pränatal, myogene Faktoren

Introduction

Skeletal muscle develops in a biphasic manner. During embryonic development, myoblasts proliferate and then line up and fuse to form a population of large primary myofibres. A larger population of smaller secondary myofibres then form on their surfaces (REHFELDT et al., 1999). As opposed to birds and mammals, whose myofibre number is fixed at birth (in most species), fish grow continuously throughout their lifespan and undergo a slightly different pattern of myogenesis involving a more prolonged hyperplasia (KOUMANS et al., 1991). The molecular events in the myogenic pathway are controlled by the MRFs, (MyoD, Myf-5, Myogenin and Mrf4) the expression of which regulates the timing of proliferation and differentiation

(REHFELDT et al., 1999). The myogenic cells of mammalian, avian and fish embryos possess a level of developmental plasticity, whereby they can respond to the manipulation of various environmental conditions. Prenatal manipulation of temperature (MALTBY et al., 2004), nutrition (BAYOL et al., 2004), hormones (CLEMMONS, 1998) and innervation (LEFEUVRE et al., 1996) can have significant effects on the timing and length of the expression of the myogenic regulators, influencing the number of pre-natal muscle fibres and nuclei formed, and subsequently altering the adult muscle phenotype.

Nutrition

Studies on intra-litter variation in the pig have shown that the nutritionally disadvantaged smallest fetus develop fewer secondary myofibres (in complete sections of *M. semitendinosus*) than the largest fetus that was well-nourished. However, primary fibre numbers are not affected (BEDI, 1982; WIGMORE and STICKLAND, 1983a). The difference in secondary myofibre numbers may be due to the reduced surface area of primary fibres (PENNEY et al., 1983; WIGMORE and STICKLAND, 1983b) in smaller fetuses, resulting from maternal undernutrition (MILLER et al., 1975; MADGEWICK, 1991; DWYER and STICKLAND, 1994) and/or uterine position (McLAREN and MICHIE, 1960; PERRY and ROWELL, 1969), therefore supporting less secondary fibres (WIGMORE and STICKLAND, 1983a). WIGMORE and STICKLAND (1983b) also showed that the larger fetuses contained more muscle DNA. A low fibre number predisposes the pig to poor long-term catch-up growth as shown by HANDEL and STICKLAND (1988).

The gestational time-point at which this undernutrition is targeted is critical for the development of secondary myofibres. Nutritional supplementation of pregnant sows from 2.5kg/day to 5kg/day during 25-50days of gestation (before the onset of secondary myofibre hyperplasia) enhanced the mean number of secondary myofibres by 9-13% in developing pig fetuses (DWYER et al., 1994), thus reducing the variation in myofibre development between the fetal size-range. A study of guinea pig offspring by DWYER et al., (1995) showed that feed restriction within the first half of gestation caused the same decrease in secondary myofibre number as feed restriction throughout gestation. Fetal muscle development is unaffected by late gestational maternal undernutrition as shown in a pig study (after 100 days gestation) by EZEKWE and OPOKU (1988).

A number of nutritional trials have shown an influence of prenatal nutrition on postnatal growth parameters without a significant influence on muscle fibre number in rats (BAYOL et al., 2004) and sheep (GREENWOOD et al., 1999; McCOARD et al., 2000). However, the myofibre number in sheep was differentially affected by season (McCOARD et al., 1997). In sheep, low levels of maternal nutrition resulted in fetuses with a higher connective tissue content (Figure 1), a factor that is also observed when intralitter comparisons are made in the pig (STICKLAND et al., 2000).

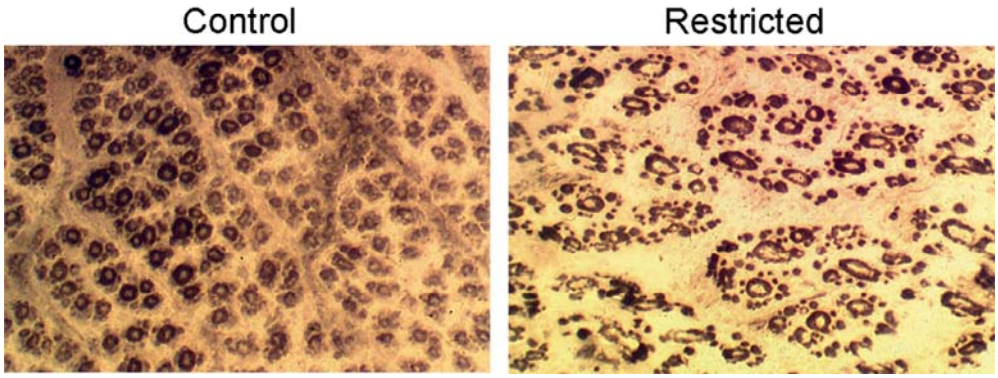

Fig. 1: A cross section of the semitendinosus muscle of control (left) and nutritionally restricted (right) sheep fetuses illustrating the higher amount of connective tissue in the restricted animal

Temperature

Different rearing temperatures can alter the cellularity of muscle tissue from fish embryos and larvae. In some fish species at hatch (e.g. Atlantic salmon; *Salmo salar L.*), higher incubation temperatures result in less but larger myofibres (Figure 2) and fewer nuclei in a complete larval cross-section compared to larvae reared at lower temperatures (STICKLAND et al., 1988; USHER et al., 1994). In herring embryos (*Clupea harengus L.*) a higher rearing temperature leads to more but smaller myofibres (VIEIRA and JOHNSTON, 1992). Muscle differentiation occurred later in rainbow trout (*Oncorhynchus mykiss*) embryos incubated at colder temperatures (XIE et al., 2001). This may indicate a longer proliferation phase which leads to more muscle fibres. Atlantic salmon reared at 5°C up to hatching grew significantly better post-hatch, up to 3 weeks, than fish reared at 11°C. The post-hatch temperature was the same for all experimental salmon (NATHANAILIDES et al., 1995). Fish with more muscle fibres appear to grow faster in the first few weeks post-hatch. MATSCHAK et al. (1995) showed the importance of physiological hypoxia as a potential driving force behind the temperature effects on muscle development. These fish studies have demonstrated the importance of pre-hatch developmental temperature on pre- and post-hatch myogenesis and the implications these environmental alterations have on the growth potential of the fish. Fish grown at lower temperatures (near ambient level) not only have more and smaller myofibres, but they also have more nuclei thus suggesting increased capacity for hyperplasia and hypertrophy through a greater number of precursor cells.

Experiments with turkeys have shown that a small manipulation of incubation temperature (+/-1 to 2°C for 4 days during the early stages of incubation) can significantly alter the post-hatch muscle phenotype up to 3 weeks of age (MALTBY et al., 2004). The prenatal effects observed included an up-regulation of MyoD and PCNA, occurring during the period of secondary fibre formation. These changes in pre-hatch gene expression appeared to have an impact on post-hatch muscle development. An increase in fibre and nuclear number (Figure 3) was observed in the birds that underwent the temperature increase in early embryogenesis. The evidence in the turkey experiments suggest that increased muscle fibre number is associated with increased proliferation and delayed differentiation within the developing muscles.

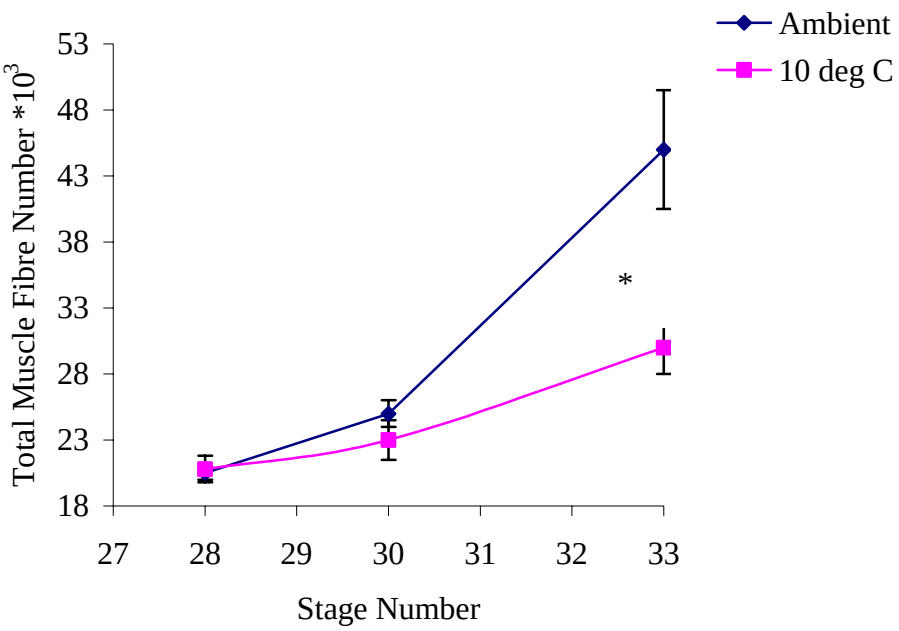

Fig. 2: Total fibre number in a complete embryonic cross sectional area. The fish reared at 10°C have lower total muscle fibre numbers than those at the ambient temperature. Asterisk indicates a significant difference at P < 0.05. (STICKLAND et al., 1988).

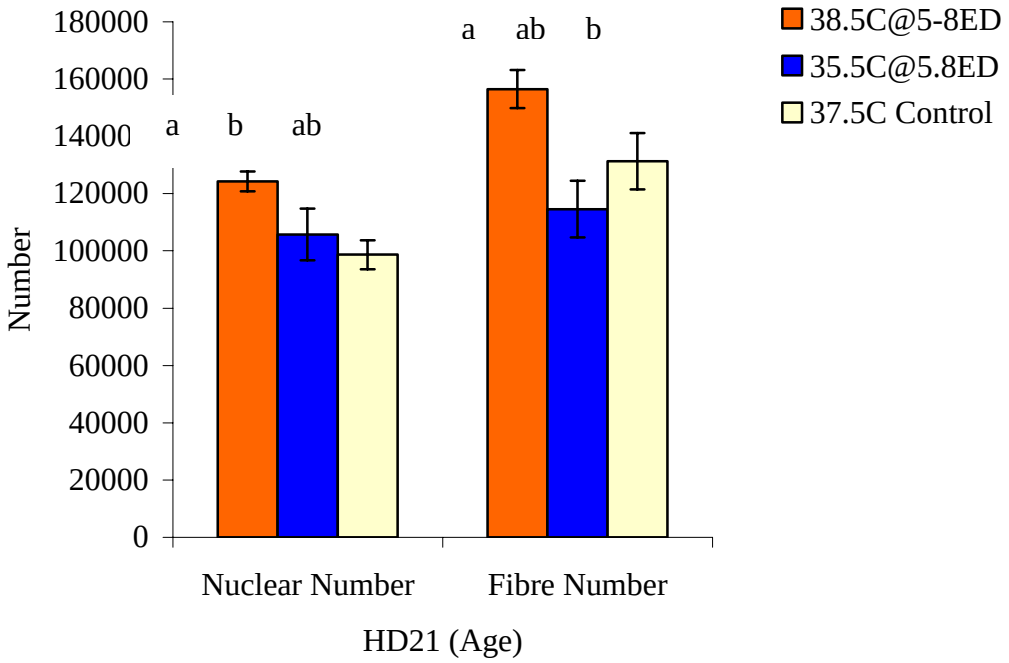

Fig. 3: The total fibre and nuclear number present in the cross sectional area of the semitendinosus muscle of 3-week old turkeys exposed to 3 different temperatures between embryonic day 5 and embryonic day 8. (MALTBY et al., 2004)

Movement

Stimulation of movement *in ovo* in chicken embryos has also yielded similar changes in the muscle phenotype. 4-aminopyridine administered during a key point in muscle development (i.e. myoblast proliferation) induces increased

movement. Histological examination of the semitendinosus muscle revealed that fibre and nuclear number is greater in the stimulated embryos immediately pre-hatch compared to the control embryos (McENTEE et al., 2004). Also, higher level of succinic dehydrogenase activity (a marker of oxidative capacity) was observed immediately pre-hatch (embryonic day 20). The increase in muscle fibre and total nuclear number seen in the treated embryos is possibly as a result of a longer period of proliferation and may be due to a temporary suppression of myogenin (WALTERS et al., 2000; MALTBY et al., 2004). Initial results exploring how movement affects the temporal gene expression of key factors during myogenic proliferation and differentiation suggest that both the timing and level of expression of myogenin, myostatin and the IGFs is indeed altered by neuromuscular stimulation *in ovo*. The prolonged effects of 4-AP may also be attributable to the maintenance of polyneuronal innervation. Gene expression of neurogenic factors such as neural cell adhesion molecule (NCAM) and nicotinic acetylcholine receptor (nAChR) also appear to be significantly up-regulated in treated embryos during the later stages of embryogenesis (embryonic day 16 to 18). This suggests an increased level of innervation may be contributing to the observed increased proliferation.

Conclusions

The results of these experiments suggest that prenatal environmental factors (e.g. temperature, maternal nutrition movement, uterine position) influence muscle development in a number of different ways. There may be an influence on the balance between proliferation and differentiation within given cell lineages (including secondary myofibres), which may also be affected by altered expression of IGFs, IGFBPs and IGF receptor and the timing of the expression of the myogenic regulatory factors. There may also be an influence on the commitment of early stem cells to particular lineages (i.e. muscle cells, satellite cells, side population cells and connective tissue cells) within developing muscles. The manipulation of these prenatal myogenic factors potentially has a fundamental role in the determination of meat quality and quantity in mammals, poultry and fish.

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Prenatal events that determine the number of muscle fibres are important for lean growth and meat quality in pigs

Abstract

Prenatal myogenesis is considered as one of the most important events that determine postnatal growth, lean accretion and meat quality. During myogenesis stem cell commitment and the extent of muscle cell multiplication largely determine how many muscle fibres are formed. Piglets of low birth weight have formed a low number of muscle fibres by genetic or maternal reasons. Postnatally, these pigs exhibit the lowest growth performance and the lowest lean percentage at slaughter. In addition, they tend to develop extremely large muscle fibres (giant fibres) and poor meat quality. Prenatal growth/myogenesis is under the control of various genetic and environmental factors. It is mainly dependent on the nutritional supply/utilization and under the control of hormones and growth factors, which in turn are closely interrelated with each other. It has been shown that the maternal somatotropic axis plays a significant role in the control of myogenesis. Thus, treatment of sows with growth hormone (GH) until mid gestation is able to increase the number of muscle fibres in progeny which is associated with changes in the determination and/or proliferation of myoblasts as seen by increases in the expression of *Myf 5* and *MyoD* transcripts, in creatine phophokinase activity, DNA and protein concentrations. The effects are associated with increased nutrient availability to the embryos in terms of glucose and free fatty acids and changes in regulatory proteins of the GH- IGF axis. The increases in muscle fibre number are most pronounced in small littermates disadvantaged by insufficient nutrient supply. Cellular and molecular mechanisms controlling basic events of myogenesis are worthy of further investigation.

Key Words: myogenesis, birth weight, growth hormone, muscle fibre, pig

Zusammenfassung

Titel der Arbeit: **Die pränatale Determination der Muskelfaseranzahl ist von Bedeutung für Muskelwachstum und Fleischqualität**

Die Myogenese ist einer der wichtigsten pränatalen Vorgänge, die das postnatale Wachstum, den Fleischansatz und die Fleischqualität beeinflussen. Während der Myogenese sind es die Determination der Stammzellen und die Vermehrung der Muskelzellen, die weitgehend bestimmen, wie viele Muskelfasern gebildet werden. Ferkel mit geringem Geburtsgewicht besitzen auf Grund genetischer und/oder maternal bedingter Ursachen eine geringe Anzahl von Muskelfasern. Postnatal zeigen diese Schweine die geringste Wachstumsleistung und den niedrigsten Magerfleischanteil im Schlachtkörper. Außerdem tendieren sie zur Ausprägung extrem großer Muskelfasern (Riesenfasern) und schlechter Fleischqualität. Pränatales Wachstum und Myogenese werden durch verschiedene genetische- und Umweltfaktoren kontrolliert. Sie hängen vorwiegend von der Nährstoffversorgung und -ausnutzung ab und unterliegen der Steuerung durch Hormone und Wachstumsfaktoren, Prozesse, die wiederum in enger Wechselwirkung stehen. Es ist gezeigt worden, dass die maternale somatotrope Achse eine bedeutende Rolle in der Steuerung der Myogenese spielt. So kann eine Behandlung von Sauen mit Wachstumshormon (GH) bis zur mittleren Trächtigkeit die Anzahl der Muskelfasern der Nachkommen erhöhen. Dieses geht mit Veränderungen in der Determination und/oder Proliferation von Myoblasten einher, zu ersehen aus Zunahmen in der Expression von *Myf 5* und *MyoD* Transkripten, in der Aktivität der Kreatinphosphokinase sowie in DNA und Proteinkonzentrationen. Diese Effekte sind mit einer erhöhten Nährstoffverfügbarkeit (Glukose, freie Fettsäuren) für die Embryonen sowie Veränderungen in der GH-IGF Achse assoziiert. Die Zunahme in der Muskelfaseranzahl ist bei kleinen Wurfgeschwistern, die durch ungenügende Nährstoffversorgung benachteiligt sind, am deutlichsten ausgeprägt. Zelluläre und molekulare Mechanismen, welche die Basisprozesse der Myogenese steuern, sollten weiterführend untersucht werden.

Schlüsselwörter: Myogenese, Geburtsgewicht, Wachstumshormon, Muskelfaser, Schwein

1. Myogenesis determines how many muscle fibres are formed

The regulation of prenatal growth is complex and not sufficiently understood. It is mainly dependent on the nutritional supply to the embryo/foetus and its ability to utilize the available substrates. Nutritional partitioning and utilization in turn are under the control of hormones and growth factors but, conversely, nutrition may also

influence the hormonal status (THISSEN et al., 1994; STRAUS, 1994; BRAMELD, 1997; BRAMELD, et al. 1998; BREIER, 1999). Also prenatal muscle development (myogenesis) is under the control of nutrients as well as hormones and growth factors affecting its basic events via changes in metabolism and (or) at the level of transcription and translation of regulatory and structurally important genes. They interact with a series of transcription factors, as for example the well-known myogenic regulatory factors (MRFs).

The basic events of myogenesis are stem cell commitment, proliferation, and apoptosis of myoblasts as well as differentiation and fusion of myoblasts and finally maturation of muscle fibres. All these events decide over the number of muscle fibres which are formed prenatally and, as we will see later, also over the degree of postnatal fibre hypertrophy. The importance of apoptosis (programmed cell death) within myogenesis has largely been neglected in the past, although it definitely affects the number of myoblasts. Apoptosis of muscle cells occurs under a variety of physiological and pathological conditions and it is a well-known event that eliminates a number of undesired cells (WEBB, 1972; FIDZIANSKA and GOEBEL, 1991). During myogenesis apoptosis correlates positively with cell cycle activity, however, is also increasing with growth factor withdrawal.

It has been multiply shown that animals selected on high growth, which resulted in increased muscle mass, exhibit higher numbers of muscle fibres and higher muscular DNA content resulting from higher proliferation rates (see REHFELDT et al., 2000). We used the highly growth-selected mouse line DU-6, which is currently the heaviest known mouse line in the world, to look at muscle fibre number, muscle fibre size, proliferation, and apoptosis of myogenic cells that were derived from this line and an unselected control line (Figure 1). The results suggest that increased cell proliferation and decreased basal apoptosis may contribute to higher muscle mass in growth-selected animals.

(a) (b) (c)

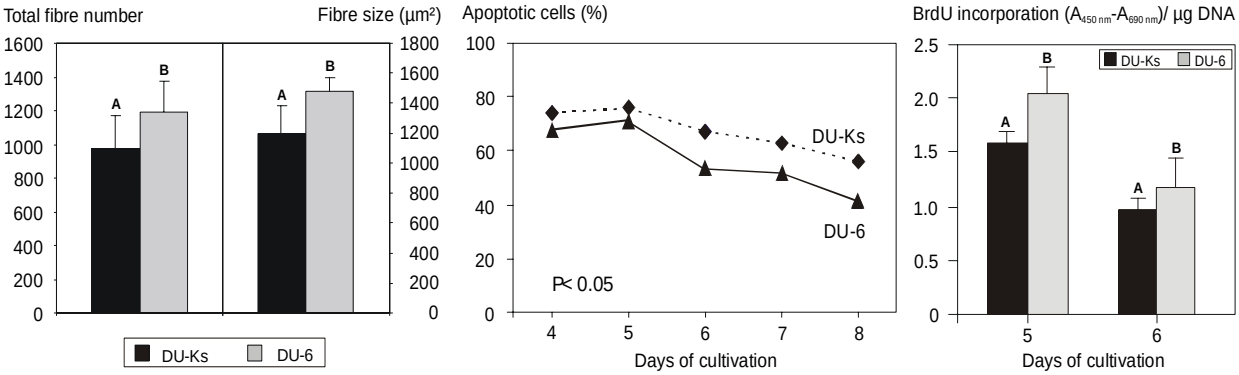

Fig. 1: **(a)** Muscle fibre number in Extensor digitorum longus muscle (generation 40) **(b)** frequencies of apoptosis and **(c)** DNA synthesis rate in cultures of myogenic cells (gen 70) derived from a long-term growth selected mouse line (DU-6) and an unselected control line (DU-Ks) (REHFELDT and BÜNGER, 1990; REHFELDT et al., 2004a). Different letters indicate significant differences (P<0.05).

2. Individual muscle fibres grow more slowly in size when the number of muscle fibres is high

In mammals, prenatal development (time shortly after birth in rodents) ends with a given number of fibres in most skeletal muscles that does not further increase.

Thereafter, the increase in skeletal muscle mass results mainly from an increase in muscle fibre size (hypertrophy), which in turn is limited by genetic and physiological reasons (Figure 2a).
In addition, the number of prenatally formed muscle fibres decides, at least in part, over the rate of fibre hypertrophy. During postnatal development the individual muscle fibres generally increase in size more slowly when the number of fibres is high and conversely fibres grow rapidly when the number of fibres is low (see REHFELDT, et al. 2000). This has been shown for several species such as mouse, chicken, pig, and cattle by negative phenotypic and genetic correlation coefficients ranging from – 0.3 to – 0.8 (Figure 2b). On the other hand both fibre number and fibre thickness are positively correlated with muscle cross sectional area. The largest loin areas can be achieved with very high fibre numbers at low size and vice versa.

(a) (b)

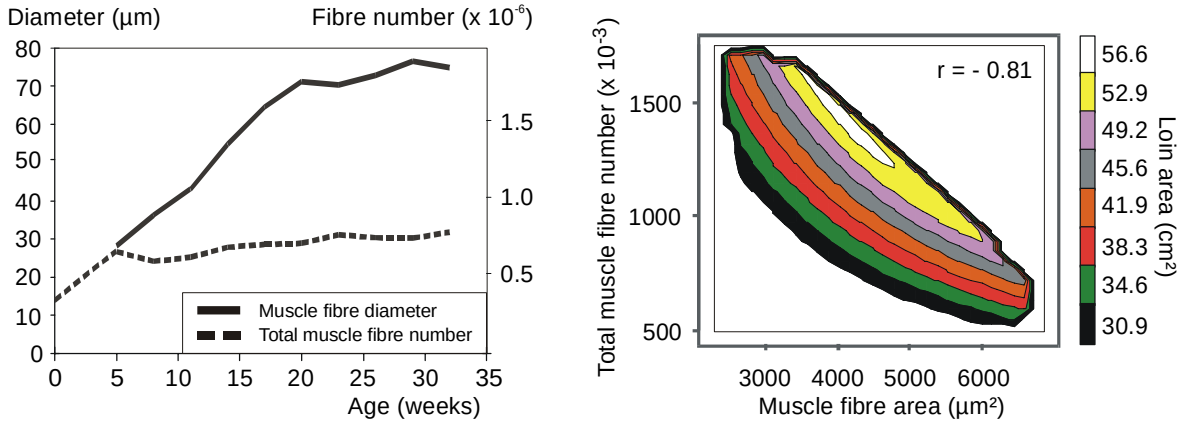

Fig. 2: **(a)** Postnatal development of fibre diameter and total fibre number per muscle cross section in the Semitendinosus muscle of German Landrace pigs **(b)** Contour plot on the relationship between Longissimus muscle fibre number and fibre area with showing different levels of loin area in German Landrace pigs (n=260); REHFELDT et al. (1987; 2004b)

3. Muscle fibre number is important in the relationship of birth weight with carcass quality

In multiparous species such as the pig there is an intralitter variation in body size and muscle fibre number (WIGMORE and STICKLAND, 1983; HANDEL and STICKLAND, 1987; REHFELDT et al., 2001a). So-called runts are commonly excluded from rearing. Foetal growth retardation in pigs may be caused by an insufficient nutrient supply, which may depend on the position of the foetus within the uterus or on litter size. Other constrains that are caused by maternal environmental and genetic factors may also play a role. HANDEL and STICKLAND (1988) showed that only piglets with high fibre numbers were able to exhibit postnatal catch-up growth.
Recently, we examined the influence of foetal growth retardation resulting in low birth weight/low fibre number on postnatal growth and carcass composition at slaughter in pigs (KUHN et al., 2002). In this experiment, piglets of 16 sows of German Landrace were assigned to 3 birth weight groups (BWG): 25% low weight (LW; < 1.20 kg), 50% middle weight (MW); 25% heavy weight (HW; >1.62 kg). Per litter 3 neonates were selected with the lowest, middle and highest birth weight for the analysis of body composition and muscle fibre characteristics (runts < 800g excluded). The remaining piglets were reared until slaughter. They were weaned at d 28 of age, subsequently fed

ad libitum and kept in groups. At slaughter, pigs were randomly selected for carcass and muscle fibre analysis (n=58).

Piglets of low birth weight grew clearly slower as compared to piglets of high birth weight as seen by daily gains and resulting live weights at slaughter (Figure 3). The ranking in weight was the same at birth and at slaughter.

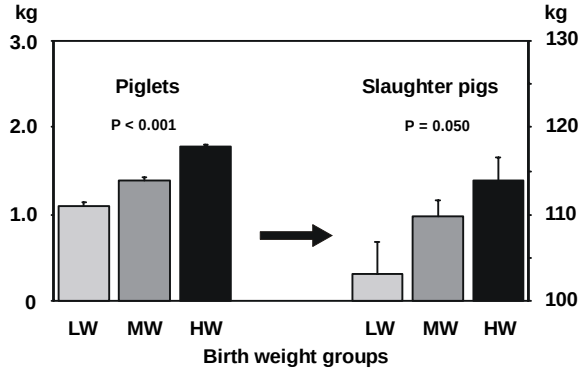

Fig. 3: Weights of neonatal piglets and of slaughter pigs (d 182) of different birth weight groups

Table 1
Body composition of neonates of low (LW), middle (MW) and heavy birth weight (HW)

	Birth weight groups (BWG)			
	LW	MW	HW	P_{BWG} <
Birth weight, kg	1.06	1.44	1.72	0.001
Internal organs, %	14.4	13.68	13.51	0.05
Muscle tissue, %	42.8	44.7	45.4	0.001
Subcutaneous fat, %	8.74	8.73	8.65	0.94
Bones, %	37.2	35.7	35.2	0.001
Skin, %	10.7	10.3	10.2	0.05
Water, %	80.11	79.71	79.34	0.05
Protein, %	14.88	15.28	15.52	0.05
Fat, %	1.01	1.13	1.15	0.001

Already at birth, significant differences in the body composition were found (Table 1). LW piglets exhibited higher percentages of internal organs, bones and skin, whereas the percentage of muscle tissue was smaller than in HW piglets. By chemical analysis of the whole body the LW piglets contained less fat and protein and more water indicating their relative immaturity. Correspondingly, significant differences were observed in the weight, cross sectional area and length of the Semitendinosus muscle that we used to determine muscle fibre characteristics. The low weight piglets had formed a significantly lower number of muscle fibres ($P<0.05$) during foetal development. This resulted mainly from lower numbers of secondary fibres ($P=0.08$) (Figure 4). The linear correlation coefficient between birth weight and the Semitendinosus fibre number is appr. +0.5 indicating that muscle fibre number is important in determining birth weight. Muscular protein concentration was lowest in LW piglets and the activity of creatine kinase (CK) as a marker of muscular differentiation was also markedly lower (Table 2). The DNA concentration was highest in LW piglets, but total DNA content was lowest as well. Consequently, in LW piglets both cell proliferation and protein accretion in skeletal muscle are far below the average. In addition, LW piglets tended to exhibit the lowest blood glucose concentration (77% of HW; correlation with BW was r= +0.40), indicating that they were not adequately supplied *in utero*. Surprisingly, the highest circulating IGF-I concentration (170% of HW, $P=0.01$) was found in LW piglets.

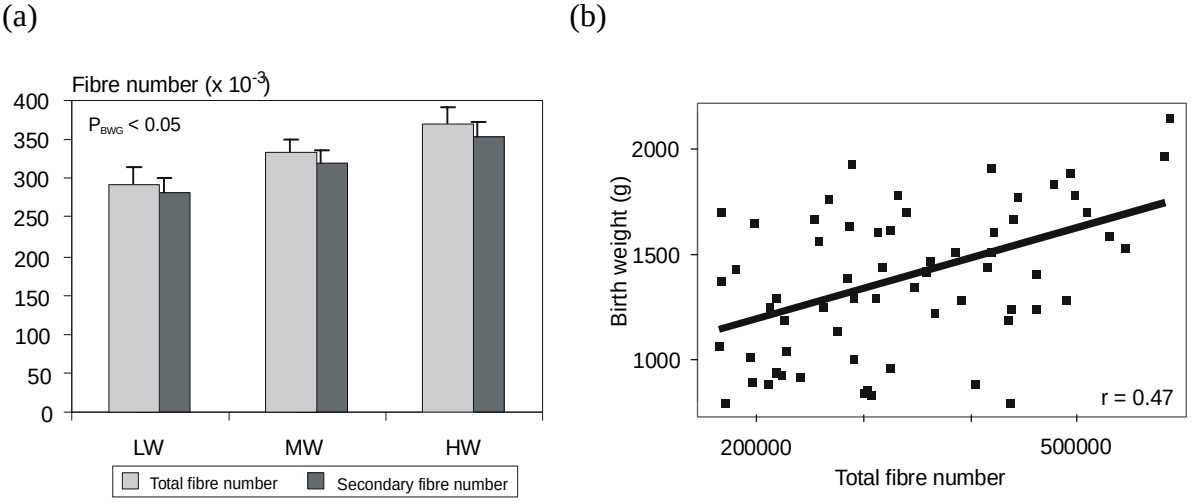

Fig. 4: Relationships of Semitendinosus muscle fibre number with birth weight **a)** Total and secondary fibre number in different birth weight groups **b)** phenotypic correlation coefficient between birth weight and total muscle fibre number

Table 2
Analysis of muscle and plasma of newborn piglets of different birth weight groups

	Birth weight groups (BWG)			
	LW	MW	HW	P_{BWG} <
Muscle				
Semitendinosus weight (g)	1.99	3.13	3.98	0.0001
Psoas major weight (g)	2.65	3.91	4.54	0.0001
CK/protein (IU/mg)*	3.62	4.11	4.29	0.01
DNA (mg/g)*	1.94	1.84	1.87	0.01
DNA total ST (mg)	3.87	5.53	6.89	0.0001
Protein (mg/g)*	80.1	83.8	85.5	0.06
Plasma				
Glucose (mmol/l)	3.79	4.85	4.93	0.12
IGF-I (ng/ml)	185.2	134.6	107.6	0.05

*Average of Semitendinosus, Psoas major, Longissimus, Biceps femoris muscles

From the principles of muscle fibre growth (see above, Figure 2), we would expect a faster fibre growth in low birth weight piglets, and that the plateau of fibre growth is attained earlier than in high BW piglets. Actually, we found the largest fibres in slaughter pigs of low birth weight both in the Semitendinosus and in the Longissimus muscle. The ranking of fibre number was found to be the same as at birth with low fibre numbers in LW and high numbers in HW pigs (Figure 5a). No differences were observed in the frequencies of different fibre types such as slow twitch oxidative, fast twitch oxidative, and fast twitch glycolytic fibres (data not shown) suggesting that postnatal fibre type differentiation is not dependent on birth weight.

What we observed instead were differences in the percentage of giant fibres in both Semitendinosus (HW vs LW: 0%; 0.07%; P=0.06) and Longissimus (HW vs LW: 0.07%; 0.44%; P<0.05) muscle. These are structurally abnormal fibres of extreme size that appear in post mortem muscle and are considered to arise from hyper-contraction. Extreme fibre size and higher frequencies of giant fibres have been shown to be associated with poor meat quality in pigs and poultry. Close genetic relationships between the frequency of hyper-contracted giant fibres and various meat quality characteristics have been found in pigs (Table 3). Probably, in the muscles of LW pigs more fibres of extreme size occur, which is not compatible with normal fibre function.

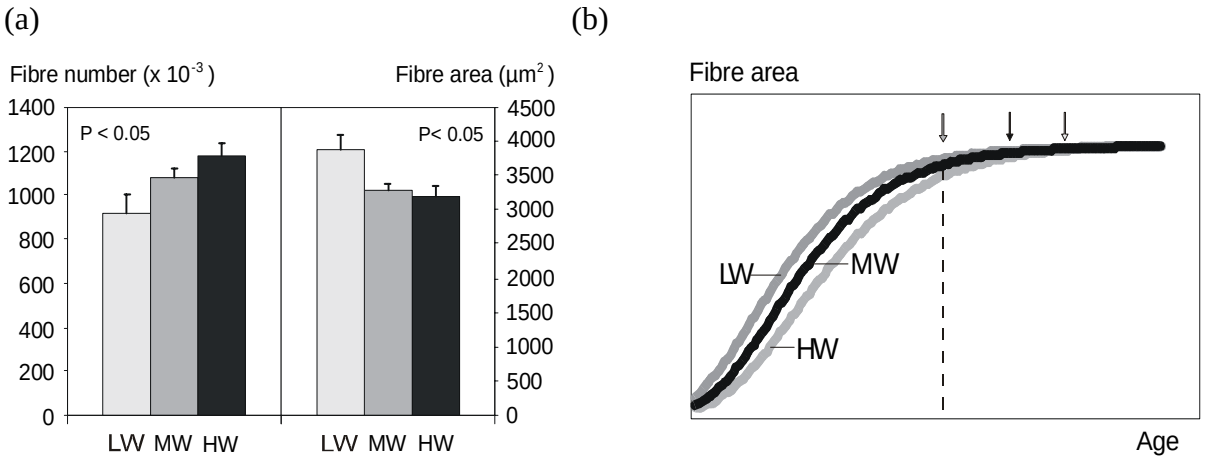

Fig. 5: **(a)** Fibre number and fibre area of Semitendinosus muscle in slaughter pigs (d 182) **(b)** Model of muscle fibre growth in dependence of birth weight groups

Table 3
Genetic correlation coefficients ($r_G \pm SE$) of Longissimus muscle characteristics with traits of growth and pork quality estimated from data of half- and full-sib groups (n = 1997) from 514 sires and 1078 dams of four pig genotypes (FIEDLER et al., 2004)

Trait	Fibre number	Fibre diameter	Percentage of giant fibres
Average daily gain (g/d)	0.46 ± 0.15	0.03 ± 0.19	0.47 ± 0.18
Back fat thickness (mm)	- 0.05 ± 0.11	- 0.12 ± 0.18	0.24 ± 0.23
Lean meat (%)	0.38 ± 0.12	0.52 ± 0.10	0.06 ± 0.00
Drip loss (%)	- 0.05 ± 0.19	0.64 ± 0.25	0.77 ± 0.17
Reflectance (%)	- 0.05 ± 0.14	0.32 ± 0.14	0.79 ± 0.11
pH 45 min p.m.	0.13 ± 0.14	- 0.37 ± 0.19	- 0.78 ± 0.11

Which carcass quality did the pigs of the different birth weight groups develop at slaughter? Pigs of low birth weight had lower carcass weights, meat percentages, and loin muscle areas at slaughter than pigs of high birth weight (Table 4). Also the weight of the heart was significantly lower. On the other hand, the internal fat percentage was higher. Likewise, the fat percentage by chemical analysis was numerically highest in LW pigs. The drip loss as one of the indicators for poor meat quality was highest in LW and lowest in HW piglets. Thus, animals having the largest fibres and the lowest fibre number exhibit the lowest meat percentage. This may also explain, why the phenotypic correlation coefficients between lean growth and fibre diameter are sometimes low or show a strong variation. Meat quality, on the other hand, is poorest in those pigs, which have the largest fibres and the highest percentage of giant fibres.
Finally, the growth model of postnatal fibre hypertrophy may explain the connection of fibre number with lean growth and meat quality (Figure 5b). The middle curve is representing the average fibre growth. In LW pigs, the increase in fibre size is faster because of the low fibre number, and the plateau of fibre growth is therefore attained earlier (see arrows). Consequently, nutritional energy cannot be longer used for muscle accretion, but will be mainly used to deposit fat. In contrast, pigs with high muscle fibre number (HW) attain this plateau later and have therefore a higher potential of muscle accretion. In addition, LW pigs develop more fibres of extreme size as they are probably at the plateau of fibre growth at slaughter, whereas the MW and HW pigs are

slaughtered before the fibres can grow to extreme size. This may be the reason that LW pigs tend to develop the poorest meat quality.

Table 4
Carcass quality of slaughter pigs of different birth weights (n = 58; d 182 of age)

	Birth weight groups			
	LW	MW	HW	$P_{HW:LW}$
Carcass weight, kg	84.2	90.6	95.5	0.07
Lean meat, %	54.8	56.2	56.5	0.09
Loin area, cm²	44.9	48.7	49.1	0.08
Heart, %	0.34	0.37	0.39	0.02
Internal fat, %	2.78	2.63	2.44	0.08
Fat (analytical), %	25.7	22.4	23.5	0.34
Drip loss, %	6.63	4.47	4.49	0.08

Our results are in agreement with studies from BEE (2004) who observed larger muscle fibres and increased percentage of adipose tissue in slaughter pigs of low birth weight. GONDRET et al. (2003) observed similar differences in fibre number and fibre size but no differences in selected carcass and meat quality traits. Extreme adipose tissue growth, hyperphagia, hyperglycemia and hyperinsulinaemia have been observed in rats born to undernourished dams (WOODALL et al., 1996; VICKERS et al., 2000). However, signs of obesity have also been observed in primiparous sheep in response to maternal feed restriction or at low birth weights (GREENWOOD et al., 1998; BISPHAM et al., 2003; SYMONDS et al., 2003). Although muscle fibre number was not affected, muscle DNA content was clearly lower as has been observed in our (GREENWOOD et al., 2000) and other studies with pigs (ROBINSON, 1969; BUITRAGO et al., 1974). The amount of satellite cells, which are essential for postnatal muscle fibre growth and repair, may be another limiting factor for postnatal muscle growth.

4. Manipulation of muscle fibre number by maternal growth hormone in pigs

Pituitary growth hormone (GH) is the principal hormone involved in regulating growth and metabolism. Numerous studies have shown the efficacy of GH in stimulating lean growth and inhibiting adipose tissue growth in postnatal pigs. Furthermore, research of the last decade has shown that circulating maternal GH plays a role in the prenatal development of the progeny (REHFELDT et al., 2004c). Exogenous porcine GH (pGH) given to pregnant sows in a sufficient dose affects the metabolic status by increasing maternal nutrient concentrations (glucose, free fatty acids) and providing an endocrine environment (increased IGF-I, insulin) that may stimulate placental and foetal growth. GH administered to sows during gestation is capable to stimulate placental growth and to modify the expression of regulatory proteins of the GH-IGF axis in placental tissues (FREESE et al., 2002). It is also capable to increase nutrient availability to the embryo/foetus and to induce short- and long-term changes in serum IGF-I concentrations in the progeny. As a consequence foetal growth may be accelerated by maternal GH treatment. However, advantages in growth gained by GH treatment during early and mid-gestation are not maintained until birth, whereas GH treatment during late or greatest part of gestation may result in heavier piglets. If maternal GH level is increased by treatment during early gestation, only littermates that are disadvantaged by maternal constraints are able to grow faster until birth and to

improve their body composition towards leanness, which results in a higher balance within litters.

It has been shown that these effects of maternal GH were related to changes in pig myogenesis. We studied the influence of pGH treatment during early gestation (d 10 to 24 or 27; REHFELDT et al., 1993; 2001ab; 2002). Individual muscle weights and/or cross sectional areas were not affected in foetuses, neonatal piglets and pigs at weaning or at slaughter. Summarizing the data from two experiments with 46 sows, maternal pGH treatment did not significantly change the average total muscle fibre number, but tended to increase the number of primary muscle fibres in Semitendinosus muscle (Table 5). However, consistent with the findings for birth weight, an increase in total fibre number has been observed in LW littermates (Figure 6). When the total fibre number was higher (LW piglets), the numbers of both primary and secondary muscle fibres were increased ($P<0.05$; $P<0.10$). The increase in secondary fibre number occurred as late as during the last trimester and was accompanied by a higher expression of the myogenic regulatory factors MyoD and Myf-5 indicating that the muscle contained a higher proportion of proliferating myoblasts. It remains unclear, whether secondary fibre formation was induced by more effective placental IGF action and better placental supply or by any unknown mechanism triggered by the higher number of primary fibres. As seen for fibre number a tendency for a pGH x BWG interaction ($P=0.11$) was found for the specific creatine kinase (CK) activity in muscle with CK being a marker for muscle specific differentiation. CK activity determined as average of four muscles was increased in low weight and middle weight but decreased in heavy weight piglets. In addition, the average muscle DNA and protein concentration was significantly increased independently of birth weight group ($P<0.05$). Increases in total muscle fibre number can also be derived from another study (GATFORD et al., 2003). The authors found increased Semitendinosus muscle cross sectional areas at unchanged fibre number per mm² (fibre density) in 61-d-old female progeny of sows treated with pGH during the second quarter of gestation. The results of both studies suggest that increased maternal glucose, at least partly, mediates the effect of maternal pGH treatment on muscle growth.

Table 5
Semitendinosus muscle cross sectional area and numbers and ratios of secondary and primary muscle fibres in newborn pigs in response to maternal pGH treatment (6 mg/d) from d 10 to 27 of gestation (LSmeans ± SE) (REHFELDT et al., 2004c).

Trait	Control (n = 64)	pGH (n = 69)	P	P (BWG x pGH)
Muscle area (cm²)	1.02 ± 0.04	1.04 ± 0.04	0.69	0.15
Total fibre number ($x10^{-3}$)	319.7 ± 11.9	334.9 ± 11.4	0.36	0.11
Primary (P) fibre number ($x10^{-3}$)	13.74 ± 0.45	14.69 ± 0.43	0.13	0.19
Secondary (S) fibre number ($x10^{-3}$)	305.7 ± 11.1	320.3 ± 11.1	0.37	0.14
S/P ratio	22.93 ± 0.72	21.72 ± 0.69	0.23	0.42

Treatment with pGH during early gestation changed the body composition of neonatal piglets (REHFELDT et al., 2001b; KUHN et al., 2004). Selective increases in the proportions of the intestinal tract, internal organs and skin and a slight decrease in the percentage of muscle tissue were observed. Bones and subcutaneous fat remained unchanged. More information was available by the interactions of the BWG x pGH treatment (Table 6). The percentage of muscle tissue was increased in LW piglets and decreased in MW and HW piglets. For subcutaneous fat and fat determined by

chemical analysis the opposite changes were found; i.e. decreases in LW and increases in MW and HW piglets.

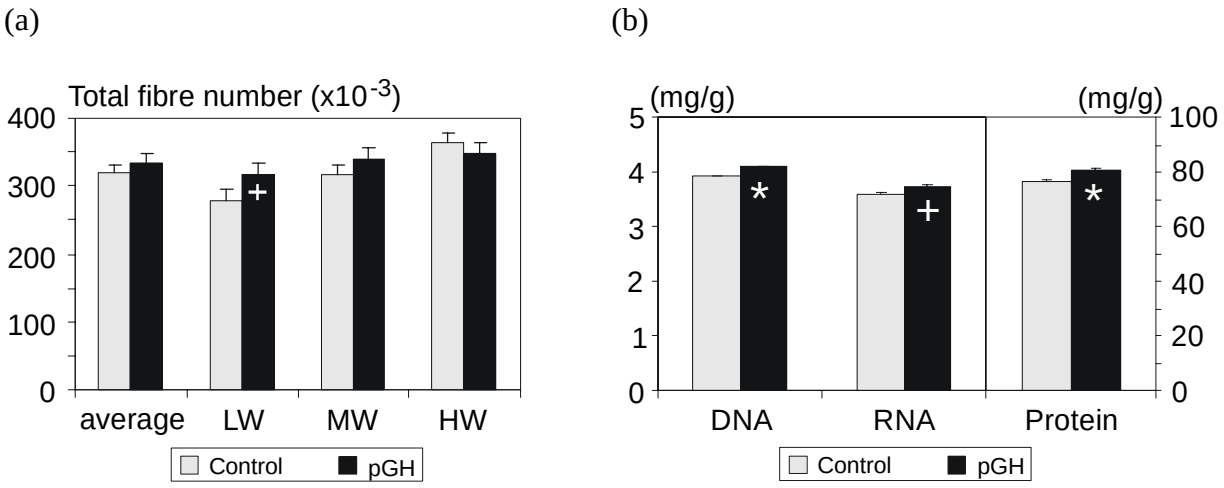

Fig. 6: **(a)** Total muscle fibre number in Semitendinosus muscle of neonatal piglets born to gilts treated with 6 mg pGH per d (n=69) or placebo (control) (n=64) from d 10 to 27 of gestation (REHFELDT et al., 2004c). There was a pGH x birth weight group interaction for total fibre number (P=0.11). $^{+}$P=0.09 for differences between pGH and control. **(b)** Muscular DNA, RNA, and protein concentrations (average of 4 muscles). *P<0.05, $^{+}$P=0.12

Table 6
Birth weight group (BWG) x pGH interactions for traits of neonatal body composition in response to maternal pGH treatment (6 mg/d) from d 10 to 27 of gestation (REHFELDT et al., 2001b; KUHN et al., 2004)

Trait	LW	MW	HW	P (BWG x pGH)
Muscle tissue, %	+	-	-	0.03
Subcutaneous fat, %	-	+	+	0.03/ 0.06
Fat (analytical), %	-	+	+	0.05

These changes are consistent with those found for muscle fibre number in the piglets. Likewise, changes in lean percentage recorded in the offspring at slaughter (d 182) depended on birth weight. Except the increased percentage of internal organs (P=0.09), there were only marginal changes in the average carcass composition. However, there was a tendency for a pGH treatment by BWG interaction for muscle meat percentage with decreases in pigs that were born as HW (-1.09% units) and MW (-0.77% units) and an increase in pigs that were born as LW piglets (+0.59% units), which again is consistent with results obtained in neonates. Also the ranking for muscle fibre number was the same as at birth with increases seen in originally LW piglets.

Unlike the above findings, treatment with pGH during mid and late gestation was not able to induce an increase in the cross-sectional area or total Semitendinosus muscle fibre number in neonatal piglets (REHFELDT et al., 1993). This suggests that increased birth weights in response to higher maternal GH in late gestation are not related to increased muscle fibre numbers. In this case, the increases in birth weight result mainly from higher percentages of total body lipid, which has been explained by the diabetogenic stage of the dams (KVERAGAS et al., 1986; ETIENNE et al., 1992) and is in good analogy with macrosomia of infants born to diabetic mothers. In a recent study GATFORD et al. (2004) have recently reported that maternal long-term

treatment with pGH from d 25 to 100 of gestation increased progeny size at birth, but information on muscle structure traits was not available.

Conclusions

Foetal growth retardation in pigs cannot be compensated during postnatal growth. Pigs of low birth weight exhibit the lowest lean growth and poorest meat quality at slaughter. The number of muscle fibres formed prenatally is positively correlated with birth weight and it plays a significant role in the relationship of birth weight with muscle accretion and meat quality. Piglets with high muscle fibre numbers are requested for efficient pig production. Treatment of sows with growth hormone until mid gestation is able to increase birth weight and the number of muscle fibres in the offspring by stimulating the determination and/or proliferation of myoblasts. This effect is only pronounced in small littermates disadvantaged by insufficient nutrient supply and it is associated with increases in lean percentage in neonates and at slaughter. Cellular and molecular mechanisms controlling basic events of myogenesis are worthy of further investigation.

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Combining QTL- and expression-analysis: identification of functional positional candidate genes for meat quality and carcass traits

Abstract

In order to identify genes controlling meat quality and carcass traits we are aiming to combine two approaches: (1) the QTL analysis (2) and expression profiling. Here results of QTL analyses in different porcine resource populations and current attempts to identify functional candidate genes for meat quality traits are reviewed and own efforts are presented: A QTL scan for meat quality and carcass traits was conducted in two resource populations, the DUMI resource population based on Duroc and Berlin Miniature Pig and the DUPI resource population based on Duroc and Pietrain. This analysis provides positional information on QTL for these traits and confirms and supplements results of numerous other QTL scans. Differential display was used to compare transcription profiles of fetal (presumptive) M. longissimus dorsi tissue at 7 key stages of myogenesis in Pietrain and Duroc breeds. Furthermore differential display and microarray analyses were conducted of muscle and liver of discordant sibpairs with regard to body composition. Expression profiling offers a list of functional candidate genes for meat quality and carcass traits with trait-, breed-, and stage-associated expression of various physiological networks. The application of bioinformatic tools and the use and generation of mapping information of the differentially expressed genes combined with QTL information reveal segregating functional positional candidate genes. These will be selected for further association and molecular genetic analyses to provide statistical and functional evidence for their impact on meat quality and carcass traits and to elucidate prenatal events affecting postnatal growth performance and meat quality.

Key Words: pig, meat quality, carcass traits, QTL, expression analysis

Zusammenfassung

Titel der Arbeit: **Kombinierte Anwendung von QTL- und Expressionsanalysen: Identifizierung von funktionellen positionellen Kandidatengenen für Fleischbeschaffenheits- und Schlachtkörpermerkmale**

Um Gene mit Einfluss auf Fleischbeschaffenheits- und Schlachtkörpermerkmale zu identifizieren, können zwei Ansätze kombiniert angewandt werden: QTL-Analysen, und (2) Expressionsanalysen. Hier wird ein Überblick über derzeitige QTL- und Expressionsstudien hinsichtlich Fleischqualitätsmerkmale gegeben sowie eigene Untersuchungen dargestellt. Eigene Untersuchungen umfassen QTL-Scans in zwei porcinen Ressourcen-Populationen DUMI, basierend auf Duroc und Berlin Miniaturschwein, sowie DUPI, basierend auf Duroc und Pietrain. Die Ergebnisse bestätigen und ergänzen eine Reihe früherer Untersuchungen hinsichtlich der gefundenen QTL-Positionen. Vergleiche der Transkriptome von M. longissimus dorsi Gewebeproben von sieben pränatalen Zeitpunkten der Myogenese wurden mittels Differential display bei Pietrain und Duroc gemacht. Weiter wurden Differential display und Mikroarray-Analysen von Muskel und Leber von in Merkmalen der Körperzusammensetzung diskordanten Geschwisterpaaren durchgeführt. Die Expressionsprofile zeigen eine Reihe von funktionellen Kandidatengenen für Fleischqualitäts- und Schlachtkörpermerkmale aus Merkmals-, Rassen- und Stadien-assoziierten physiologischen Netzwerk an. Die Anwendung bioinformatorischer Analysen und Kartierung der differentiell exprimierten Gene kombiniert mit QTL-Informationen ermöglicht die Identifizierung segregierender, funktioneller, positioneller Kandidatengene. Diese werden molekulargenetisch weiter untersucht hinsichtlich der Effekte auf Fleischqualität und Schlachtkörpermerkmale.

Schlüsselwörter: Schwein, Fleischqualität, Schlachtkörpermerkmale, QTL, Expressionsanaylsen

Breeders' issues

Growth in general is controlled by many endo- and exogenous factors and characterised mainly by the deposition of adipose and connective tissue, bone and muscle. Muscle tissue accounts for most of the body mass and daily energy

consumption, while liver largely contributes to the over all metabolic status of the organism. Skeletal muscle becomes meat after slaughter. It consists of three different types of muscle fibres each characterised by certain biophysical, biochemical and metabolic properties. The number, size and proportion of different muscle fibres affect growth performance and are endogenous factors on post mortal meat quality traits (LENGERKEN et al., 1994). The number and proportion of muscle fibres are to a large extent determined during the prenatal development.
There are currently three major production problems that breeders encountered when attempting to breed for improved performance, body composition and meat quality. Firstly, these traits are to some extent negatively correlated. Secondly, most of the traits, especially body composition and meat quality traits can only be measured at post mortem, which make prior breeding selection difficult. Thirdly, the consumers´ as well as the meat processing industries´ conception of high meat quality is not uniform and their expectations are changing.
The identification of genes that regulate fattening, body composition and meat quality traits will assist efficient meat production and facilitate resolving existing production problems.

Map-based and function-driven approaches to identify QTL
Researchers have used different strategies to detect such genes. One is the positional cloning approach that includes the observation of co-segregation of a large number of markers and a trait (“genome scan“) and fine mapping of the suspect regions approaching the unknown gene more and more closely. Candidate genes may be identified based on knowledge of physiology, biochemistry or pathology, which clearly indicates the mechanism of the trait ("direct candidate" approach) or based on findings in other species (comparative mapping approach). More recent approaches to detect candidate genes are based on the analyses of differences of the expression profile of particular subsets of cells and/or individuals with certain phenotypes. These are functional candidate genes because of their temporo-spatial distribution of expression. The positional candidate approach combines linkage information for a particular trait and mapping information of a candidate gene, which may be identified because of its specific expression pattern (functional candidate). The functional candidate approach benefits from the fact that it only deals with cDNA, devoid of intronic and intergenic sequences, which represent only a few percent of the total genome (about 3% in mammals). Differential expression screening approaches are therefore more closely associated to gene function.

Genome scans
Most of the successful approaches to detect QTL were based on genome scans performed in resource populations. The first QTL in pig detected by this approach was reported by ANDERSSON et al. (1994). A QTL accounting for 10% of phenotypic variation for average backfat and abdominal fat on chromosome 4 was detected. Subsequently these results were supplemented and confirmed. QTL for meat and carcass quality traits based on reverse genetics were also reported (Table 1).

Table 1
Summary of results of current QTL analyses for traits related to meat quality (Überblick über Ergebnisse von QTL-Analysen für Fleischqualitätsmerkmale)

Trait	Pop	Sscr	Pos [cM] or marker interval	Sig	Var	Model	Reference
conductivity1$_{ham}$	M x P	6	98	**	23,7	line cross	Yue et al. 2003a
		13	74	†	4,1		Yue et al. 2003c
	WB x P	1	221	†	4,4		Beeckmann et al. 2003a
		6	85	**	44,7		Yue et al. 2003a
conductivity24$_{ham}$	M x P	5	161	†	3,1		Lee et al. 2003b
		6	97	**	24,3		Yue et al. 2003a
	WB x M	7	104	†	4,2		Yue et al. 2003b
		13	83	†	3,8		Yue et al. 2003c
	WB x P	2	0	†	3,3		Lee et al. 2003a
		6	85	**	44,9		Yue et al. 2003a
		x	0	†	4		Cepica et al. 2003b
conductivity1$_{loin}$	M x P	6	97	**	30,9	line cross	Yue et al. 2003a
	WB x M	13	62	†	3		Yue et al. 2003c
	WB x P	6	78	**	52,3		Yue et al. 2003a
		7	160	†	4,1		Yue et al. 2003b
		8	0	†	3,5		Beeckmann et al. 2003c
conductivity24$_{loin}$	M x P	6	96	**	36,9	line cross	Yue et al. 2003a
		16	56	†	4,6		Pierzchala et al. 2003
	WB x M	5	175	†	3,6		Lee et al. 2003b
		13	72	*	5,2		Yue et al. 2003c
	WB x P	4	81	†	3,9		Cepica et al. 2003a
		6	77	**	59,1		Yue et al. 2003a
pH1$_{ham}$	M x P	6	98	**	20,2	line cross	Yue et al. 2003a
		x	6	**	8,7		Cepica et al. 2003b
	WB x M	3	54	†	3,4		Beeckmann et al. 2003b
	WB x P	6	82	**	32,8		Yue et al. 2003a
	M x L, LW	18	31	†		line cross	de Koning et al. 2001
		14	1	†		sex specif.	
		X	1	†			
		11	54	†		paternal	
		14	96	†			
pH24$_{ham}$	M x P	2	73	*	5,8	line cross	Lee et al. 2003a
		18	55	*	5,3		Dragos-Wendrich et al. 2003
	WB x M	3	30	*	5,8		Beeckmann et al. 2003b
	WB x P	16	110	*	5,9		Pierzchala et al. 2003
		x	81	†	4,3		Cepica et al. 2003b
	B xY	6	53	†	2,9		Malek et al. 2001
		14	110	†	3,59		
		15	72	*	4		
pH1$_{loin}$	M x P	6	101	**	30,5	line cross	Yue et al. 2003a
	WB x P	1	137	†	4,1		Beeckmann et al. 2003a
		6	78	**	47,3		Yue et al. 2003a
		16	33	†	4,1		Pierzchala et al. 2003
pH24$_{loin}$	M x P	2	107	†	4,2	line cross	Lee et al. 2003a
	WB x P	6	84	†	4,9		Yue et al. 2003a
		7	114	†	3,6		Yue et al. 2003b
	B xY	5	113	*	4,85		Malek et al. 2001
		15	76	**	5,61		
	G x L	3	33	†	6,4		Ovilo et al. 2002

† - suggestive significance=less than 1 false positive per genome scan; * - significant at 5% genome-wide threshold; ** - significant at 1% genome-wide threshold
Duroc, D; Berkshire, B; Yorkshire, Y; Guadyerbas, G ; Landrace, LR ; Pietrain, P; Large White, LW; Meishan, M; Wild Boar, WP; Mangalitza, MG; Hampshire, H; Meishan Synthetic, MS; comXY, komerziellXY

We have conducted a genome scan in two porcine resource populations, the DUMI and the DUPI resource population. The first is a three-generation porcine F2 resource

population based on reciprocal crossbreeding of Duroc and Berlin Miniature Pig (HARDGE et al., 1999). The DUPI is based on reciprocal crossbreeding of Duroc and Pietrain. Performance testing was done according to the guidelines of expert commission of German pig breeding organisations at the performance test station of the Institute of Animal Breeding and Genetics, University of Bonn. Traits taken into account here are $pH1_{loin}$, $pH24_{loin}$, $pH1_{ham}$ and $pH24_{ham}$ as well as $conductivity1_{loin}$, $conductivity24_{loin}$, $conductivity1_{ham}$ and $conductivity24_{ham}$ (pH-values and conductivity measured 45 min and 24 hours post mortem in M. longissimus dorsi at $13^{th}/14^{th}$ rib and M. semimembranosus, respectively) plus meat colour (Opto: meat colour 24 hours post mortem in M. longissimus dorsi at $13^{th}/14^{th}$ rib; Opto star).

Animals were genotyped at 76 type II and 9 type I markers distributed over autosomes. QTL analysis was performed using interval mapping by regression under the line cross and half sib model (QTL-Express, SEATON et al., 2002). Phenotypic data were adjusted for systematic effects by fixed models taking into account effects of sire, dam, sex, parity and slaughter weight and slaughter date, as appropriate.

Under the line cross model QTL with genome wide significance were found on Sscr 3 and 15 for meat colour and on Sscr 4 for $conductivity24_{ham}$. Furthermore QTL with chromosome wide significance are on Sscr 1, 2, 4, 6, 13, 15 and 18 (Table 2). Halfsib analyses revealed the same QTL for meat colour on Sscr3 and additional QTL for the various technological meat quality traits with genome wide significance on Sscr 2, 3, 5, 6, 12, 13, 15, 16, 17 (Table 3). Preliminary results obtained in the DUPI resource population provide a highly significant QTL for pH24ham on Sscr 1. Our and other published results (Table 1) show that - as might be expected - loci affecting meat quality are distributed over most autosomes but obviously do not segregate in all populations.

For a subset of animals of the DUMI resource population also muscle fibre characteristics were determined by quantitative histochemical methods (WIMMERS et al., in preparation). Interestingly, QTL for pH and conductivity on Sscr 2 and 4 match QTL identified for diameter of STO and FTG fibres on Sscr 2 as well as proportion of STO and giant fibres on Sscr 4, respectively. These results indicate that probably these QTL for technological parameter of meat quality are related to QTL effecting muscle fibre proportion and characteristics.

Table 2
QTL for meat quality traits obtained in the DUMI resource population under the F2 line cross model (QTL für Fleischqualitätsmerkmale identifiziert in der DUMI Ressourcen-Population im F2-Modell)

Trait	Sscr	Pos [cM]	F-value	add. genet. eff.	dom. eff.	Var
$pH24_{loin}$	1	21	6.91**	-0.03±0.01	-0.01±0.01	1.80
$conductivity1_{ham}$	2	54	5.26*	0.14±0.05	0.10±0.11	1.40
$meat\ colour_{OPTO}$	3	0	13.36****	-1.74±0.35	0.57±0.51	3.55
$conductivity1_{loin}$	4	26	5.42*	0.02±0.04	-0.25±0.08	1.49
$conductivity24_{ham}$	4	109	8.13****	0.03±0.12	0.75±0.19	2.18
$pH24_{loin}$	4	90	6.44*	-0.02±0.01	-0.02±0.01	1.72
$conductivity24_{loin}$	6	138	6.06*	-0.27±0.08	-0.29±0.18	1.53
$pH1_{loin}$	13	76	5.88*	0.04±0.01	0.02±0.02	1.57
$meat\ colour_{OPTO}$	15	117	7.99***	-1.27±0.37	-0.84±0.50	2.16
$pH24_{loin}$	15	63	5.70*	-0.03±0.01	0.02±0.02	1.52
$pH24_{ham}$	15	117	5.13*	0.02±0.01	-0.03±0.01	1.33
$pH24_{loin}$	18	66	4.83*	-0.02±0.01	-0.25±0.01	1.29

Table 3
QTL for meat quality traits obtained in the DMI resource population under the halfsib model (QTL für Fleischqualitätsmerkmale identifiziert in der DUMI Ressourcen-Population im Halbgeschwister-Modell)

Trait	Sscr	pos [cM]	F-value	Var
$pH1_{loin}$	1	99	3.53*	2.07
$conductivity1_{loin}$	2	24	3.77**	1.82
$conductivity24_{ham}$	2	76	4.68***	2.72
$meat\ colour_{OPTO}$	3	0	7.61****	5.61
$pH1_{loin}$	3	24	2.95*	1.72
$pH1_{loin}$	4	12	3.25*	1.89
$meat\ colour_{OPTO}$	5	9	6.62***	4.87
$meat\ colour_{OPTO}$	6	170	4.48*	3.30
$conductivity24_{loin}$	6	24	4.55*	2.66
$conductivity24_{ham}$	6	0	16.35****	9.52
$pH1_{loin}$	8	128	3.33*	1.95
$pH1_{loin}$	11	72	2.99*	1.74
$conductivity24_{loin}$	11	36	3.63*	2.14
$conductivity24_{ham}$	12	92	4.89***	2.83
$meat\ colour_{OPTO}$	13	43	7.98****	5.87
$pH24_{loin}$	13	0	3.35*	1.97
$conductivity24_{loin}$	14	120	3.89*	2.22
$pH24_{loin}$	15	48	5.39***	3.14
$conductivity1_{loin}$	15	88	3.00*	1.36
$meat\ colour_{OPTO}$	16	42	4.47*	3.29
$conductivity1_{loin}$	16	20	4.48***	2.28
$meat\ colour_{OPTO}$	17	0	3.80*	2.80
$pH1_{loin}$	17	68	3.07*	1.80
$conductivity24_{loin}$	17	0	5.19***	3.02

Genome-wide scans cannot resolve the location of a QTL more precisely than 10-30 cM. As this is equivalent to a region containing 5-30 MB of deoxyribonucleic acid (DNA) and several hundreds of genes it is a major task to fine map the QTL region in order to finally positional clone the gene. The identification of the polymorphic genes within the QTL regions detected by reverse genetics is the aim of many current studies. Furthermore it has to be proven that the QTL segregate and show any effect in other commercial and experimental populations than the resource population used to identify the QTL region.

Expression analyses

In the livestock field several attempts to use of gene expression profiling techniques have been made to elucidate tissue-specific differential gene expression in relation to particulate developmental stages and/or exhibition of certain phenotypes. The results will significantly improve and enlarge the list of candidate genes for economic important traits including growth, carcass composition and meat quality for which muscle is the mayor target tissue.

There are currently several efforts to apply microarrays to obtain genes that are differentially expressed in muscle tissue either between different developmental stages/ages or breeds or housing/feeding conditions in the pig. Using a porcine skeletal muscle cDNA macroarray containing 327 cDNAs derived from whole embryo and skeletal muscle 48 genes were identified being differentially expressed in two or more of the four muscle tissue targets derived from 75- and 105-d foetal hind limb muscles, and 1- and 7-wk postnatal semitendinosus muscles (ZHAO et al., 2003). Hybridisation of a cDNA microarray of more than 700 clones of normalized skeletal muscle cDNA

libraries from hind limb muscle of pigs at 45 and 90 d of gestation, birth, 7 wk and 1 year of age with targets derived from total RNA from skeletal muscle of pigs at 60 d of gestation and 7 wk of age revealed 55 clones that were over expressed by at least 2-fold (41 by at least 2.5-fold) in 60-d foetal skeletal muscle as compared to 7-wk postnatal muscle in all four experiments (ERNST et al., 2002). To monitor gene expression throughout skeletal muscle development, SEO and BEEVER (2001) aimed to construct filter array of clones of cDNA libraries of muscle tissue from several gestational (early [29, 35, 43, 49 days], middle [56, 64, 70, 78 days], late [84, 93, 99, 106 days]) and postnatal stages (160 days of age). A comprehensive study of muscle expression profiles in pigs by microarray technology was published by BAI et al. (2003). A cDNA microarray made up of 3500 and 2000 randomly chosen moderately to weakly expressed clones from a 50-day-old foetal M.longissimus dorsi and a 3-day-old piglet M. gastrocnemius cDNA-library, respectively, was hybridised with transcripts of red (M. psoas) and white muscle (M. longissimus dorsi). Seventy genes more highly expressed in M. psoas and 45 more highly expressed in M. longissimus were sequenced. These included mitochondrial genes and genes involved in gluconeogenesis, transcription, translation and signal transduction on the one hand and genes coding for sarcomeric structural proteins and enzymes of glycolysis on the other hand as well as novel genes. The loci represent candidate genes that could influence muscle phenotype.

ZHAO et al. (2001) used the mRNA differential display technique (DD-RT-PCR) to identify differentially expressed genes in longissimus muscle between Duroc and Erhuanlian breeds. RNA pools were made from three animals in each breed. Five 3' anchored primers in combination with 10 different 5' arbitrary primers were used and nearly 2000 bands were examined, among which 12 differentially displayed cDNAs were found. Two bands uniquely expressed in Erhualian pigs were cloned and sequenced. One band showed significant homology to the porcine myosin heavy chain gene. Semiquantitative reverse transcription PCR confirmed its differential expression. Another band is homologous to porcine NADH4 and no difference was found in its expression level between the two breeds. By differential display MAAK et al. (2001) identified genes that are differentially expressed between healthy and splay leg newborn piglets in order to identify candidate genes for this inherited disorder.

We used the carcass trait `eye-muscle area´ with its high heritability as a valuable model case to apply the approach of analysing discordant sib pairs of the breed German landrace and of the F2 of the DUMI resource population by differential display in order to obtain candidate genes. These groups of animals also differed by one standard deviation in traits related to meat quality. The comparison of about 4000 differential display band obtained with the anchored primer (d)T_{11}VA (V:A,C,G) and 26 arbitrary primers revealed 27 bands with differential expression between discordant sib pairs. Out of these 7 bands were analysed further and trait-associated differential expression was confirmed by semiquantitative RT-PCR for six fragments. Two clones showed high homology to known genes, two were homologous to an EST and a SINE sequence. Two clones did not show any homology (PONSUKSILI et al., 2000). These loci represent candidate genes for traits related to muscularity and also meat quality. The discordant sib pairs of the DUMI resource population were also used for expression analyses using a custom made liver cDNA microarray and liver cDNA as target in order to identify functional candidate genes for traits related to muscularity

and body composition. Also among these genes some are likely to affected meat quality (mainly in terms of fat contend and distribution).
An European initiative, PorDictor (QLK5_2000_01363; coordinator K. Wimmers), aims to detect prenatal differential expressed muscle transcripts for the Pietrain and Duroc breeds at seven key developmental stages (d14, d21, d35, d49, d63, d77, and day 91 in order to obtain genes affecting meat quality (WIMMERS et al., 2002).
Several techniques for expression profiling, i.e. cDNA-microarrays, Differential Display-RT-PCR, construction of stage-specific muscle cDNA libraries and subtractive hybridisation, are applied. We generated expression profiles of the seven stages during porcine myogenesis using a total of 88 differential display primer combinations. Comparisons between breeds or between stages revealed 445 fragments varying either in their intensity or in their presence. Among these are 144 fragments showing differences between breeds and 301 fragments differentially displayed between stages (Tables 4 and 5).

Table 4
DD-RT-PCR-fragments showing stage and breed specific patterns of expression (DD-RT-PCR-Fragmente mit Stadien- und Rassen-spezifischen Expressionsmustern)

group by stage specificity														# of bands	breed specificity: appeared	# of bands
	14		21		35		49		63		77		91			
1	1		0		0		0		0		0		0	85	only in Pi	38
2	0		1		1		1		1		1		1	51	only in Du	30
3	0		1		0		0		0		0		0	26	stronger in Pi	38
4	1		1		0		0		0		0		0	19	stronger in Du	12
5	0		1	<	1		1		1		1		1	20	earlier in Pi	11
6	0		0		1		1		1		1		1	38	earlier in Du	5
7	0		0		0		1		1		1		1	7	longer in Pi	4
8	0		0		0		0		1		1		1	9	longer in Du	6
9	1	>	1		1		1		1		1		1	12		
10	0/1	<	1	<	1	<	1	<	1	<	1	<	1	6		
11	1	>	1	>	1	>	1	>	1	>	1	>	1	3		
singleton	-		-		-		-		-		-		-	25		

Table 5
Summary of the function of sequenced breed and stage specific expressed gene (Überblick über die Funktionen der Rassen- und Stadien-spezifisch exprimierten Gene)

Function / Homology	breed-specific	stage-specific
EST	4	7
genomic sequence	1	3
gene with unknown funktion	1	2
structural gene	1	6
metabolism	-	2
transcription	2	2
differentiation	1	-
proliferation	3	1
autocrine / paracrine factor	1	-
extrac. matrix / cell adhesion	1	1
other / without homology	16	1

Combining results of QTL and expression analysis

The genome scans provide positional information of regions containing QTL for meat quality that are segregating within the resource populations analysed. The expression analyses provide a list of functional candidate genes for these traits based on their temporo-spatial and/or phenotype-associated expression. We are aiming to combine the positional, genetic information and the functional evidence for impact on the traits of interest of the candidate genes by using existing (also comparative) and generating mapping information. In order to select genes out of the list of functional candidate genes for further analysis we therefore took into account the mapping position of the genes found to be regulated during myogenesis on top of other criteria like consistency and reproducibility of expression pattern, breed specificity and function.

Furthermore we are aiming to extent the integration of map-based and function-driven approaches to identify QTL by (1) generating QTL-genotype associated expression profiles and by (2) construction and application of region-specific microarrays.

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Molecular characterisation of the porcine skeletal myosin heavy chain cluster and a major candidate regulatory domain

Abstract

Members of the myosin heavy chain (MyHC) gene family are subjected to temporal regulation (gene switching) during development. One strategy to the identification of *cis*-acting regulatory elements that are involved in temporal or fibre-type specific regulation is to undertake a comparative analysis of the MyHC gene family between the pig, an important target species, and other mammals, like human whose entire genome has been recently sequenced. Towards this end, we report on the isolation, and characterisation of the porcine cardiac (MyHC slow/ß and α) and skeletal MyHC (embryonic, 2a, 2x, 2b and perinatal) gene clusters, and their structural comparisons with mouse and human clusters. The genome organisation of both clusters in the pig, human and mouse is conserved as having the same gene order, similar intergenic distances, and the same head-to-tail orientation. For a period of pre-natal muscle growth (at least from gestation day 35 to 77), the relative expression of MyHC isoforms, as determined by TaqMan real-time RT-PCR, was in the same order as the gene arrangement in the skeletal MyHC cluster (embryonic > 2a > 2x > 2b), suggesting the possible presence of major DNA elements on the same side as the MyHC embryonic gene that direct temporal regulation. This putative regulatory domain could also be a candidate region for genetic variation analysis associated with muscle characteristics.

Key Words: myosin, gene expression, gene cluster, pig

Zusammenfassung

Titel der Arbeit: **Molekulare Charakterisierung des Klusters der Gene für die schweren Ketten der Myosin Moleküle und wichtiger regulativer Domainen**

Die Expression der Gene für die schweren Ketten des Myosins (MyHC, Myosin heavy chain) ist entwicklungs-abhängig reguliert. Zur Identifizierung der cis-aktiven regulativen Elemente, die die Entwicklungs- und Fasertyp-abhängige Expression steuern, erfolgte zunächst die Isolierung der porcinen MyHC des Herz- und Skelettmuskels sowie ein Sequenzvergleich mit Mensch und Maus. Die genomische Organisation der Regionen ist hinsichtlich der Reihenfolge der Gene, ihrer Abstände und Richtung zwischen den Spezies konserviert. Während der pränatalen Entwicklung (zwischen Tag 35 und 77) entspricht die relative Expression der MyHC-Isotypen ihrer Anordnung im Genkluster (embryonal > 2a > 2x > 2b). Dies weist auf ein regulierendes Element hin, das die temporale Expression koordiniert. Diese regulative Domaine ist eine Kandidatenregion für genetische Variation mit Effekt auf Muskeleigenschaften.

Schlüsselwörter: Myosin, Genexpression, Genkluster, Schwein

Introduction

The expression of a particular myosin heavy chain (MyHC) isoform in a fibre defines its biochemical and functional phenotype, and reflects the co-ordinated pattern of gene expression in that fibre (SCHIAFFINO and REGGIANI, 1996). MyHCs are the major structural proteins of myofibrillar thick filaments that are able to convert chemical energy to mechanical energy for muscle contraction. As for other structural muscle proteins, MyHCs are encoded by a highly conserved multigene family, of which 8 isoforms are known in mammals (2a, 2x, 2b, embryonic, perinatal, slow/β, extraocular and α), each encoded by a separate gene which displays distinct temporo-spatial regulation (WEISS et al., 1999; WEISS and LEINWAND, 1996).

In pre-natal mammalian muscles, the embryonic, perinatal and slow/β MyHC isoforms represent the 3 dominant skeletal muscle fibre types in the developing foetus. Shortly

after birth, the expression of embryonic and perinatal MyHC genes is down regulated and replaced by the postnatal MyHC isoforms (2a, 2x and 2b). Thus, in adult mammalian muscles (rodents and pigs) there are 4 major fibre types characterised by the expression of the slow/β, 2a, 2x and 2b MyHC gene isoforms. Interestingly, in human skeletal muscle, there are only 3 major postnatal fibre types, with the conspicuous absence of 2b fibres. Even though MyHC 2b mRNA is found in some human cranial muscles, no MyHC 2b protein is ever detected (BOTTINELLI and REGGIANI, 2000; HORTON et al., 2001). Metabolic, biochemical and biophysical characteristics, such as oxidative and glycolytic capacities, fibre size, colour, glycogen and lipid contents, have been found to vary between MyHC fibre types (KARLSSON et al., 1999; KLONT et al., 1998; SCHIAFFINO and REGGIANI, 1996). The slow/β and fast 2b fibres, also known as slow oxidative and fast glycolytic respectively, represent two extreme metabolic profiles. The fast 2a and fast 2x fibres are intermediate fast oxidative-glycolytic fibres. Fast 2a fibres are more closely related to slow/I fibres, and fast 2x are more similar to fast 2b fibres. Hence the composition of fibre types in a muscle is a major determinant of its phenotypic properties.

All known genes encoding the skeletal and cardiac MyHC isoforms have been cloned from laboratory rodents and human (WEISS and LEINWAND, 1996). Members of the MyHC gene family are organised into 2 clusters in the genome: cardiac and skeletal. The cardiac cluster is composed of the slow/β and α MyHC gene isoforms, and is responsible for the only 2 MyHC isoforms expressed in mammalian heart (WEYDERT et al., 1985). The skeletal muscle cluster comprises 6 members of the skeletal MyHC family (embryonic, perinatal, 2a, 2x, 2b and extraocular), spanning a distance of about 300kb. The linear relationship of each isoform in the cluster had been recently described in mouse (chromosome 11) and human (chromosome 17) (SHRAGER et al., 2000; WEISS, SCHIAFFINO, and LEINWAND, 1999). As a target farm animal species, the 5'-end cDNAs and genomic DNA (λ genomic clones) of the MyHC 2a, 2x, 2b, perinatal, embryonic, and slow/β genes from the pig were recently cloned and characterised (CHANG et al., 1993; CHANG et al., 1995; CHANG and FERNANDES, 1996; CHANG and FERNANDES, 1997; DA COSTA et al., 2000). Fibre type composition is an important factor in the determination of meat quality and quantity (LOBLEY et al., 2000; WEGNER et al., 2000). Therefore, insights into the molecular mechanisms governing the temporal or isoform specific regulation of MyHCs are of fundamental and strategic importance to farm animal muscle research. Presently, mechanisms governing the temporal activation and repression of individual skeletal MyHC genes are largely unknown. One strategy that could further our understanding of temporal regulation or fibre type specific regulation relevant to farm animals is to undertake a comparative genomic study of the MyHC gene family between pig and other mammals, like human whose entire genome has been recently sequenced (VENTER et al., 2001). Towards this end, we report on characterisation of the porcine cardiac and skeletal MyHC clusters and the temporal expression of individual members during prenatal development.

Materials and methods

Isolation of porcine MyHC BAC clones

Details of the isolation and molecular characterisation of porcine MyHC BAC clones were previously described (SUN et al., 2003).

Quantitative real-time RT-PCR

TaqMan quantitative real-time RT-PCR was performed to detect the transcriptional expression of 6 MyHC gene-isoforms (MyHC embryonic, MyHC perinatal, MyHC slow/I/β, MyHC 2a, MyHC 2x and MyHC 2b), β-actin and glyceraldehyde 3-phosphate dehydrogenase (GAPDH), using primers and TaqMan probes in conditions as previously described (SUN et al., 2003). Each cDNA template was prepared from pooled *longissimus dorsi* (LD) muscles derived from 3 pig foetuses, for gestational time points 35, 49, 63, 77 and 91 days (gestation 115 days). The control post-natal muscle was taken from the *supraspinatous* muscle of a 6-week-old pig.

Immunohistochemistry

Unfixed cryostat serial sections (10 μm thickness) were generated from the same foetal muscles as those used in the generation of cDNA templates for real time-PCR. Details of immunohistochemistry were performed as previously described (DA COSTA et al., 2003).

Results and Discussion

Porcine cardiac and skeletal MyHC clusters

Isolated porcine BAC clones were subjected to subcloning, genome walking, long range PCR, pulse field electrophoresis, restriction mapping and Southern hybridisation to localise the cardiac and skeletal MyHC clusters. The porcine cardiac MyHC cluster comprises two gene isoforms, slow/β and α, separated by an intergenic distance of 6.7 kb (Figure 1A). The porcine MyHC slow/β was previously mapped to chromosome 7 (DAVOLI et al., 1998), and by inference the cardiac MyHC cluster is on the same chromosome. In human and mouse, the cardiac MyHC cluster is sited on chromosome 14 with a 4.5 kb intergenic region (SACZ et al., 1987; WEISS and LEINWAND, 1996; WEYDERT et al., 1985). More is known about the regulation of the cardiac MyHC cluster than the skeletal MyHC cluster. Enhancer-like elements have been reported in the cardiac intergenic region and in the upstream regulatory region of the MyHC slow/β gene (RINDT et al., 1995; TSIKA et al., 1990; WRIGHT et al., 1999).

In the porcine skeletal MyHC cluster, gene members are arranged in the sequential order of embryonic, 2a, 2x, 2b and perinatal (Figure 1B). The porcine MyHC 2b and 2x genes have been mapped to chromosome 12 (DAVOLI et al., 1998; ZIJLSTRA et al., 1998), which imply that the porcine skeletal MyHC cluster is located on the same chromosome. The genome organisation of the porcine, human and murine cluster is arranged in the same order and in the same head-to-tail orientation. Three predominantly postnatal MyHC isoforms (2a, 2x and 2b) are flanked by two prenatal isoforms (embryonic and perinatal) (Figure 1B). The respective MyHC intergenic regions between the three species are comparable in size. The intergenic distances of MyHC β/α, MyHC 2a/2x, and MyHC 2x/2b in all three species are under 10 kb. However, the intergenic regions of MyHC embryonic/2a and MyHC 2b/perinatal are substantially larger. In the pig their sizes are estimated at 59.8 kb and 43.3 kb, respectively. In the human, their respective sizes are inferred as 80.6 kb and 21.6 kb (http://www.ensembl.org/Homo_sapiens/). The MyHC extraocular gene isoform was not identified in the porcine skeletal cluster. It a minor isoform whose expression is mainly confined to the cranial region (BRIGGS and SCHACHAT, 2000).

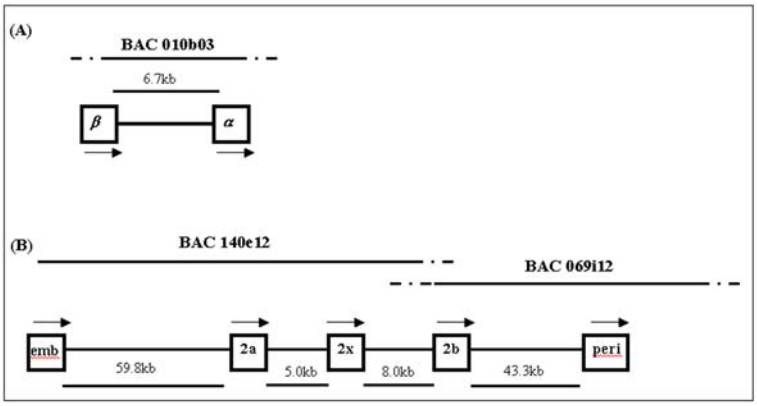

Fig.1: The porcine cardiac MyHC cluster comprises two gene isoforms, slow/β and α, separated by an intergenic distance of 6.7 kb (A). In the porcine skeletal MyHC cluster, gene members are arranged in the sequential order of embryonic, 2a, 2x, 2b and perinatal (B).

Transcriptional changes of MyHCs during pre-natal development

TaqMan real time PCR was performed to profile the quantitative and/or qualitative changes of all 6 major skeletal MyHCs (embryonic, perinatal, slow/I, 2a, 2x and 2b) at 7 pre-natal developmental time points (14, 21, 35, 49, 63, 77 and 91 days of gestation) (Figures 2 and 3). MyHC embryonic isoform was the first to be detected at day 14 at low levels (Table 1). MyHC embryonic and slow were the dominant isoforms for most part of gestation. Postnatal MyHC isoforms 2a, 2x and 2b were readily detectable by 35 d day gestation. In the early phase of prenatal growth, at least from days 35 to 77, the relative abundance of the three fast postnatal isoforms were in the order of 2a > 2x > 2b (Figure 3).

Table 1
Qualitative differences in sarcomeric gene expression between 14 and 21 days of gestation. PCR cycle (C_T) at which target was detected in a 40 cycle real time PCR reaction (average of 3 separate PCRs). By day 21, all major pre-natal and post-natal MyHC isoforms (except 2x) were detected.

	β-actin	**α-actin**	**GAPDH**	**MyHC embryonic**	**MyHC perinatal**	**MyHC slow/I**	**MyHC 2a**	**MyHC 2x**	**MyHC 2b**
14-day old foetus	16.7	-	20	37.1	-	-	-	-	-
21-day old foetus	17.3	23	19.4	24.2	34.6	21.4	35.8	-	35.3

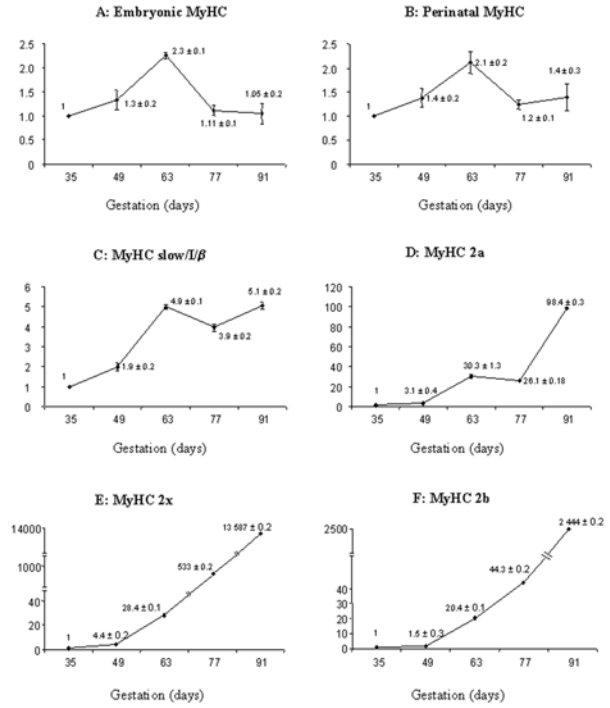

Fig. 2:
Relative quantitative mRNA changes of each major pre- and post-natal MyHC isoform from 35 days to 91 days of gestation. Expression level was expressed as fold difference relative to day 35. Expression of MyHC embryonic (A) and perinatal (B) was steady throughout the period with a small peak at day 63. MyHC slow (C) showed modest increase in expression with time. The increases of post-natal MyHC 2a (D), 2x (E), and 2b (F) were more substantial, with MyHC 2x showing the most dramatic increase.

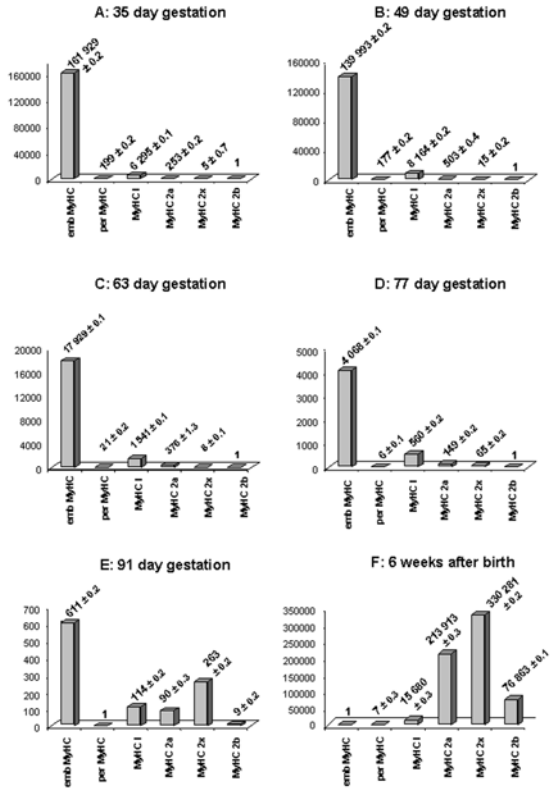

Fig. 3:
Relative mRNA levels of all major pre- and post-natal MyHC isoforms at gestational time points of 35 days (A), 49 days (B), 63 days (C), 77 days (D), 91 days (E), and 6 weeks after birth (F). MyHC embryonic isoform was the most abundant isoform for most of gestation. At late gestation, MyHC 2x became the second most abundant isoform. Control post-natal *supraspinatous* muscle (F) demonstrates the down-regulation of embryonic and perinatal MyHC mRNAs to insignificant levels. The relative levels of fast post-natal isoforms were consistent with a red muscle. Expression levels are relative to the lowest expressing isoform within each developmental time point.

Detection of MyHC proteins in pre-natal muscles

To determine the extent of coincident MyHC mRNA and protein expression in pre-natal muscles, immunohistochemistry was performed on the same batches of foetal samples as those used for real time PCR. Six different monoclonal antibodies (fast MyHC-specific antibody M-32; slow MyHC-specific antibody NOQ7.5.4D; perinatal and/or embryonic MyHC-specific antibody 4C10; embryonic MyHC-specific antibody F1.652; fast 2a and 2x MyHC-specific antibody SC-71; and fast 2b MyHC-specific antibody BF-F3) were used to detect the presence of pre- and post-natal MyHC isoforms during pre-natal development (Figure 4).

No MyHC protein was detected at day 14 of gestation at the start of somite formation. Postnatal MyHC isoforms showed increasing protein levels with gestation. Co-expression of more than one MyHC protein in a myotube was a common feature.

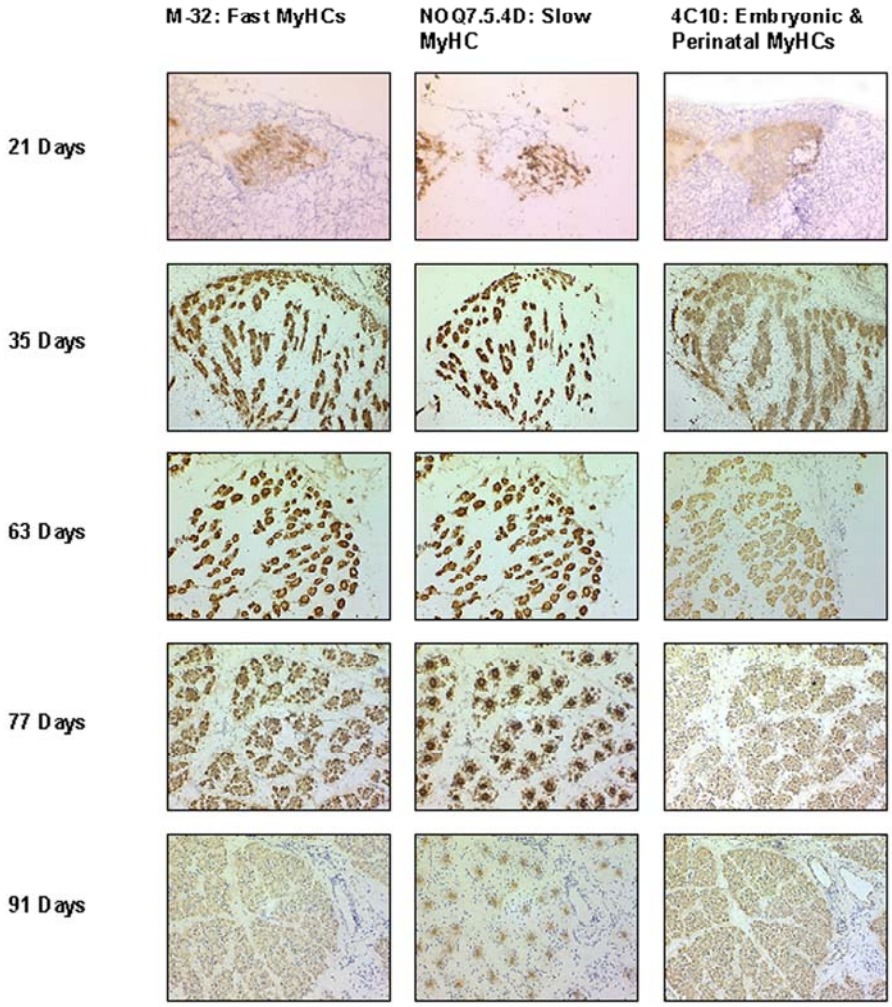

Fig. 4: Profiling the protein expression of pre- and post-natal MyHC isoforms at days 21, 35, 63, 77 and 91 of gestation by immunohistochemistry on serial sections with 6 different monoclonal antibodies specific for (A) fast pre- and post-natal MyHCs; slow MyHC only; embryonic and perinatal MyHCs; (B) embryonic MyHC only; 2a and 2x MyHCs; and 2b MyHC only. There was a high degree of co-expression of pre- and post-natal MyHC isoforms during pre-natal muscle growth. Note that at day 21, post-natal antibodies SC-71 and BF-F3 showed no cross reactivity with MyHC embryonic or slow. Compared with other isoforms, MyHC 2b showed a more restricted pattern of expression. Fibre segregation was most pronounced for the MyHC slow isoform where it was concentrated in the central primary fibres from day 77 of gestation. From day 63 onwards, smaller secondary myotubes become increasingly evident. Results shown are representative of at least 3 repeated experiments.

Conclusions

1. Post-natal MyHC isoforms (2a, 2x and 2b) were expressed in the pig at a much earlier stage of gestation (by 35 days gestation) than previously found in small mammals, and that there was a high degree of co-expression of pre- and post-natal MyHC isoforms, at both mRNA and protein levels, for much of pre-natal muscle growth.

2. During a window of pre-natal muscle growth, when expression of embryonic MyHC was at its highest (at least from days 35 to 77), the relative expression of the post-natal MyHC isoforms was in the order of 2a > 2x > 2b. Hence the order of relative

expression seemed to correlate with the same gene order as found in the skeletal MyHC cluster (embryonic > 2a > 2x > 2b).

3. It is tempting to speculate on the presence of *cis*-acting elements on the same side of the cluster as the embryonic isoform that direct temporal regulation of the skeletal cluster during pre-natal development. Indeed, with the recent publication of the entire human genome sequence (VENTER et al., 2001), we found that of all MyHC isoforms in the skeletal cluster, the region surrounding the MyHC embryonic gene shows the highest GC content and the most number of predicted CpG islands (SUN et al., 2003). CpG islands are important signatures of many activated mammalian genes and are usually considered to be unmethylated (BALLESTER and WOLFFE, 2001; GARDINER-GARDEN and FROMMER, 1987). We speculate that such elements could function to direct temporal regulation of the skeletal MyHC cluster, which, in turn, is an important candidate region for genetic variation that could affect fibre type composition.

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The influence of increased maternal nutrition on offspring muscle development, growth and meat quality, and endocrine concentrations of IGFs and IGFBPs in both sow and foetuses

(Einfluss von erhöhter, mütterlicher Ernährung auf Muskelentwicklung, Wachstum und Fleischqualität der Nachkommen, und die endokrinen Konzentrationen von IGF und IGFBP sowohl bei der Sau als auch bei den Embryos)

The objective of this study was to examine the effects of increased maternal feed intake during early to mid gestation on foetal muscle development and offspring postnatal growth performance and meat quality (pH_{24}, colour, drip loss and pigmentation), and on maternal and foetal endocrine concentrations of the IGFs and IGFBPs. Pregnant sows were either fed restrictively (2 kg/d) throughout gestation (C) or fed ad libitum from d 25-50 (A_{25-50}) or d 25-70 (A_{25-70}) and as C in the remaining periods. Offspring were slaughtered litter wise at around 100 kg live body weight, and the lightest (LW), middle (MW) and heaviest (HW) weight pig by slaughter weight of each sex within litter was analysed. Muscle fibre number, fibre area and concentration and content of DNA and RNA in the semitendinosus muscle (ST) of the offspring were not significantly affected by increased maternal feed intake. The weight of ST and muscle deposition rate was significantly lower in A_{25-50} pigs compared to C, and also daily gain was numerically lower. Interactions between maternal nutrition and pig weight was found, where the LW A_{25-50} pigs had a lower muscle mass and muscle deposition rate then the LW C pigs. It was also found that the intra-litter variation in postnatal growth performance could be explained by variation in both muscle fibre number and muscle fibre growth rate. We found no effect of maternal nutrition on meat quality traits of the offspring. Another twenty sows were fed in groups of 5 the same diets as in the above-described trial, but the sows were slaughtered at d 50 or d 70 to obtain endocrine profiles of maternal and foetal IGFs and IGFBPs. Maternal IGF-I was significantly higher and IGFBP-3 and –4 numerically higher in ad libitum than restrictively fed sows. There was no effect of maternal feed intake on concentrations of IGFs and IGFBPs in foetal serum.

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The influence of dietary energy level in double-muscled Belgian Blue cows during the indoor period on calf birth weight and development

Abstract

One hundred twenty-six Belgian Blue double-muscled cows were involved in an experiment to investigate the effect of energy level (100, 90, 80 or 70% of their requirements) during the indoor period (140 d) on calf birth weight and development. All cows were turned out on pasture in similar circumstances during the summer period (re-alimentation period). Half of the cows were suckled by their calves, while the other cows were dried after calving and colostrum collection. The calves of these cows were managed as rearing calves. Beside the milk diet, the same concentrate and hay were available for reared as well as for suckling calves. Calf birth weight averaged 52 kg, and was not diminished by the reduced energy intake of the dams during the indoor period. There was no effect on daily live-weight gain from birth to 16 weeks of age (0.83 kg/d), neither in suckling nor in reared calves. However, suckling calves gained faster (0.95 kg/d) than reared calves (0.71 kg/d, $P < 0.05$). Within suckled cows, milk production was similar for the different energy levels. Individual daily live-weight gain of suckling calves was closely related to milk intake ($r = 0.801$; $P < 0.001$). A tendency for an increased mortality rate was observed for the offspring of dams fed the lowest energy level. Therefore, an energy restriction of 30% of the requirements of Belgian Blue double-muscled cows during a period of 140 days should be dissuaded.

Key Words: Beef cows, energy restriction, calf, birth weight, live weight gain

Zusammenfassung

Titel der Arbeit: **Einfluss des Nahrungs-Energieniveaus von Weißblauen Belgier Kühen während der Stallperiode auf Geburtsmasse und Entwicklung ihrer Kälber**

Der Einfluss des Energieniveaus (100, 90, 80 oder 70% des Energiebedarfs) wurde an 126 Weißblauen Belgier Kühen während der Stallperiode (140 Tage) hinsichtlich der Auswirkung auf die Geburtsmasse und Entwicklung ihrer Kälber untersucht. Alle Kühe, wurden im Sommer, auf der Weide, unter vergleichbaren Bedingungen, gehalten (Kompensationsperiode). Die Hälfte der Kühe säugte ihre Kälber, während die andere Hälfte nach dem Abkalben von ihren Kälbern getrennt wurde. Das Kolostrum wurde gesammelt. Die Kälber dieser Kühe wurden getränkt. Neben der Milchration, erhielten alle Kälber Kraftfutter und Heu. Die Geburtsmasse betrug durchschnittlich 52 kg und war durch das Energieniveau während der Stallperiode nicht reduziert. Es gab keinen Einfluss auf die Lebendmassezunahme von Geburt bis zum Alter von 16 Wochen (0,83 kg/Tag), weder bei gesäugten noch bei getränkten Kälbern. Jedoch wuchsen gesäugte Kälber schneller (0,95 kg/Tag) als getränkte Kälber (0,71 kg/Tag, $P < 0,05$). Die Milchproduktion war durch das Energieniveau bei den säugenden Kühen nicht beeinflusst. Die individuelle Lebendmassezunahme der gesäugten Kälber war eng verbunden mit der Milchaufnahme ($r = 0,801$; $P < 0,001$). Es existierte eine Tendenz für eine höhere Mortalität bei Nachkommen von Kühen, die nur 70% des Energiebedarfs erhielten. Deshalb wird von einer Energiebeschränkung von 30%, während einer Periode von 140 Tagen, bei Weißblauen Belgier Kühen abgeraten.

Schlüsselwörter: Fleischrinder, Energiebeschränkung, Kalb, Geburtsmasse, Lebendmassezunahme

Introduction

Energy intake in beef cows may be restricted during the indoor period by limiting the amount of feed consumption, but mostly by feeding a low quality diet (SINCLAIR and AGABRIEL, 1998). In these circumstances, cows may mobilize body reserve tissue, which is restored during the subsequent period of herbage growth (PETIT et al., 1992). Several authors showed that this feeding strategy can be successfully applied (SHELL et al., 1995; FREETLY et al., 2000).

Double-muscled animals are characterised by a high lean meat content and a low fat content in their carcasses (FIEMS et al., 1995) and a reduced intake capacity (FIEMS et al., 1999). These properties may aggravate feed restriction, provoking a detrimental effect on animal performance. VERMOREL et al. (1976) reported a negative effect on gain and feed efficiency in double-muscled Charolais bulls, compared to non-double-muscled Charolais and Friesian bulls, when intake was restricted at 75% of ad libitum level. HORNICK et al. (1999) found that double-muscled animals failed to compensate when growing has been interrupted. Furthermore, nitrogen digestibility and efficiency were reduced. Double-muscled animals are known to be more stress susceptible (HOLMES et al., 1973). The repercussions of nutritional stress in this type of animals on the performance of their offspring is not known, as far as we are aware.
This report deals with the effect of a restricted energy intake in double-muscled cows during the indoor period on performance of the offspring.

Materials and methods

One hundred and twenty six double-muscled Belgian Blue cows were involved in an experiment from end 1998 to spring 2004. They were divided in comparable groups at the start of the experiment, based on age, parity, live weight and number of days in gestation, to investigate the influence of energy level (E) on calf birth weight and development. E was restricted at 100, 90, 80 or 70% of their requirements during the indoor period (140 days, restriction period). Cows were confined in tie stalls. The basic diet consisted of maize silage and straw (80/20 on a dry matter basis) and 0.5 kg of a mineral-vitamin-premix. It was supplemented with urea and soybean meal to fulfil protein requirements. All cows were turned out on pasture in similar circumstances during the summer period (re-alimentation period). No oestrus synchronisation was applied, so that parturitions were spread over the year. Calves born during the restriction period as well as those born during the re-alimentation period were involved in this study.
Half of the cows were suckled by their calves, while the others were dried off after calving and colostrum collection. Individual milk intake of suckling calves was measured every four weeks by the weighing-suckling-weighing technique. Calves were abruptly weaned at 16 weeks of age. The calves from the non-lactating cows were managed as rearing calves up to 16 weeks of age, with a limited access to a milk diet (10% of their birth weight). Beside the milk diet, the same concentrate and hay were available for both groups of calves. The amount of colostrum (first milking after parturition) was measured by hand-milking of a limited number of cows (n = 46) and the immunoglobulin content was estimated, using a colostrometer.
The effect of E on calf performance was tested using analysis of variance. Data were presented as least square means. Regression analysis was applied to study the relationship between dam milk production and calf live-weight gain. A χ^2 test was used to verify the influence of dam dietary E on calf mortality.

Results

Mean calf birth weight averaged 52±7 kg and was not affected by E (Table 1). Calf birth weight amounted to 50 and 55 kg for female and male calves, respectively, and was significantly affected by sex. No significant effect of parity was found, while there were no significant interactions.

Table 1
Effect of dam energy level (E), dam parity (P) and calf sex (S) on birth weight

	Dam energy level (%)					Significance
	100	90	80	70	Ø	
Male calves	55	56	56	54	55	
1st calving	57	54	53	53	54	E: 0.260
2nd calving	54	58	56	52	55	P: 0.283
3 and more calvings	55	57	58	58	56	S: < 0.001
Female calves	48	52	50	50	50	E x P: 0.155
1st calving	49	51	48	50	50	E x S: 0.485
2nd calving	48	53	50	48	50	P x S: 0.765
3 and more calvings	46	52	51	53	50	E x P x S: 0.994
All calves	51	54	53	52		

Calf management clearly affected growth rate (Table 2).

Table 2
Effect of calf management (M), dam energy level (E), dam parity (P) and calf sex (S) on daily live-weight gain of calves (birth weight as covariate)

	Dam energy level (%)					Significance
	100	90	80	70	Ø	
Suckling calves	0.96	0.90	0.96	0.94	0.94	M: < 0.001
Male calves	0.94	0.91	0.99	0.95	0.95	E: 0.889
1st calving	0.84	0.89	1.04	-	0.92	P: 0.133
2nd calving	0.93	0.89	1.02	0.89	0.93	S: 0.721
3 and more calvings	1.02	0.95	0.91	1.01	0.98	M x E: 0.401
Female calves	0.98	0.90	0.93	0.93	0.93	M x P: 0.162
1st calving	0.96	0.73	0.80	0.91	0.85	M x S: 0.172
2nd calving	1.00	0.89	1.03	0.92	0.96	E x P: 0.414
3 and more calvings	0.97	1.08	0.95	0.96	0.99	E x S: 0.595
						P x S: 0.317
Rearing calves	0.69	0.72	0.71	0.72	0.71	M x E x P: 0.245
Male calves	0.67	0.71	0.71	0.68	0.69	M x E x S: 0.640
1st calving	0.71	0.73	0.69	0.66	0.70	M x P x S: 0.572
2nd calving	0.66	0.70	0.72	0.70	0.69	E x P x S: 0.371
3 and more calvings	0.67	0.70	0.72	0.67	0.69	M x E x P x S: 0.491
Female calves	0.71	0.73	0.71	0.76	0.73	
1st calving	0.75	0.72	0.68	0.71	0.72	
2nd calving	0.63	0.71	0.73	0.80	0.73	
3 and more calvings	0.71	0.77	0.71	0.76	0.74	
All calves	0.82	0.81	0.83	0.82		

Suckling calves gained faster than rearing calves (0.94 vs. 0.71 kg/d; $P < 0.001$). Live-weight gain was not affected by energy level and sex. There was a tendency for a lower growth rate in calves from primiparous than from multiparous cows. No significant interactions occurred between the different factors. The somewhat lower daily gain of suckling calves born out of primiparous cows can be partly explained by a lower milk production. Significant differences were found between primiparous cows, cows with two parturitions and cows with three or more parturitions (6.2, 7.3 and 7.5 kg/d, respectively; $P = 0.004$; birth weight as covariate).
A tendency was also found for a lower milk production for male than for female calves (6.7 vs. 7.3 kg/d; birth weight as covariate; $P = 0.071$), although growth rate was not different between male and female calves. Daily live-weight gain in suckling calves was closely related to average daily milk production ($r = 0.803$). The Figure shows the effect in female and male calves, respectively.

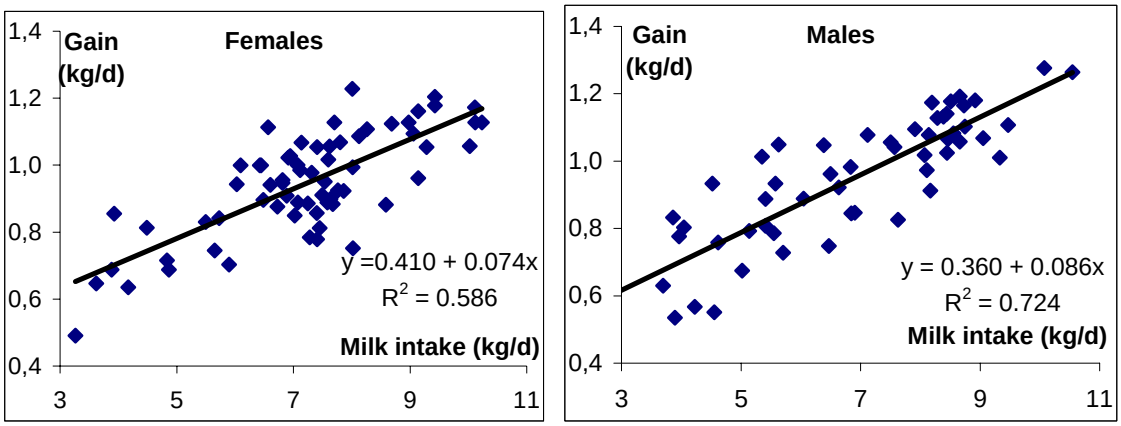

Figure: Relationship between mean daily milk intake and daily gain from birth to 16 weeks

Eleven out of the 213 born calves died in the course of the first 16 weeks of life: 1 calf was stillborn, 5 calves died within 24 h after birth and 5 died later on. Six calves belonged to E = 70%; 2 to each of E = 90% and 80% and 1 to E = 100%. Eight females and 3 male calves died. One suckling male calf from E = 90% was crushed by the dam on the first day of life. A χ^2 test showed a tendency for higher losses for E = 70% (P = 0.075) when the crushed calf was omitted. This higher mortality rate could not be explained by a lower colostrum production or immunoglobulin content (Table 3).

Table 3
Effect of energy level on yield and immunoglobulin content of colostrum

	Number of measurements	Dam energy level (%)				S.D.
		100	90	80	70	
Yield (kg)	46	2.6[a]	3.1[a]	3.2[ab]	5.0[b]	2.3
Immunoglobulin (mg/ml)	110	98[a]	96[a]	99[a]	104[a]	33

[ab] values with the same superscripts are not different (P > 0.05)

Excluding the crushed calf, birth weight of the calves who died (51.1 kg) did not differ from that of the surviving calves (52.3 kg; P = 0.537; sex as covariate). There was a tendency for a higher mortality rate in the offspring of older cows (P = 0.056). Dams of calves dying before the age of 16 weeks had a lower body condition score at calving (1.45; see AGABRIEL et al. (1986) for a description of the body condition score) than dams whose calves survived (1.75; P = 0.044; E and parity as covariates).

Discussion

Birth weight was relatively high in comparison with most literature data (CORAH et al., 1975; HOUGHTON et al., 1990; SPITZER et al., 1995; FREETLY et al., 2000). It was not affected by energy level, unlike results of CORAH et al. (1975) and HOUGHTON et al. (1990), where birth weight was reduced by a pre-partum energy restriction of 50 or 30%, respectively. SPITZER et al. (1995) and FREETLY et al. (2000) reported a lower birth weight when cows lost body condition prior to parturition. These findings were not confirmed by our results. The discrepancy between our results and the literature data may be due to the fact that the restriction period did not always coincide with the gestation period in our experiment. However, even if we only consider calves born during the last two months of the restriction

period (data not shown), we did not find an effect of energy level. SHELL et al. (1995) also found that a restricted cow diet fed at 70% of energy requirements did not affect calf birth weight.

No effect of a decreasing energy level on live-weight gain or 16-week live weight was observed, which is in accordance with a similar milk production for the different energy levels. The similar milk production among groups means that energy level itself has no effect on growth rate. CORAH et al. (1975) found a lower weaning weight when dams were restricted at 50 % of the recommended energy level during the last 100 days prior to scheduled parturition, which may be attributed to a lower milk production. Suckling calves gained faster than reared calves, as reported by BOUCQUÉ et al. (1978). It may be worthwhile to look at a possible effect on growth rate and meat quality when the offspring becomes older. Calves exhibiting a lower growth rate during the first months of life did not realize a compensatory gain afterwards (BOUCQUÉ et al., 1978, HENNESSY and MORRIS, 2003). A transitory effect on muscle growth path was reported, without a carryover effect of early nutrition on meat quality (ALLINGHAM et al., 2001; HENNESSY and MORRIS, 2003) .

As milk is considered to be most important in early life, a good relationship between milk production and calf performance can be expected. The positive correlation was in agreement with the findings reported by NEVILLE (1962; r = 0.81). However, most authors reported correlation coefficients that were lower than in our experiment (ROBISON et al., 1978: r = 0.63; DALEY et al., 1987: r = 0.36 – 0.45; LEWIS et al., 1990: r = 0 to 0.51).

SHELL et al. (1995) did not find an effect of pre-partum energy level (70 vs. 110% of the requirements) on colostrum yield, but the low level doubled the immunoglobulin G content. CORAH et al. (1975) reported 3 to 10% more stillborn calves when energy was restricted at 50% of the recommended level during the last 100 days prior to predicted calving. This coincided with a lower fat cover of the cows at calving. Our results demonstrated that a low dam body condition score at parturition is an indication of nutritional stress with a detrimental impact on calf survival. It is not clear why more calves died for E = 70%, since colostrum yield and immunoglobulin content were not negatively affected by this energy level. Similar findings have been reported in the literature (HALLIDAY et al., 1978; BLECHA et al., 1981; BURTON et al., 1984; HOUGH et al., 1990). The higher mortality rate may be due to a worse absorption of immunoglobulins, causing a lower calf plasma immunoglobulin content. STOTT (1980) suggested that pre-partum cow stress can affect immunoglobulin absorption by the calf.

Conclusion

Energy restriction up to 70% of requirements of the cow during the indoor period did not effect birth weight and live-weight gain of the calves during the first 16 weeks of live. However, this energy level is not acceptable because of a higher calf mortality. A dam body condition score at parturition approaching 2 may be desirable for a minimal calf loss.

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Effect of sow feed intake during gestation on the growth performance of progeny to slaughter

Abstract

It was hypothesised that a strategic increase in sow's gestation feed levels may increase the growth potential of pigs. Following insemination using semen from meat-line sires, 46 parity two to four L x LW sows were fed at 30 MJ DE/d. Sows were blocked on parity and weight and assigned to one of two nutritional treatments: 30 MJ DE/d or 60 MJ DE/d between day 25 and 50 of gestation. From day 50 to 110 of gestation sows were fed at 30 MJ DE/d. A gestation diet (6.2 g lysine/kg; 12.8 MJ DE/kg) was used. From day 110 of gestation to weaning a lactation diet (8.6 g lysine/kg; 14 MJ DE/kg) was fed to appetite. Progeny were weaned at 27 ± 0.1 days of age (mean ± SE) and one mixed sex pair of pigs of average weight was penned from each litter. In total, 46 mixed sex pairs of pigs were offered *ad-libitum* an identical sequence of diets to day 47 post-weaning. From day 47 to slaughter at 131 days post-weaning, pigs were formed into single sex groups of 12 pigs. They were offered the same finisher diet *ad-libitum* using a transponder feeding system and individual feed intakes were recorded. The number born alive (10.9 ± 0.75 pigs) and the number born dead (0.9 ± 0.32 pigs) were similar for both treatments. Pig performance from birth to slaughter was not significantly affected by treatment. Days taken from birth to slaughter were 158.5 and 158.1 ± 1.16 (P>0.05) and daily gain from birth to slaughter was 602 and 600 ± 7.9 g (P>0.05) for progeny of sows fed 30 MJ DE/d and 60 MJ DE/d, respectively. In conclusion, these data do not support the original hypothesis.

Key Words: Pregnancy, Nutrition, Intake, Daily gain, Lean, Feed conversion efficiency

Zusammenfassung

Titel der Arbeit: **Effekt der Futteraufnahme tragender Sauen auf die Wachstumsleistung der Nachkommen bis zur Schlachtung**

Es wurde die Hypothese aufgestellt, dass sich das Wachstumspotential von Schweinen durch strategisches Ansteigen des Fütterungsniveaus der Muttersau während der Trächtigkeit erhöht. Nach Besamung mit einer fleischbetonten Rasse wurden 46 Sauen (Landrasse x Large White) in der zweiten bis vierten Trächtigkeit mit 30 MegaJoule Verdauliche Energie/Tag (MJ VE/Tag) gefüttert. Die Sauen wurden nach den Kriterien Anzahl der Trächtigkeiten und Gewicht unterteilt und gleichsam einem von zwei Ernährungsplänen zugewiesen: 30 MJ VE/Tag oder 60 MJ VE/Tag zwischen dem 25. und 50. Tag der Trächtigkeit. Alle Sauen wurden vom 50. bis zum 110. Trächtigkeitstag mit 30 MJ VE/Tag gefüttert. Eine Trächtigkeitsdiät wurde verfüttert (6,2 g Lysin/kg, 12,8 MJ VE/kg). Vom 110. Trächtigkeitstag bis zur Entwöhnung der Ferkel wurde eine Laktationsdiät (8,6 g Lysin/kg, 14 MJ VE/kg) nach Appetit gefüttert. Die Nachkommen wurden am 27. ± 0,1 Lebenstag entwöhnt (Durchschnittsergebnis ± Standardabweichung) und es wurden aus jedem Wurf jeweils zwei Schweine unterschiedlichen Geschlechts und durchschnittlichen Gewichts zusammen aufgestallt. Insgesamt wurden 46 getrenntgeschlechtlichen Schweinepaaren identische Futtersequenzen *ad-libitum* bis zum 47. Tag nach der Entwöhnung angeboten. Vom 47. Tag bis zur Schlachtung am 131. Tag nach der Entwöhnung wurden die Nachkommen in gleichgeschlechtlichen Gruppen von 12 Schweinen aufgestallt. Allen wurde dasselbe Endmastfutter angeboten und die Fütterung erfolgte *ad-libitum* mit einem Transponderfütterungssystem, durch das die individuelle Futteraufnahme aufgezeichnet wurde. Die Anzahl der Lebendgeborenen (10,9 ± 0,75 Schweine) und die Anzahl der Totgeborenen (0,9 ± 0,32 Schweine) war für beide Ernährungspläne gleich. Die Mastleistung der Schweine vom Zeitpunkt der Geburt bis zur Schlachtung wurde durch den Ernährungsplan nur unbedeutend beeinflusst. Für Nachkommen der Muttersauen, die mit 30 MJ VE/Tag bzw. für jene, deren Mütter mit 60 MJ VE/Tag ernährt wurden, ergaben sich folgende Resultate: Tage zwischen Geburt und Schlachtung waren 158,5 bzw. 158,1 Tage ± 1,16 (P>0,05), Tageszunahmen von Geburt bis Schlachtung waren 602 bzw. 600 ± 7,9 Gramm (P>0,05). Resultierend hieraus ergibt sich, dass die ursprünglich erstellte Hypothese nicht bestätigt wird.

Schlüsselwörter: Trächtigkeit, Ernährung, Aufnahme, Tageszunahme, mager, Futterumwandlungseffizienz

Introduction

Muscle fibre number determines the lean content of the growing pig (STICKLAND and GOLDSPINK, 1975; STICKLAND, 1996). Faster growing strains of pigs have more muscle fibres than slower growers. Meat quality also tends to be better in pigs

with more muscle fibres as fibres are thinner thus allowing better nutrient diffusion (STICKLAND, 1996).
Muscle fibres in pigs occur in two forms, primary and secondary. Primary fibres are formed early in pregnancy and their number is thought to be largely genetically determined. Secondary fibres are formed between days 54 and 90 of pregnancy. They group around the primary fibres and are vulnerable to environmental factors including nutrition (STICKLAND, 1996). The effect of nutrition may be mediated through an effect of insulin like growth factor on the placenta (MUSSER et al., 1997a). Muscle fibre type and size, but not number, can be affected by nutrition and exercise in the postnatal period (STICKLAND, 1996). As fibre number is determined prenatally the meat producing potential of a pig is also determined prior to birth.
Sows during gestation are normally only fed one third to half of their voluntary feed intake. However, doubling the feed allowance between day 25 and 80 of pregnancy has been shown to increase the number of muscle fibres in an indicator muscle *(M. semitendinosus)* of progeny. Subsequently, in the period from 70 days of age to slaughter at 80 kg growth rate was found to increase (10%) and FCE to improve (8%) in progeny (DWYER et al., 1994). It was suggested that similar results might be achieved by increasing feed intake for a shorter period between day 25 and 50 of gestation. Other studies have found this to be the case (MUSSER et al., 1997b; PENNY and VARLEY, 1999)
Growth rate from birth to 25 kg is positively correlated with birthweight (CAMPBELL and DUNKIN, 1982; DWYER et al., 1993) but not with the total number of muscle fibres (DWYER et al., 1993). This is most likely related to feed intake differences between light and heavy pigs (STICKLAND, 1996). However, growth rate from 25 kg to slaughter is positively correlated with muscle fibre number but not birth weight (DWYER et al., 1993). Though the smaller pigs in a litter, at birth, tend to have fewer muscle fibres, those small pigs with relatively high fibre numbers are capable of exhibiting catch-up growth (HANDEL and STICKLAND, 1988).
The objective of this experiment was to test the hypothesis that increasing maternal feed intake between day 25 and 50 of gestation would improve the growth performance of the progeny.

Materials and Methods

Sows

Forty-six parity 2 to 4 sows (Landrace x Large White) of PIC (Pig Improvement Company, Oxford, UK) origin were selected at weaning. Sows were fed *ad-libitum* from weaning to oestrus when they were inseminated twice at 24 hour intervals using Hylean Landrace semen (Hermitage AI, Co. Kilkenny, Ireland).
The ingredient composition and the nutrient content of the diets are shown in Table 1. Sows were liquid fed (3.5:1 water to feed DM) a dry sow diet (diet 1), 4 sows per valve, by a computerised feeding system (Big Dutchman, Vechta, Germany). From service to day 25 of gestation sows were fed at 30 MJ DE per sow per day. At which time sows were weighed and backfat depth measured ultrasonically 6 cm from the midline at the level of the last rib (Scanoprobe, Scanco Inc., Ithaca, New York.). Sows were then blocked on the basis of parity and weight and assigned at random to one of the following treatments; (1) Standard feed level at mid-pregnancy (30 MJ DE per sow per day) and (2) Increased feed level at mid-pregnancy (60 MJ DE per sow

per day). After day 50 of gestation all sows were fed at 30 MJ DE per sow per day up to farrowing.
On entry to the farrowing house sows were liquid fed (3.4:1 water to feed DM) a lactation diet (diet 2). A lactation feed curve increasing from 25 MJ DE/kg at farrowing to 95 MJ DE/kg at farrowing was used. After each farrowing, number born alive and number born dead was recorded. Each pig was individually weighed, crown to rump length measured and tagged for identification purposes at birth. Litter size was standardised across treatments by cross fostering. Creep feed (diet 3) was fed from day 12 to weaning to all litters and intakes recorded.

Progeny
Pigs were weaned at 26-28 day at which time they were individually weighed. One mixed sex pair of average weight pigs was taken from each litter. Intake and growth rate of these pigs was recorded to slaughter. In total 92 pigs (46 male and 46 female) were selected and each was fed 2 kg starter diet (diet 3), 5 kg link diet (diet 4) followed by weaner diet (diet 5) as dry pellets to day 48 post-weaning. A dry pelleted finisher diet (diet 6) was fed during the period from day 48 post-weaning to slaughter at day 130 post-weaning. Pigs were fed 3 times daily in the first week and *ad-libitum* thereafter, with care being taken to avoid feed wastage and spoilage. Intakes were recorded weekly.

Housing
Dry sows were accommodated in individual basket stalls (2.4 x 0.6m; O'Donovan Engineering, Coachford, Ireland) up to day 110 of gestation. Temperature was maintained at 20 ^{o}C.
From day 110 of gestation until weaning, sows were accommodated in farrowing rooms with 8 pens room. NPD type farrowing crates (O'Donovan Engineering) were used. Water was available *ad-libitum* from one nipple drinker per pen and supplemental water was provided by lever valve where necessary. Temperature was maintained at 20 ^{o}C except around farrowing when temperature was increased to 24 ^{o}C for 48 hours.
Pigs were penned as mixed sex pairs from weaning to day 48 post-weaning in fully slatted (Faroex, Manitoba, Canada) pens 1.2 m x 0.9 m. Water was available *ad-libitum* from one drinking bowl (BALP, La Buvette, Charleville Nord, France) per pen. Temperature was maintained at 28 ^{o}C in the first week and reduced by 2 ^{o}C per week to 22 ^{o}C in the fourth week after weaning.
From day 48 post-weaning, pigs were penned in eight fully slatted pens (3.7 m x 2.9 m). Feeding was by a Hunday FIRE (Osborne (Europe), Newcastle, UK) transponder feeding system as groups of 10-12. Water was available *ad-libitum* from one drinking bowl (BALP) per pen. Temperature was maintained at 20 ^{o}C.

Slaughter
Pigs were slaughtered at approximately 95 kg liveweight. They were transported 14 km to the abattoir and killed by bleeding after CO_2 stunning. Backfat thickness was measured at 6 cm from the edge of the split back at the level of the 3rd–4th last rib using a Hennessy grading Probe (Hennessy and Chong, Auckland, New Zealand). Lean content was estimated according to the following formula (Department of Agriculture and Food, 1994):

Estimated lean meat content (g/kg) = 534.1 – 7.86x + 2.66y
where x = fat depth (mm); y = muscle depth (mm)

Carcass weight was estimated by multiplying the weight of the hot eviscerated carcass, (minus tongue, bristles, genital organs, kidneys, flare fat and diaphragm) 45 minutes after slaughter by 0.98. Kill-out proportion (g/kg) was calculated as carcass weight/slaughter weight.

Feed Analysis.

The methods of feed analysis were previously described by LAWLOR et al. (2002). The ingredient composition and chemical composition of diets is shown in Table 1.

Table 1
Ingredient composition and nutrient content of experimental diets (g/kg)

Diet no.	**1**	**2**	**3**	**4**	**5**	**6**
Diet type	**Dry Sow**	**Lactating Sow**	**Starter**	**Link**	**Weaner**	**Finisher**
Steam flaked wheat			229.5	481		
Wheat		459			542.2	303.9
Steam flaked maize			200	81.5		
Barley	859	300			200	450
Dried whey			120	75		
Dried skim milk powder			110			
Herring meal			95	100	75	
Soya 50	100	170			150	215
Full fat soya			175	220		
Soya oil			50	20		
Tallow	10	40			10	10
Mineral and vitamins[1]	1.5	1.5	4.0	4.0	3	1
Lysine HCl[2]		1	4.0	3.0	3	2.5
DL-Methionine			1.5	1.5	1	0.75
L-Threonine[2]			2.0	2.0	1.5	0.75
Di-calcium phosphate	12	10	5.0	5.0		
Limestone flour	13	14	2.0	5.0	11	13
Salt	4	4	1.0	1.0	3	3
Crina premix[3]			1.0	1.0		
Antibiotic feed additive[4]	+	+	+	+	+	
Phytase 5000 iu/g	0.1	0.1			0.1	0.1
Analysed chemical composition (g/kg)						
Dry matter	880	875	910	889	878	873
Crude Protein	145	170	235	223	208	189
Fat	32	58	92	84	37	31
Crude fibre	42	32	21	26	30	49
Ash	42	48	62	59	46	44
Lysine[5]	6.2	8.6	17.4	15.0	13.6	11.2
Digestible energy [5] (MJ/kg)	12.8	14	16.1	15.3	14.0	13.5

[1]Provided per kilogram of complete diet:
Diets 1 and 2: Cu, 38 mg; Fe, 70 mg; Mn, 62 mg; Zn, 80 mg; I, 0.6 mg; Se, 0.2 mg; vitamin A, 10000 IU; vitamin D_3, 1000 IU; vitamin E, 100 IU; vitamin K, 2 mg; vitamin B_{12}, 15µg; riboflavin, 5 mg; nicotinic acid, 12 mg; pantothenic acid, 10 mg; choline chloride, 500 mg; Biotin, 200µg; Folic acid, 5 mg; vitamin B_1, 2 mg and vitamin B_6, 3 mg.
Diets 3, 4 and 5: Cu, 175 mg; Fe, 140 mg; Mn, 47 mg; Zn, 120 mg; I, 0.6 mg; Se, 0.3 mg; vitamin A, 6000 IU; vitamin D_3, 1000 IU; vitamin E, 100 IU; vitamin K, 4 mg; vitamin B_{12}, 15µg; riboflavin, 2 mg; nicotinic acid, 12 mg; pantothenic acid, 10 mg; choline chloride, 250 mg; vitamin B_1, 2 mg; vitamin B_6, 3 mg; and endox, 60 mg.
Diet 6: Cu, 100 mg; Fe, 40 mg; Mn, 31 mg; Zn, 80 mg; I, 0.3 mg; Se, 0.2 mg; vitamin A, 2000 IU; vitamin D_3, 500 IU; vitamin E, 40 IU; vitamin K, 4 mg; vitamin B_{12}, 15µg; riboflavin, 2 mg; nicotinic acid, 12 mg; pantothenic acid, 10 mg; vitamin B_1, 2 mg and vitamin B_6, 3 mg.
[2]Synthetic amino acids
[3] Flavouring agent. Crina, Akzo Nobel, Chemin de la Combe 15, CH-1196 Gland, Switzerland.
[4]Diets 1 and 2 contained 400mg/kgStafac 100 (Smithkline Beecham) which provided 40 mg virginiamycin per kg of finished feed
Diets 3 and 4 contained 100 mg olaquindox per kg of feed provided from Enterodox 100 BMP (Nor-feed UK Ltd, Northallerton, UK.).
Diet 5 contained 40 mg Avilamycin per kg of feed provided by Maxus G200 premix (Elanco Products)
[5] Calculated from standard book values for ingredients.

Management

Pigs were weighed at the commencement of the trial, day 27 and day 48 post-weaning and at slaughter at approximately day 130 post-weaning. Feed intake was recorded for the periods day 0 to 27, day 27 to 48 and day 48 to 130. After slaughter the carcass weight, fat depth, muscle depth, and lean meat content were recorded for each pig.

Statistical Analysis

The data was statistically analysed using the General Linear Model (GLM) procedure of SAS (1996) for a completely randomised design. The pig was the experimental unit and the model included the effect of pen in the finishing period.

Results and Discussion

Sows

The effect of gestation feeding on sow weight and breeding performance is shown in Table 2. By day 50 of gestation sow weight was greater ($P<0.01$) for sows fed 60 MJ DE/day than for sows fed 30 MJ DE/day. However, there was no significant difference in sow live weight between treatments at day 110 and at weaning. Sow back-fat thickness was similar for both treatments at day 25, day 50, day 110 and at weaning ($P>0.05$). The number of pigs born alive and the number born dead were similar for both treatments ($P>0.05$).

Progeny

The effect of gestation feeding on pig performance from birth to slaughter is shown in Table 3. As the interaction effects between treatment and sex were not significant only the main effects are discussed. Pig performance at any period from birth to slaughter was not significantly affected by treatment.

Previous studies have found that growth rate was increased and FCE improved in the finisher period as a result of over-feeding sows in mid-gestation (DWYER et al., 1994; PENNY and VARLEY, 1999). In the present experiment, there was no significant treatment effect on intake or growth rate between day 48 post-weaning and slaughter at day 130 post-weaning.

Treatment did not effect days taken from birth to slaughter ($P>0.05$), daily gain from birth to slaughter ($P>0.05$), lean meat yield ($P>0.05$) or kill out proportion ($P>0.05$).

Male pigs were heavier than females at birth ($P=0.07$). Growth rate was higher ($P<0.05$) and FCE more efficient ($P<0.001$) for males than females. Females had a higher lean meat yield ($P<0.05$) and kill-out proportion ($P<0.01$) than males.

Table 2
Effect of Gestation feeding programme on sow weight and breeding performance

Treatment	1	2	s.e.	P-value
Number per treatment	22	24		
Parity	3.6	3.6		
Gestation intake (kg/day)	2.32	2.73		
Days				
Gestation	114.5	114.5	0.19	0.87
Lactation	27.5	27.8	0.28	0.39
Weaning to service	5	5	0.04	100
Intake				
Lactation feed intake (kg/day)	5.83	5.47	0.193	0.19
Sow weight (kg)				
Service	210	216	5.1	0.44
Day 25	223	225	4.4	0.82
Day 50	226	244	5.0	0.01
Day 110	264	271	6.2	0.42
Post farrowing	246	255	6.1	0.31
Weaning	235	240	5.4	0.54
Back fat (mm)				
Day 25	19.4	18.8	0.68	0.54
Day 50	20.5	20.5	0.70	0.95
Day 110	21.3	22.2	0.69	0.34
Weaning	17.0	17.5	0.49	0.48
Gain (kg)				
Pregnancy	42.9	59.1	3.63	0.05
Lactation	-28.6	-30.9	3.94	0.67
Progeny				
Total born (no.)	12.2	11.5	0.74	0.47
Born alive (no.)	11.1	10.6	0.75	0.66
Born dead (no.)	1.1	0.8	0.32	0.51
Birth weight (g)	1537	1473	47.8	0.35

Table 3
Effect of Gestation feeding and sex on pig performance from birth to slaughter at c.96 kg

					s.e.	P-value	
						Treatment	Sex
Treatment	**1**	**2**					
Sex			**Female**	**Male**			
Number/treat	**42**	**50**	**46**	**46**			
Age at slaughter (days)	158.5	158.1	160	156.7	1.16	0.43	0.04
Crown to rump length (cm)	29.0	29.0	28.8	29.1	0.35	0.88	0.57
Weight (kg)							
Birth (g)	1554	1527	1486	1596	42.1	0.69	0.07
Weaning	8.0	8.2	8.0	8.2	0.18	0.38	0.41
Day 27	20.0	19.9	19.5	20.3	0.41	0.85	0.19
Day 48	35.6	35.3	34.8	36.1	0.63	0.90	0.17
Day 130	96.9	95.9	95.4	97.5	1.01	0.54	0.18
Dead (day 130)	73.2	72.4	72.7	73.0	0.87	0.54	0.91
Daily feed intake (g/day)							
Day 0 to 48	819	819	813	825	13.4	0.71	0.57
Day 48 to 130	1928	1901	1935	1893	30.1	0.93	0.26
Day 0 to 130	1525	1503	1533	1495	20.1	0.74	0.14
Average daily gain (g/day)							
Day 0 to 48	577	567	561	583	11.6	0.63	0.18
Day 48 to 130	738	740	716	761	13.1	0.56	0.02
Day 0 to 130	679	676	660	694	9.7	0.87	0.02
Birth to day 130	602	600	588	614	7.9	0.89	0.02
Carcass (Day 48 to 130)	604	605	593	615	11.9	0.66	0.20
Lean (birth to 130)	269	267	268	268	4.6	0.89	0.89
Feed conversion efficiency (g/g)							
Day 0 to 48	1.47	1.45	1.48	1.44	0.033	0.77	0.42
Day 48 to 130	2.63	2.59	2.71	2.50	0.034	0.43	0.0001
Day 0 to 130	2.25	2.24	2.32	2.16	0.026	0.64	0.0001
Carcass (Day 48 to 130)	3.22	3.17	3.28	3.11	0.046	0.59	0.008
Carcass							
Lean meat (g/kg)	584	580	589	575	4.3	0.69	0.04
Fat depth (mm)	11.9	12.7	12.0	12.6	0.43	0.27	0.46
Muscle depth (mm)	54	54.8	56.2	52.6	1.05	0.46	0.02
Kill out (g/kg)	756	754	762	748	3.0	0.71	0.002

Conclusion

In conclusion, these data do not support the original hypothesis. It could be speculated that the period of increased feeding used in the present study was not sufficient to show a treatment response. It should also be noted that the sows used in the present trial were of a genotype that had been much more highly selected for leanness than those used in the study by DWYER et al. (1994). This may also help explain the absence of a treatment response in the present study.

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Non-invasive measurement of muscle development in lambs postnatally – implications for meat quality

Abstract

In recent years scientists have investigated the potential of multiple electrode arrays in electromyography recording and analysis, and whilst many systems remain rather complex, the technique of small surface electrodes arranged at specific distances has shown potential as a means of non-invasively assessing changes in muscle morphology and function. This project investigates the use of double-differentiated evoked surface electromyography recordings in lambs during the early postnatal period to assess the development of *m. Longissimus dorsi* and *m. Biceps femoris*. Findings are discussed in terms of parameters that have been shown to predict muscle fibre composition and in relation to meat quality indicators.

Key Words: surface electromyography, CMAP, small ruminants, stimulation

Zusammenfassung

Titel der Arbeit: **Muskelentwicklung und Fleischqualität bei Lämmern**

In den letzten Jahren haben Forscher die Möglichkeiten von „Multiple Electrode Arrays" in elektromyographischen Aufzeichnungen und Analysen untersucht. Während viele Systeme noch weiterhin ziemlich komplex sind, liegt in der Technik von kleinen, in spezifischen Abständen angeordneten Oberflächenelektroden eine Möglichkeit einer „non-invasiven" Methode, mit der sich Veränderungen in der Muskelmorphologie und -funktion feststellen lassen. Dieses Projekt untersucht die Anwendung von doppelt-differenziert beeinflussten oberflächen-elektromyographischen Aufzeichnungen zur Darstellung der Entwicklung von *m. Longissimus dorsi* und *m. Biceps femoris* bei Lämmern in der frühen postnatalen Periode. Die Ergebnisse werden anhand von Parametern, die die Muskelfiberzusammensetzung vorhersagen und in Verbindung mit Fleichqualitätsindikatoren diskutiert.

Schlüsselwörter: Oberflächenelektromyography, CMAP, kleine Wiederkäuer, Stimulation

Introduction

The morphology and physiology of striated muscle has always been of great interest to man. Improved methods of studying structure and function, have increased our knowledge of both normal and abnormal muscle so that a number of muscle disorders can now be identified and easily monitored.

Surface electromyography (EMG) is now being used successfully to give information about skeletal muscle properties in a non-invasive fashion. Surface EMG is a technique that records electrical signals in active muscle fibers through the skin. Each muscle comprises many fibers each of which consists of repeating chains (myofibrils) of contractile units, which create the force of muscle action. Each muscle fiber is innervated by an α motor neuron which when stimulated chemically activates the muscle fiber at its neuromuscular junction. This develops an electrical charge which is transmitted along the length of the fiber activating voltage gated Na^+-channels located on the fiber surface. It is this electrical transmission, which is recorded as the EMG signal. The EMG signal is described as random as it does not have a consistent waveform when recorded from voluntary contractions, however it does have repeating spikes (action potentials) which vary in amplitude and duration when muscles are directly field stimulated at set frequencies and with defined pulse durations.

Using the equations of KUPA et al. (1995), derived from isolated and electrically stimulated rat skeletal muscles, preliminary *in vivo* findings in our laboratory (shown below) obtained with human hand muscles have been found to give very precise predictions of muscle fiber type proportions (JOHNSON et al., 1973). These data illustrate the potential to predict fiber type composition in livestock by means of EMG signal analysis.

% SO type I fibres = -1.55 x IMF(133) – 0.55 x ΔMF(77) + 310 = 61.5% cf. published values of 52-74% with a mean of 63.0%

(where IMF = Initial Mean Frequency, ΔMF=Delta Mean Frequency between maximal and final values)

It is hypothesised that the amplitude and duration of the spikes reflect the physiological state of the muscle, and in that sense can be a valuable tool in the study of muscle development and meat quality. The present study has used non-invasive electromyography to investigate the post natal development of *m.Longissimus dorsi* and *m.Biceps femoris* in lambs and to discuss the use of the method as a tool to predict meat quality.

Material and methods

Twenty two lambs from eleven twin-bearing adult Shropshire ewes mated with two different Shropshire rams, were included in the study. The ewes were kept on grass until 6 weeks prior to lambing, whereafter they were housed indoor and fed silage (56% Dry Matter (DM), 6.1% ash, 7.9 % Crude Protein (CP), 1% fat) *ad libitum* and 200 g barley (88.7% DM, 2.2% ash, 10.4% CP, 2.3% fat) and 200 g of a commercial supplement (89.5% DM, 5.68% ash, 45.4% CP, 5.1% fat) the last 6 weeks of gestation, before lambing in february. After parturition the supplementation was changed and increased gradually to 1 kg/ewe/day of a commercial lactating diet for sheep (88.8% DM, 6.8% ash, 14% CP, 3.2% fat). A total of 22 lambs was included in the experiment and raised indoor by the ewes.

Evoked CMAP recordings

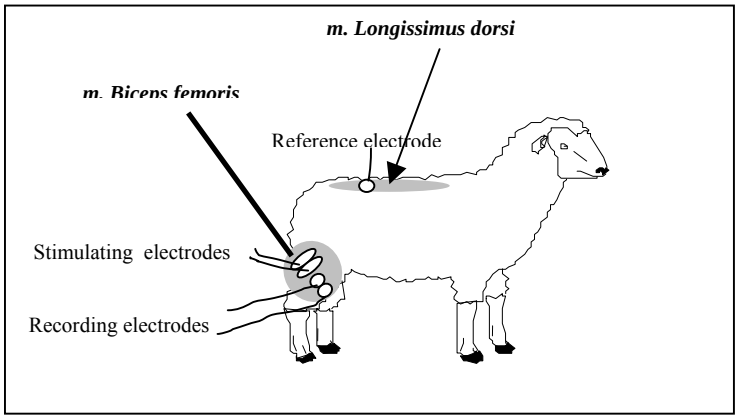
We used the guidelines laid out in the *European Recommendations for Surface ElectroMyoGraphy* as detailed by the SENIAM project (HERMENS et al., 1999), which document the optimal placement of sensors, including sensor size, individual muscle locations and recording and analysis procedures. In preparation for surface electromyography recordings lambs were shorn between the mid and caudal region of *m. Longissimus dorsi* (LD) adjacent to the spine and on the right side of the animal. Lambs were also shorn over the majority of *m. Biceps femoris* (BF) and surface evoked electromyography recordings were made on both muscles (see Figure 1A) and measured 8 times in the period from day 2 to day 32 *post partum*.

Measurements were done by a double differential electrode configuration, with disposable electromyography electrodes (N-00-S; Blue Sensor, Medicotest, Denmark) of 0.5 cm and 3 mm in length and width, respectively.

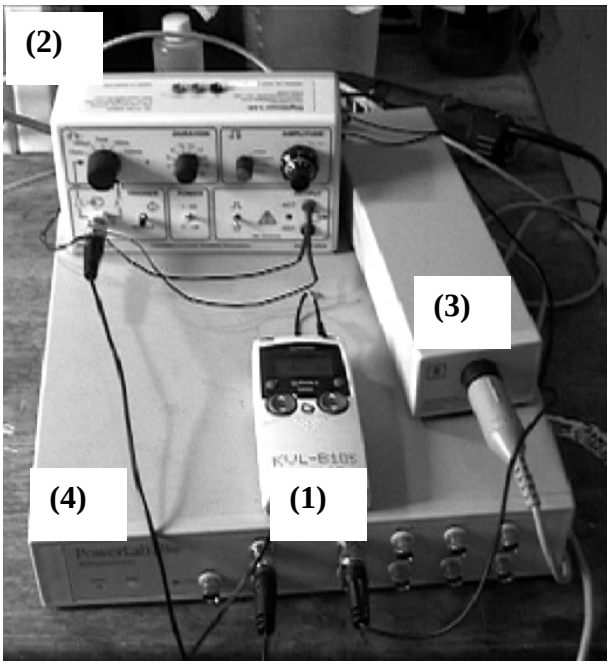
Legend of Figure 1 (page 58)

Fig. 1: **(A)** Placement of reference, stimulating and recording electrodes on *m. Longissimus* and *m. Biceps femoris*, measuring CMAP. **(B)** ELPHA II 3000 stimulator (1), Digitimer, DS3 isolated stimulator (2), ML132 amplifier (3), ML780 Powerlab/8s A/D converter (4) used to record the CMAP signal. **(C)** Recorded CMAP signal and the parameters analysed.

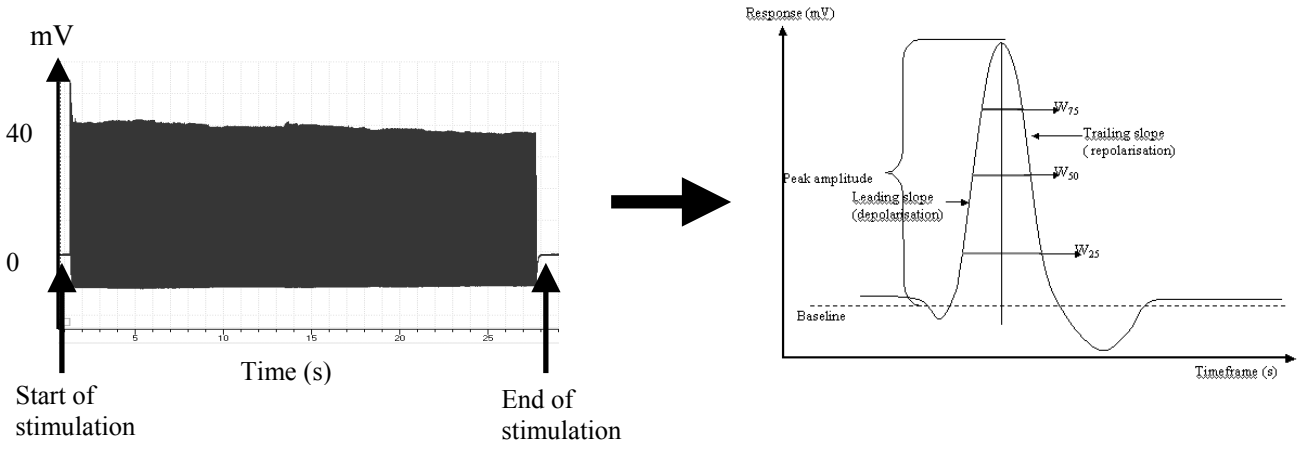
A


m. Longissimus dorsi
m. Biceps femoris
Reference electrode
Stimulating electrodes
Recording electrodes

B


(2)
(3)
(4)
(1)

C


mV
40
0
Time (s)
Start of stimulation
End of stimulation
Response (mV)
W_{75}
Trailing slope (repolarisation)
W_{50}
Peak amplitude
Leading slope (depolarisation)
W_{25}
Baseline
Timeframe (s)

Stimulation was at 40 Hz (day 2 to 6; ELPHA II 3000 stimulator, Danmeter, DK), 140 Hz (days 8-14; Digitimer DS3 isolated stimulator, Digitimer Ltd, UK), and 200 Hz (day 21-32; Digitimer DS3 isolated stimulator, Digitimer Ltd, UK) using 200 ms pulses of 9.3 mA via Palsflex electrodes (Danmeter, DK). Signals were recorded via an ML132 amplifier to a ML780 PowerLab/8s A/D converter connected to an iMac running Chart v. 3.6.3/s Software (AD Instruments, Australia) (Figure 1B). Input impedance was 200 MΩ differential and a high and low pass filter of 3 Hz and 500 Hz, respectively, was used. Sampling speed was set to 40,000 data samples per second. The method resulted in an exitation of muscle fibers in LD and BF both through stimulation of the dorsal branches of the intercostals and lumbar nerves and the caudal gluteal and sciatic nerves, and *via* direct stimulation at the muscle fiber level.
Compound Muscle Action Potentials (CMAP) were analysed using ”Peak Parameters” software (ADInstruments, UK) with emphasis being placed on such parameters as peak area, the signal peak corrected for baseline values (Corr Peak), both leading and trailing slopes and finally the width at 50% of peak max. (W50) (see Figure 1C).

Statistical analysis

All results were assessed for statistical significance within groups by two-way analysis of variance, with class variables including paternal genetics and sex and time as the repeated unit using the statistics package PROC MIXED in SAS (Version 8.2) (LITTEL et al., 1996). This statistical procedure enables multivariable repeated analysis to compare treatment means over time. Data were found to be normally distributed and of equal variance. Values are presented as means ± SEM. Differences showing a P value <0.05 were considered significant.

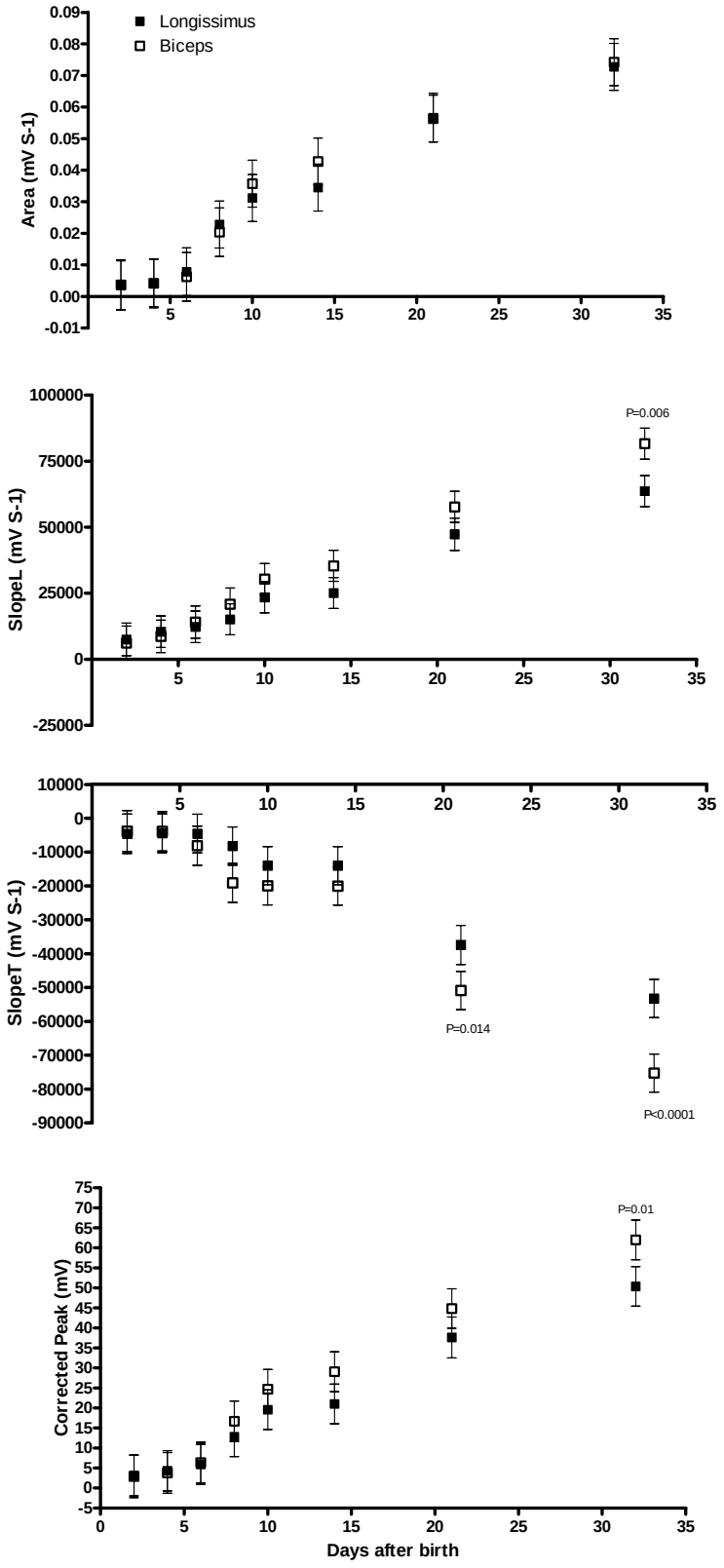
Results

The EMG-signals showed no difference between the two rams at anytime during the observation period, likewise there were no differences between sexes. All parameters analysed showed a significant increase from day 2 to 32 *post partum*, showing development of the surface evoked EMG signal (Figure 2). Leading and trailing slopes and Corrected peak were significantly different in the two muscles measured on day 32 *post partum* (Figure 2). BF had a steeper leading slope and a greater corrected peak at day 32 than LD. This trend was numerically present from day 7 *post partum*. None of the other parameters analysed were different between the two muscles.

Discussion

The present study has shown that the postnatal development of two diverse skeletal muscles in the lamb can be followed non-invasively using surface electromyography with clear identification of a number of CMAP signal parameters. Of particular importance, however, is the fact that subcutaneous fat layers are known to have a low conductivity (DISSELHÔRST-KLUG et al., 1998). Hence, high levels of subcutaneous fat will reduce the CMAP signal compared with animals with a lower level of subcutaneous fat deposition. Since the present study is non-invasive in nature, scanning has been used to assess any clear differences in subcutaneous fat depth and muscle size that may have existed between animals, and thereby affected the interpretation of the recorded CMAP signal. Our measurements showed that no such differences were found between the groups (data not presented).

Fig. 2: Recorded CMAP area, leading (SlopeL) and trailing slope (SlopeT), width at 50% of maximum peak amplitude(W50), corrected peak amplitude (Corrected peak) in lambs the first 32 days *post partum* (n=22)



Meat

Development of meat quality *post mortem* is dependent on the physiological state of the muscles at the time of slaughter since such factors as glycogen stores and muscle protein turnover may affect the pH development and proteolytic potential, respectively. These factors ultimately determine the quality traits such as water binding capacity, juiciness and tenderness. Thus, methods which can predict the state of muscles prior to slaughter are very valuable tools in terms of producing high quality meat.

The question now arises, as to whether information gained from surface EMG can be used in predicting meat quality? It is well known that different muscles on the carcass have different meat qualities, which can be explained by the differences in functionality, connective tissue content and fibre type distribution and size.

In rat muscle a difference in protein turnover has been demonstrated between muscles rich in slow- and fast-twitch fibres (EL HAJ et al., 1986; GARLICK et al., 1989). Likewise, a higher concentration of RNA and a greater μ-calpain activity have been found in *m. Supraspinatus* compared with *m. Longissimus* from Holstein calves (THERKILDSEN et al., 2002), suggesting both a greater protein synthesis and protein degradation capacity, respectively in *m. Supraspinatus* rich in slow-twitch fibres compared with *m. Longissimus* rich in fast-twitch fibres. Similar findings of higher activities of the calpain system in muscles containing mainly slow- *cf*. fast-twitch fibers have been reported by others (OUALI and TALMANT, 1990; WHIPPLE and KOOHMARAIE, 1992). The possible link between muscle protein degradation in the living muscle and the rate of *post mortem* tenderisation mediated *via* the calpains and their inhibitor, has been reviewed by KOOHMARAIE and colleagues (2002), and suggests that animals with a high muscle protein degradation at the time of slaughter have a potential towards a greater degree of tenderisation *post mortem*. Indeed, muscles rich in slow-twitch fiber types and exhibiting a high rate of protein turnover are expected to be superior with regard to the development of tenderness *post mortem*. However, even though meat quality differences between muscles may to some extent be explained by differences in fibre type frequency, results describing meat quality traits of the same muscle but with variation in fibre type frequency are variable. Some authors have found no correlation between fibre type frequency and tenderness (VESTERGAARD et al., 2000) and some have found negative correlations between type II frequency and tenderness (CALKINS et al., 1981; HENCKEL et al., 1997) and positive correlations between type I fibres and tenderness (CALKINS et al., 1981).

Thus, there are indications of some relation between fibre type frequency and quality aspects, which suggest that the surface EMG method could be used to study this relation. In particular, this non-invasive method would be valuable in a study aimed at linking the random variation in fibre type frequency in muscles with *post mortem* meat quality. This is a particularly important aspect as most studies are designed to explain other questions, which may influence any conclusions drawn regarding the link between fibre type frequency and meat quality.

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Effects of pre- and postnatal exposure to maternal low and high dietary protein levels on body mass development and energy expenditure in rat progeny

Abstract

Recent data from rodent models is presented on the relation between low and high protein intake during pregnancy with birth weight, pre- and post-weaning body mass gain and body fatness, as well as glucose metabolism. Numerous studies in rodents show that inadequate nutrient supply during pregnancy leads to fetal growth retardation and inappropriate organ growth. This is associated in some cases to a decreased birth weight and subsequent disorders of growth, glucose metabolism, and development of increased body fatness throughout life. The hypothalamo-pituitary-adrenal axis is thought to play a role in fetal programming.

Key Words: maternal low protein, pregnancy, growth, macronutrient metabolism, energy metabolism, fetal programming

Zusammenfassung

Titel der Arbeit: **Einfluss von maternaler und früh-postnataler Niedrig- oder Hochproteinernährung auf die Körpermasseentwicklung und den Energiestoffwechsel bei Ratten**

Neuere Daten im Rattenmodell weisen auf eine Beziehung zwischen der Höhe der Proteinzufuhr während der Trächtigkeit und dem Geburtsgewicht, der Körpergewichtsentwicklung, dem Körperfettgehalt sowie dem Glukosestoffwechsel hin. Zahlreiche Studien beim Nager zeigen, dass unangepasste Nährstoffzufuhr zu fötaler Wachstumsretardierung und ungenügendem Organwachstum führt. Dies ist in manchen Fällen verbunden mit verringertem Geburtsgewicht, Störungen des postnatalen Wachstums und des Glukosestoffwechsels sowie verstärkter Körperverfettung. Es wird angenommen, dass die hormonelle Achse Hypothalamus-Hypophyse-Nebenniere hier eine Rolle spielt.

Schlüsselwörter: Maternale Niedrigproteinernährung, Trächtigkeit, Wachstum, Makronährstoffstoffwechsel, Energiestoffwechsel, Fötale Programmierung

Introduction

In many populations worldwide epidemiological evidence relates low birth weight to increased risk for syndrome X, coronary heart disease, and high blood pressure in adult age (ERIKSSON et al., 1999; HALES and BARKER, 1992; LAW and SHIELL, 1996; MI et al., 2000). On the basis of these observations it was suggested that low birth weight is causally related to fetal under- or malnutrition and subsequent fetal growth retardation or disproportionate growth which permanently affects cardiovascular health, glucose tolerance and insulin sensitivity (GODFREY and BARKER, 2000). Obesity and type 2 diabetes are the most common metabolic diseases in Western societies, together affecting a large proportion of the adult population. Evidence is emerging that nutritional programming during fetal development might be involved in the world wide increasing obesity epidemic. To date research on the effects of maternal nutrition on adiposity and glucose metabolism in farm animals is scarce.

Results and Discussion

Human data

There is some but not unequivocal evidence that birth weight and body fatness in humans are inversely related (ref. METGES, 2001; JAQUET et al., 2000). A meta-

analysis of the relationship between birth weight and adult body fatness showed that linking evidence for association between low birth weight and high adult body fatness (body mass index; kg BW /m^2) is contradictory (MARTORELL et al., 2001). On the contrary, it appears that in humans high birth weight (> 4.8 kg) is linked to adult body fatness (MARTORELL et al., 2001). This is somewhat surprising because excess adipose tissue, leads to reduced insulin sensitivity in metabolically responsive tissues, which in turn is frequently reported to be associated with low birth weight. Possibly, adiposity is a less sensitive outcome of fetal growth retardation than other metabolic disorders, at least in humans. Another possibility is that birth weight as a measure of the relative success of pregnancy, i.e. fetal growth, is useful but incomplete because a raise in birth weight via nutritional factors is not necessarily associated with an increase in perinatal survival or infant health, and long-term metabolic health implications are not considered. Perhaps the critical outcome of poor fetal nutrition is not birth weight but may be accelerated post-natal catch-up growth, and/or disproportional post-natal organ growth.
In the pig low birth weight (of unknown genesis) is linked to lower daily gain, a higher body fat mass, and a decreased meat quality (KUHN et al., 2002).

Low and high maternal protein model
A widely established model to study fetal programming by maternal malnutrition and exploring mechanisms responsible for the development of adult degenerative disease is the maternal low protein (MLP) model in rodents. Usually an isocaloric casein-based semi-synthetic diet of about 40-50 % protein restriction during pregnancy is compared with an adequate maternal diet of 180-200 g protein/kg diet. However, there are a number of subtle but possibly meaningful differences in experimental design. For example, because of an increased requirement of rodents casein-based diets are widely supplemented by methionine. In some studies the supplemental methionine:protein ratio is balanced (e.g. BENNIS-TALEB et al., 1999), but in others methionine is supplemented independently of the dietary protein content (REES et al., 1999; GARDNER et al., 1997). Another issue is the energy intake in the MLP model. Because in most studies pregnant rats were fed ad libitum it is possible that dams on low protein diets reduced their food intake (although this was either not measured or not reported in most of the respective studies). LANGLEY and JACKSON (1994) have shown that protein-restricted rat dams reduced their energy intake. Consequently, it cannot be ruled out that some effects may be attributable to protein-energy malnutrition. Further, in some reports offspring was exposed to low protein diet in utero and during lactation (DESAI et al., 1997), whereas in others the use of foster mothers on control diet throughout pregnancy and lactation allowed separation of protein effects between prenatal and early postnatal phase (BENNIS-TALEB et al., 1999). Others have provided MLP already 2 wk before mating (REES et al., 1999). We found recently, that body mass gain during the suckling period differs between offspring of mothers exposed to low protein diet in utero and during lactation, or in utero only (DAENZER, PETZKE, METGES, KLAUS, unpublished). This indicates that there are effects of food composition during pregnancy (i.e. protein intake) on milk quality during early lactation.
In numerous studies it has been shown that a low protein intake throughout pregnancy in rats and mice results in altered body and organ growth, hepatic glucose output, age-

related loss of glucose tolerance and insulin resistance, and hypertension (OZANNE et al., 1996, 2001, 2003; DESAI et al., 1997; GARDNER et al., 1997; KHATTABI et al., 2003). Even a low protein diet during the last week of pregnancy only led to an impaired pancreatic beta cell mass (BERTIN et al., 1999). There are only few studies in which postnatal body mass gain and body fat mass was investigated in offspring born to mothers fed low protein diets during pregnancy. OZANNE et al., 2004, recently reported in mice that this phenotype was characterized, although small at birth, by increased catch-up growth during lactation and post-weaning and higher body weight at age 10 wk. Data on body fat were not reported. This appears to be in contrast to observations in pigs where a lower birth weight was related to a lower body mass gain (KUHN et al., 2002).

Not only protein malnutrition has been shown to result in low birth weight in rats, also isocaloric maternal high protein diet during pregnancy was followed by a reduction of body weight at day of life 2 but a higher body weight than controls up to wk 6 in the offspring (DAENZER et al., 2002).

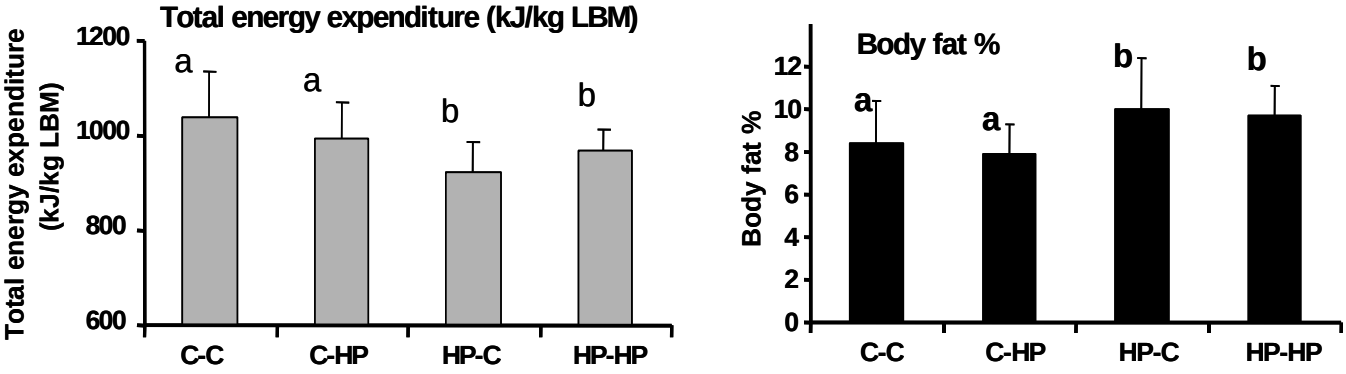

Figure: Twenty-four hour energy expenditure (left) and body fat (right) in offspring of rats fed a control or a high protein diet during pregnancy (DAENZER et al., 2002).

Exposure to high protein diets during pregnancy and lactation resulted in a decreased body weight of pups until weaning (GAMBARDELLA et al., 1987; DAENZER et al., 2002). Furthermore, the offspring from maternal high protein feeding had a reduced total energy expenditure and a higher body fatness at wk 9 (Figure). In contrast, postnatal protein overnutrition only did not lead to an obese phenotype (DAENZER et al., 2002). This suggests that upon prenatal high protein exposure offspring overcompensated in terms of catch-up growth which was followed by increased body fat in young adults, which provides first evidence that in utero high protein exposure can predispose offspring to adult adiposity.

Potential Mechanisms

So far there is no single mechanism identified explaining how maternal nutritional factors change the intrauterine environment to lead to metabolic alterations in adult offspring. However, it was suggested that due to inadequate maternal nutrient supply the maternal-fetal nutrient (e.g. glucose, amino acids) and hormonal transfer leads to placental adaptations and subsequently alters the intrauterine environment with parallel changes of the hormonal setpoints in the fetus, due to a nutrient supply inadequate to the respective genetically determined time window of development.

In the case of maternal protein undernutrition the fetus receives a reduced amino acid but an increased glucose supply. In the rat this was shown to be related to a placental down regulation of 11ß-hydroxysteroide reductase gene expression which is associated with a decreased inactivation of the biologically active cortisol (BERTRAM et al., 2001). This leads to an increased fetal exposure to maternal glucocorticoids but an upregulation of glucocorticoid receptors in several fetal tissues, persistent throughout post-natal life. A post-natal hyperactivity of the hypothalamo-pituitary-adrenal axis might be related to increased proteolysis and gluconeogenesis, followed by decreased insulin sensitivity. Cortisol is also known to affect the GH/IGF-1-axis and thereby growth and glucose homeostasis.

Investigations in porcine fetus show an inverse association between plasma cortisol and fetal weight on days 65 and 100 of gestation. In ovine fetus a high cortisol concentration was linked to a reduced IGF-1 gene expression (ASHWORTH et al., 2001).

Thus, because the hypothalamo-pituitary-adrenal axis plays a role in fetal programming which postnatal effects are also of relevance for animal production traits (i.e. body fatness, insulin sensitivity) and eventually animal performance, future studies need to identify if and how dietary conditions and specific nutrients during gestation regulate fetal development in farm animals. Further, it needs to be clarified whether phenotypes caused by fetal (i.e. maternal) imbalanced nutrition described in the rodent model is equally important in farm animal species.

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The long-term influences of birth weight on muscle characteristics and eating meat quality in pigs individually reared and fed during fattening

Abstract

This study aims to determine whether a low weight at birth may influence muscle characteristics and meat quality traits in pigs at market weight. At 68 days of age, a total of 30 female piglets were assigned within litter to one of the two experimental groups (n = 15 per group): low birth weight (LW: 1.05 ± 0.04 kg) or heavy birth weight (HW: 1.89 ± 0.02 kg). Pigs were penned individually and fed ad libitum up to slaughter. At 111.8 ± 0.8 kg, LW pigs were 12 days older ($P < 0.001$) than HW pigs. Feed conversion ratio was deteriorated in LW pigs compared to HW pigs (+10%, $P< 0.001$). A higher proportion of subcutaneous fat (+29%, $P < 0.005$) and a lower lean meat content (-1.85 point, $P < 0.01$) were observed in LW carcasses compared with HW ones. Myofiber cross-sectional area was 14% higher in both *semitendinosus* and *longissimus* muscles of LW pigs than in HW pigs. Total fiber number was 19% lower ($P < 0.01$) in the *semitendinosus* muscle of LW pigs. Lipid content was 25% higher in the *semitendinosus* muscle of LW pigs compared with HW pigs, but did not differ in the *longissimus* muscle. The LW pigs exhibited a lower score for loin meat tenderness compared with HW pigs. Tenderness score tended to be negatively correlated (r = -0.34, $P = 0.07$) with mean myofiber size of the *longissimus* muscle. Then, birth-weight effect on meat tenderness may be partly attributed to its influence on myofiber hypertrophy.

Key Words: pigs, birth weight, muscle fiber, sensory tenderness, carcass composition

Zusammenfassung

Titel der Arbeit: **Langzeiteffekte des Geburtsgewichts auf Muskeleigenschaften und Fleischqualität beim Schwein**

Es wurde untersucht, ob beim Schwein niedrige Geburtsgewichte Einfluss auf die Muskeleigenschaften und Fleischqualität zum Zeitpunkt der Schlachtung haben. Bei 68 Tagen Lebensalter wurden 30 weibliche Schweine innerhalb Wurf zwei Experimentalgruppen (n=15) zugeordnet: Niedriges Geburtsgewicht (LW: 1,05 ± 0,04 kg) und hohes Geburtsgewicht (HW: 1,89 ± 0,02 kg). Die Tiere wurden einzeln aufgestallt und ad libitum gefüttert. Bei einem Lebengewicht von 111,8 ± 0,8 kg waren LW-Tiere 12 Tage älter ($P < 0,001$) als Tiere der HW-Gruppe. Die Futterverwertung war bei LW-Tieren verschlechtert (+10%, $P< 0,001$). Tiere der Gruppe LW hatten einen höheren Anteil an subkutanem Fett (+29%, $P < 0,005$) und einen geringeren Magerfleischanteil (-1,85 Punkte, $P < 0,01$). Muskelfaserquerschnitte waren bei Tieren der Gruppe LW sowohl im *M. semitendinosus* als auch *M. longissimus* 14 % großer als bei HW-Tieren.. Die Faseranzahl im *M. semitendinosus* war 19 % niedriger ($P < 0.01$). Der Fettgehalt im *M. semitendinosus* war 25% höher bei LW als HW; der Unterschied trat im *M. longissimus* nicht auf. Schweine der LW-Gruppe erreichten schlechtere Bewertungen der Zartheit. Die Bewertung war tendenziell negativ korreliert (r=-0,34, $P = 0,07$) mit der Muskelfasergröße im *M. longissimus*. Der Einfluss des Geburtsgewichtes auf die Zartheit kann demnach z.T. auf die Effekte auf die Muskelfaserhypertrophie zurückgeführt werden.

Schlüsselwörter: Schweine, Geburtsgewicht, Muskelfaser, Zartheit, Schlachtkörperzusammensetzung

Introduction

Selection for sow's ability to give birth to a higher number of piglets has led to an increased within-litter variation in piglet birth weight (TRIBOUT et al., 2003). A critical birth weight of 950 g has been proposed, below which the development of myofibers and lipids may be modified (HEGARTY and ALLEN, 1978; POWELL and ABERLE, 1980; 1981). An individual birth weight below 2.5 standard deviation of the mean litter weight has been further suggested to constitute a better sensitive threshold,

below which the individual's lean growth potential will be limited (HANDEL and STICKLAND, 1987). A precise delineation however probably depends on piglet genotype, placenta and litter size, and sow milk production capacity (LE DIVIDICH, 1999 for a review). In addition, POWELL and ABERLE (1980) have clearly evidenced that small pigs encouraged to grow by foster-nursing or by individual rearing and feeding from birth to slaughter displayed both a fatter carcass and a higher muscle lipid content compared with normal littermates. This strongly suggests behavioral and competitive effects on fat tissue development in small piglets. Supplemental milk replacer during lactation was however ineffective in changing carcass composition at slaughter in piglets of small birth weight (WOLTER et al., 2002). The aim of this study was to determine whether body composition, myofiber traits at slaughter, and sensory meat tenderness may differ between pigs having being small or heavy at birth, when individually reared and fed during the growing-finishing period only.

Material and methods

Thirteen litters [(Large White x Landrace) x Pietrain halothan negative pigs) were considered during 6 successive blocks at the experimental unit of INRA (UMRVP, 35590 Saint-Gilles, France). Piglets were weaned at 28 days of age, and then moved to nursery with their respective littermates (10-12 pigs per batches). At the end of the post-weaning period (68 days), a total of 30 females were assigned within litter to one of the two following groups (n = 15 per group): low birth weight (LW: 0.75-1.25 kg) or heavy birth weight (HW: 1.75-2.05). Pigs were then moved to the growing-finishing house, where they were penned individually to eliminate all effects of competition between littermates, and they were fed ad libitum. Feed consumption data were recorded weekly during the growing-finishing period. Pigs were weighed at birth, weaning, end of post-weaning, and then weekly until slaughter. Average daily gain from birth to slaughter was calculated.

Pigs were slaughtered at 111.8 $\pm$ 0.8 kg, after an overnight fast. The hot carcass and the perirenal fat were weighed. Backfat and muscle depth were assessed on the day of slaughter with an invasive probe to estimate lean meat content. Three muscles of different anatomical location were rapidly excised from carcass. They were the entire *semitendinosus* (a mixed muscle with a red oxidative deep part and a white glycolytic superficial part), the entire *rhomboïdeus* (a slow-twitch oxidative muscle), and the *longissimus* at the last rib level (a fast-twitch glycolytic muscle). Histological determinations were performed in muscle samples, as previously described (GONDRET et al., 2004). Thirty min. after slaughter, 2 g of each muscle were also taken, immediately frozen in liquid nitrogen, and stored at –70°C. The pH (pH_1) was measured after muscle homogenization in iodo-acetate, using a combined glass electrode and a portable pH meter.

After 24 h of chilling, ultimate pH (pH_{24}) was measured in the *longissimus* muscle, using the apparatus described above. Total lipid content was determined according to FOLCH et al. (1957). The loin was then excised from the right carcass side, allowed to mature 3 days more, and stored at –20°C. After thawing at room temperature, the loin was cooked, and sensory meat tenderness was scored on a 1-10 scale by a trained panel (12 members).

Data were analyzed by variance analysis using the PROC GLM procedure of SAS (SAS Inst. Inc., Cary, NC), with birth group and block as main effects. For sensory traits, the model included the main effects of birth group, panel member, session, and their interactions.

Results

Mean weight in LW pigs was 55% of that in HW pigs at the day of birth. Compared with their larger littermates, the LW pigs needed 12 more days to reach the same slaughter weight than HW pigs (Table 1).

Table 1
Growth performance and carcass traits in pigs with a low (LW) or high (HW) weight at birth

	HW	LW	*P* value
Growth performance			
Birth weight, kg	1.89 ± 0.02	1.05 ± 0.04	0.001
Slaughter weight, kg	111.6 ± 0.9	111.9 ± 0.8	NS
Age at slaughter, d	159.5 ± 2.4	171.1 ± 2.5	<0.001
Total average daily gain, g/d	690 ± 12	650 ± 9	0.008
Feed consumption, g/d	2.25 ± 0.06	2.28 ± 0.05	NS
Feed conversion ratio, g/ g	2.49 ± 0.04	2.70 ± 0.06	0.01
Carcass			
Hot carcass weight, kg	89.5 ± 0.6	90.2 ± 0.7	NS
Perirenal fat weight, g	945 ± 49	1226 ± 69	0.002
Backfat depth, mm	15.0 ± 0.4	18.2 ± 0.7	0.002
Estimated lean meat content	63.0 ± 0.2	61.1 ± 0.4	0.002

There was no significant difference among birth weight groups in the feed consumed per day, whereas feed conversion ratio was 8% higher in LW pigs than in HW pigs (Table 1). Perirenal fat weight and backfat thickness at slaughter were increased by 30% and 21%, respectively, in LW pigs compared with HW pigs, whereas estimated lean meat content was reduced by 2 points in the former pigs compared to their larger littermates.

Table 2
Weight and histological muscle traits in pigs with a low (LW) or high (HW) weight at birth

	HW	LW	*P* value
Longissimus			
Mean CSA of fibers, µm²	6217 ± 211	7069 ± 347	0.04
Semitendinosus			
Mean CSA of fibers, µm²	7496 ± 318	8485 ± 509	0.06
Total fiber number, $x10^3$	739.6 ± 34.2	601.8 ± 19.7	0.003
Rhomboideus			
Mean CSA of fibers, µm²	4375 ± 123	4983 ± 215	0.03
Total fiber number, $x10^3$	83.4 ± 3.2	76.3 ± 4.5	NS

For the three muscle studied, the mean cross-sectional area of myofibers was about 14% larger in LW pigs compared with HW pigs (Table 2). Total fiber number was reduced by 19% in LW pigs in *semitendinosus* muscle, but the difference between groups did not attain significant level in *rhomboideus* muscle (-9%, $P = 0.17$).

Intramuscular lipid content was not influenced by birth weight in the *longissimus* muscle. Values of pH_1 and pH_{24} did not differ between groups (Table 3).

On the opposite, loin meat of LW pigs showed a lower score for eating tenderness compared with that of HW pigs (Table 3). Overall, sensory meat tenderness tended to

be negatively correlated (r = -0.34, *P* = 0.07) with mean cross-sectional area of myofibres in *longissimus* muscle. It tended to be positively correlated (r = 0.31, *P* = 0.10) with total fiber number in the *semitendinosus* muscle

Table 3
Meat quality traits in *longissimus* of pigs with a low (LW) or high (HW) weight at birth

	HW	LW	*P* value
Lipid content, g/100 g	1.4 ± 0.1	1.6 ± 0.1	NS
pH_1	6.11 ± 0.03	6.13 ± 0.04	NS
pH_{24}	5.48 ± 0.01	5.49 ± 0.02	NS
Tenderness	4.6 ± 0.1	4.0 ± 0.1	0.002

Discussion

It is widely accepted that light pigs at birth required a greater number of days to reach the same market weight than did their heavier littermates (WOLTER et al., 2002; GONDRET et al., 2004, current results). The main cause of a low birth weight is recognized as fetal under-nutrition; thereafter, light piglets at birth also consume less milk due to competition for the access to the udder with heavier littermates (LE DIVIDICH, 1999 for a review). The current finding of a similar daily feed consumption in LW pigs and HW pigs when individually reared and fed during fattening period, suggests that the level of perinatal nutrition does not imprint the appetite. On the opposite, feed: gain ratio was altered in LW pigs compared with HW littermates, in close-agreement with previous findings of bad feed efficiency in runt pigs (POWELL and ABERLE, 1980). This may be due to inadequate digestion or impaired utilization of nutrients, as suggested in rats (ROEDER and CHOW, 1972).

Both a higher body fat and a lower lean meat content was currently demonstrated in LW pigs compared with HW pigs at same weight. In agreement, some authors have reported that carcasses of low birth weight piglets (< 1470g) were fatter at the same weight (CAUGANT and GUÉBLEZ, 1983). Similarly, runt pigs that were penned and fed individually from birth to slaughter displayed a higher backfat thickness and a lower estimated muscle percentage than control littermates (POWELL and ABERLE, 1980). On the opposite, other reports indicated that carcass fat depth and/or carcass fat proportion were rather similar among birth weight groups (POWELL and ABERLE, 1980; WOLTER et al., 2002; GONDRET et al., 2004). Discrepancies on the effects of birth weight on carcass composition probably arise from differences in genotype, weight delineation, level of behavioral competition, and feeding scale. The results of the present study then suggest that the level of nutrition during fattening is crucial for fat tissue growth, for small piglets especially. No difference in total lipid content in the *longissimus* muscle was however evidenced among birth weight groups.

Since total fiber number of one muscle is indicative of that of other muscles in the body (STICKLAND and GOLDSPINK, 1973), it is generally admitted that birth weight effect on muscle fiber number is common to all skeletal muscles. We reported a lower total fiber number in *semitendinosus* muscle from LW piglets compared to HW littermates, however this effect did not reach significance level in *rhomboideus* muscle. The current observation of the lowest total fiber number in *semitendinosus* muscle from pigs having being small at birth is in agreement with studies comparing pigs categorized by fetal weight (WIGMORE and STICKLAND, 1983), birth weight (REHFELDT et al., 2003; GONDRET et al., 2004), weight at 5 weeks (DWYER and STICKLAND, 1991), or carcass weight at same age (NISSEN et al., 2004). Other

reports did not evidence any effects of birth weight (runts excluded) on total fiber number (HANDEL and STICKLAND, 1987; DWYER et al., 1993). These discrepancies may be explained by the fact that muscle fiber number is a better indicator of postnatal growth performance than of birth body weight solely (HANDEL and STICKLAND, 1987). Because total fiber number is fixed before birth in pigs, the low total fiber number in LW pigs compared to HW littermates was probably assigned to fetal nutrition level (DWYER et al., 1994). The current results of a higher cross-sectional area of fibers in the three muscles of LW group compared to HW group are in agreement with other findings in pigs (HEGARTY and ALLEN, 1978; POWELL and ABERLE, 1981; GONDRET et al., 2004). In support, REHFELDT et al. (2000) underlined that hypertrophy of individual myofibers may partly compensate for an apparent decrease in the number of myofibers.

A large myofiber size and/or a low total number of fibers in pig muscle has been suggested to lead to a lower pH_1 (LENGERKEN et al., 1997). However, both halothan positive and negative pigs are considered in the study mentioned above, which gene is known to affect pH_1 and drip loss (SELLIER, 1998). We did not observe any differences between LW and HW pigs in pH values, despite clear variations in myofiber characteristics between groups. In agreement, NISSEN et al. (2004) did not observe any difference in pH_{24} among halothan-negative littermates categorized by carcass weight at same age, despite a reduced total fiber number in the lightest pigs. We suggest that variations in myofiber size at slaughter were associated with a lower sensory score for loin meat tenderness in LW pigs compared with HW pigs. This disagrees with previous reports of lack of relationship between myofiber size and eating tendernes (MALTIN et al., 1997; HENCKEL et al., 1997). It remains to determine whether other muscle components, such as quantity and heat-solubility of collagen, may be also partly responsible for differences in tenderness between the two birth-weight groups.

Conclusion

This study presented evidence for an altered muscle cellularity in pigs having being small at birth compared with larger littermates at the same weight at slaughter. For the first time, sensory test attributed a lower score of tenderness for the loin meat of light birth weight. Differences in tenderness may be attributed, at least partly, to birth-weight effect on postnatal myofiber hypertrophy.

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Influence of maternal nutrition levels on growth rate and muscle gene expression in the offspring in the rat

(Einflüsse maternaler Ernährung auf das Wachstum und die Genexpression der Nachkommen bei der Ratte)

This study examined the influence of two levels of gestational under-nutrition on the post-natal growth rate, skeletal muscle cellularity and the expression of genes involved in muscle growth, in offspring at weaning. Rat pups from the 50% restricted diet group had an increased growth rate compared with pups fed 40% of ad lib. The growth rate of the control group (ad lib) was intermediate between the 50% and 40% groups. The two restricted diets did not influence muscle fibre numbers in the semitendinosus muscle of the offspring but nuclei numbers were reduced in the 40% group compared with controls. The lightest littermates at birth from the 50% group had elevated muscle IGF-1, IGFBP-5 and PCNA mRNAs compared with both control and 40% groups while the heaviest littermates at birth had increased IGFBP-4, PCNA and M-cadherin mRNAs. There were no correlation between growth rates and IGF-1R, myostatin and MyoD transcript levels. The pups from the 40% group exhibited reduced muscle IGF-1 mRNA but all other transcripts were similar to controls. This study suggests that the increased post-natal growth rate associated with milder foetal under-nutrition (50%), may be due to a more active local muscle IGF system and increased muscle cell proliferation.

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Expression of the myosin light chains 1 and 2 in the developing fast muscle of gilthead sea bream (*Sparus aurata*)

(Die Expression der Gene der leichten Ketten 1 und 2 des Myosins in der Entwicklung der schnellen Muskulatur der Goldbrasse (*Sparus aurata*))

Myosin, the major component of striated muscle, is a complex molecule of heavy and light chains, which undergo continuous replacement to meet developmental and environmental demands. A range of myosin isoforms are expressed in early developmental stages and are of special interest as they offer information about muscle formation and function early in life. In addition, they can act as markers for the study of prenatal events with an effect on postnatal growth performance. In this study, the spatial and temporal expression of embryonic myosin light chains 1 (MLC1) and 2 (MLC2) was studied in sea bream larvae post-hatch by *in situ* hybridization using riboprobes. The expression pattern of the transcripts was studied in transverse sections (5μm) of whole larvae samples, 4, 8, 10, 15, 20, 25, 34, 51 and 80 days post-hatch. MLC1 and MLC2 exhibited overlapping expression patterns at the early stages of sea bream development. Both MLCs were expressed exclusively in white muscle and no expression was observed in the superficial red muscle layer. On day 4 the expression of both transcripts was strong throughout the epaxial and hypaxial musculature. From day 10 onwards two distinct germinal zones appeared in the dorsal and ventral side of the larvae, characterized by small diameter muscle fibers, while fiber diameter gradually increased from the lateral germinal zones towards the horizontal myoseptum. An increase in fiber diameter of the deep white muscle layers next to the notochord was observed, indicative of high hypertrophic activity. At the same time, MLCs' expression became restricted to the periphery of the maturing muscle fibers and it was predominant at the germinal zones. This pattern persisted up to day 51, when the germinal zones disappeared and expression of MLCs was observed only in cells situated between the mature white fibers, most probably satellite cells.

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Selection for growth rate alters the expression of rapid myosin heavy chain isoforms in chicken breast muscle

(Selektion auf schnelle Wachstumsrate beeinflusst die Expression des Gens der schweren Kette der Myosin-Isoform schneller Muskelfasern im Brustmuskel beim Huhn)

Differences in growth rate are likely to affect the contractile differentiation of muscle fibres. We have used chickens from two genotypes divergently selected for high or low growth rate, which differ in their overall body size. At hatch, chicks of the High Growth (HG) line show a higher number of muscle fibres (about 20%) than those of the Low Growth line (LG), without differences in fibre size. As growth proceeds at different rates, the muscle fibres differ in size within a few days post-hatch. To assess if these differences could be associated with differences in the contractile properties of the muscle we have measured the expression of the mRNAs encoding for three developmental rapid myosin heavy chains (MHC) isoforms: the embryonic 3, neonatal and adult rapid MHC. Birds have been weighted, sacrificed and Pectoralis major muscles have been collected at 15 and 18 days *in ovo*, and at 0, 2, 4, 7 and 43 days post hatch. Total RNAs have been prepared. Specific primers have been chosen from the published sequences and their use validated to specifically amplify and quantify each of the three MHC isoforms using real time RTPCR. The maximal levels of each mRNA were observed at day 18 *in ovo* for embryonic 3 MHC, at day 7 post-hatch for neonatal MHC and at day 43 post-hatch for the rapid MHC. The comparison between genotypes showed higher values in the LG genotype at 7 and 43 days of age for the neonatal MHC mRNA. This suggests that the differentiation of muscle fibres in the breast muscle can be altered with growth rate.

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Isolation and identification of two slow Troponin T genes in the *Sparus aurata*: *In Silico* comparative genomic analysis with *Fugu rubripes*

(Isolierung und Identifizierung zweier Gene für Troponin T der langsamen Muskelfasern bei der Goldbrasse (*Sparus aurata*): Vergleichende genomische *in silico* Analyse mit dem Kugelfisch (*Fugu rubripes*))

Troponin T (TnT) is a component of the thin filament of striated muscle. Besides anchoring the Troponin complex (Tn), which also includes Troponin C (TnC) and I (TnI), to tropomyosin, it plays an important role in muscle contraction. Three TnT genes exist in mammals and birds which encode slow skeletal, fast skeletal or cardiac muscle TnTs. In mammals a single gene encodes slow skeletal muscle TnT (sTnT) which in mouse and human is composed of 14 exons. However, in contrast to the genes for other TnT isoforms only a few splice variants of sTnT have been identified in mouse and human and none of them have developmental specificity (Perry, *J. Muscle Res. Cell Motil.* **19(6)**, 1998). Relatively few studies of TnT exist in fish and those that do are confined to a single species, the zebrafish (*Danio rerio*) and are unlikely to be representative of the 25,000 teleosts identified. The gene for sTnT has been described in zebrafish (*Danio rerio*) and is intronless, in contrast to the terrestrial vertebrates (Hsiao et al. *Dev. Dyn.* **227**, 2003).

A modified cDNA library screening strategy was implemented using two [α-^{32}P]dCTP-labelled probes simultaneously to screen a sea bream (*Sparus aurata*) larval cDNA library. Screening was carried out at low stringency with a halibut fTnT probe against a conserved region in all TnTs and at high stringency using a 3'UTR probe for the sea bream fTnT gene. Tissue specificity of the isolated TnTs was determined by northern blot using Tri-extracted total RNA from sea bream adult white and red muscle, heart and liver. The isolated sea bream TnT sequences where used to BLAST against GenBank (www.ncbi.nlm.nih.gov), Fugu (http://fugu.hgmp.mrc.ac.uk) and Medaka databases (http://medaka.lab.nig.ac.jp*)* and phylogenetic classification of the sea bream TnT genes inferred using the maximum parsimony method with 1000 bootstrap analysis with the PAUP* Version 4.0b software package. *In silico* analysis of the putative genomic organization of Fugu and sea bream sTnT genes was carried out by aligning the sea bream sequences against the Fugu mayffolds using Spidey mRNA to Genome software package (www.ncbi.nlm.nih.gov).

In the present study the cDNA for two slow TnTs (sTnT1sb: 1,257bp; and sTnT2sb: 1,024bp) were cloned and characterised in the important aquaculture species, the sea bream. The sequences obtained shared only 43% similarity at the nucleotide level. The sTnT1sb and sTnT2sb clones found coded for different proteins, of, respectively, 268 and 240 amino acids. Northern blot analysis revealed a single transcript for each gene in sea bream adult red muscle tissue. Phylogenetic analysis showed that the two sTnT genes from sea bream clustered in independent groups. While sTnT1sb clustered

closely with tetrapod sTnT sequences, sTnT2 clustered with a group of fish specific sequences (*Fugu*, Medaka and *S. trutta*) constituting a new and not previously identified sTnT group which appears to be teleost specific. Analysis of the putative genomic organisation of Fugu/sea bream sTnT revealed they had a similar organisation to that found in mammals and both genes were composed of exons and introns, in direct contrast to the zebrafish in which the isolated sTnT is intronless (Hsiao et al. *Dev. Dyn.* **227**, 2003). Notably, the complete genomic organization of Fugu/sea bream sTnT2 was determined with this gene spanning in Fugu for ~2kb and presenting 12 exons and 11 introns.

In conclusion, at least two sTnT genes exist in sea bream, in contrast to a single gene in tetrapods. Moreover, the putative genomic structure of the Fugu/sea bream sTnT1 and sTnT2 resembles the tetrapod genomic organisation and is clearly distinct of the zebrafish intronless sTnT gene (Hsiao et al. *Dev. Dyn.* **227**, 2003). The two sTnT cDNA isolated in sea bream appear to have arisen from a teleost specific genomic duplication event (Jaillon et al. *Nature*. **431**, 2004).

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Insulin accelerates mitochondrial gene expression during L6 cell myogenesis

(Insulin stimuliert die Genexpression in Mitochondrien von L6-Zellen während der Myogenese)

Cellular energy needs are largely met by the mitochondrial oxidative phosphorylation system. The components of this system are encoded by both the nuclear and mitochondrial genomes. Given the greater energy needs of proliferating cells, it is not surprising that mitogenic factors trigger increased expression of mitochondrial genes while induction of cell quiescence has the opposite effect. An increase in both mitochondrial genome copy number and RNA level observed during terminal differentiation of myogenic cells to myotubes may be part of a myogenic differentiation program and may reflect mitochondrial response to metabolic factors that accompany their differentiation.

Insulin is the most potent anabolic hormone; its primary effect is to maintain blood glucose levels, but it also activates lipogenesis, protein synthesis and the mitogenic response. The cells most sensitive to insulin are muscle cells, liver cells and adipose cells. The stimulating effect of insulin on the mitochondrial capacity for oxidative phosphorylation may in part reflect increased expression of mitochondrial genes in skeletal muscle *in situ*.

Aim. The goal of this study was to quantify mitochondrial gene expression over the course of cellular quiescence and differentiation.

Materials and methods. Rat L6 myoblasts and HTC-IR hepatocytes were grown in DMEM supplemented with 10% FBS. Then, L6 cell differentiation was induced by culturing the cells in the presence of 2% FBS, and HTC-IR cells were made quiescent by lowering serum concentration in the medium to 1% FBS. RNA concentrations were quantified by RT-real time PCR. RNA was reverse transcribed using SuperScript II RT and random hexamers. The real-time PCR reactions were carried out with DNA-specific dye SYBR Green I. Myoblasts differentiation was analyzed by morphological markers (cells were considered fused if they contained at least three nuclei within one cytoplasmic continuity) and biochemical markers of myogenesis (immunostaining with antibodies to myogenin and myosin heavy chain). Cell proliferation was assayed by the incorporation of ^{3}H-thymidine.

Results. Low growth factor concentration in medium decreased proliferation of both cell types and induced differentiation of myoblasts. The expression of all mitochondrial genes decreased in quiescent hepatocytes whereas it increased in quiescent differentiated myotubes, as compared with proliferating cells, similarly to reflecting the expression of the *insulin receptor* gene, both in myoblasts and hepatocytes. The kinetics of mitochondrial RNA levels were similar to the expression patterns of two nuclear genes, *subunit e of mitochondrial ATP-synthase* and *uncoupling protein-2*, however they did not reflect changes in mitochondrial DNA

content. Insulin accelerated myogenesis and expression of both mitochondrial and nuclear genes in differentiated myotubes but not in quiescent hepatocytes.
Conclusion. Our studies prove that myogenesis may require the orchestrated transcriptional activation of both mitochondrial and nuclear genes and provide additional evidence confirming the regulatory impact of insulin on the function of muscle mitochondria. Although the exact mechanisms controlling mitochondrial gene expression remain to be established, these studies support the interesting hypothesis that circulating insulin may play an important role in maintaining the physiological function of mitochondria in different cell types, including skeletal muscles. Conversely, altered insulin secretion may affect cellular energy balance and result in development of several pathophysiological conditions as a consequence of mitochondrial dysfunction.

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Associations between polymorphism of some candidate genes and growth rates, feed intake and utilisation, slaughter indicators and meet quality in cattle

Abstract

We showed that the leucine/valine substitution at amino acid position 127 of the bovine growth hormone (GH) affects growth rate, feed intake/feed conversion as well as carcass traits in dairy and beef cattle. This indicates the GH gene as a potential *locus* of quantitative traits (QTL) in cattle. Moreover, associations were studied between the polymorphism at GH receptor (*GHR*), leptin (*LEP*), STAT5A, and PIT-1 *loci* and traits related to meat production in the growing Polish Friesian and beef bulls. Statistically significant differences were found between various genotypes at the *loci* studied and feed consumption, meat production, and carcass quality traits. Analysis of beef bulls revealed differences in meat quality between calpain (*CAPN1*) genotypes; the animals of the GC genotype had highest cooking loss and darker color of meat; a difference was also observed between genotypes in the total content of hem pigment of meat.

Key Words: candidate gene, meat quality, cattle

Zusammenfassung

Titel der Arbeit: **Zusammenhang zwischen Polymorphismen einiger Kandidatengene und Wachstumsrate, Futteraufnahme und -verwertung, Schlachtkörper und Fleischbeschaffenheit beim Rind**

Es wurde gezeigt, dass die Leuzin/Valin-Substitution der Aminosäure 27 im bovinen Wachstumshormon (GH) Effekte auf die Futteraufnahme und –verwertung sowie Schlachtkörpermerkmale beim Fleisch- und Milchrind hat. Weiter haben wir die Assoziation zwischen den Genorten GH-Rezeptor (GHR), Leptin (LEP), STAT5A und PIT-1 und Wachstumsmerkmalen bei Polnischen Friesian und Fleischbullen untersucht. Statistisch signifikante Unterschiede zwischen den Genotypen und Futteraufnahme, Fleischansatz und Schlachtkörpermerkmalen wurden gefunden. Untersuchungen bei Fleischrindern zeigten ferner Unterschiede in Fleischqualitätsmerkmalen zwischen Calpain (*CAPN1*) Genotypen; Tiere mit dem Genotyp GC hatten die höchsten Kochverluste und dunklere Fleischfarbe; auch Unterschiede im Gehalt an Hämpigment wurden beobachtet.

Schlüsselwörter: Kandidatengen, Fleischqualität, Rind

Introduction

Genes coding for growth hormone and other hormones and factors of the "somatotropic axis" are considered promising candidates for markers of economically important quantitative traits (QTLs). The biological effects of growth hormone (GH) involve a variety of tissues and the metabolism of all nutrient classes: carbohydrates, lipids, proteins, and minerals. These co-ordinated changes in tissue metabolism alter nutrient partitioning and thus play a key role in increasing growth performance or milk yield (ETHERTON and BAUMAN, 1998). Therefore, there is a great interest in using growth hormone to improve production traits in cattle. Moreover, the gene encoding for GH was considered a promising candidate as a marker for selection purposes (GROCHOWSKA et al., 2002). Pituitary transcription factor PIT-1, which belongs to a large POU domain family, is a positive regulatory factor for synthesis of growth hormone, prolactin, and thyrotropin β-subunit in the mammalian pituitary. Therefore, the gene encoding PIT-1 was chosen as a candidate to investigate its association with growth, carcass traits, and lactational performance in cattle (STANCEKOVA et al.,

1999). Leptin, the product of the *ob* gene, is secreted from white adipocytes and regulates food intake and whole-body energy metabolism (FRIEDMAN and HALAAS, 1998). Leptin is an important regulator of energy metabolism, adiposity and reproduction, and is perhaps linked to meat quality determinants such as marbling (HOSSNER, 1998). Leptin is also involved in the regulation of body weight and can, probably, be considered as one of the best biological markers reflecting total body fat in both animals and humans. STAT5 is a key intracellular mediator of prolactin signalling and can activate transcription of milk protein genes in response to prolactin (WAKAO et al., 1994). STAT5 is also known as a main mediator of growth hormone (GH) action on target genes (ARGETSINGER and CARTER-SU, 1996).
The calpain protease system comprised several molecules: two Ca^{2+}-dependent proteases, μ-calpain and m-calpain, and a third polypeptide, calpastatin, whose only known function is to inhibit the two calpains. This proteolytic system plays a key role in the tenderisation process that occurs during *post-mortem* storage of meat under refrigerated conditioning. The polymorphism of genes encoding these proteases is examined from the point of view of its effect on corresponding production traits. The calpain genes are investigated as a potential candidate for quantitative trait loci (QTLs) affecting meat tenderness.

Material and methods

One hundred forty five young Friesian bulls were progeny of 24 A.I. bulls, and were born in herds of 4500-5000 kg milk yield. The number of half-sibs varied from 3 to 9. The bulls were housed in a tie-stall and fed *ad libitum* on silage, hay and concentrate, while during the growth performance-testing period (28 days from 7^{th} to 8^{th} month of age) received *ad libitum* a full concentrate diet. All bulls were slaughtered at the age of 15 months, after 24 hours of fasting. The carcasses were chilled for 24 hours at 4^{o} C. Right carcass-sides were dissected. The estimation of slaughter value was based on cold carcass weight, dressing percentage, weight of valuable cuts in carcass-side (round, shoulder, best ribs, fore ribs sirloin).
Blood samples for DNA genotyping were collected from jugular vein. Blood was collected on K_2EDTA and stored at -25^{o}C for few weeks or at -75^{o}C up to several months. The isolation of DNA from whole blood was done with the method described by KANAI (1994).
The GH genotypes were determined according to Lucy et al. (1993). The primer sequences were: 5'-CCGTGTCTATGAGAAGC-3' and 5'-GTTCTTGAGCAGCGCGT-3'. 30 amplification cycles included:94 ^{o}C-30s; 60 ^{o}C –1 min; 72 ^{o}C –30s. The amplified DNA was digested with *AluI* restriction nuclease.
Genetic variants of the PIT-1 gene were identified according to MOODY et al. (1995). The sequences of primers were: 5'-CAATGAGAAAGTTGGTGC-3' and 5'-TCTGCATTCGAG-ATGCTC-3'. Initial cycle of 95°C - 2 min, 55°C - 1 min and 72°C – 2 min was followed by 29 amplification cycles: 94^{o}C - 45 sec.; 55^{o}C - 1 min; 72^{o}C - 1 min., and concluded with a final extension at 72°C for 2 min. The amplified 1355-bp-long DNA fragment was digested with *Hinf*I restriction nuclease.
Leptin (LEP) genotypes were identified according to POMP et al. (1997). The 1820-bp fragment of the bovine leptin gene was amplified using following primers: 5´-GTCACCAGGA-TCAATGACAT-3´ and 5´-AGCCCAGGAATGAAGTCCAA-3´.

The PCR amplification cycles were: 95^{o} C– 1 min; (95^{o}C-1min, 60^{o}C-2min, 72^{o}C-3 min) x 32 cycles; 72^{o}C – 7 min. The PCR product was digested with *Sau3*AI nuclease. The following PCR primers were designed for STAT5A genotyping: 5'-CTGCA-GGGCTGTTCTGAGAG-3'; 5'-TGGTACCAGGACTGTAGCACAT-3'. The polymerase chain reactions were performed using a PCR-mix with: primers, both at concentration 5.0 pmol/ml, 1 U Taq polymerase (Sigma), 1 µl Taq polymerase buffer four dNTPs, each at final concentration of 0.2 mM, ca 100 ng of genomic DNA, and H_2O up to 10 µl. The following PCR protocol was used: 1 min at 94^{o}C, 1 min at 61^{o}C and 1 min at 72^{o}C - 34 cycles. PCR products were digested in 10-µl with 10 U of *Ava*I restriction nuclease (BioLabs, New England, USA) for 3 hours at 37^{o}C.
The 670 bp DNA fragment (exon 14 to exon18 - the region coding for protein large subunit of domain IV, and including introns) in the *CAPN1* gene was amplified. PCR amplified using the following primers: CAPN1: 5`-TTCAGGCCAATCTCCCCGACG–3' (exon 14); CAPN2: 5`-GATGTTGAACTCCACCAGGCCCAG -3`(exon 18). The following PCR protocol was used: 30 s at 94°C, 45 s at 62°C and 45 s at 72°C – 32 cycles. PCR produced amplified DNA product was digested with 10 U of *Fok*I restriction nuclease.
All PCR reactions were performed in MJ Research TETRAD thermal cycler. The restriction fragments were subjected to electrophoresis in 2% agarose/ethidium bromide gels (Gibco, BRL, England) in 1 x TBE buffer (0.09 M Tris-boric acid, 0.002 M EDTA). Gels were visualised under UV light and documented in FX Molecular Imager apparatus (Bio-Rad). The studied marker *loci* were expressed either as genotype classes or as regressions on the amount of presence of the alternative allele and dominance. The reference genotypes were LL, LV, VV for *locus* GH, AA, AB, AC for LEP, AA, AB, BB for PIT-1, CC, CT for STAT5 and CC, CT TT for CAPN1 *locus*.

Results

The allele frequencies at the studied *loci* were: 0.65/0.35 for GH L/V variants, 0.85/0.07/0.08 for LEP A/B/C variants, 0.25/0.75 for PIT-1 A/B variants, 0.90/0.10 for STAT5 C/T variants and 0.27/0.73 for CAPN1 T/C variants. Body weight and feed intake capacity were strongly dependent on the GH genotype. The LV heterozygotes were heaviest and consumed most. It also may be concluded that the feed intake was very much a function of body weight. The GH genotype affected only the meat amount (kg) in the carcass. The LL homozygotes and LV heterozygotes proved to be related to higher meat deposition (kg) than VV homozygotes; the differences attained 3.20 kg (LL *vs* VV) and 2.56 kg (LV *vs* VV) and were both significant. There was no direct effect of GH genotype on carcass bone and fat as well as weight of valuable cuts.
The polymorphism of leptin *locus* (*LEP*) significantly influenced carcass traits measured in this study. The weight of carcass side was the highest for the AA homozygotes. The AA homozygotes also had a higher fat deposition (19.2 kg) than AC heterozygotes (17.9 kg); the difference was significant. The per cent of valuable cuts was the highest for the AC genotype bulls – 62.7 as compared to 61.9 for AB and 62.2 for AA animals. In general, bulls of AA LEP genotype consumed more feed than AB or AC heterozygotes.

In the present study the effect of PIT-1 genotype was observed on carcass measurements traits only. The AA homozygotes had a higher girth and depth of chest and girth of round, but BB homozygotes had a higher round dimension. In the 28–days test period the bulls AB at the PIT-1 *locus* consumed more dry matter, protein, protein digested in small intestine (PDI) and feed units for maintenance and meat production (UFV) as compared to AA or BB genotypes.
The CT genotype of the STAT5A gene - was associated with higher weight of bone in best ribs (4.2 kg *vs* 3.8 kg) and in sirloin (1.6 kg *vs* 1.3 kg) and oblique carcass length (112 cm *vs* 110 cm). The CC genotype was associated with the significantly faster growth rate from 8 to 15 months (1.04 kg daily *vs* 0.97 kg).
The TT genotype of CAPN1was significantly associated with higher lean share in valuable cuts. The advantage over CC genotype was 4.5%. Statistically significant differences were found between various genotypes at the *loci* studied and feed consumption, meat production, and carcass quality traits. Moreover, the analysis revealed differences in meat quality between the animals of different calpain (*CAPN1*) genotypes; the animals of the GC genotype had highest cooking loss and darker color of meat; a difference was also observed between genotypes in the total content of hem pigment of meat.

Discussion

The results on genetic action of particular *loci* may be biased due to failing to account for the sire in the model. In the present study the structure of data was very close to paternal half sib group design. The best approach would than be to analyse the data within sire. Yet, some of the sires had only one progeny in the sample, and the sires themselves had not been genotyped for the studied *loci*. With small progeny groups not all the possible genotypes were present within sire. On the other hand, placing a sire in the model explains half of the fixed genotype at a particular *locus*, while correcting for the random part of his genotype.
Hormones, growth factors, and other regulatory proteins associated with so called "somatotropic axis" are candidate markers for quantitative traits in farm animals. Genes encoding for growth hormone (GH), GH receptor (GHR), transcription factor PIT-1 (activating expression of GH and prolactin genes in the anterior pituitary), insulin-like growth factor-I (IGF-1), and perhaps presently unknown genes coding for GH signal transduction pathways, could contribute to the progress in genetic selection of farm animals. In spite of this, only few studies have been carried out of the effect of these genes on growth performance and carcass traits in the cattle. SCHLEE et al. (1994) showed a significant effect of GH L/V genotype on meat breeding values of Simmental bulls. In their study the heterozygous LV genotype was superior to LL and VV heterozygotes in both carcass gain and meat value. However, if classification score was considered, the LL genotype was significantly better than LV and VV. In the same breed, CHRENEK et al. (1998) found that bulls with VV genotype had a lower body weight in comparison to the bulls with LL or LV genotypes. PIT-1 has been described as the critical cell-specific transcription factor responsible for activating expression of the prolactin (PRL) and GH genes in the anterior pituitary gland. To date, nine different mutations in the PIT-1 gene have been described in mammals. Four mutations have effects on DNA binding, causing GH- and-PRL gene disorders (RENAVILLE et al., 1997). Because the PRL and the GH are essential for mammary

gland development and milk yield, the PIT-1 gene has potential as a marker for genetic variation in milk yield traits. WOOLARD et al. (1994) as the first described polymorphism within bovine PIT-1 gene – RFLP detected with *HinfI* nuclease. Within Italian Holstein-Friesian cattle the allele A of the PIT-1 gene (frequency 0.18) showed a significant superiority over the allele B for milk yield, protein yield and body conformation traits (RENAVILLE et al., 1997).

In the present report the frequency of allele A in the PIT *locus* was 0.25; similar to that previously found in Polish Friesian cattle - 0.26 (KLAUZIŃSKA et al., 2000) and in Canadian Holstein bulls - 0.21 (SABOUR et al., 1996). In the study by RENAVILLE et al. (1997) and in our previous study (ZWIERZCHOWSKI et al., 2002) it was shown that allele A in the PIT *locus* positively influenced milk production traits in Friesian cattle. Only few studies have been performed of the effect of leptin gene polymorphism on performance traits in cattle. LEIFERS et al. (2002) have reported on the association between the leptin genotype and milk production traits in Holstein-Friesian cows; AB genotype was associated with more produced milk. They concluded that LEP allele B could yield a higher milk production without negatively affecting energy balance and fertility. The estimated frequency of LEP A allele in the presently studied population of Friesian cattle was 0.85, and was slightly higher then previously reported for Holstein cattle - 0.71 (POMP et al., 1997), but about the same as in another population of Polish Friesian cattle - 0.80; (KLAUZIŃSKA et al., 2000). The frequency of variant C was 0.10. This variant of the leptin gene appeared favourable for some milk production traits in Polish Friesian cattle; cows carrying this allele (genotype AC) showed higher daily yield of milk components - fat protein, and lactose – then those with AA and AB genotypes (ZWIERZCHOWSKI et al., 2002).

Only in few cases nucleotide sequence polymorphism has been detected in the bovine STAT5A gene. MCCRACKEN et al. (1997) found TG repeats of different length within the gene in the intron 12. ANTONIOU et al. (1999) found two SSCP variants of the gene fragment that encodes SH2 domain in bovine STAT5A protein. In neither case an association with production traits was studied. Recently, we identified 15 new polymorphic sites within the 5' region of the bovine STAT5A gene (FLISIKOWSKI and ZWIERZCHOWSKI, 2003; GenBank, accession number AY280369), most of them representing single nucleotide polymorphisms (SNPs). Moreover, the new nucleotide sequence polymorphism was found in the coding region of the bovine STAT5A gene - substitution C/T at position 6853 within the exon 7, recognisable by PCR-RFLP with the *Ava*I and *Dde*I restriction nucleases (FLISIKOWSKI et al., 2003). We showed that beef cattle with CC variant of the STAT5A gene were superior over CT animals for weight gain, several carcass traits, and feed conversion.

Initial statistical analysis suggests that a newly identified mutation in CAPN1 gene was associated with some carcass traits. The TT genotype of CAPN1 and possibly of T allele appeared favourable for important meat production traits in different cattle breeds. In addition, the analysis conducted to investigate the effects of the genotypes on physical and chemical traits of meat showed that CC genotype was associated with highest fat content, but CT genotype had best tenderness of meat. The sensory evaluation showed that that CT had better cooking quality of meat than CC bulls.

In conclusion, the results presented here confirm the value of GH, LEP PIT-1 loci as markers for carcass traits and feed intake in the growing cattle. Our results also showed the value of the STAT5A *locus* as a marker for carcass traits in the cattle.

Thus, we confirmed that these *loci* are candidate genes that may themselves produce differences in growth phenotypes. For the first time the association of the *CAPN*1 gene with meat quality was shown; significant differences being found between bulls of different *CAPN*1 genetic variants. The important message from these results is that these mutations can be used in further research seeking the associations between gene polymorphism and meat quality traits in cattle.

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Marker assisted introgression of the *Compact* mutant *myostatin* allele: $Mstn^{Cmpt\text{-}dl1Abc}$ into a mouse line with extreme growth: effects on body composition, muscularity and skeletal muscle cellularity

Abstract

Myostatin is a negative regulator of muscle growth, and mutations in this gene are associated with muscular hypertrophy and reduced fat in mice, humans and cattle. Marker assisted introgression (MAI) was used to introduce a murine *myostatin* mutation, $Mstn^{Cmpt\text{-}dl1Abc}$ (*Compact, C*), into a high growth mouse line (DUHi) Objective: To investigate the effects of myostatin-deficiency in a high growth background on growth, body composition and, using samples from *M. rectus femoris* and *M. longissimus dorsi*, on muscle histology.
Results: *Inter se* matings between C/+ animals up to generation 10 produced a total of 838 genotyped pups, 29% +/+, 63% +/*C*, and only 8% *C*/*C*. This highly significant distortion of the segregation ratio shows that *Compact* is associated with lower fitness on the DUHi- background. Homozygous (*C/C*) mice were, compared to wildtype (+/+) mice, c4-5% lighter, had c7-8% shorter tails, increased muscle weights (e.g. *M. quadriceps* in males was 59% heavier), an increased 'dressing percentage' (c49 vs. 39%) and the weights of several organs (e.g. liver, kidney, heart) were significantly reduced (12 - 20%). Total body fat content was higher in +/+ animals (c17.5%) than in *C/C*-animals (10-12%). Total muscle fibre number was increased by 24%, whereas fibre size was not significantly affected. Protein and DNA concentrations, DNA/protein ratios and specific CK activity remained unchanged, indicative of increases in the contents of total DNA and muscle specific protein with increased muscle mass. Fibre type distribution was shifted to white glycolytic muscle fibres (+c16% units) at the expense of red oxidative fibres. Capillary density was substantially lower in *C/C* than in +/+ mice, with lower number of capillaries per fibre (-35%) and larger fibre area per capillary (+77%).

Key Words: myostatin, mutation, muscle growth, muscle histology, mouse, model animal

Zusammenfassung

Titel der Arbeit: **Marker gestützte Einkreuzung des mutierten *Compact myostatin* alleles: $Mstn^{Cmpt\text{-}dl1Abc}$ in einer Mauslinie mit extremem Wachstum: Effekte auf die Körperzusammensetzung, Muskelentwicklung und Mikrostrukturmerkmale der Skelettmuskulatur**

Myostatin ist ein negativer Regulator des Muskelwachstums, und Mutationen in diesem Gen führen zur Muskelhypertrophie und zu einem reduzierten Fettansatz, wie es für Mäuse, Menschen und Rinder gezeigt wurde. Mittels markergestützter Einkreuzung wurde die murine *myostatin* Mutation, $Mstn^{Cmpt\text{-}dl1Abc}$ (*Compact, C*), in eine auf hohes Wachstum selektierte Mauslinie (DUHi) eingekreuzt, um den Effekt eines Myostatinmangels auf dem genetischen Hintergrund einer Linie mit hoher Wachstumsveranlagung auf das Wachstum, die Körperzusammensetzung und Muskelstruktur zu untersuchen. Proben des *M. rectus femoris* und des *M. longissimus dorsi* aller drei Genotypen dienten der Beurteilung von Einflüssen auf die Miskrostruktur der Skelettmuskulatur. Ergebnisse: *Inter se* Paarungen zwischen *C*/+ Tieren bis Generation 10 erbrachten insgesamt 838 genotypisierte Jungtiere: 29% +/+, 63% *C*/+, und nur 8% *C*/*C*. Diese hoch signifikante Störung des Segregationsverhältnisses zeigt, dass *Compact* zu einer verminderten reproduktiven Fitness in der Linie DUHi führt. Homozygote (*C/C*) Tiere waren im Vergleich zu den nicht mutierten Wildtyp-Tieren (+/+) etwa 4-5% leichter, hatten ca. 7-8% kürzere Schwänze, höhere Muskelgewichte (z.B. war der *M. quadriceps* der männlichen Tiere um 59% schwerer) sowie eine um 10% höhere Schlachtausbeute. Das Gewicht verschiedener Organe (z.B. Leber, Nieren, Herz) war signifikant reduziert um 12 bis 20%. Der Fettanteil von +/+ Tieren war höher (ca. 17.5%) als bei *C/C*-Tieren (10-12%). Die Gesamtanzahl der Muskelfasern nahm um 24% zu, während die Muskelfasergröße nicht signifikant beeinflusst war. Die Protein- und DNA-Konzentrationen, das DNA/Protein Verhältnis sowie die spezifische Creatinkinase-Aktivität im Muskel blieben unverändert. Das weist darauf hin, dass bei erhöhter Muskelmasse die Gesamtgehalte von DNA und muskelspezifischem Protein erhöht waren. Die

Faserverteilung war in Richtung weißer, glykolytischer Muskelfasern (+ ca. 16% Punkte) zu Lasten der roten, oxidativen Fasern verschoben. Die Dichte der Blutkapillaren von *C/C*- Tieren war wesentlich geringer als die von +/+ Mäusen, was sich in einer geringeren Zahl von Kapillaren pro Faser (-35%) und in einer größeren Faserfläche pro Kapillare (+77%) niederschlug.

Schlüsselwörter: Myostatin, Mutation, Muskelwachstum, Muskelstruktur, Maus, Modelltier

Introduction

Myostatin is a TGF-β family member that acts as a negative regulator of muscle growth. Mice, humans and cattle deficient for myostatin have a widespread and dramatic increase in skeletal muscle mass (MCPHERRON and LEE, 1997; ARNOLD et al., 2001; SCHUELKE et al., 2004). 'Double- muscling' (DM) caused by natural mutations in the *myostatin* gene has been seen in several cattle breeds, in humans and in one mouse line. In some breeds of cattle, the tremendous increase in muscle fibre number is associated with higher muscle mass of at least 20% and decreases in fat (SHAHIN and BERG, 1985; WEGNER et al., 2000). DM results in excessive muscle fibre formation (hyperplasia), which is a prenatal event. Skeletal muscles of DM cattle contain almost double the number of fibres of other cattle breeds, whereas fibre size is similar or slightly larger when animals get older (OUHAYOUN and BEAUMONT, 1968; WEGNER et al., 2000). In different transgenic mouse models with myostatin modifications, either muscle fibre hyperplasia (NISHI et al., 2002) or hypertrophy (ZHU et al., 2000; GROBET et al., 2003) or both (MCPHERRON et al., 1997) contributed to increased skeletal muscle mass. For DM cattle, at the level of muscle tissue a shift to glycolytic metabolism has been shown by higher proportions of fast-twitch glycolytic (white) fibres and these fibres are associated with paler meat (HOLMES and ASHMORE, 1972; WEGNER et al., 2000). Whether the decreased animal endurance and higher anaerobic muscle capacity is also related to changes in muscular capillary supply has not been investigated. Although DM is associated with many positive effects in cattle, e.g. on meat quantity and quality, there are reports of potential and observed negative side effects in reproductive traits and stress resistance, with substantial evidence of epistatic gene interaction (WIENER et al., 2002), suggesting it may be possible to select for the beneficial effects while minimising the impact of the negative effects. Since the discovery and mapping of myostatin it has become the subject of much research, and breeding companies in the meat producing sector (pigs, poultry, sheep, and also in fish) are devoting considerable effort to screening for mutations in this gene using high-throughput methods with the hope of finding myostatin variants associated with increased muscularity. However, there are reports on negative side effects from studies on cattle and on our mice. Therefore, it is important that the impact of variation in this gene on a wide range of production and welfare traits is fully explored before it becomes a focus of selection for breeders of meat producing livestock.

Mouse model. In 1997 the mode of inheritance of a new hypermuscular mouse mutation termed ''C*ompact*'' (*Cmpt*) (VARGA et al., 1997) was reported. This mutation arose or became visible in a selection line named here BEH (**Be**rlin **H**igh, BÜNGER et al., 2001a) in the course of a selection experiment on protein amount conducted at the Technical University of Berlin (WENIGER et al., 1974). Linkage mapping, fine mapping and sequencing in a BEH-based population revealed a single locus on chromosome 1 and finally a 12-bp deletion in the myostatin gene (*Mstn*$^{Cmpt-}$

^{dl1Abc}) was found to be the causative mutation responsible for the hypermuscularity in BEH mice (SZABO et al., 1998).
The objectives of the model experiment with mice described here were to utilise marker assisted introgression of *Mstn*$^{Cmpt\text{-}dl1Abc}$ to estimate its effects on muscle characteristics and body composition on the genetic background of an extreme high growth line, and to prepare the ground for future investigations of side effects on reproduction and welfare traits in this model multiparous species.

Material and methods

Animals. The BEHi (BEH inbred) line homozygous for *Cmpt* was derived in the Edinburgh lab by inbreeding from BEH (BÜNGER et al., 2001a). A heavy long-term growth selected and inbred mouse line (DUHi, BÜNGER et al., 2001b) was used as the recurrent backcross (BC) partner and *Mstn*$^{Cmpt\text{-}dl1Abc}$ was introgressed by marker assisted introgression (MAI) into this line. The F1 was followed by 5 generations of repeated backcrossing to the DUHi line. The genotyping for *Mstn*$^{Cmpt\text{-}dl1Abc}$ for the MAI was described earlier (SZABO et al., 1998). Heterozygous animals of this line were mated *inter se* in generations 5 (= BC4) and 6 (=BC5) to give homozygous wild-type (+/+), heterozygous (denoted *C*/+) and homozygous (denoted *C*/*C*) animals for the *Compact* (*Mstn*$^{Cmpt\text{-}dl1Abc}$) mutation. These animals were used for the dissection at 70d of age with details given elsewhere (BÜNGER et al., 2004).
Cellular and metabolic characteristics of skeletal muscle. These were examined by histological/ histochemical and biochemical analyses of *Rectus femoris* (RF) and/or *Longissimus dorsi* (LD) muscles. Details of the methods to determine total muscle fibre number, fibre size, fibre type distribution, capillary density, DNA and protein concentrations, and creatine kinase activity are given by REHFELDT et al. (2005).
Statistical methods. The data were subjected to analysis of variance with GLM using the SAS System for Windows Release 6.08 (SAS Institute Inc., Cary NC 27513 USA). Data were adjusted for generation effects but body weight was not included as a co-variable.

Results

Genotype frequencies (inter se matings). In total 838 pups from *inter se* matings were genotyped. Of those 244 (29.1%), 528 (63.0%), and 66 (7.9%) were homozygous wildtype (+/+), heterozygote (*C*/+), and homozygous for *Compact* (*C*/*C*) respectively, indicative of a significant distortion of the segregation ratio (for details see BÜNGER et al., 2004).
Body weight and dimensions (Compact *C*/*C* vs. wildtype +/+ homozygotes). Homozygous *C*/*C* animals of both sexes were c.4-5% lighter than their +/+ littermates (significantly only in males, Table). *Compact* females had significantly shorter bodies, whereas there was no significant difference for males, but both sexes had significantly shorter tails (8-9%). The maximal width in the lumbar region ("belly width") of *Compact* animals was significantly reduced in both sexes by over 11-12%. The increased muscularity in *Compact* animals of both sexes resulted in e.g. increased leg measures, which were 13 to 20% higher in *C*/*C* animals.
Organ and tissue weights. Heart, kidney, liver and the digestive tract in *C*/*C* animals were significantly reduced in both sexes by 16 to 23%, but the weights of the spleen were significantly reduced only in males. The lung was not affected.

Muscling traits. *Compact* animals had, as expected, much heavier carcasses and these differences amount to 18% (f) and 19% (m). This had substantial effects on the ratio of the carcass to the total body weight: those for +/+ and *C*/*C* were 39.8 and 49.1% in females and 38.7 and 48.7% in males. The weight of the whole left leg in *C*/*C* animals was increased by 27% and 29% and that of the quadriceps by 47% and 54% in females and males respectively.

Fatness traits. Homozygous *Compact* animals of both sexes had significantly smaller amounts of total body fat, reduced in females by 42% and in males by 34%, resulting in substantial changes of fat content (%). Whereas +/+ females and males contained 17.5 and 17.4% fat, respectively, the values for *C*/*C* animals were 9.5 and 11.6%. Similarly, the measured individual fat depots were reduced by 61 to 70% in females and by 45 to 54% in males with a higher reduction in perirenal and retroperitoneal fat.

Cellular and metabolic characteristics of skeletal muscle. In generation 6, homozygous *C/C* mice were 12% lighter than their +/+ littermates (data not shown). The heterozygous *C*/+ animals were significantly heavier than both homozygotes, which is consistent with the results for the whole data set from generations 5 and 6 (see above). Although the body weight was lower in *C/C* than in +/+ animals, the QF muscle group was 38% heavier, consistent in both sexes. The ***cross sectional area*** of the constituent RF ***muscle*** (MCSA) was on average 21% larger, with an 18% and 23% increase in females and males, respectively (data not shown). This difference in MCSA resulted mainly from high ***total number of fibres*** per muscle cross section, which was 24% higher in *C/C* than +/+ mice (Figure 1A). Although genotype by sex interactions were not significant, the increase in fibre number was greater in males (35%) than in females (13%) (data not shown). For the *C/C* genotype, females had only 81% of the male fibre number ($P < 0.05$). Dominance effects of the $Mstn^{Cmpt\text{-}dl1Abc}$ allele, which are essentially a comparison between heterozygotes (*C*/+) and the unweighted mean of the two homozygotes, were not observed for total fibre number. The average ***size of*** all RF ***muscle fibres***, measured as fibre cross sectional area (FCSA), did not differ between the genotypes (Figure 1A). However, the white fibres were significantly smaller in *C/C* than +/+ mice (-15%), resulting from significant decreases in males and a corresponding tendency in females (data not shown). There were no differences in the FCSA of red and intermediate fibres between the genotypes over the sexes; however genotype by sex interactions revealed smaller fibres in males, but larger fibres in females in the *C/C* than the +/+ group. In LD and RF, the number of muscle fibres/mm² that can be taken as another, but inverse, estimation of the average FSCA, was not significantly different between the genotypes (data not shown) and thereby consistent with the results of direct measurements of the FCSA in RF.

Estimates of the ***fibre type composition*** for the muscles of the +/+ wild-type mice were approximately 60% white and equal proportions (20%) of red and intermediate fibres (Figure 1B). The percentage of white fibres was markedly higher in homozygous *C/C* (RF and LD 79%) than +/+ mice (RF 63%; LD 62%), whereas that of red, oxidative fibres was lower (RF 7% vs. 21%; LD 9% vs. 19%). The percentage of intermediate fibres was also lower in *C/C* animals, significantly so only in LD muscle. The effects of the $Mstn^{Cmpt\text{-}dl1Abc}$ allele on the percentage of white muscle fibres were partially recessive as the *C*/+ animals were closer to the +/+ than to the *C/C* animals. Fibre type distribution was largely independent of sex (data not shown). The ***distribution of capillaries*** was examined in RF muscle. The average number of capillaries per muscle

fibre was 35% lower and the muscle fibre area associated with one capillary was up to 77% greater in homozygous *C/C* than wild-type +/+ animals (Figure 1C). The *Mstn*$^{Cmpt\text{-}dl1Abc}$ allele was found to be completely recessive for fibre area per capillary ($P<0.05$), while a corresponding tendency was observed for capillaries/fibre ($P=0.16$). Further data (not presented) show that more capillaries are associated with red fibres than with intermediate or white fibres. These relationships were the same within the genotypes; while the differences between the genotypes described above were, at least numerically, observed for each fibre type. Finally, the distribution of capillaries was independent of sex and no significant sex by genotype interactions were observed, although differences were more pronounced in males than in females (data not shown). Neither genotype nor sex was found to affect muscular ***protein and DNA concentrations,*** the ***DNA/protein ratio*** (data not shown),or the ***activity of*** the muscle specific ***creatine kinase*** was the same for all genotypes and both sexes. As muscle weights were substantially higher in *C/C* than in +/+ mice unchanged concentrations reveal increases in the total DNA and protein contents and total CK activity.

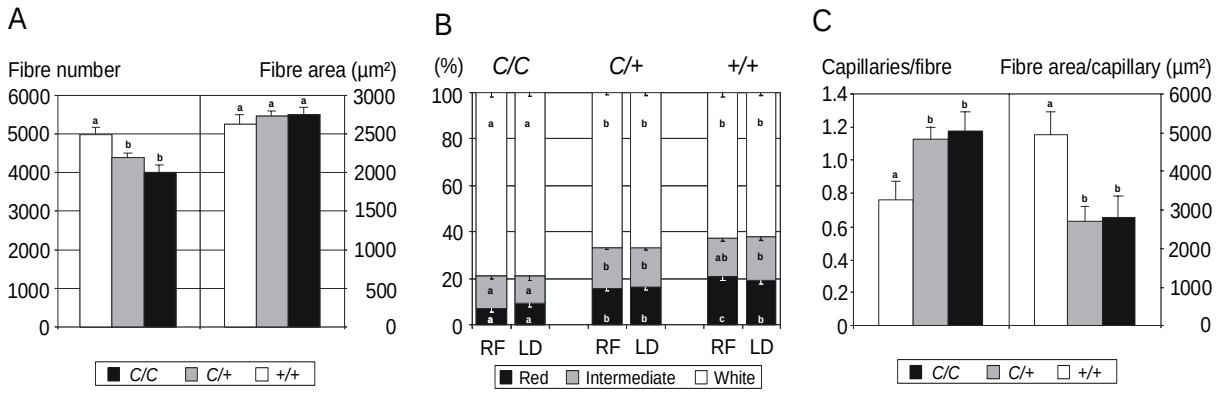

Figure 1: A. Muscle fibre number and muscle fibre size of rectus femoris (RF) muscle. B. Distribution of metabolic muscle fibre types in RF and longissimus (LD) muscles. C. Density of blood capillaries in RF muscle. Bars showing a common character are not significantly different ($P > 0.05$)

Discussion

The *Mstn*$^{Cmpt\text{-}dl1Abc}$ *mutation* (*Compact*) had significant effects on all measured traits except the lung on the DUHi background. Essentially the animals were much leaner, slightly lighter, shorter, especially in the tail, had reduced maximal width in the lumbar region and increases in measures reflecting or presenting hypertrophic muscularity like leg measures, carcass weight (g and %), muscle and leg weights. These effects promoting the development of muscle tissue were associated with substantial reductions in the weight of almost all organs. This effect becomes even stronger when organ weights were expressed as a ratio to the body weight as *C*/*C* animals were somewhat lighter. The effects of sex were significant for all traits but fat%. No significant sex by genotype interactions were found, except for the weight of the quadriceps, indicating that both sexes generally reacted in a similar way to the myostatin deficiency (Table), when values are not corrected for body weight.

Myostatin deficiency caused by the murine mutation *Mstn*$^{Cmpt\text{-}dl1Abc}$ does not result in additional body growth on a genetically high growth background and suggests that in *C*/*C* animals the priority of muscle protein is increased over other tissues like fat, some other organs and possibly bone.

Table
Weights, dimensions and body composition of 70 d old mice homozygous (*C*/*C*), heterozygous (*C*/+) and wildtype (+/+) for *Mstn*$^{Cmpt\text{-}dl1Abc}$

		females				males					
		C/*C*	*C*/+	+/+	*C*/*C* vs. +/+	*C*/*C*	*C*/+	+/+	*C*/*C* vs.	sd	S G X
trait	n	8	67	35	(%)	12	74	56	(%)		
body weight and dimensions											
body weight	(g)	59.7^{e}	65.4^{d}	62.0^{e}	-3.7	76.8^{c}	85.2^{a}	81.0^{b}	-5.2	7.28	S G -
body length	(cm)	11.2^{e}	11.8^{c}	11.6^{d}	-3.1	11.8^{b,c}	12.1^{a}	11.9^{b}	-0.4	0.335	S G -
tail length	(cm)	10.2^{e}	10.8^{d}	11.0^{c}	-7.9	10.5^{e}	11.3^{b}	11.6^{a}	-9.4	0.527	S G -
width lumbar region-max	(cm)	3.96^{c}	4.60^{b}	4.49^{b}	-11.7	4.47^{b}	5.04^{a}	5.04^{a}	-11.2	0.407	S G -
width upper rear leg	(cm)	1.85^{b,c}	1.73^{d}	1.64^{e}	12.7	2.03^{a}	1.86^{b}	1.78^{c,d}	14.3	0.140	S G -
width lower rear leg	(cm)	1.06^{b}	0.96^{c}	0.89^{d}	19.6	1.16^{a}	1.03^{b}	0.97^{c}	19.2	0.072	S G -
organ and tissue weights											
heart	(g)	0.243^{c}	0.307^{b}	0.293^{b}	-17.2	0.326^{b}	0.384^{a}	0.388^{a}	-16.2	0.061	S G -
kidney	(g)	0.640^{d}	0.740^{c}	0.759^{c}	-15.7	0.937^{b}	1.171^{a}	1.160^{a}	-19.2	0.131	S G -
liver	(g)	3.12^{c}	3.99^{b}	3.89^{b}	-19.8	3.84^{b}	4.86^{a}	4.88^{a}	-21.4	0.619	S G -
lung	(g)	0.919^{b,c}	0.981^{b,c}	0.932^{c}	-1.3	0.953^{b,c}	1.09^{a}	1.05^{a,b}	-9.1	0.237	S - -
spleen	(g)	0.244^{a,b}	0.244^{a,b}	0.258^{a}	-5.4	0.176^{c}	0.234^{b}	0.227^{b}	-22.5	0.058	S G -
digestive tract	(g)	8.2^{c}	10.7^{b}	10.5^{b}	-21.9	9.9^{b}	12.9^{a}	12.9^{a}	-23.2	1.63	S G -
muscling traits											
carcass weight	(g)	28.9^{d}	27.3^{d}	24.6^{e}	17.7	37.4^{a}	35.0^{b}	31.3^{c}	19.4	2.91	S G -
carcass weight	(%)	49.1^{a}	41.9^{b}	39.8^{d}	23.6	48.7^{a}	41.1^{c}	38.7^{e}	25.8	2.18	S G -
left leg	(g)	4.45^{c}	3.95^{d}	3.51^{e}	26.9	5.71^{a}	5.05^{b}	4.42^{c}	29.4	0.459	S G -
m. quadriceps (right)	(g)	0.593^{b}	0.473^{c}	0.405^{d}	46.6	0.756^{a}	0.590^{b}	0.493^{c}	53.5	0.062	S G X
fatness traits											
total body fat	(g)	6.4^{c}	10.8^{b}	11.0^{b}	-42.2	9.4^{b}	13.7^{a}	14.2^{a}	-33.7	3.75	S G -
total body fat	(%)	9.5^{b}	16.2^{a}	17.5^{a}	-45.9	11.6^{b}	15.6^{a}	17.4^{a}	-33.6	4.76	- G -
epididymal fat	(g)	0.627^{d}	2.05^{b}	2.07^{b}	-69.7	1.36^{c}	2.45^{a}	2.49^{a}	-45.3	0.837	S G -
perirenal and retroperitoneal fat	(g)	0.50^{c}	1.44^{b}	1.28^{b}	-61.1	0.78^{c}	1.66^{a}	1.69^{a}	-53.9	0.523	S G -

Means sharing a common character in their superscript a not significantly different (P > 0.05). sd – standard deviation pooled over all groups. *S G X*: tests for sex, genotype and sex by genotype interaction; '-' indicates the effect is not significant (P > 0.05)

This experiment has clearly shown that *Mstn*$^{Cmpt\text{-}dl1Abc}$ has substantial negative effects on fatness and positive effects on muscle hypertrophy even though the mouse line into which it was introgressed is the heaviest line selected to date (BÜNGER et al., 2001b). *Mstn*$^{Cmpt\text{-}dl1Abc}$ is associated with a similar, possibly less drastic phenotype than associated with mice with ablated *myostatin* expression created using transgenic technology (MCPHERRON et al., 1997). Thus *Mstn*$^{Cmpt\text{-}dl1Abc}$ can be considered as another allele affecting *myostatin* activity and like other deletions, knockouts and substitutions, results in extreme muscularity. Although the precise molecular mechanisms are unknown and might differ between these different mutations, the impact of perturbing *myostatin* expression is similar on the phenotype and *Mstn*$^{Cmpt\text{-}dl1Abc}$ is therefore an immediately available and valuable model for other mammalian species, especially farm animals, and will help to provide information on the consequences of the widespread use of such natural mutations or transgene knockouts in livestock.

The introgression of *Compact* into the growth-selected DUHi mouse line was shown to affect the cellular characteristics of skeletal muscle in a substantial way. ***Hyperplasia or hypertrophy?*** The higher muscle mass found in *Compact* homozygous DUHi resulted exclusively from increases in muscle fibre number (hyperplasia) but not in size (hypertrophy), at least for RF and LD muscle. The observations that heavier muscle masses were coupled with similar DNA and protein concentrations, DNA:protein ratios, and creatine kinase activities suggest that hypermuscularity in *C/C* animals was associated with substantial and balanced increases in myonuclear proliferation and muscle specific protein accretion. During postnatal development the individual muscle fibres generally increase in size more slowly when the number of fibres (formed prenatally) is high and *vice versa* (REHFELDT et al., 2000). However, the individual fibres of *C/C* mice were not smaller but similar in size to those of wild-type mice despite the substantial increase in fibre number. During prenatal myogenesis the number of proliferating myoblasts is important in determining the number of muscle fibres (hyperplasia), which, in mammals, remains almost unchanged after birth (for review see REHFELDT et al., 2000; STICKLAND et al., 2004). Postnatally, mitotically active satellite cells are the source of new myonuclei and essential for postnatal fibre hypertrophy and regenerating processes (MOSS and LEBLOND, 1971; SCHULTZ, 1974). It has been suggested that proliferation of either myoblasts (prenatally) or satellite cells (postnatally) are under the control of myostatin (THOMAS et al., 2000; TAYLOR et al., 2001; LANGLEY et al., 2002; AMTHOR et al., 2002). In summary, a deficiency of myostatin, which acts as a negative regulator of myoblast/satellite cell proliferation and differentiation, is predicted to increase both hyperplasia and hypertrophy of muscle cells. Hyperplasia may be seen as the major effect of myostatin deficiency in mice and cattle due to the particularity of skeletal muscle development, in that muscle fibre hyperplasia precedes hypertrophy and prenatal hyperplasia correlates inversely with postnatal fibre hypertrophy. Only if the myostatin gene was inhibited postnatally, would the response be hypertrophy (GROBET et al., 2003). When muscle fibre number is high, muscle fibres grow more slowly and therefore take longer to reach their maximal size. Fat deposition in pigs is lower in low birth weight piglets with low muscle fibre numbers (KUHN et al., 2002; REHFELDT et al., 2004), which could also contribute to lower fatness in myostatin deficient mice.

The ***distribution of fibre types*** in muscle between red, intermediate, and white fibres is characteristic of the predominant metabolic pathway providing the energy for their contraction. Both muscles examined in this study consist of predominantly white (fast-twitch glycolytic) fibres. A substantial additive genetic effect on metabolic fibre types was associated with myostatin genotype on the DUHi genetic background, the proportion of white fibres in rectus femoris and longissimus muscle both being c.16% higher in *C/C* than +/+ mice. This indicates a marked shift to the glycolytic muscle metabolism, and is consistent with responses observed in other long-term growth selection programs. The differences in the proportion of white fibres in *C/C* vs. wild-type mice are similar to differences observed between DM and normal cattle phenotypes (HOLMES and ASHMORE, 1972; WEST, 1974; WEGNER et al., 2000). Thus, hypermuscularity caused by genetic myostatin deficiency seems to be associated with a shift in the metabolic pathway of energy production towards glycolysis. This may explain, in part, the higher sensitivity of double muscled cattle to environmental changes (HOLMES et al., 1973). ***Capillary supply.*** Oxidative metabolism is largely dependent on oxygen supply via an extensive capillary bed. The mean capillary density per fibre was 0.2 capillaries lower and the fibre area supplied by one capillary 1080µm² higher in animals homozygous for *Compact* vs. wild-type DUHi mice. This reduced density of capillaries in myostatin deficient mice may be one reason why their muscle fibres produce energy by glycolysis, which is less reliant on oxygen supply. Capillary growth and density correlate with physiological function such as increased aerobic capacity in endurance type exercise (e.g. JENSEN et al., 2004; TERJUNG et al., 2002), but changes in capillary density of muscles from DM and normal cattle have not yet been compared. The mutated allele was found to be partially recessive for both fibre type distribution and capillary density, suggesting that these traits are closely associated and that the use of heterozygous animals could limit the negative consequences in stress resistance.

Conclusions

Myostatin deficiency caused by the *compact* mutation also has substantial effects on a high growth background: *C/C* animals are leaner and have reduced organ weights, but animals are not heavier. There is evidence of epistatic effects, as the phenotypic effects of *Compact* depend very much of the genetic background into which it is introgressed. On the high growth genetic background used here, strong negative effects on the fitness of *C/C* animals/gametes was found. *C/C* animals had a high degree of muscularity reflected by increased muscle mass and substantially increased carcass to body weight ratios. This hypermuscularity caused by the mutated myostatin gene introgressed into the high-growth genetic background results from muscle fibre hyperplasia rather than hypertrophy, and from balanced increases in myonuclear proliferation and protein accretion. Myostatin deficiency leads to an excessive prenatal muscle fibre formation, which is related to postnatal muscle function. Capillary supply is adversely affected and muscle metabolism shifted towards glycolysis, which could have negative consequences for physical fitness. From a practical breeding standpoint it could be significant that these phenotypic effects appear to be partially recessive . The results of introgressing the *Compact* gene onto a different genetic background clearly demonstrate that the defect in the myostatin gene is directly related to the phenotypic expression of muscle fibre hyperplasia, shift to glycolytic muscle

metabolism, and lowered capillary supply. This study and others in cattle show that the effects of deficiencies in the myostatin gene depend greatly on the genetic background.

Note: A detailed presentation of all aspects of this experiment is given elsewhere: BÜNGER et al., (2004) and REHFELDT et al., (2005).

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The influence of divergent selection for body weight at 63 days of age in rabbits on muscle characteristics at a same age or at similar weight

Abstract

Two lines of rabbits divergently selected for body weight at 63 days during 6 generations (H: high line; L: low line) and a cryo-preserved control group (C) were used to assess the effects of growth rate on muscle characteristics, either at a fixed age (63 days) or at the same weight (2297 ± 10 g). In the two series of slaughter, body weight at weaning (28 days) was decreased in the rank order L < C < H. At 63 days of age (n = 20 per line), the decrease in slaughtering body weight between the three lines (L: 2.32 kg < C: 2.62 kg < H: 2.87 kg) induced a proportional decrease in the weight and total cross-sectional area of the *semitendinosus* (ST) muscle. Mean cross-sectional area of ST myofibers was the lowest in the L line (-15%, P < 0.001), but it did not differ among H and C lines. Total number and type frequency of the myofibers were similar in the three groups. At a same weight (n = 20 per line), the decrease in slaughter age in the order H < C < L (51 d, 57 d, and 63 d, respectively) did not influence ST myofiber histological characteristics, however ultimate pH was slightly lower in the L line compared with the two other groups. In conclusion, divergent growth rate selection had asymetrical effects on myofiber size in rabbits at a same age, whereas it did not influence myofiber traits at a same weight.

Key Words: rabbit, growth selection, muscle fibers, carcass composition

Zusammenfassung

Titel der Arbeit: **Der Einfluss divergenter Selektion auf Körpergewicht am 63. Lebenstag auf Muskeleigenschaften beim Kaninchen bei gleichem Schlachtgewicht oder Schlachtalter**

Zwei Kaninchenlinien, über sechs Generationen divergent auf Körpergewicht bei 63 Tagen Lebensalter (H: high line; L: low line) selektiert, und eine kryokonservierte Kontrolllinie wurden zur Schätzung des Effekts der Wachstumsrate auf Muskeleigenschaften, zum einen bei gegebenem Schlachtalter (63 Tage) und zum anderen bei gegebenem Schlachtgewicht (2297 ± 10 g), untersucht. Für das Körpergewicht beim Absetzen (28 Tage) zeigte sich die folgende Rangierung der Testgruppen L < C < H; für das Gewicht bei 63 Tagen (n = 20 per Linie) L: 2,32 kg < C: 2,62 kg < H: 2,87 kg. Gewicht und Muskelfläche des M. *semitendinosus* (ST) waren proportional unterschiedlich. Die durchschnittlichen Muskelfaserquerschnitte des ST waren in der L-Linie am niedrigsten (-15%, P < 0,001), aber zwischen H und C nicht unterschiedlich. Die Gesamtanzahl an Muskelfasern war bei den drei Gruppen ähnlich. Für Schlachtungen bei gleichem Schlachtgewicht (n = 20 per Linie), zeigte sich für das Schlachtalter folgende Rangierung H < C < L (51., 57., bzw. 63. Lebenstag). Es war kein Einfluss auf histologische Muskelfasermerkmale zu erkennen. Der ultimative pH war geringfügig niedriger bei der L-Gruppe. Demnach hat Selektion auf Wachstumsrate beim Kaninchen asymmetrische Effekte auf die Muskelfasergröße bei gegebenem Schlachtalter aber nicht bei konstantem Schlachtgewicht.

Schlüsselwörter: Kaninchen, Selektion auf Wachstum, Muskelfasern, Schlachtkörperzusammensetzung

Introduction

Changing animal growth rate is an important goal to ensure economically viable rabbit meat industry. However, for any method aimed to increase processing efficiency, the effects of genetic improvement in growth rate have to be evaluated in terms of carcass composition, muscle characteristics, and meat quality traits. The relationships between postnatal weight gain and carcass composition in rabbits have been previously studied using various breeds of different adult weights (dwarf, giant), native breeds, or lines selected for different criteria (e.g., growth vs. reproductive traits, PLA et al., 1996;

1998). Few within-breed studies have also examined the effects of selection for an increased growth rate on carcass quality traits (PILES et al., 2000; RAMIREZ et al., 2004) and indicators of meat quality. For instance, negatively correlated phenotypic responses of selection for rapid growth rate on myosin heavy chain type I percentage and instrumental texture properties of the muscle *longissimus* have been demonstrated in rabbits at a fixed age (RAMIREZ et al., 2004). However, the consequences of a selection for growth rate on other determinants of meat quality, such as total number, size and type frequency of the muscle fibers, are unknown. Furthermore, other problems for production quality may arise in rabbits differing in growth rate and mature size but slaughtered at the same live weight. The aim of this study was to compare histological, and biochemical muscle characteristics in rabbits divergently selected for growth performance and slaughtered at a same age or at similar weight.

Material and methods

Two divergent lines were settled for either a high (H) or a low (L) 63-days body weight (BW) during five generations of selection (LARZUL et al., 2005). Cryopreserved embryos from the founder generation were thawed and implemented in rabbit does at generation four, to be contemporary to the fifth generation (control population, [C]). Young rabbits were weaned at 30 days of age, and were then given ad libitum access to a standard diet until slaughter. Body weight changes were recorded from weaning to slaughter for the calculation of average daily gain. Rabbits were slaughtered either at 63 days of age (n = 40 in each group), or at the same live weight (2.3 ± 0.7 kg, n = 40 per group). They were electrically stunned, and bled. Commercial dressing procedures were followed (BLASCO et al., 1993). The carcass was divided into fore-, back- and hind-parts; all parts were weighed and expressed relatively to chilled carcass weight.

Just after slaughter, the *semitendinosus* muscle (ST) was excised from the left carcass side in one-half of the animals (n = 20 per group at each stage). After weighing, a slice (entire cross-section) was taken in the mid-belly of the muscle, cooled at 4°C during 1 h after removal, and then frozen at –20°C for determination of muscle cross-sectional area. Another slice was taken and immediately frozen in liquid nitrogen, for measurements of activity levels of enzymes representing oxidative pathway (isocitrate dehydrogenase, [ICDH]) or glycolytic pathway (lactate dehydrogenase, [LDH]). For the evaluation of myofiber characteristics, two ST samples (200 mg/sample) were taken at fixed points, one in the red deep part, and one in the superficial white part. Transverse cryostat-cut serial sections were stained with azorubin. Total fiber number was determined by extrapolation from the number of fibres counted over the six fields of known size and from muscle cross-sectional area. Mean cross-sectional area (CSA) of myofibers was also assessed by image analysis in red and deep samples, after inter-fiber network extraction. Another section was stained for the actomyosin ATPase activity to identify types I, IIA or IIB/X fibers in each muscle part (BROOKE and KAISER, 1970). The remaining mid-part of ST sample was cut into small pieces and frozen in liquid nitrogen, to evaluate lipid content (FOLCH et al., 1957).

After 48 h of carcass chilling, the entire ST was excised from the right side of carcasses and crushed in a solution of sodium iodo-acetate to measure ultimate pH (pH_u).

Results and discussion

Growth performance and carcass traits

In the two slaughtering series, weaning weight was ranked in the order L < C < H groups (Table 1). At a same final body weight, ADG from birth to slaughter was 14% lower and 18% higher in L rabbits and H rabbits, respectively, compared with C group. This gave rise to an increase of 5 to 11 days of age at slaughter in L group compared with C rabbits and H animals, respectively. At 63 days of age, the difference was 12 g/d for ADG and 450 g for body weight between the divergently selected lines, the controls being intermediary. Altogether, these results suggest that divergent selection for 63-d BW affected both pre- and post-weaning events, probably through variations in the levels of anabolic hormones, catabolic hormones and/or levels of response of response of the target tissues (CLUTTER et al., 1995; DUCLOS et al., 1996; WEILER et al., 1998).

Rapid growing rabbits had a 7% lower feed conversion (FC) ratio compared with controls at the same weight, however feed efficiency did not significantly differ between H line and C group at 63 days of age. Conversely, decreasing ADG by selection caused an augmentation in the amount of feed needed per gram of gain achieved in L rabbits compared with controls slaughtered at the same weight (+11%) or age (+3%). Overall, these observations match with the negative genetic association previously reported between feed efficiency and ADG in rabbits (MOURA et al., 1997).

Table 1
Growth performance and carcass traits in rabbits from the low (L) and high (H) lines and the control group (C)

	Constant weight				Constant age			
Item	L	C	H	*P*	L	C	H	*P*
Growth performance								
Weight at weaning, g	726^{a}	795^{b}	903^{c}	***	793^{a}	820^{b}	861^{c}	**
Final live weight, g	2308	2311	2300	NS	2423^{a}	2684^{b}	2880^{c}	***
Final age, d	63.3^{a}	58.0^{b}	52.2^{c}	***	63	63	63	-
ADG, g	47.2^{a}	55.0^{b}	65.2^{c}	***	50.0^{a}	55.4^{b}	62.1^{c}	**
FC ratio, g/g	3.18^{a}	2.83^{b}	2.64^{c}	***	3.05^{a}	2.96^{ab}	2.90^{b}	*
Chilled carcass, g	1358^{a}	1314^{b}	1313^{b}	***	1273^{a}	1453^{b}	1562^{c}	***
Carcass composition, %								
Dressing out	58.6^{a}	56.8^{b}	56.7^{b}	***	54.41^{a}	55.67^{b}	55.53^{b}	*
Hind part	31.4^{a}	30.3^{b}	30.3^{b}	**	-	-	-	
Fore part	34.1	34.3	34.2	NS	-	-	-	
Back part	17.2	16.8	16.7	NS	-	-	-	
Perirenal fat	1.76	1.76	1.64	NS	1.62^{a}	1.92^{b}	2.04^{b}	***
Lipid content in *semitendinosus* muscle, %	2.1	2.3	2.2	NS	1.78^{a}	2.10^{b}	2.02^{ab}	*

Whatever slaughtering series, asymmetrical responses to selection were observed for carcass conformation with responses in the L line only. Many causes may generate asymetrical responses between divergent lines, such as inbreeding depression, maternal effect, genes with large effects, and some form of genotype by environment interaction (FALCONER, 1981). At a same body weight, chilled carcass weight, dressing out, and hind part proportion in the carcass were the highest in L rabbits (Table 1). Since selection for 63-d BW had undesirable effect of changing adult weight (GONDRET et al., 2002), it is likely that such a better carcass quality in L rabbits

compared with H rabbits was associated to improved degree of physiological maturity in the low line at fixed slaughter weight. On the opposite, carcass weight and carcass yield were slightly lower in L line than in the two latter groups, when rabbits were killed at a same age. In close-agreement with our results, dressing out was found to correlate positively with ADG in rabbits at a fixed age (SZENDRO et al., 1988; PILES et al., 2000). The relative proportion of perirenal fat depot in the carcass did not differ among growth rate groups at a constant weight at slaughter, whereas fat proportion was significantly decreased in L rabbits compared with other groups at the same age. Variations in ST muscle lipid content closely paralleled those reported above for carcass fatness. Altogether, these observations suggest that decreasing growth rate by selection led to a reduced genetic ability for fat deposition in rabbits. Elsewhere, both decreased (PILES et al., 2000) and increased (RAMIREZ et al., 2004) carcass fat depots have been reported in rapid-growing rabbits compared to within-breed controls at a fixed slaughter age.

Table 2
Myofiber characteristics and energy metabolism in *semitendinosus* muscles of rabbits from the low (L) and high (H) lines and control group (C)[1]

	Constant weight				Constant age			
Item	L	C	H	*P*	L	C	H	*P*
Total fiber number	76708	75692	79949	NS	75446	74044	80278	NS
Mean CSA, µm²	2883	3036	2885	NS	2786[a]	3374[b]	3222[b]	***
Type frequency, %								
Inner part								
I	15.9	14.9	17.3	NS	17.41	17.95	17.03	NS
IIA	15.2	16.4	16.1	NS	15.47	16.93	15.68	NS
IIB/X	68.9	68.7	66.6	NS	67.12	65.12	67.29	NS
Superficial part								
I	1.0	0.7	1.2	NS	0.12	0.54	0.37	NS
IIA	5.4	5.0	6.6	NS	4.17	4.71	3.12	NS
IIB/X	93.6	94.3	92.2	NS	95.71	94.63	96.63	NS
Enzyme activities of energy metabolism, µmol.min^{-1} per g								
LDH	1113	1106	984	NS	-	-	-	-
ICDH	11.7	12.3	11.6	NS	-	-	-	-
pH, 48 h *post-mortem*	6.13[a]	6.10[a]	6.02[b]	***	6.13	6.13	6.09	NS

[1]Abbreviations used: CSA = cross-sectional area; LDH = lactate dehydrogenase; ICDH = isocitrate dehydrogenase.

Myofiber characteristics

In the current study, the total number of the myofibers in ST muscle did not differ among groups, whatever rabbits were slaughtered at the same weight or at a same age (Table 2). The lack of variation in total myofiber number in response to growth rate selection does not match with other reports in mice (LUFF and GOLDSPINK, 1970; HANHARAN et al., 1973) and chicken (RÉMIGNON et al., 1994), showing that a higher growth rate is related to a greater number of myofibers. However, maternal factors have been also suggested as significant determinants of total fiber number (REHFELDT et al., 2000). They might be of particular importance in rabbit species, in which total myofiber number is set only around 17 days postnatal (NOUGUÈS, 1972). Mean cross-sectional area (CSA) of myofibers did not differ among groups at a constant weight at slaughter (Table 2). On the opposite, myofiber CSA was the lowest in the L line at a same age, whereas controls and H rabbits displayed myofibers of

similar CSA. The effect of body weight on mean CSA or diameter of myofibers in the rabbit species is generally greater than the effect of age (MEISTER et al., 1974; REDDY et al., 1990; GONDRET et al., 2002), which could explain the lack of responses to selection in rabbits of same weight. The lower myofiber CSA in the L line compared to the H group at a same age was in agreement with previous observations in chicken divergently selected for growth rate (RÉMIGNON et al., 1994).

Whatever weight or age at slaughter, the type frequency of the myofibers in ST muscle did not differ with growth rates. In addition, the aerobic capacity, as indicated by ICDH activity, and the anaerobic capacity, as indicated by LDH activity, were similar in muscles of both groups, at least when rabbits were slaughtered at the same weight (Table 2). By contrast, ultimate pH value raised in ST muscle after 2 days of maturation was slightly lower in H line compared to L and C groups at a constant weight, whereas it did not differ among rabbit lines at the same age. The lack of variation in fiber type frequency according to growth rate was in agreement with other reports in divergently growth-selected chicken (RÉMIGNON et al., 1994, 1995) and mice (REHFELDT and BÜNGER, 1990). In addition, no significant changes in the metabolic equilibrium of ST muscle, as measured by enzyme activities representative of oxidative (ICDH) or glycolytic (LDH) pathways, could be found to explain differences in ultimate pH among lines at a same weight, an observation closely similar to that reported in chicken selected for body composition (BERRI et al., 2001). *Post mortem*, glycogen is converted to H+ and lactic acid, resulting in the pH decrease (HOCQUETTE et al., 1998 for a review). OKSBJERG et al. (2000) identified a slightly larger amount of glycogen in muscles from fast-growing pigs than slow-growing pigs at a same weight. Therefore, it remains to determine whether the lowest pH_u in ST muscles from H rabbits was related to differences between groups in muscle glycogen content. Finally, the effects of growth rate on pH_u in rabbit muscles are rather controversial, since muscles of rapid-growing rabbits had either a higher pH_u (PLA et al., 1998), a lower pH_u (CABANES-ROIRON and OUHAYOUN, 1994), or the same pH_u (HERNANDEZ et al., 1997) than muscles of rabbits undergoing a slow growth rate.

Conclusion

This study did not support the idea that selection for a rapid growth rate has a negative impact on carcass composition and myofiber characteristics. Rabbit meat quality seems to be more robust to improved growth rate by selection than did other productions as poultry and pork.

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Role of mitochondria in insulin-dependent perinatal muscle development

(Die Rolle der Mitochondrien auf die Insulin-abhängige Muskelentwicklung)

Insulin is a key anabolic hormone of pleiotropic activity providing hyperplasia and hypertrophy in several tissues. Just recently, some of the molecular mechanisms of insulin action related to growth regulation have been revealed. Since some of insulin targets in signaling pathway leading to myogenesis include energy sensors (mTOR) (DENNIS et al., 2001; SUMITANI et al., 2002) we assumed that this hormone might affect mitochondrial functions, main source of ATP. Myogenesis is a well described process of muscle formation, although detailed mechanisms of its regulation remain elusive. We decided to examine how insulin affect myogenesis from 1-day old C2C12 mouse skeletal muscle cells where functions of mitochondria are inhibited and vice versa, how insulin signaling inhibitors affect myogenesis and mitochondrial functions. Certain inhibitors of respiratory chain enzyme complexes (oligomycin, rotenone, myxothiazol) and insulin signaling (LY294002, PD98059) were used to retard either energy formation or propagation of insulin signal, respectively. Additionally, synthesis of mitochodrial proteins was reduced by chloramphenicol to asses their impact on muscle fiber formation. The effect of insulin was studied at the level of proteins crucial both for insulin pleiotropic actions (PKB/Akt) and muscle cell differentiation (myogenin). Cell viability (potency to generate reducing equivalents) and other molecular studies crucial for both insulin action and muscle differentiation were performed. Preliminary experiments were focused on dose-response and time-course (5 days) to determine non-toxic concentrations of selected inhibitors. Rotenone (complex I inhibitor) and oligomycin (complex V inhibitor) slightly reduced viability of myotubes at the final stage of myogenesis. Neither during chloramphenicol nor during oligomycin treatment myogenin protein expression was detected, whereas the latter was markedly reduced by rotenone or myxothiazol. In contrast, insulin dose-dependently elevated myogenin protein expression. Similarly to inhibitors of mitochonrial functions, blockade of insulin signaling at the level of PI-3K (LY294002) but not MEK (PD98059) diminished myogenin protein levels. In conclusion, we suggest that by targeting mitochondria insulin plays crucial role in myogenesis and support viability of muscle cells. Inhibition of respiratory chain enzyme complexes or alternatively blockade of insulin signaling at PI-3K level led to retarded myogenesis.

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The influence of mitochondrial function on meat quality in turkey and swine

Abstract

In several farm animals like turkey or pigs altered meat quality has been described. Some of these alterations are related to changes in the energy and calcium metabolism of the muscle cells. With regard to this, dysfunctions in the mitochondria which supply most of the ATP and contribute to calcium haemostasis might be related to altered meat quality.
In the first study the mitochondrial respiration rates and the citrate synthase (CS) activities were determined in M. pectoralis (MP) biopsies of two turkey strains, which differ clearly in their growth characteristics. The probes were obtained after 10 and 20 weeks of fattening. The ADP dependent state 3 respiration rates decreased during the growth period in both strains as well as the CS-activities. Interestingly, the slow growing turkeys showed tendentially and significantly higher state 3-respiration rates and activities of the CS. Comparison of the MP, a "white" muscle, with the "red" Musculus iliotibialis (MIT) resulted in significantly higher state 3 respiration rates and CS activities in the MIT. With regard to the meat quality we showed that – independent of the strain – in the MPs that showed rapid pH decline shortly after slaughter (pH<5.6) the ability to respire pyruvate decreased tendentially.
In another study the mitochondrial respiration in M. longissimus (ML) biopsies of two pig breeds with and without a mutation of the RyR gene was determined. The probes were obtained during fattening and after stress induction. During growth the state 3 respiration of the substrates glutamate and succinate decreased, whereas after stress induction only a reduced pyruvate respiration was determined. All state 3 respiration differences were independent of the RyR-status of the pigs, only an increased state 4 respiration was determined in MHS-positive pigs assuming an altered integrity of the mitochondria. Interestingly, it could be shown again that -independent of the RyR-status – muscle probes with low $pH_{45'}$ (<5.8) values have reduced pyruvate respiration rates after stress induction.
Both studies indicate a strong relation between bad meat quality and mitochondrial dysfunction in turkeys and pigs.

Key Words: meat quality, muscle, turkey, pig, mitochondria,

Zusammenfassung

Titel der Arbeit: **Der Einfluss der mitochondrialen Funktion auf die Fleischqualität von Pute und Schwein**
Bei mehreren Nutztieren, wie Pute oder Schwein, wurden Fleischqualitätsmängel beschrieben. Viele dieser Abweichungen sind mit Veränderungen im Energie- und Kalzium-Stoffwechsel der Muskelzelle verbunden. Diesbezüglich müsste eine Dysfunktion der Mitochondrien, die den größten Teil des ATP liefern und auch zur Kalzium-Homoöstase beitragen, in veränderter Fleischqualität resultieren.
In der ersten Untersuchung wurden die mitochondrialen Atmungsraten und die Citratsynthase (CS)-Aktivitäten im M. pectoralis (MP) von zwei Puten-Herkünften, die sich in ihren Wachstumseigenschaften deutlich unterschieden, bestimmt. Die Proben wurden nach 10 und 20 Wochen Mast entnommen. Die ADP abhängigen State 3-Atmungsraten sanken während der Wachstumsphase in beiden Herkünften genauso wie die CS-Aktivitäten. Interessanterweise zeigten die langsam wachsenden Puten tendenziell und signifikant höhere State 3-Atmungsraten und Aktivitäten der CS. Beim Vergleich des MP, einem „weißen" Muskel, mit dem „roten" M. iliotibialis (MIT) zeigte sich, dass die State 3-Atmungsraten und die CS-Aktivitäten im MIT signifikant höher waren. In Bezug auf die Fleischqualität zeigte sich, dass – unabhängig von der Herkunft- in MPs mit einem beschleunigten pH-Abfall kurz nach der Schlachtung (pH<5,6) die Fähigkeit zur Veratmung von Pyruvat tendenziell reduziert war.
In einer anderen Untersuchung wurde die mitochondriale Atmung in M. longissimus (ML)-Biopsien von zwei Schweinerassen mit oder ohne Mutation im RyR-Gen analysiert. Die Proben wurden während der Mastzeit und nach Stressinduktion entnommen. Während des Wachstums sank die State 3-Atmung mit den Substraten Glutamat und Succinat, wohingegen nach Stressinduktion nur eine reduzierte Pyruvat-Atmung bestimmt werden konnte. Die Unterschiede in der State 3-Atmung waren unabhängig vom RyR-Status der Schweine, nur bei der

State 4 Atmung konnte bei den MHS-positiven Schweinen eine Reduktion festgestellt werden. Letzteres lässt auf eine veränderte Integrität der Mitochondrien schließen. Interessanterweise konnte wiederum gezeigt werden, dass –unabhängig vom RyR-Status- in Muskelproben mit niedrigem $pH_{45'}$ (<5,8)-Wert nach der Stressinduktion die Pyruvat-Atmungsraten reduziert waren. Beide Studien deuten auf einen klaren Zusammenhang zwischen schlechter Fleischqualität und mitochondrialer Dysfunktion in Puten und Schweinen hin.

Schlüsselwörter: Fleischqualität, Muskel, Pute, Schwein, Mitochondrien

Introduction

Meat quality traits like appearance, tenderness, juiciness or aroma are important criteria influencing the buying behaviours of consumers. In contrast to this reduced water holding capacity, increased drip loss, pale/ heterogeneous colour or toughness are meat failures which are less acceptable.

In the last decades pig and poultry are severely selected for quick growth, leanness and muscularity. This “extreme” breeding resulted in morphological changes (e.g. muscle fibre hypertrophy, higher glycolytic to oxidative fibre ratio) that were accompanied with an increasing amount of animals showing meat quality failures after slaughter. An important genetic variation that had been clearly related to bad meat quality is the malignant hyperthermia syndrome (MHS) in pigs (HAMILTON et al., 2000). MHS is a disease originally found in humans which is caused by mutations in the gene of a calcium-release-channel (CRC) of the sarcoplasmic reticulum (SR) (FUJII et al., 1991; MELZER and DIETZE, 2001). Animals and humans with the defective CRC show increased cytoplasmic Ca^{2+}-concentrations in the muscle cells (ENZMANN et al., 1998). In normal situations this dysfunction could be compensated by the activity of the SR-Ca2+-ATPase (SERCA) but in stress situations (e.g. halothane anaesthesia) the system could decompensate resulting in overshooting calcium dependent effects (KÜCHENMEISTER et al., 1999). These include increased muscle contraction, heat production, rapid glyco(geno)lysis, lactacidosis causing premature denaturation of muscle proteins as well as impaired water-holding capacity (WHC) and meat color. In pigs an important symptom of MHS after slaughter is pale, soft and exsudative (PSE) meat (MELZER and DIETZE, 2001; HAMBRECHT et al., 2004). This alteration in meat quality has also been demonstrated in turkeys (BARBUT, 1997) assuming a similar pathogenesis just like in pigs (CHIANG et al., 2004).

Recapitulating, known factors influencing the development of meat quality failures in pigs and turkey are biochemical effects like increased muscle contraction, rapid glycol(geno)lysis, lactacidosis, defective calcium homeostasis and morphological alterations like increased glycolytic to oxidative fibre ratio, reduced mitochondria to muscle fibre ratio and reduced capillary to muscle fibre area ratio.

Interestingly, most of these effects are related to the energy and calcium metabolism of the cells and thereby to the function of the mitochondria (WICKE et al., 2000; GUNTER et al., 2004; OPALKA et al., 2004). Mitochondria supply most of the ATP required for the cellular work (e.g. muscle contraction, active transport systems) and contribute to the regulation of the cellular Ca2+ homeostasis (GUNTER et al., 2004). Several mitochondrial dysfunctions are known which could be accompanied with “mitochondrial diseases” (GELLERICH et al., 2003).

Data about mitochondrial function of skeletal muscles in farm animals used for meat production are very rare. From other fast growing animals, for instance rats (TOSTLEBE et al., 2001), reduced oxidative capacities with increasing age have been documented. In pigs and turkeys different strains with different growth and meat

quality characteristics exist. With regard to this it could be suggested that the functional properties of the mitochondria differ between the strains.
Aim of the study was the characterization of the mitochondrial function in the musculature of different pig and turkey strains to elucidate the influences of the mitochondria on the development of meat quality failures.

Material and Methods

Material

Male turkeys of two different strains differing in their growth properties were purchased from a commercial hatchery at day one of age and grown up under standardized indoor conditions. Biopsies were obtained from the Musculus (M.) pectoralis (MP) at week 10 and 20 of the fattening period and at week 20 from the M. iliotibialis (MIT). The probes were investigated for mitochondrial respiration and frozen for determination of the activity of citrate synthase and histological determination of muscle structure.
In a second experiment MHS-positive (Piétrain) and -negative (German Landrace) pigs were fattened under standardized indoor conditions. Biopsies were obtained from the M. longissimus (ML) after 60 to 90 (time I), 120 to 150 (time II) and 180 to 200 days (time III) of fattening. Before removing the last biopsies the pigs were stressed by nose-sling-treatment for 10 minutes. The probes were investigated for mitochondrial respiration.

Methods

After slaughtering the turkeys (10 and 20 weeks) and pigs (180 to 200 days) pH-values were determined in the MP of the turkeys and ML of the pigs.
All porcine and a part of the turkey biopsies were used for determination of the mitochondrial respiration. After careful dissection and chemical or mechanical permeabilisation (chemical only with the poultry probes) the skinned-fiber bundles were analysed on respiratory activity in an OROBOROS® oxygraph (Innsbruck, Austria). Changes in the oxygen consumption rates were determined with the substrates pyruvate/ malate, glutamate/ malate and succinate/ rotenone after addition of ADP (state 3-respiration) and the inhibitor of the adenine-nucleotid-translocator atractylate (state 4-respiration). The respiratory control index (RCI) was calculated by the ratio between state 3 and state 4-respiration values (GELLERICH et al., 2002).
The citrate synthase activities were measured spectrophotometrically in skeletal muscle homogenates of the turkeys. Enzymatic activities were related to the protein concentration. Citrate synthase (CS) activities were determined with DTNB (5,5'-Dithiobis-2-nitrobenzoic acid) at 412 nm (SHEPERD and GARLAND, 1969).
For the histological investigation the turkey samples were cut into slices of 12 µm thickness, stained by the combined SDH/ATPase staining method and classified into “slow twitch oxidative” (STO), “fast twitch oxidative” (FTO) and “fast twitch glycolytic” (FTG) fibers (OPALKA et al., 2004)
Each parameter was tested for normality by the Kolmogornow-Smirnov test. If this criterion was fulfilled, statistical significance was tested with the student’s t-test for unpaired variables. Otherwise, the Wilcoxon Test (Mann-Whitney U Test) was applied. A P value below 0.05 was considered to be significant.

Results

Mitochondrial respiration in the poultry probes (Tab. 1 and 2)

In the MP of 20 weeks old turkey strains the state 3 respiration rates were up to 45 % lower than at week 10. The slow growing strain showed slightly higher respiration rates significantly different ($P<0.05$) for glutamate at week 10 and pyruvate at week 20 (Tab. 1).

Table. 1
Mitochondrial respiration rates with different substrates in the M. pectoralis (MP) of fast- and slow-growing turkeys dependent on the age (10 and 20 weeks) and strain of the animals (OPALKA et al., 2004)

		Fast-growing Strain		Slow-growing Strain	
	Age⇒	**Week 10**	**Week 20**	**Week 10**	**Week 20**
State 3-Respiration [nmole/ min*mg s.w.]	**Glut. / Mal.**	1.55 ± 0.26 */#	0.99 ± 0.30 #	1.62 ± 0.32 */#	1.08 ± 0.28 #
	Pyr. / Mal.	1.06 ± 0.16 #	0.63 ± 0.17 */#	1.17 ± 0.24 #	0.75 ± 0.15 */#
	Suc. /Rot.	1.32 ± 0.18 #	0.89 ± 0.21 #	1.43 ± 0.27 #	1.02 ± 0.19 #
State 4-respiration [nmole/ min*mg s.w.]	**Glut. / Mal.**	0.15 ± 0.04	0.13 ± 0.04	0.15 ± 0.04	0.14 ± 0.04
	Pyr. / Mal.	0.14 ± 0.03	0.13 ± 0.06	0.14 ± 0.02	0.13 ± 0.05
	Suc. /Rot.	0.32 ± 0.20 #	0.21 ± 0.04 #	0.35 ± 0.28 #	0.23 ± 0.06 #
Respiratory Control Index (RCI)	**Glut. / Mal.**	10.76 ± 3.59 #	8.44 ± 3.79 #	11.25 ± 3.73 #	8.23 ± 2.61 #
	Pyr. / Mal.	8.10 ± 1.93 #	5.98 ± 3.01 #	8.71 ± 2.76 #	6.68 ± 2.60 #
	Suc. /Rot.	4.29 ± 0.98	4.39 ± 0.91	4.35 ± 0.99	4.66 ± 1.03

Mean ± S.D., * - significant difference ($P<0.05$) between strains at the same age; # - significant difference ($P<0.05$) between age within the same strain

Table 2
Mitochondrial respiration rates with different substrates in the M. pectoralis (MP) and the M. iliotibialis (MIT) of fast- and slow-growing turkeys (20 weeks old) dependent on the muscle type and strain (OPALKA et al., 2004)

		Fast-growing Strain		Slow-growing Strain	
	Muscle type⇒	**MP**	**MIT**	**MP**	**MIT**
State 3-Respiration [nmole/ min*mg s.w.]	**Glut. / Mal.**	1.23 ± 0.17 #	2.38 ± 0.48 #	1.34 ± 0.17 #	2.45 ± 0.35 #
	Pyr. / Mal.	0.84 ± 0.08 */#	2.71 ± 0.50 #	0.96 ± 0.15 */#	2.48 ± 0.33 #
State 4-respiration [nmole/ min*mg s.w.]	**Glut. / Mal.**	0.10 ± 0.07 #	0.39 ± 0.12 #	0.11 ± 0.05 #	0.43 ± 0.26 #
	Pyr. / Mal.	n.d.	n.d.	n.d.	n.d.
Respiratory Control Index (RCI)	**Glut. / Mal.**	18.17 ± 12.4 #	6.72 ± 2.36 #	15.84 ± 9.64 #	7.66 ± 4.81 #

Mean ± S.D., * - significant difference ($P<0.05$) between strains; # - significant difference ($P<0.05$) between muscle type within the same strain; n.d.= not determined

After addition of atractylate (state 4-respiration) the pyruvate- and glutamate-dependent respiration rates in the MP showed no age- or strain-related differences. This results in reduced RCI values at week 20 due to the concurrent reduction of the state 3 respiration rates. In contrast to this, addition of the substrate succinate resulted in decreased state 4 respiration rates after 20 weeks and in nearly identical RCI values (Tab. 1).
Analysis of the two different turkey muscles MP and MIT resulted in two times higher state 3 respiration rates in the MIT independent of the substrate (Tab. 2).
The state 4 respiration in the MIT was up to four times higher in comparison to the MP. This resulted in significantly ($P<0.05$) lower RCI values in the MIT (Tab. 2).

Mitochondrial respiration in the porcine probes (Tab. 3)
The biopsies of the MHS negative pigs obtained after 60 to 90 and 120 to 150 days showed significantly ($P<0.05$) lower state 3 respiration rates for the substrates glutamate and succinate whereas in MHS positive animals this effect occurred only with the substrate succinate. However, no significant differences in the state 3 respiration rates could be determined between the investigated pig strains with regard to the particular collection time.
The state 4 respiration decreased significantly ($P<0.05$) between the first and second collection time in both strains, but was significantly higher ($P<0.05$) in the MHS positive pigs indicating an uncoupling of the muscular mitochondria.
The investigation of the probes obtained after nose sling treatment resulted in both strains in significant lower ($p<0.05$) state 3 respiration rates with the substrate pyruvate in comparison to the second collected biopsies. No differences could be determined between the pig strains.

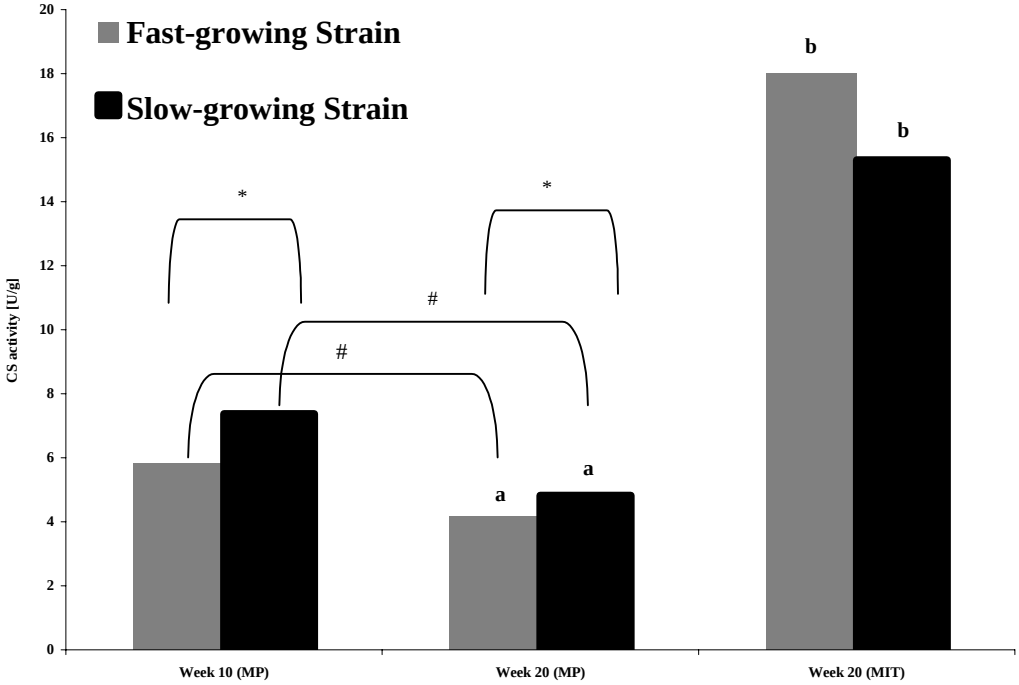
Table 3
Mitochondrial respiration rates with different substrates in the M. longissimus (ML) of MHS positive and negative pigs after 60 to 90 days and 120 to 150 days of fattening and after stress induction by nose sling treatment (180 d) (WICKE et al., 2000)

		German Landrace (MHS negative)			**Pietrain (MHS positive)**		
	Age⇒	**60-90 d**	**120-150 d**	**After nose sling treatment**	**60-90 d**	**120-150 d**	**After nose sling treatment**
State 3-Respiration [nmole/ min*mg s.w.]	Glut./Mal.	0.76 ± 0.13 #	0.61 ± 0.2 #	0.68 ± 0.13	0.77 ± 0.18	0.63 ± 0.25	0.65 ± 0.17
	Pyr./Mal.	0.79 ± 0.2	0.79± 0.11 *	0.6 ± 0.09 *	0.74 ± 0.15	0.71 ± 0.24 *	0.57 ± 0.23 *
	Suc. /Rot.	0.67 ± 0.16 #	0.45 ± 0.24 #	0.33 ± 0.14	0.73 ± 0.17 #	0.48 ± 0.16 #	0.42 ± 0.14
State 4-respiration [nmole/ min*mg s.w.]		0.11 ± 0.03 #/a	0.08 ± 0.02 #/a	0.07 ± 0.02	0.17 ± 0.04 #/b	0.11 ± 0.04 #/b	0.09 ± 0.02
Respiratory Control Index (RCI)		7.2 ± 2.7 a	11.4 ± 8.78 a	7.46 ± 2.63	4.13 ± 1.12 b	6.83 ± 3.62 b	6.94 ± 3.3

Mean ± S.D., a,b - significant difference ($P<0.05$) between strains; # - significant difference ($P<0.05$) between age within a strain; * - significant difference ($P<0.05$) between treatment within a strain

Enzyme kinetic results of the poultry probes (Fig.)
The activity of the mitochondrial marker enzyme citrate synthase (CS) decreased in the MP significantly ($P<0.05$) from week 10 to 20 in both strains.

After 20 weeks of growth in both strains the CS activity of the MIT was three to four times higher than the activity in the MP.
The significant (P<0.05) higher CS activity rates in the slow-growing strain in comparison to the fast-growing confirm the slightly and significantly higher state 3 respiration results of the respirometric experiments.



Mean ± S.D., # - significant difference (P<0.05) between age within the same strain, * - significant difference (P<0.05) between strains at the same age, [a,b] – colums with different letters between the muscle types differ significantly (P<0.05).

Figure: Citrate synthase (CS) activities in the M. pectoralis (MP) and M. iliotibialis (MIT) of fast- and slow-growing turkey strains dependent on the age and the strain of the animals and the investigated muscle type (OPALKA et al., 2004).

Mitochondrial function and meat quality parameters

In turkeys prone to develop acid intramuscular pH values (<5.6) shortly after slaughtering, which is an indicator for impaired meat quality, tendentiously lower rates of pyruvate oxidation were found. The opposite was observed for glutamate respiration rates resulting in a significant (P<0.05) lower rates of glutamate-related pyruvate oxidation.
In pigs showing acid pH values (< 5.7) shortly after slaughtering no reduced pyruvate oxidation was determined in comparison to pigs with normal meat quality. However, induction of stress by nose sling treatment resulted in significant (P<0.05) lower pyruvate respiration values in pigs with meat quality failures. The state 4 respiration in pigs with meat failures was significantly higher.

Histological results of the poultry probes

Both investigated turkey strains differed significantly (P<0.05) in the average fibre area after 10 and 20 weeks with increasing dimensions at the later point of time. The fibers in the MP of the fast-growing turkeys were significantly larger (3616 ± 952 µm^2 (10 weeks), 7042 ± 2398 µm^2 (20 weeks)) than those of the slow-growing ones (3025

± 536 µm^2 (10 weeks), 5862 ± 1567 µm^2 (20 weeks). The MP of the investigated strains consisted of more than 99% fast twitch glycolytic fibres (FTG) after 10 and 20 weeks whereas the MIT is predominantly slow twitch oxidative (STO).

Discussion

The intensive breeding of farm animals improves meat production effectiveness with respect to growth rates and meat yield. The major disadvantages of this development are an increasing number of reports regarding impaired meat quality and the striking susceptibility of some of these animals to stress (OWENS et al., 2000; HAMBRECHT et al., 2004). These alterations originally affect pigs but in recent time comparable meat quality failures were also found in turkeys (BARBUT, 1997). Reduced meat quality is correlated with changes in the cellular morphology (e.g. muscle fiber hypertrophy) and homeostasis (e.g. lactacidosis, hypercalcaemia, heat production) (WICKE et al., 2000). Since many of these effects are related to the mitochondria in the present study mitochondrial functions were investigated in the musculature of pigs and turkeys focusing on growth, muscle type and meat quality.

A broad spectrum of strains and races with different growth rates, lean meat, musculature development characteristics and pronesses to meat quality failures among turkeys and pigs exist. In the present study we compared two turkey strains with diverse growth characteristics and two pig races with different stress susceptibilities.

In the turkeys one strain was selected for rapid growth and high breast muscle development. The other animals were only moderately selected with regard to these criteria. Unfortunately, no unselected wild type strain was available in an adequate amount of chicks. In pigs we compared MHS-negative animals of the race German Landrace with MHS-positive of the Piétrain race.

It could be shown that the oxidative capacities in the muscle cells decreased during growth in turkeys and pigs. This means that the ability to regenerate ATP via the oxidative metabolism in these tissues changed during ageing. During high energy consumption possible complications of this aerobic-to-anaerobic shift could be the increased lactatacidosis due to the reduced NADH regeneration capacities and the development of an intracytoplasmic hypercalcaemia caused by the lack of energy for the active re-transport of calcium to the sarcoplasmatic reticulum. A consequence of the latter is the induction of calcium dependent effects like induction of muscle contraction and glycolysis that support the maintenance of the “vicious circle” (LAMBERT et al., 2001; BINKE, 2004).

With respect to this it could be assumed that the older turkeys - especially the fast-growing turkey strains - and pigs are more sensitive to rapid changes in the ATP consumption rates and “stress” e.g. by loading or transport. This conclusion is supported by the result that after nose-sling-treatment the pigs react in the reduction of the respiration rates. However, the MHS-positive pigs react similar to the MHS-negative indicating that the nose-sling treatment is a less effective method to clarify differences in the stress susceptibilities of the animals. Due to this further investigations are necessary.

The differences in respiration rates and the CS activities between the turkey strains could be related to the muscle structure of the animals. It could be suggested that the bigger fibers in the fast-growing strain lack oxygen supply by lower capillary-fiber-ratio. This results in the reduction of the mitochondrial density and the oxidative

capacity. The decrease in the capillary-muscle area-ratio was already shown in pigs (FIEDLER et al., 1993). In current experiments in our laboratory the latter results should be proved by determination of the capillary density in turkey breast muscles.
Increases in the state-4-respiration rates indicate an uncoupling of the mitochondria accompanied with a proton leak along the inner mitochondrial membrane (HAN et al., 2004). Since the state-4-respiration rates in the MHS-positive pigs differ significantly from those of the MHS-negative animals a partial uncoupling of the mitochondria could be assumed. Uncoupling of mitochondria is a physiological process in brown adipose tissue of young or hibernating animals (nonshivering heat production) (DE MEIS, 2003) but is unwanted in nearly all other animals due to the reduced ATP production and the possible heat production (HAN et al., 2004). Further investigations are necessary to prove if these mechanisms affect the stress susceptibility of the MHS-positive pigs.
Reduced meat quality is mainly affected by alterations in the cell homeostasis close to the slaughter of the animals. With regard to the mitochondrial function it could be assumed that in animals, who show a reduced oxidative capacity, every premortal activation of the ATP consumption e.g. by “stress” (e.g. loading, transport, anaesthesia) is extensively accompanied with a delay in ATP regeneration and metabolic acidosis. Without the adequate resting period before slaughter these animals were slaughtered with lactacidosis resulting in a rapid pH-decline and negative effects on the meat quality (e.g. PSE, reduced WBC).
In conclusion, this study provides data on the mitochondrial function of turkey and pig skeletal muscles. From these results mechanisms that lead to impaired meat quality in turkey breast muscle and pig back muscle could be assumed. In the almost glycolytic turkey MP and pig ML the oxidative capacities decreased throughout life with a shift from partly oxidative to a mainly glycolytic metabolism. This is very objective in those animals showing a rapid pH-decline shortly after slaughter. Since metabolic activation e.g. by stress leads to a release of large amounts of pyruvate due to glycogen breakdown in muscles, in tissues with low oxidative and high glycolytic capacities the lactacidosis is more severe than in muscles that metabolize pyruvate via the oxidative pathways (MIT). This acidosis not only affects the welfare of the living animal, but is also related to the muscle-to-meat-transition after slaughter. Although further studies are needed to fully understand the conditions under which impaired meat quality develops, the techniques introduced here can be applied to characterise *in vivo* biopsy specimen with predictive value for the meat quality post mortem. Especially the combination of high resolution respirometry and measurement of mitochondrial enzyme activities allowed a sensitive description of mitochondrial function in turkey and pig skeletal muscle.

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Muscle development in cultured blackspot seabream *Pagellus bogaraveo*: preliminary histochemical and immunohistochemical data on the fibre types

(Muskelentwicklung beim Graubarsch, *Pagellus bogaraveo*: Erste histochemische und immunohistochemische Daten zu Muskelfasertypen)

Muscle ontogeny and fibre type characteristics have been studied in a new species for commercial aquaculture: the blackspot seabream. Myosin ATPase and SDH histochemistry and immunohistochemistry with a panel of antibodies to myosin isoforms and parvalbumin were tested at different ontogenetic stages. In general, deep white muscle was parvalbumin-positive, and superficial 'red' muscle was parvalbumin-negative at all ages examined. At 6 days of age (transition from endogenous to exogenous feeding) three layers of muscle fibres were observed with different antimyosin reactivities: superficial monolayer, presumptive slow red (present only as a small group of fibres adjacent to the lateral line nerve), and presumptive fast-white (forming the bulk of the muscle). The superficial monolayer and presumptive slow fibres were positive for SDH. At 60 days of age (transition from live to artificial feeding) an additional fibre type was identified: a typical 'pink' or intermediate layer. In juveniles, the axial muscle consisted mainly of fast white fibres covered by a slow-red layer and between them a pink layer. Surprisingly, the red layer could be resolved into 2 distinct types by myosin immunostaining. Red fibres were also present along the horizontal septum, near the notochord. Both red and white muscle layers showed a mosaic appearance, which was confirmed by ATPase reaction.

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Insulin and insulin-like growth factors in muscle growth in trout and sea bream

(Insulin und Insulin-ähnliche Wachstumsfaktoren im Muskel von Forelle und Seebarsch)

Insulin and insulin-like growth factors (IGFs) play an important role in metabolism and growth regulation in vertebrates. However, the role of IGFs in muscle proliferation, differentiation and quality in fish remains to be established.
We studied the response of insulin and IGFs during compensatory growth in trout and sea bream. Juvenile trout and sea bream were deprived of food for 1, 2-3 or 4 weeks, and subsequently fed *ad libitum* for one or two months. The decrease in weight, condition factor, plasma glucose and insulin induced by fasting was recovered after re-feeding, but only the group fasted for 2 weeks showed partial compensatory growth. Insulin and IGF-I binding in trout white muscle increased during fasting. The expression of IGF-I in trout liver was studied by Northern blot and real-time-PCR. A transcript of the expected length was detected in control but not in fasted fish. mRNA levels decreased for both IGFs in all fasting periods, but after re-feeding expression was similar to control. Expression of IGF-II was maximum in the re-fed group fasted for 2 weeks. All this suggests a significant role of the insulin-IGF system in the process of compensatory growth.
Additionally trout myocytes were isolated and cultured. IGF-I and IGF-II stimulated the glucose uptake more strongly than insulin. IGF-I increased L-alanine uptake more potently than insulin, and IGF-I also stimulated thymidine uptake, while insulin did not. IGFs and insulin showed stimulatory effects on MAPK and AKT pathways. However, MAPK expression and activity were higher during proliferation, but decreased once myocytes had differentiated, while AKT maintained its presence throughout myoblast differentiation. This is an interesting area to investigate in the search for the best markers of satellite cells as indicators of the growth potential and quality of fish muscle.

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Pattern of muscle fiber formation in Large White and Meishan pigs

Abstract

The objective of the present study was to compare myofiber typing and myogenesis in skeletal muscle between two pig breeds exhibiting dramatic differences in postnatal muscle growth potential and meat quality, i.e. the Large White (LW) and Meishan (MS) breeds. Experiments were carried out at 62 kg body weight on the longissimus muscle (LM), and at birth and 75 days of gestation on the semitendinosus muscle (SM). The first experiment reported a striking shift from myosin heavy chain IIb to IIx, and to a lesser extend to IIa, in LM of MS compared to LW pigs, in accordance with a shift towards a less glycolytic and more oxidative metabolism. Besides differences in fiber typing, the total number of muscle fibers (TNF) was found to be dramatically lower in SM of MS than LW pigs, to a larger extend in the future white than red portion of the SM. The underlying mechanisms seemed to differ between muscle portions. Thus, the reduced TNF in MS pigs resulted mostly from a lower number of primary myotubes in the future white portion, and from a decreased secondary/primary ratio in the future red portion.

Key Words: muscle fiber, myosin heavy chain, enzyme activity, myogenesis, pig, breed

Zusammenfassung

Titel der Arbeit: **Abläufe der Muskelfaserbildung bei Large White und Meishan Schweinen**

Die Untersuchungen zielen auf den Vergleich der Muskelfasertypen-Zusammensetzung und Myogenese des Skelettmuskels zweier Schweinerassen, Large White (LW) und Meishan (MS), mit extremen Unterschieden im postnatalen Wachstumspotential und in Fleischqualitätsmerkmalen. Untersucht wurden der M. longissimus dorsi (LM) von Tieren mit 62 kg Körpergewicht sowie der M. semitendinosus (SM) zum Zeitpunkt der Geburt und am Tag 75 der pränatalen Entwicklung.

Im ersten Experiment zeigt sich eine auffällige Verschiebung in der Fasertypenverteilung von Typ IIb (Myosin heavy chain IIb, MyHC IIb) nach Typ IIx und im geringeren Umfang auch nach Typ IIa im LM von MS im Vergleich zu LW, in Übereinstimmung mit einem weniger glykolytisch und mehr oxidativem Stoffwechsel. Neben diesen Unterschieden in der Fasertypenverteilung zeigte sich eine drastisch niedrigere Gesamtfaseranzahl (TNF) im SM von MS verglichen mit LW. Dieser Unterschied war ausgeprägter im späteren weißen als im roten Anteil des SM. Die zugrundeliegenden Mechanismen sind in beiden Muskelanteilen unterschiedlich: Die geringere TNF bei MS-Schweinen resultiert im wesentlichen aus einer geringeren Anzahl an primären Muskelfasern im späteren weißen Muskelanteil und aus einem kleineren Verhältnis sekundärer zu primären Myotuben im späteren roten Muskelanteil.

Schlüsselwörter: Muskelfaser, Myosine schwere Kette, enzymatische Aktivität, Muskelentwicklung, Schwein, Geschlecht

Introduction

Eight isoforms of myosin heavy chains (MyHC), each encoded by a separate gene, are located in two clusters in skeletal muscle of mammals (SHRAGER et al., 2000; WEISS et al., 1999). In the pig, one cluster contains the α- and type I (or β) MyHC on chromosome 7, whereas the other one contains the embryonic, IIa, IIx, IIb, neonatal and extraocular MyHC on chromosome 12 (DAVOLI et al., 2002). In postnatal growing pigs, only types I, IIa, IIx and IIb MyHC are expressed in skeletal muscle (LEFAUCHEUR et al., 2002). Most studies classify fibers in three types by histochemistry (BROOKE and KAISER, 1970; ASHMORE and DOERR, 1971), and do not take into account the existence of four MyHC isoforms. The first experiment will re-examine fiber typing in pig skeletal muscle in the Meishan (MS) and Large

White (LW) breeds. The MS pigs exhibit lower growth rate, poorer feed efficiency and lower lean meat content than conventional western pig breeds (BIDANEL et al., 1990; BONNEAU et al., 1990; WHITE et al., 1995), but sensory quality of their meat is superior (SUZUKI et al., 1991; TOURAILLE et al., 1989). The total number of fibers (TNF) is fixed before birth in pig (WIGMORE and STICKLAND, 1983), and post-natal muscle growth depends on their hypertrophy. Thus, the second experiment will deal with the comparison of the TNF between MS and LW pigs at birth. Because the TNF results from two successive generations of myofibers, i.e. a primary generation up to 50-55 days of gestation (dg) followed by a secondary generation up to 90 dg, the last experiment will aim at better describing the genesis of primary and secondary fibers between MS and LW pigs during the fetal period.

Material and methods

Experiment 1: Eight LW and eight MS females were fed under the same standard conditions until an average body weight of 62 kg, corresponding to 131 and 142 d of age in LW and MS animals, respectively. Within 30 min of slaughter, the longissimus muscle (LM), a predominantly fast-twitch glycolytic muscle, was sampled adjacent to the last rib level, and frozen in 2-methylbutane (isopentane) pre-chilled by liquid nitrogen. Histological fiber typing was based on acto-myosin ATPase staining (BROOKE and KAISER, 1970), and MyHC polymorphism using in situ hybridization and immunocytochemistry as previously described (LEFAUCHEUR et al., 2004). Differences were also quantified by real time RT-PCR. Activities of lactate dehydrogenase (LDH, E.C. 1.1.1.2.7), citrate synthase (CS, E.C. 1.1.3.7) and β-hydroxy-acyl-CoA dehydrogenase (HAD, E.C. 1.1.1.35) were used as markers of glycolytic metabolism, global oxidative capacity (tricarboxylic cycle) and lipid β-oxidation potential, respectively. Enzyme activities were expressed as µmol of substrate degraded $\cdot min^{-1} \cdot$g of fresh $muscle^{-1}$(LEBRET et al., 1999).

Experiment 2: Six LW and 9 MS one day old piglets were used. The semitendinosus muscle (SM) was excised, weighed, tied at resting length, and frozen in isopentane pre-chilled by liquid nitrogen. Histological examination was used to determine the TNF, as well as the number of primary and secondary fibers, in the future red and white portions of the SM. Activities of LDH, CS and HAD were also determined.

Experiment 3: The SM was taken from 6 LW and 6 MS 75 day old foetuses and processed for histology as described in experiment 2.

Results and discussion

Experiment 1: No difference in fiber type composition (I, IIA, IIB) between LW and MS breeds was observed using conventional acto-myosin ATPase staining (BROOKE and KAISER,1970) (Table 1).

In contrast, a decrease in MyHC IIb to the advantage of MyHC IIx, and to a lesser extend IIa, was observed in the MS breed at the protein and mRNA levels. Figure 1 illustrates differences of IIx and IIb MyHC expressions between the LW and MS breeds at the mRNA level by in situ hybridization.

Table 1
Proportions of myosin heavy chain I, IIa,IIx and IIb and enzyme activities in longissimus muscle of Large White (LW) and Meishan (MS) pigs

	LW	MS	RSD[a]	P[b]
Histoenzymology[c] (number, %)				
I	7.9	7.5	2.2	NS
IIA	5.5	5.3	2.7	NS
IIB	86.6	87.3	3.0	NS
Histoimmunology[d] (area,%)				
I	7.2	8.0	1.4	NS
IIa	8.6	11.4	2.1	*
IIx	24.5	62.5	20.8	**
IIb	74.6	57.9	7.0	***
In situ hybridation[e] (area, %)				
I	6.4	7.3	1.5	NS
IIa	9.4	14.1	4.2	*
IIx	25.5	67.5	10.9	***
IIb	77.5	56.8	7.2	***
RT-PCR (%)				
I	6.2	8.3	2.9	NS
IIa	7.5	13.4	3.3	**
IIx	23.4	61.1	11.9	***
IIb	62.9	17.1	10.6	***
Enzyme activities[f]				
LDH	2529	2086	264	***
CS	6.41	8.30	0.86	***
HAD	3.48	5.08	0.54	***
LDH/CS	405	252	64	***
HAD/CS	0.55	0.61	0.05	*

[a]Residual standard deviation within each muscle.
[b]Statistical significance: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$, NS, not significant.
[c]Conventional amATPase typing (BROOKE and KAISER, 1970), numerical percentages.
[d]Percentage area stained by each monoclonal antibody.
[e]In situ hybridization (percentage area labeled by each riboprobe).
[f]Activities expressed as µmol substrate·min^{-1}·g of fresh muscle^{-1}. LDH, CS and HAD denote lactate dehydrogenase, citrate synthase and β-hydroxy-acyl-CoA dehydrogenase, respectively.

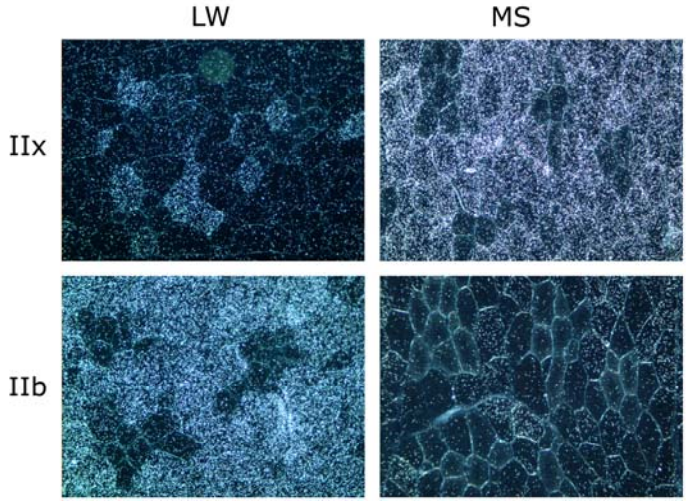

Figure: In situ hybridation using ^{35}S labelled MyHC IIx and IIb riboprobes in longissimus muscle of Large White (LW) and Meishan (MS) pigs.

Thus, MyHC IIb is the prominent isoform in LM of LW, whereas it is IIx in LM of MS pigs. Present results clearly show that the classification in types I, IIA, and IIB is insufficient to type myofibers in pig longissimus, and that MyHC IIb expression is not

restricted to small species as previously suggested (PETTE and STARON, 2000). Besides, MS pigs exhibited a lower LDH and higher CS and HAD activities than LW. Thus, the shift towards a slower phenotype in MS pigs was consistent with a less glycolytic and more oxidative metabolism, potentially using more lipids as fuel.
The striking differences of IIx and IIb MyHC expressions between LW and MS breeds raises the question of their specific implication in muscle growth and meat quality. Because many other muscle characteristics differ between LW and MS pigs, further experiments are needed to specifically address this point.
Experiment 2: At birth, MS animals exhibited smaller SM in absolute terms as well as relative to body weight (Table 2). The TNF was 38% lower in whole SM of MS than LW pigs. However, the decrease was stronger in the future white (-41%) than red (-33%) portions of the muscle. The clusters consisting in groups of slow fibers surrounded by fast fibers could only be accurately identified in the red portion. In this red portion, the number of clusters, corresponding to the number of primary fibers, did not differ significantly between breeds, whereas the number of fibers per cluster, i.e. the secondary/primary ratio, was drastically reduced in MS pigs (-28%, $P<0.001$). Mean fiber cross sectional area tended to be larger in MS pigs only in the future red portion. Therefore, the smaller SM in MS animals at birth is the result of a lower TNF, in particular in the future white portion of the muscle.

Table 2
Comparison of histochemical characteristics and enzyme activities between Large White (LW) and Meishan (MS) semitendinosus muscle (SM) at birth

	LW	MS	RSD[a]	P[b]
Birth weight (g)	1311	983	215	**
SM weight (g)	2.74	1.61	0.41	***
SM weight (mg/g BW))	2.15	1.58	0.13	***
Histochemistry				
Whole muscle				
TNF[c] (10^{-3})	425	263	41	***
Red portion				
TNF (10^{-3})	193	128	29	*
Primary fibers	7722	6877	1206	NS
Secondary/primary	26.6	19.2	3.1	***
White portion				
TNF (10^{-3})	232	136	29	***
Enzyme activities[d]				
LDH	52.1	45.0	8.2	NS
CS	10.7	12.2	0.7	***
HAD	7.27	8.28	0.56	***

[a]Residual standard deviation.
[b]Statistical significance: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$, NS, not significant.
[c]Total number of fibers.
[d]Activities expressed as µmol substrate·min^{-1}·g of fresh muscle^{-1}. LDH, CS and HAD denote lactate dehydrogenase, citrate synthase and β-hydroxy-acyl-CoA dehydrogenase, respectively.

Experiment 3: At 75 days of gestation (dg), all primary myotubes are present, whereas the secondary generation will be fully differentiated only from 90 dg onwards (WIGMORE and STICKLAND, 1983). At this stage, it is possible to distinguish primary from secondary myotubes in both the future red and white portions of SM. Interestingly, primary myotubes stained positively with an anti-slow MyHC antibody and negatively with an adult anti-fast MyHC antibody in the future red portion, whereas the opposite was observed for primary fibers in the future white portion (LEFAUCHEUR et al., 1995). The number of primary myotubes in the future white

portion was drastically reduced in MS pigs (-40%, P<0.001, Table 3). The difference went in the same direction in the future red portion but did not reach significance. The secondary/primary ratios did not differ between breeds, and were similar in the future red and white portions (17.8). This suggests that the rate of secondary fiber differentiation around each primary myotube was similar in both breeds and both muscle portions. Therefore, the higher secondary/primary ratio in LW (26.6) than MS (19.2) pigs in the future red portion at birth (Table 2) could be due to a longer period of secondary fiber differentiation in LW than MS pigs. Based on the number of primary fibers found in the future white portion of ST muscle at 75 dg (Table 3), the secondary/primary ratio in the future white portion at birth was calculated to be 22.0 and 21.7 in LW and MS pigs, respectively. Therefore, the strikingly lower TNF in MS than LW pigs in the future white portion of the SM would entirely result from a lower number of primary fibers, in contrast to the future red portion in which mostly the secondary/primary fiber ratio was affected between breeds.

Table 3
Comparison of semitendinosus muscle (SM) histochemical characteristics between Large White (LW) and Meishan (MS) pigs at 75 days of gestation.

	LW	MS	P[a]
Body Weight (g)	294	245	***
SM weight (g)	0.63	0.35	***
SM weight (mg/g BW)	1.91	1.44	***
Histochemistry			
Whole muscle			
TNF[b] (10^{-3})	283	200	**
Red portion			
TNF (10^{-3})	116	91	NS
Primary fibers	6520	5200	NS
Secondary/primary	17.9	17.4	NS
White portion			
TNF (10^{-3})	167	109	**
Primary fibers	9580	5800	***
Secondary/primary	17.4	18.7	NS

[a]Statistical significance: **, P < 0.01; ***, P < 0.001, NS, not significant.
[b]Total number of fibers.

In conclusion, the present study shows that the lower potential for postnatal muscle growth of MS than LW pigs is already present at birth via a reduced TNF, a factor fixed before birth. Both primary and secondary fibers are involved but their respective influence seems to depend on the muscle type. Postnatally, additional striking differences between breeds still occur for contractile and metabolic maturation of myofibers. The significance of all these differences between breeds for muscle growth and meat quality deserves further studies

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Genetic foundation of meat quality traits of station tested slaughter pigs in North Rhine-Westphalia (Germany). A status report

Abstract

Within the German herdbook organisations several meat quality parameters were measured on station tested slaughter pigs in order to improve pig meat quality genetically. PSE meat is characterized by pH (pH_1), conductivity ($C_{1,24}$) and meat colour (Opto) measurements recorded in the loin at the $13^{th}/14^{th}$ rib cut 1 or 24 h after slaughter. In addition pH is measured in the ham and loin 24 h post mortem ($pH_{24,Loin/Ham}$), which are used as indicators of DFD meat.

Genetic parameters of these traits were estimated by using carcass data of purebred Piétrain gilts (Pi, N=5346), German Large White or Landrace castrates (Dam, N=4676) and Pi×F1 female crossbreds (Pi×F1, N=4009), station tested during the years 1999 to 2004. Within the Pi-group, 2118 and 1013 pigs were homozygote carriers (Pi_{PP}) or non carriers (Pi_{NN}) of the stress MHS-allele P. REML-variance components were estimated within the breeds and MHS-status groups by using bivariate animal models, which comprise the fixed effects MHS-genotype and day of slaughter.

With the exception of C_{24} in the Pi_{PP} (h^2: 0.31) and Pi×F1 (0.32) groups the h^2 of $C_{1,24}$ did not exceed 0.2. Slightly higher h^2 were estimated for pH_1 ranging from 0.16 (Dam) to 0.44 (Pi_{PP}). As expected high genetic correlations ($r_g > |0.75|$) were estimated between pH_1, C_1 and C_{24}. However, within the stress robust Dam- and Pi_{NN}-groups the r_g between $C_{1,24}$ and pH_1 were considerably lower (r_g between -0.12 and -0.48) indicating that different genes are possibly involved in the expression of these traits.

Heritabilities for meat colour (Opto) were highest within the Dam group (h^2: 0.35), whereas in all other breeds these estimates range between 0.11 and 0.21. Only within the Pi breed moderate r_g were estimated between Opto and pH_1 or $C_{1,24}$ (rg: |0.44| to |0.48|), whereas in the Dam- and Pi×F1-lines these relationships were close to zero.

For all breeds, estimated h^2 for the DFD indicators $pH_{24,Loin/Ham}$ vary from 0.24 to 0.28.

Key Words: meat quality, pigs, driploss, genetic foundation

Zusammenfassung

Titel der Arbeit**: Genetische Parameter von Fleischbeschaffenheitsmerkmalen von stationär geprüften Schlachtschweinen in Nordrhein-Westfalen. Ein Status Bericht**

An den Schlachtkörpern stationär geprüfter Schweine werden in der deutschen Schweineherdbuchzucht zu züchterischen Zwecken eine Reihe von Merkmalen zur Beurteilung von Fleischbeschaffenheitsmängeln erfasst. PSE-Fleischeigenschaften sollen mit den 1 oder 24 h nach der Schlachtung an der Anschnittstelle 13./14. Rippe gemessenen Merkmalen pH (pH_1), Leitfähigkeit ($C_{1,24}$) und Fleischfarbe (Opto) charakterisiert werden. Zusätzlich wird der pH-Wert, 24 h post mortem als Indikator von DFD-Fleisch im Schinken gemessen.

Für diese Merkmale wurden auf der Basis der in den Jahren 1999 bis 2004 erfassten Schlachtköperdaten von Reinzucht Pietrain Sauen (Pi, N=5346), Börgen der Rassen Deutsches Edelschwein und Deutsche Landrasse (Dam, N=4676) und Pi×F1 Kreuzungssauen (Pi×F1, N=4009) genetische Parameter geschätzt. Innerhalb der Pi-Gruppe waren am MHS-Genlocus 2118 bzw. 1013 Schweine homozygote Träger (Pi_{PP}) bzw. Nichtträger (Pi_{NN}) des Stressallels P. REML-Varianzkomponenten wurden innerhalb der Linien und MHS-Gruppen auf der Basis einer Reihe bivariater Tiermodelle geschätzt, die die fixen Modellfaktoren MHS-Genotyp und Schlachttag beinhalteten.

Mit der Ausnahme von C_{24} in der Pi_{PP} (h^2: 0,31) und Pi×F1 (0,32) Gruppe lagen die h^2 von $C_{1,24}$ nicht über 0,2. Unwesentlich höhere h^2 wurden für das Merkmal pH_1 mit Werten zwischen 0,16 (Dam) bis 0,44 (Pi_{PP}) geschätzt. Wie erwartet wurden hohe genetische Korrelationen ($r_g > |0,75|$) zwischen pH_1, C_1 und C_{24} ermittelt. Innerhalb der Stress robusten Mutterlinien- and Pi_{NN}-Gruppe waren hingegen die r_g zwischen $C_{1,24}$ and pH_1 wesentlich schwächer ausgeprägt (r_g zwischen –0,12 und –0,48). Dies deutet daraufhin, dass möglicherweise unterschiedliche Gene an der Expression dieser Merkmale beteiligt sind.

Die höchsten Heritabilitäten für das Merkmal Fleischfarbe (Opto) wurden in der Mutterlinien-Gruppe (h^2: 0,35) geschätzt, während in allen anderen Herkünften diese Schätzwerte nur zwischen 0,11 and 0,21 rangierten. Nur in der Pi Herkunft wurden moderate r_g zwischen den Merkmalen Opto und pH_1 oder $C_{1,24}$ (rg: |0,44| to |0,48|)

festgestellt, während in den Mutterlinien- und Pi×F1-Herkünften sich diese Beziehung nur unwesentlich von 0 unterschieden. In allen Herkünften variierten die geschätzten h^2 für die DFD Indikatoren $pH_{24,Loin/Ham}$ zwischen 0,24 to 0,28.

Schlüsselwörter: Fleischqualität, Tropfsaftverlust, genetische Fundierung

Introduction

In order to genetically improve pig meat quality, traditionally several meat quality parameters are measured on station tested slaughter pigs within the German herdbook organisations. Pale, Soft and Exudative (PSE) meat is characterized by pH, conductivity and meat colour measurements recorded in a loin cut 1 or 24 h after slaughter. In addition, to be used as indicators of DFD meat, pH is measured in the ham and loin 24 h post mortem. Conductivity measured 24 h (northwest part of Germany) or pH measured 1 h after slaughter (southern part of Germany) are included into the aggregate genotype used for selection within the herdbook nucleus dam and sire breeds.

While within the dam lines Large White and Landrace the stress causative MHS allel P is completely removed, this allel is still segregating within the sire line Pietrain. However, currently efforts are in progress to eliminate the P allel also within the Pi-breed.

In consideration of these ongoing developments two questions arise: a) Is there still a genetic foundation of pH, conductivity or meat colour measurements which allows a sufficient genetic progress? b) Do these indirect meat quality parameters offer sufficient information to the improvement of direct meat quality parameters like drip loss, which is of major importance for the slaughter industry?

Material and methods

The analyzed data set comprises on station tested 4676 castrates of the dam lines German Landrace (LR), German Edelschwein (LW) and crosses of type LW×LR (F1), 5346 sows of the sire line Piétrain (Pi) and 4009 female slaughter pig of type Pi×F1. These pigs were housed in groups of two full sibs which had ad libitum access to feed and water. The wheat-barley-soybean meal diets contained 16% CP, 1% lysine and 13.0 MJ/kg ME. The fattening period started at 35 kg and ended at a live weight of 105 kg (carcass weight of approximate 85 kg). Along with several carcass composition traits, the meat quality traits pH and conductivity were recorded immediately 1h and 24h after slaughtering with the LF- and pH-star devices (Company Matthäus, Klausa). These recordings were performed in the loin at the $13^{th}/14^{th}$ rib cut and in the ham at three different positions according to the rules of stationary performance testing in Germany (ALZ, 2003). Meat colour, defined as the L-value of the Cielab system was measured at the loin cut 24 h post mortem using the Opto-Star equipment (Company MATTHÄUS, KLAUSA).

In addition to these classical meat quality traits, the drip loss of a longissimus dorsi meat sample was recorded by the EZ-DripLoss method developed by the Danish Meat Research Institute (CHRISTENSEN, 2003). These recordings were only performed in a subset of slaughter pigs as shown in table 1.

Genetic REML-parameters were estimated within the breeds and MHS-status groups by using a series of bivariate animal or sire models covering 5 generation of pedigree information. A sire model was used within the Pi×F1 breed because no pedigree

information of the F1 sows was available. As fixed environmental effects the model includes the day of slaughter (meat quality traits), month of slaughter (carcass lean content, daily gain) and slaughter weight (carcass lean content). Genetic analysis were performed with the VCE 4 software (NEUMAIER and GROENEVELD, 1998). Because of the small data set correlations between EZ-DripLoss and other traits were only calculated phenotypically.

Results and discussion

Table 1 shows the well known differences between the different breeds. Pi sows show a clear superiority in the carcass lean content up to 10% relative to the LW/LR castrates, but a distinct inferiority with respect to daily gain and all meat quality traits. The commercial crosses Pi×F1 take a medial position between sire and dam breeds in all traits.

Table 1
Lean content, daily gain and meat quality traits depending on breed and MHS-status

Breed	Pi						LR/LW		Pi×F1	
MHS-status	NN		NP		PP		NN		NP (NN)	
N	1013		2215		2118		4676		4009	
	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s
Slaughter weight, kg	84.2	2.60	84.6	2.68	85.4	2.66	85.1	3.07	84.9	2.75
Daily Gain	816	77.1	815	86.0	804	85.9	891	94.5	837	85.4
Carcass lean content, %	64.4	1.15	64.7	1.18	65.1	1.21	55.1	3.07	59.5	2.18
Meat colour (Opto)	68.0	7.03	64.8	6.06	57.9	10.72	68.1	6.27	67.0	6.30
Conductivity, loin, 1h p.m., mS **($C_{1,loin}$)**	4.40	0.73	4.47	1.16	8.30	4.55	4.25	0.57	4.37	0.83
Conductivity, loin, 24h p.m., mS **($C_{24,loin}$)**	2.97	0.85	3.72	1.59	7.50	2.75	2.61	0.74	3.29	1.21
pH, loin, 1h p.m. **($pH_{1,loin}$)**	6.54	0.22	6.30	0.26	5.79	0.37	6.54	0.20	6.39	0.27
pH, loin, 24h p.m. **($pH_{24,loin}$)**	5.47	0.10	5.46	0.09	5.48	0.10	5.51	0.10	5.49	0.10
pH, ham, 24h p.m. **($pH_{24,ham}$)**	5.61	0.12	5.63	0.13	5.65	0.16	5.65	0.15	5.63	0.14
N (EZ-DripLoss)	293		221		143		668		374	
EZ-DripLoss	2.21	1.84	3.83	2.48	3.80	2.79	1.93	1.69	2.36	1.91

Regarding the MHS-status groups within the Pi-breed the difference between the PP and NN genotype are 0.7 %, which is approximately half a standard deviation. This relative small difference reflects the efforts of the breeders to improve the carcass composition traits particularly within the MHS NN-genotype group. Distinct differences can be observed between different PSE characterizing meat quality traits. The difference between the NN- and PP-genotypes exceeds 2 standard deviations, showing the large impact of the stress allel P on early post mortal meat quality characteristics. Similar results were published by LARZUL et al. (1997) and LAUBE et al. (2000). With respect to the EZ-DripLoss the differences between the extreme MHS-genotypes are less expressed (~1 s). However, as demonstrated for the examples $pH_{1,loin}$ and EZ-DripLoss in Figure 1a,b, differences between the MHS-status groups

are less explainable by overall mean differences. On the contrary such differences can be explained by a higher proportion of animals with extreme poor meat quality in the PP group, which is unexpected according to normal distribution theory.

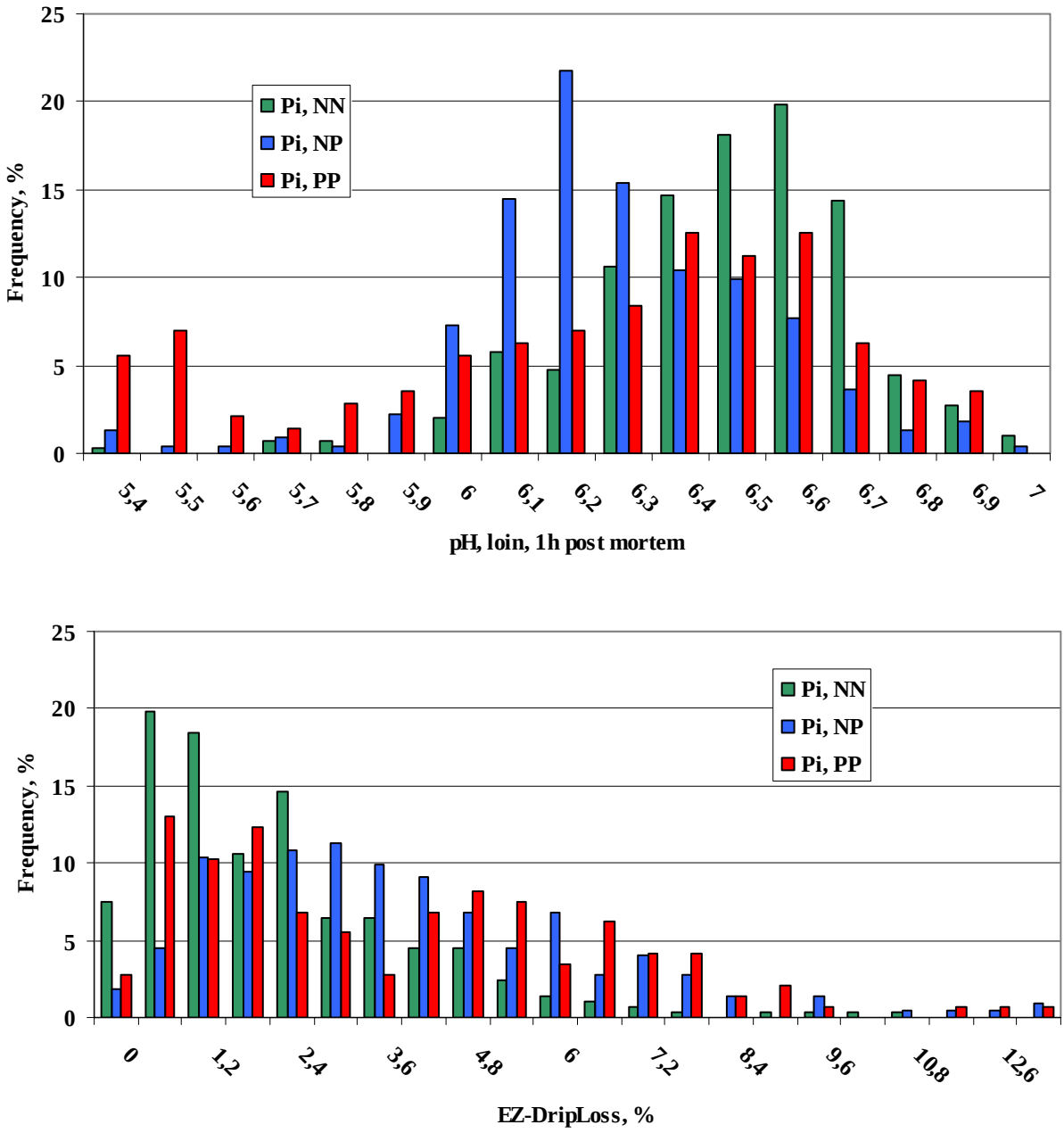

Fig. 1: Distribution of pH loin, measured 1h post mortem and EZ-DripLoss

Heritability estimates for PSE-meat characterizing traits ($C_{1,loin}$, $C_{24,loin}$, $pH_{1,loin}$) range within the NN- (LW/LR) and NP- (Pi) groups from 0.07 to 0.22. A similar range of h^2 estimates can be found in the literature, as been summarized by the study of SELLIER (1998) or KRIETER and THOLEN (2001). Within these groups $pH_{1,loin}$ offers slightly higher h^2 estimates then conductivity measurements. With the exception of $C_{1,loin}$, higher h^2 estimates for these traits were found in the Pi_{PP}- and Pi×F1 group. The relative high estimates in the Pi×F1 population can be explained by the ongoing segregation of the P-allel within that population. According to HONIKEL (1998) a

sufficient accuracy in the detection of PSE meat by conductivity measurements is possible between 2 and 50 h after slaughtering. However, within the commercial abattoir the first conductivity measurement has to be performed 1 h post mortem. The resulting measurement errors might be the causes for the low $C_{1,loin}$ h^2 estimates. As shown in table 1 almost no breed difference in the mean of the DFD characterizing meat quality traits could be observed. Nevertheless the heritability estimates for these traits are with the exception of $pH_{24,loin}$ (Pi, NN) and $pH_{24,ham}$ (Pi_{PP}) above 0.25 in all breeds, indicating a substantial within breed genetic variation for these traits. Meat colour is specifically heritable (h^2 < 0.35) within the LR/LW, Pi_{NP} and Pi×F1 crossbred population where the h^2 estimates exceed the corresponding $pH_{1,loin}$ and $C_{24,loin}$ estimates. These findings are in agreement with the results of SELLIER (1998) and HERMESCH et al. (2000a).

Table 2
Heritability (h^2) and common litter (c^2) effects depending of breed and MHS-status group

Breed		LR/LW		Pi						Pi×F1	
MHS-Status		NN		NN		NP		PP		NP / NN	
Trait		h^2	c^2	h^2	c^2	h^2	c^2	h^2	c^2	h^2	c^2
Carcass lean content		.86	.04	.49	.13	.69	.08	.71	.06	.81	.00
Conductivity, loin, 1h p.m.	**($C_{1,loin}$)**	.12	.05	.13	.11	.14	.02	.16	.10	.11	.01
Conductivity, loin, 24h p.m.	**($C_{24,loin}$)**	.07	.12	.16	.15	.15	.16	.31	.19	.31	.00
pH, loin, 1h p.m.	**($pH_{1,loin}$)**	.16	.12	.20	.10	.22	.06	.44	.15	.30	.01
Meat colour	**(Opto)**	.35	.07	.19	.01	.35	.03	.21	.12	.46	.00
pH, loin, 24h p.m.	**($pH_{24,loin}$)**	.26	.08	.14	.01	.29	.02	.29	.08	.38	.00
pH, ham, 24h p.m.	**($pH_{24,ham}$)**	.27	.06	.26	.04	.35	.00	.15	.01	.27	.01
s_h^2, s_c^2,	Min.	.02	.01	.06	.01	.04	.00	.04	.01	.04	.05
	Max.	.03	.02	.11	.05	.06	.03	.06	.03	.06	.06

In general the genetic correlation between carcass lean content and PSE characterising meat quality traits are unfavourable. This result is in agreement with previous reports (e.g. SELLIER, 1998; HERMESCH et al., 2000b; KRIETER and THOLEN, 2000). However, the correlation between carcass lean content and $pH_{1,loin}$ is substantially different from zero only within the Pi×F1. Similar low correlations were estimated for the relationship between carcass lean content and meat colour or $pH_{24,loin,ham}$. Considerable antagonistic correlations between these traits can only be observed within the PI_{NP} group.

As expected high genetic correlations (rg > |0.75|) were estimated between $pH_{1,loin}$, $C_{1,loin}$ and $C_{24,loin}$. (Table 3). However, within the stress robust Dam- and Pi_{NN} groups the genetic correlation between $C_{1,24,loin}$ and $pH_{1,loin}$ were considerably lower (rg between -0.12 and -0.46), indicating that different genes are possibly involved in the expression of these traits. Within the Pi_{NN} breed selection for low $C_{24,loin}$ and high $pH_{1,loin}$ values would lead to higher $pH_{24,Loin,Ham}$ values and visa versus, as indicated by the negative ($C_{24,loin}$) and positive ($pH_{1,loin}$) genetic correlation in the range of |0.10| to |0.41|. Within all other breeds these relationships are opposite in sign, but only within

the Pi_{PP}-breed substantially different from zero (rg: |0.28| to |0.52|). The genetic correlation between pH_{24} recorded at ham and loin position was close to unity, suggesting that the same genes are responsible for the expression of these traits.
Only within the Pi_{PP} and Pi_{NN} breed moderate genetic correlations were estimated between meat colour and $pH_{1,loin}$ or $C_{1,24,loin}$, whereas in the Dam- and Pi×F1-lines these relationships were close to zero.

Table 3
Genetic correlations between different meat quality traits, daily gain and carcass lean content, depending on breed and MHS-status

Trait	Breed	MHS Status	Conductivity, loin 1h p.m.	Conductivity, loin 24h p.m.	pH, loin, 1h p.m.	pH, loin 24h p.m.	pH, ham, 24h p.m.	Meat colour
Carcass lean content	Pi×F1	NP/NN	.12	-.04	-.21	.03	-.03	-.32
	LW/LR	NN	.29	.60	-.07	-.20	.18	.08
		NN	.17	.87	.06	-.17	.05	-.06
	Pi	NP	.24	.07	.10	-.30	-.21	-.41
		PP	-.03	.14	-.08	.18	.29	.11
Conductivity, loin, 1h p.m.	Pi×F1	NP/NN		.79	-.87	-.08	.21	-.02
	LW/LR	NN		.85	-.12	.26	.20	.28
		NN		.73	-.19	.00	.29	-.51
	PI	NP		.68	-.90	-.08	-.33	-.44
		PP		.76	-.86	.41	.45	-.63
Conductivity, loin, 24h p.m.	Pi×F1	NP/NN			-.74	-.19	.41	.18
	LW/LR	NN			-.46	.18	.31	.26
		NN			-.14	-.41	-.25	-.64
	Pi	NP			-.86	.03	-.03	-.06
		PP			-1.00	.28	.37	-.56
pH, loin, 1h p.m.	Pi×F1	NP/NN				.28	-.09	.17
	LW/LR	NN				-.22	-.10	-.02
		NN				.28	.10	1,00
	PI	NP				-.23	-.05	.08
		PP				-.41	-.52	.66
pH, loin, 24h p.m.	Pi×F1	NP/NN					.76	.49
	LW/LR	NN					.80	.57
		NN					1,00	.70
	PI	NP					.86	.76
		PP					.81	.24
pH, ham, 24h p.m.	Pi×F1	NP/NN						.46
	LW/LR	NN						.62
		NN						.81
	Pi	NP						.86
		PP						.08

Because of the small number of observations the relationship between EZ-DripLoss and other traits were estimated only phenotypically. Similar to the indirect meat quality traits described above, the correlations between carcass lean content and EZ-DripLoss is unfavourable but showed only marginal deviations from zero. The highest relationship to EZ-DripLoss provides $C_{24,loin}$ and $pH_{1,loin}$ with correlations between 0.5 to 0.7. From this follows, that selecting for $C_{24,loin}$ or $pH_{1,loin}$ would lead to reasonable improvements in EZ-DripLoss in all breeds, provided that the phenotypic and genetic correlations are similar. Relative to these relationships the genetic correlation between

EZ-DripLoss and meat colour or $C_{1,loin}$ are slightly lower or, in case of the correlation between EZ-DripLoss and $pH_{24,loin,ham}$ close to zero.

Table 4
Phenotypic correlations between EZ-DripLoss and carcass lean content or meat quality traits

Breed	Pi			LR/LW	Pi×F1
MHS-status	NN	NP	PP	NN	NP (NN)
N	293	221	143	668	374
Lean content, %	0.23	0.16	0.14	0.08	0.17
Meat colour	-0.35	-0.22	-0.52	-0.34	-0.39
Conductivity, loin, 1h p.m.	0.45	0.38	0.48	0.25	0.38
Conductivity, loin, 24h p.m.	0.62	0.64	0.67	0.47	0.57
pH, loin, 1h p.m.	-0.61	-0.54	-0.69	-0.51	-0.60
pH, loin, 24h p.m.	-0.03	-0.17	0.05	-0.24	-0.16
pH, ham, 24h p.m.	-0.03	-0.07	0.01	-0.11	-0.13

Conclusions

Genetic foundation of PSE and DFD characterising meat quality traits have a sufficient genetic foundation to achieve considerable genetic progress in these traits. This holds true particularly within populations which carry the MHS-P allel ($Pi_{PP,NP}$, Pi×F1), whereas within P-allel free populations (LR/LW, Pi_{NN}) the h^2 estimated are slightly reduced. Considerable genetic antagonistic relationships exist only within the NN breed between carcass lean content and $C_{24,loin}$. This relationship might have negative effect on meat quality traits in breeding programs, which put much selection pressure on carcass lean content. However, this antagonism is not supported by the relationship between $pH_{1,loin}$ and carcass lean content. In addition, compared to previous studies in German Pi-populations the differences between Pi_{NN} and Pi_{PP} genotype have been considerably reduced, whereas the superiority of the Pi_{NN} genotype in all meat quality traits has been remained almost unchanged.

Still questionable is the relationship between more direct meat quality traits like sensoric traits and drip loss. Preliminary results of our study suggest a high correlation (0.6) between $pH_{1,loin}$ ($C_{24,loin}$) and EZ-DripLoss. A direct selection on EZ-DripLoss could be more efficient under the assumption of sufficient h^2. However, under the current conditions of stationary performance testing in North Rhine-Westphalia, cost and labour consideration favour the recording of $pH_{1,loin}$ and $C_{24,loin}$.

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Technological quality of broiler breast meat in relation to muscle Hypertrophy

(Die Beziehung zwischen technologischen Parametern der Fleischqualität der Brustmuskulatur und Muskelhypertrophie beim Broiler)

In a previous study, we reported that selection for rapid growth and/or increased muscle development modified meat quality parameters (BERRI et al., Poult. Sci. 2001, 80:833-838). We conducted the present one to relate breast muscle development, including *Pectoralis major* muscle fibre size, to *post mortem* metabolism and further breast meat quality. Phenotypic and genetic relationships between fibre and meat traits were estimated for a total of 600 commercial broilers. For all birds, we measured muscle fibre cross-sectional area (CSA) and the proportion of connective tissue, glycolytic potential, lactate content, *post mortem* pH fall and classical meat traits (colour, drip and thawing-cooking loss, Warner-Bratzler shear force). The fibre CSA was highly related to body weight and breast muscle weight and yield. However, it did not affect the proportion of connective tissue and did show any relationship with fibre necrosis. According to both the phenotypic and genetic correlations, as the fibre CSA increased the glycogen reserve of muscle before death (glycolytic potential) decreased. As a consequence, breast muscles with the largest fibres exhibited the highest ultimate pH. Besides, they contained the lowest lactate at 15 minutes *post mortem* and thus exhibited the lowest rate of pH fall. As a consequence, they also exhibited the darkest colour, the lowest drip and thawing-cooking losses and were tenderer after cooking. According to a multiple regression test, the drip loss and paleness of meat appeared chiefly determined by the pHu and to a lower extent by the rate of pH fall. By contrast, the properties of cooked meat (thawing-cooking loss and shear force) were also partly determined by fibre CSA. In conclusion, this study shows that in broiler chickens, increased breast muscle development is associated with muscle fibre hypertrophy and improvement in processing ability of breast meat.

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Functional genomics applied to the analysis of bovine muscle Hypertrophy

(Funktionelle Genomik zur Analyse der bovinen Muskelhypertrophie)

Muscle hypertrophy is of particular interest in beef meat production as it has a strong economic importance. High muscle development is accompanied by particular characteristics in favour of improvement of meat tenderness but not of its flavour. This study was therefore conducted to identify some markers of muscle hypertrophy. We have studied two models of cattle with different origins of muscle hypertrophy: monogenic (Belgium Blue bulls double-muscled: DM) or polygenic (divergent lineages of Charolais bulls: with high (H) or low (L) muscle growth rate). Differential proteomic analysis of *Semitendinosus* muscle (ST, mixed fast glycolytic) was performed using two-dimensional gel electrophoresis (4-7pH gradient in the first dimension and 11% SDS-PAGE in the second) followed by mass spectrometric analysis of interesting spots (MALDI-TOF). A statistical analysis derived from the standard *t* test used for analysis of microarray data, the SAM method (for Significance Analysis of Microarrays) and adapted for proteomic analysis was used to detect proteins differentially expressed between two groups. Among the proteins differentially expressed between high and low muscled cattle, results revealed 17 proteins common of the two models DM and H. Eight proteins were over-expressed in high-muscled cattle. They corresponded mainly to Myosin Binding Protein H, isoforms of fast Troponin T, Myosin Regulatory light chain 2 and 3, Phosphoglucomutase. Nine other proteins such as slow Troponin T isoforms, slow isoforms of Myosin light chain 1, p20 were under-expressed in high muscled cattle. These proteins variably expressed in the different studied models could be good markers for muscle hypertrophy. Our results provide further evidence for a reinforced fast glycolytic phenotype in *Semitendinosus* muscle of high-muscled bulls. These properties have been confirmed by biochemical studies and a transcriptomic analysis of ST and *Rectus abdominis* (RA, slow oxidative) muscles of the same animals belonging to the divergent lineage of Charolais bulls.

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Transcript analysis using the microarray technology to investigate the stress response in the common carp *Cyprinus carpio* L.

(Transcript-Analyse mittels Microarray Technologie zur Untersuchung der Stressreaktion beim Karpfen *Cyprinus carpio* L.)

Understanding the nature of the physiological response of farmed fish to aquaculture related stressors is of major importance improving animal welfare and consequently increasing yields from farms. Many husbandry practices are associated with stress and result in a physiological response, which can contribute to undesirable changes in fish flesh quality. To study the effect of stress on fish flesh quality, it is necessary to understand the physiological changes in the neuroendocrine stress response following the exposure to several stressors. This stress response is regulated by the hypothalamo-pituitary-interrenal axis.

To identify genes involved in the stress response in common carp, we constructed a brain, pituitary and internal tissue-specific cDNA library. Based on this library a cDNA microarray was generated. This microarray was used to detect gene expression differences during the stress response between two isogenic strains of carp, a wild type and a mutant strain. In contrast to the wild type strain, the mutant strain E5 fails to mount a normal cortisol stress response.

Tissue samples were collected from interrenals of isogenic strains of carp after 0, 30 and 180 minutes of netconfiment stress. Total RNA was isolated from these tissues and cDNA generated and labeled with the fluorphores Cy3 and Cy5. After hybridization, the image was processed and spot intensity ratios analyzed. Differently expressed cDNAs were sequenced. Several cDNAs could be identified by homology and several as unidentified expressed sequence tags (ESTs). The differently expressed genes in the interrenals, like Cytochrome P450 21A2 (CYP21) and Steroid Acute Regulatory Protein (STAR), shared roles in the steroidogenic pathway and were upregulated during stress. A comparison between the mutant and wildtype after 0, 30 and 180 minutes netconfinement revealed that there was a higher expression of CYP21 and STAR genes in the mutant. These differences in gene expression will be further analyzed.

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Early differentiation of the fish myotome as shown by in situ hybridisation of numerous muscle-specific transcripts

(Darstellung der frühen Differenzierung von Myotomen beim Fisch durch in situ Hybridisierung mit Muskel-spezifischen Transkripten)

To learn more about the gene activations that underlie the differentiation and the diversification of embryonic fish myotomal fibres, we investigated the developmental expression of a large repertoire of muscle-specific genes in trout embryos using in situ hybridisation of the corresponding transcripts. The earliest event of muscle differentiation, at about the 25-somite stage, was the expression of a variety of muscle-specific genes including slow-twitch and fast-twitch muscle isoforms. The activation of these muscle-genes started in the deep somitic domain, where the slow muscle precursors (the adaxial cells) were initially located, and progressively spread laterally throughout the width of the myotome. This medio-lateral progression of gene expression was co-ordinated with the lateral migration of slow adaxial cells which specifically expressed the slow myosin light chain 1 and the slow myosin heavy chain. Subsequently, the fast and slow skeletal muscle isoforms precociously expressed in the course of the medio-lateral wave of muscle-gene activation became down-regulated in the superficial slow fibres and the deep fast fibres respectively. Finally, several muscle-specific genes including troponins, a slow myosin-binding protein C, tropomodulins and parvalbumin started their transcription only in late embryos indicating that the differentiation of the slow and fast muscle fibers is not completed with the lateral migration of the slow muscle cells towards the outemost part of the somite. A challenge for the future would be to determine the molecular mechanisms that regulate this complex sequence of gene expression in the maturing fish myotome.

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Analysis of gene expression in specific muscles of swine and turkey

Abstract

The meat quality is a result of properties of muscle metabolism and muscle structure at slaughter. Thus, it is a quantitative trait with a large number of genes involved. Moreover, environmental factors are modifying the gene effects at all stages of the ontogenesis. In our approach, we focussed on the expression of a set of genes with potential functions in development and growth of skeletal muscle. This set includes key regulatory factors of muscle cell development and growth as well as genes coding for major structural components of skeletal muscle. In swine, we investigated the expression of a total of 12 genes in M. biceps femoris of newborn piglets by RT-real time PCR. Aim of this investigation was the identification of genes differentially expressed between piglets suffering from a muscular disease (splay leg) and healthy controls. Differences in the expression of several Myosin Heavy Chain (MyHC) isoforms were found only when the animals were assigned to groups with different degrees of pathological alterations in the ultrastructure of the muscle as revealed by electron microscopy. In a second part, the expression of 6 genes was investigated in the M. pectoralis superficialis in different turkey lines. The analyses were done at two different ages at slaughter and the birds were assigned to the groups with normal and poor meat quality, respectively. The expression of the adult isoform of MyHC was significantly increased in Kelly BBB toms at week 12 compared to genotype BUT BIG6. There was also a trend to an increased MyHC and decreased glypican expression in toms with poor meat quality regardless of line and age. In contrast, expression of myostatin and insulin-like growth factor1 showed an age-dependent pattern with increased values at higher ages. The investigations demonstrate that analysis of gene expression contibutes to the understanding of mechanisms involved in development of muscular diseases as well as in poor meat quality.

Key Words: gene expression, skeletal muscle, swine, turkey

Zusammenfassung

Titel der Arbeit: **Analyse der Genexpression in spezifischen Muskeln von Schwein und Pute**

Die Fleischqualität resultiert aus strukturellen und metabolischen Eigenschaften der Skelettmuskulatur zur Schlachtung. Dem entsprechend ist eine große Anzahl von Genen in ihre Ausprägung als quantitatives Merkmal einbezogen, die zudem während der gesamten Ontogenese modifizierenden Einflüssen unterworfen sind. Für unseren Untersuchungen wählten wir Gene aus, deren Produkte in Entwicklung und Wachstum der Skelettmuskulatur involviert sind. Dazu gehören Regulationsfaktoren sowie Gene, die für strukturelle Bestandteile der Muskelfasern kodieren. Beim Schwein wurde die Expression von insgesamt 12 solcher Gene im M. biceps femoris von 22 neugeborenen Ferkeln mittels Real-Time-PCR untersucht. Ziel hierbei war die Identifizierung von Expressionsdifferenzen zwischen gesunden und Spreizerferkeln. Die Expression von verschiedenen Isoformen der Myosin Heavy Chain (MyHC) korrespondierte dabei mit dem Schweregrad pathologischer ultrastruktureller Befunde im Muskel, In Untersuchungen am M. pectoralis superficialis von Puten verschiedener Genotypen (BUT BIG6 und Kelly's BBB) konnten altersabhängige Expressionsmuster für Myostatin und IGF1 ermittelt werden, während die Expression von Glypican herkunftsunabhängig bei Tieren mit schlechter Fleischqualität vermindert und die von MyHC adult isoform erhöht war. Die Untersuchungen zeigen, dass Genexpressionsuntersuchungen zur Aufklärung von Ursachen für Erkrankungen der Skelettmuskulatur bzw. für abweichende Fleischqualität beitragen können.

Schlüsselwörter: Genexpression, Skelettmuskulatur, Schwein, Pute

Introduction

Variation in phenotypical traits bases on genetic differences as well as from environmental influences during growth and development of the animals. The genetic influence on these traits is founded on structural differences (polymorphisms) in the genome. However, the direct impact of such polymorphisms on the gene product e.g. changes in the amino acid sequence with subsequent functional alteration of the

protein is only one possible mode of action. By far more important are differences in time point of expression of genes, the amount of transcripts and their tissue specificity, respectively. Consequently, a combined approach of structural and functional genome analysis is necessary to understand the biological processes leading to different phenotypes. Our investigations focussed on the expression of selected genes in single muscles. Both, the genes and the muscles were chosen on their potential involvement in the investigated phenotype.

Material and methods

Samples

Pig

Total RNA was isolated from *M. biceps femoris* of each 11 healthy and splay leg piglets (German Landrace) within 24 h post partum and stored at -80°C until further analysis.

Turkey

Muscle biopsies of the *M. pectoralis superficialis* were collected from each 6 randomly chosen BUT Big6 and Kelly BBB toms immediately after stunning. The first two groups were slaughtered at 12 weeks of age and the second two groups at an age of 22 weeks. Biopsies were tranferred into RNAlater solution (QIAGEN) and stored at -20°C until RNA isolation.

Table 1
Primers used for transcript quantification by Real-Time PCR

Gene		**Primers: (5' – 3'forward)**	**(5' – 3' reverse)**
Pig			
18S ribosomal RNA	**18S**	GACCATAAACGATGCCGACT	GGTGCCCTTCCGTCA
Myogenic factor 5	**MYF5**	GCTGCTGAGGGAACAGGTGGA	CTGCTGTTCTTTCGGGACCAGAC
Myogenic factor 6	**MYF6**	CGCCATCAACTACATCGAGAGGT	ATCACGAGCCCCCTGGAAT
Myogenic factor 3	**MYOD1**	CACTACAGCGGTGACTCAGACGCA	GACCGGGGTCGCTGGGCGCCTCGCT
Myostatin	**MSTN**	CCCGTCAAGACTCCTACAACA	CACATCAATGCTCTGCCAA
ß-actin	**ACT**	CGGGCAGGTCATCACCATC	CGTGTTGGCGTAGAGGTCCTT
*Myosin Heavy chain isoform**			
embryonic	**emb**	CCCGGCTTTGGTCTGATTT	GGTGTCGGCTGAGAGTCA
perinatal	**peri**	CGAGCCCTCCTGCTTTATCTC	TGCCAGATGAAAATGCAGGTT
slow I	**slow1**	GGCCCCTTCCAGCTTGA	TGGCTGCGCCTTGGTTT
2a	**2a**	TTAAAAAGCTCCAAGAACTGTTTCA	CCATTTCCTGGTCGGAACTC
2x	**2x**	AGCTTCAAGTTCTGCCCCACT	GGCTGCGGGTTATTGATGG
2b	**2b**	CACTTTAAGTAGTTGTCTGCCTTGAG	GGCAGCAGGGCACTAGATGT
Turkey			
18S ribosomal RNA**)**	**18S**	CGAATGTCTGCCCTATCAACT	TGGATGTGGTAGCCGTTTCT
Myosin Heavy Chain (adult isoform)	**MyHC**	CCGAAAGTCAGAGAAGGAAAGAATC	GGGAGGGTTCATGGAGAAGAC
Myosin Light Chain	**MyLC**	ACCTTTGAAGAGTTCCTGCCCAT	TGCCGTTGCCTTCCTTGTC
Glypican	**Gly**	AAAGATTCAATCACAGCCAAGGT	CGCCTCTTCTTGTCCTCACTG
Ryanodine receptor I	**RYR1**	GGCGGGGCTCTACGGGAGCTT	ACGTTCAGCCGGTCGATGCAGTT
Myostatin	**MSTN**	CGCGCACTTCTGTCATCAAA	GCACTTCCTTCCTGTCCAAAA
Insulin like growth factor 1	**IGF1**	CTTCTACCTTGCCCTGTGTTTG	CCTTGTGGTGTAAGCGTCTACTG

*Primers for porcine Myosin Heavy chain isoforms according to DA COSTA et al. (2002)

Gene expression

Expression of selected genes was investigated by real time PCR. Total RNA was used for reverse transcription (TaqMan RT reagents, Applied Biosystems). Relative amounts of gene expression were measured on an ABI Prism 7000SDS (Applied

Biosystems) by the relative standard curve method (User Bulletin No.II, Applied Biosystems) using 18S mRNA for normalization. The PCR primers for the analyzed genes are given in Table 1.

Results and Discussion

Pig

There was no significant effect of gender (results not shown) or health status on the expression of all investigated genes (Table 2). Only for myostatin – a negative regulating factor of muscle growth – a trend towards lower expression in splay leg piglets was observed ($p = 0.08$).

Table 2
Comparison of the relative expression of structural and regulatory components of *M. biceps femoris* between newborn healthy and splay leg piglets (LSM ± SE). Results are derived from a triplicate analysis per piglet and gene

Class	Gene	Healthy (n=11)	Splay leg (n=11)	p-value
Regulation factors	MYF5	0.84±0.30	1.31±0.30	0.27
	MYF6	1.18±0.22	1.15±0.22	0.94
	MYOD1	0.63±0.14	0.55±0.14	0.70
	MSTN	0.75±0.06	0.58±0.06	0.08
Structural components	ACT	0.88±0.19	0.80±0.19	0.76
	Myosin Heavy Chain isoform			
	emb	0.58±0.14	0.66±0.14	0.70
	peri	0.51±0.08	0.52±0.08	0.96
	slowI	0.14±0.03	0.15±0.03	0.91
	2a	0.66±0.09	0.51±0.09	0.27
	2x	0.60±0.11	0.34±0.11	0.12
	2b	0.41±0.11	0.36±0.11	0.76

These results demonstrate that the expression of the investigated regulatory and structural components of the skeletal muscle immediately after birth is not related to the hereditary disease congenital splay leg in piglets.

Electron microscopy of muscle samples from healthy and splay leg piglets revealed in part distinct differences in ultrastructure. In healthy piglets a regular structure of the skeletal muscle with clearly visible striation, evenly distributed myofibrils and single fat vacuoles could be observed. In contrast, 2 of the 4 investigated splay leg samples showed marked pathological alterations. The main findings were a partial loss of striation, wide cytoplasmic areas between the myofibrils and an increased number and size of fat vacuoles. These findings have the characteristics of a hydropic degeneration and do not correspond to specific muscular diseases described so far. However, samples of two piglets that were affected by splay leg did not reveal any major pathological findings.

According to these results, gene expression was re-analyzed with three groups consisting of each 2 healthy piglets without pathological findings (**Healthy**), splay leg piglets with normal ultrastructure of skeletal muscle (**Splay leg N**) and splay leg piglets with affected ultrastructure (**Splay leg A;** Figure 1**)**.

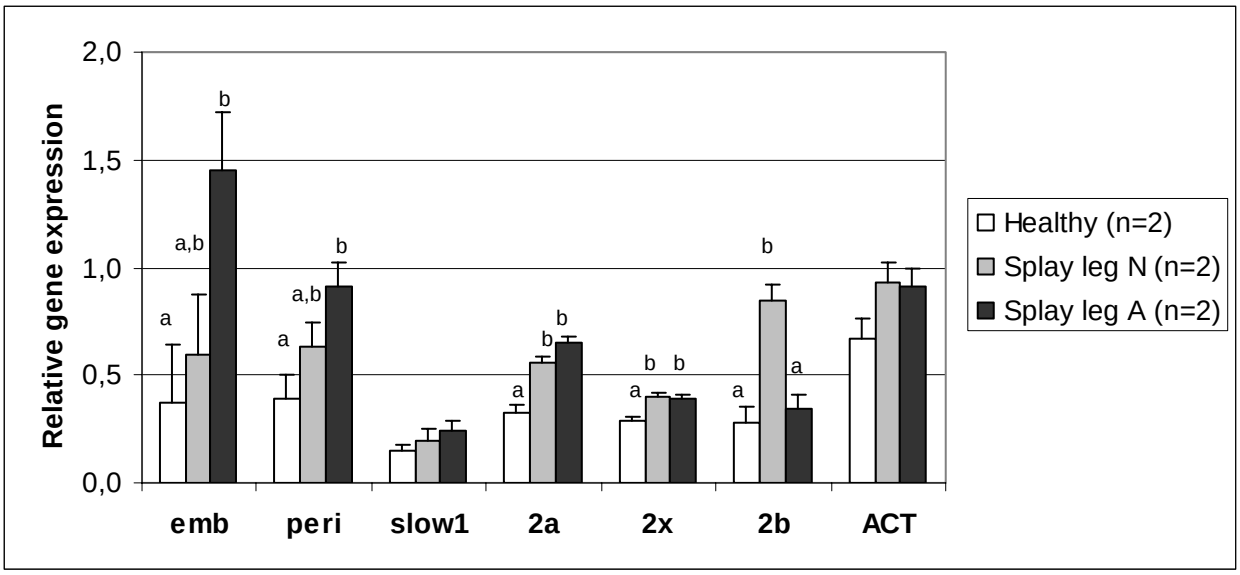

Fig. 1: Relative expression of structural genes in *M. biceps femoris* of splay leg piglets with (**Splay leg A**) and without (**Splay leg N**) pathological, ultrastructural findings compared to healthy controls (LSM ± SE). Valid comparisons are only possible within a gene. Different superscripts denote significant differences ($p < 0.05$)

The relative expression of 18S rRNA (not shown) was similar for all three groups.
Our limited material indicates a relationship between an increased expression of structural components of the skeletal muscle and the severity of the pathological findings. This could be interpreted as an ongoing repair process of the affected muscle. It can be concluded that the expression differences leading to the disease occurred in embryonic stages. Our samples characterize the result of the disease rather than the cause. Furthermore, a simple differentiation between the phenotypes healthy and splay leg is obviously not sufficient because of marked differences in the ultrastructure of the affected piglets within the disease group.

Turkey

In contrast to pig, aim of these investigations was to find a relationship between the expression of the selected genes and the meat quality characterized by the continuous traits pH value and electric conductivity.

Table 3
Relative gene expression in breast muscle of turkeys depending on line and age (LSM ± SE)

Genotype	**BUT Big 6**	**Kelly (BBB)**	**BUT Big 6**	**Kelly (BBB)**
Gene / Age (weeks)	**12**	**12**	**22**	**22**
MyHC	1.01 ± 0.22^{a}	2.76 ± 0.26^{b}	0.86 ± 0.21^{a}	1.12 ± 0.26^{a}
MyLC	1.21 ± 0.50^{a}	1.61 ± 0.57^{a}	1.64 ± 0.47^{a}	1.79 ± 0.57^{a}
Gly	1.56 ± 0.18^{a}	1.13 ± 0.21^{a,b}	0.95 ± 0.17^{b}	1.38 ± 0.21^{a,b}
RYR1	0.99 ± 0.47^{a}	0.77 ± 0.17^{a}	0.40 ± 0.09^{a}	0.82 ± 0.17^{a}
MSTN	0.33 ± 0.19^{a}	0.24 ± 0.22^{a}	1.84 ± 0.18^{b}	1.91 ± 0.22^{b}
IGF1	0.57 ± 0.24^{a}	0.92 ± 0.29^{a}	1.60 ± 0.29^{b}	2.01 ± 0.29^{b}

Different superscripts denote significant differences ($p < 0.05$)

The expression of the Myosin Heavy Chain gene (MyHC) as a major structural component of the skeletal muscle differed significantly between both lines at an age of

12 weeks (Table 3). There is a trend toward a decrease in MyHC expression with increasing age whereas Myosin Light Chain expression tends to increase. Glypican 1 as a potential regulator of muscle growth via the FGF2 signaling pathway is significantly less expressed in BUT BIG6 toms at week 22 compared to week 12 whereas the expression in Kelly BBB toms shows no major changes.
Myostatin is a potent inhibitor of muscle growth and is subsequently significantly higher expressed at higher ages in both lines. However, the same development was observed for the growth stimulating IGF1 in both lines. The Glypican gene expression was significantly related to meat quality. Its expression is lower in turkeys with poor meat quality (pH 20 min < 5.8) compared to normal meat quality (Figure 2).

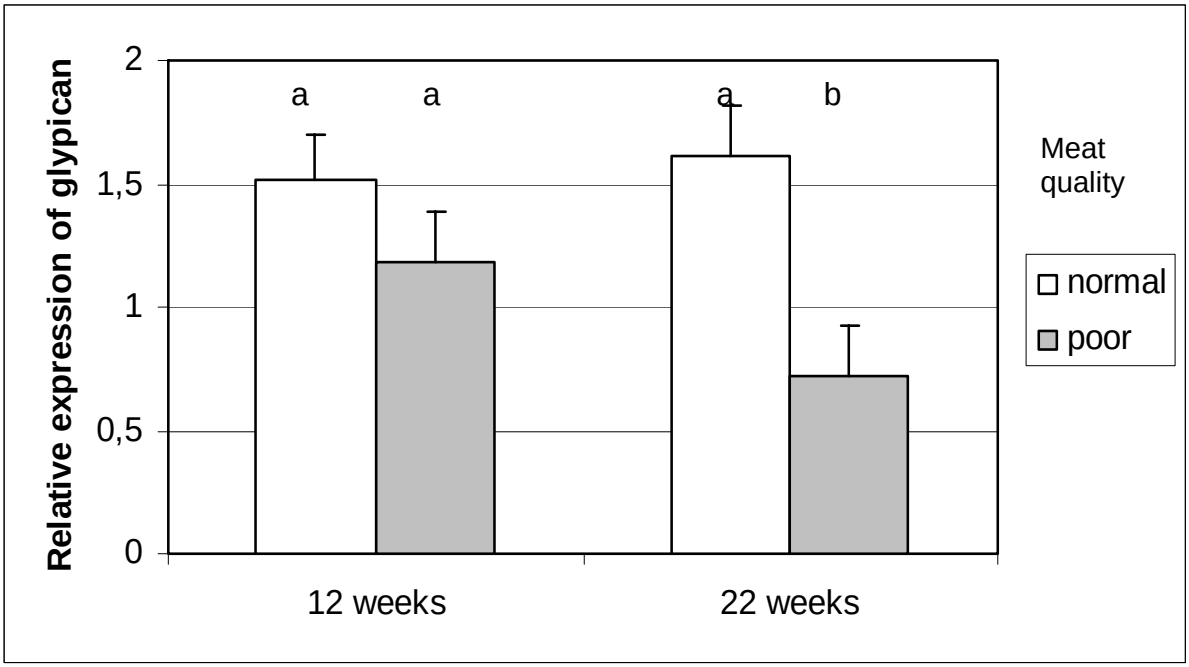

Fig. 2: Relative expression of glypican in *M. pectoralis superficialis* of turkey depending on age at slaughter and post mortem meat quality (LSM ± SE). Different superscripts denote significant differences within an age

Glypican is a component of the extracellular matrix and may influence meat quality by altering water binding capacity of the muscle (VELLEMAN, 2000).
The line specific profile of gene expression may be an indicator for differences in the growth curves of both lines.
Our investigations in swine and turkey demonstrate that the analysis of gene expression at the time point of phenotype sampling reveals differences that characterize the phenotype itself. However, in order to find expression differences related to the genetic cause of the phenotypes, it is necessary to monitor expression profiles during both prenatal and postnatal periods especially, as far as phenotypes basing on properties of the skeletal muscle are concerned.

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Muscle transcriptomes of Duroc and Pietrain pig breeds during prenatal formation of skeletal muscle tissue using microarray technology

Abstract

Mammalian myogenesis is an exclusive prenatal process regulated by the muscle regulatory factor gene family, which itself is regulated by numerous other genes. We developed a microarray consisting of the clones of two muscle-specific cDNA libraries with the addition of 500 genes with known function in myogenesis and energy metabolism. Tissue samples were collected of Duroc and Pietrain prenatal litters of 14 and 21 days of age (complete embryos) and 35, 49, 63, 77, and 91 days of age (longissimus muscle tissue) and RNA was isolated. Microarrays were hybridised with pools of six RNA samples. For each age comparisons between Duroc and Pietrain breeds were made, and transcriptome profile changes in time were made for Duroc pigs.

Comparison of Duroc and Pietrain prenatal muscle transcriptome expression profiles revealed differences in myogenesis regulating genes, suggesting differential timing of myogenesis between the two pig breeds. The differential development of the expression of the muscle structural genes strengthens this conclusion. Furthermore, differences in the expression of the energy metabolism genes were found. The results also suggest that the differential fat content between the Duroc and Pietrain pig breeds already starts to develop during early prenatal development.

The changes in the muscle transcriptome expression profiles during Duroc prenatal muscle development shows a profile of waves of expression of (i) myoblast proliferation stimulating genes,(ii) followed by myoblast proliferation inhibiting and differentiation stimulating genes during the primary muscle fibre development, which is repeated with lower magnitude during secondary muscle fibre development. Furthermore, expression of energy metabolism genes reaches a nadir when differentiation of myoblasts into myotubes takes place.

Microarray expression profiles were validated with five genes showing differential expression in the Duroc – Pietrain comparison, and in the Duroc development in time studies using 18S rRNA for normalisation. The real time PCR confirmed the microarray results.

Key Words: pig breeds, prenatal development, myogenesis, transcriptome profile, differential expression

Zusammenfassung

Titel der Arbeit: **Analyse des Muskeltranskriptoms während der pränatalen Entwicklung des Skelettmuskels bei den Rassen Duroc und Pietrain mittels Mikroarray Technologie**

Die Myogenese beim Säugetier ist ein pränataler Prozess, der durch die Mitglieder der Genfamilie der Muskel regulierenden Faktoren gesteuert wird, die selbst durch zahlreiche andere Gene reguliert werden. Ein Microarray bestehend aus Klonen von zwei Muskel-spezifischen DNA Bibliotheken sowie 500 Genen mit bekannter Funktion in der Myogenese und dem Energiehaushalt wurde konstruiert. Fetale Gewebeproben wurden bei den Rassen Duroc und Pietrain gesammelt (Tag 14 und 21 komplette Embryonen; Tag 35, 49, 63, 77 und 91 Gewebe des Musculus longissimus dorsi) und RNA wurde isoliert. Mikroarrays wurden mit Pools von je sechs RNA-Proben hybridisiert. Für jeden Zeitpunkt der pränatalen Entwicklung wurden Vergleiche zwischen den Rassen vorgenommen und für die Rasse Duroc Vergleiche zwischen den Entwicklungszeitpunkten.Unterschieden in der Expression der Myogenese-steuernden Gene zwischen Duroc und Pietrain weisen auf einen unterschiedlichen zeitlichen Verlauf der Myogenese bei den beiden Rassen hin. Unterschiede in der Expression von Muskelstrukturgenen bestätigen dies. Auch Differenzen in der Expression von Genen des Energiehaushalts wurden gefunden. Die Ergebnisse deuten zudem darauf hin, dass sich Unterschiede im Fettgehalt bei den Rassen Duroc und Pietrain bereits während er frühen pränatalen Entwicklung herausbilden. Die Änderungen der Genexpression während der pränatalen Muskelentwicklung bei der Rasse Duroc erfolgt in Wellen mit während der Bildung der primären Muskelfaser (i) zunächst Genen, die die Myoblastenproliferation stimulieren (ii) gefolgt von Genen, die die Myoblastenproliferation hemmen und ihre Differenzierung fördern und (iii) Wiederholung dieser Abfolge während der Bildung der sekundären Muskelfasern. Die Expression von Genen des Energiestoffwechsel ist minimal in den Phasen der Differenzierung. Die Ergebnisse der Mikroarray-Analyse wurden für fünf Genen mittel Echtzeit-PCR bestätigt.

Schlüsselwörter: Schwein, pränatale Entwicklung, Myogenese, Transkriptom, Expressionsprofil, Differentielle Expression

Introduction

The formation of muscle fibres (myogenesis) from precursor cells (myoblasts) is an exclusive prenatal process in mammals (REHFELDT et al., 2000). Myogenesis proceeds in two highly regulated waves following the specification and amplification of myoblasts in somites. During the primary wave myofibres are formed de novo from myoblasts while secondary myofibres are formed using the primary myofibres as a template (WIGMORE and STICKLAND, 1983).

During the last decades pigs have been selected for increased skeletal muscle mass on their carcasses (meat percentage). Skeletal muscle mass mainly relates to muscle fibre numbers (hyperplasia) and thickness (hypertrophy). While hypertrophic growth is mainly taking place postnatal, hyperplastic growth is exclusive prenatal. Pig breeds differ for myofibre hyperplasia, hypertrophy, and myofibre typing. Pig muscle composition is of high economic importance. Thus, the study of the prenatal myogenesis may highlight important fundamental processes that can be used in pig breeding to improve pig meat production. In the pig primary muscle fibre formation takes place in a period around day 35, and secondary muscle fibre formation is taking place in a period at approximately day 65 of gestation (WIGMORE and STICKLAND, 1983).

The muscle regulatory factors (MRF) gene family takes a central position in the regulation of myogenesis (WEINTRAUB et al., 1991). The MRF gene family consists of four genes, with MyoD and myf-5 expressed during the proliferation of myoblasts, myogenin expressed during the differentiation (i.e. fusion of myoblasts to form multi-nucleated myofibres) and with MRF4 mainly expressed in the muscle fibres to maintain their differentiated status (WEINTRAUB et al., 1991). The MRF genes are transcription factors regulating the transcriptome of the myoblasts to induce proliferation or fusion. However, their correct spatial and temporal expression requires strict regulation of their expression too. Over the last decade several proteins and pathways have been discovered involved in the correct regulation of the expression patterns of the MRF genes. Using microarray technology we studied both the expression patterns of the genes involved in the regulation of the MRF genes, and the muscle cell (myoblasts and myofibres) transcriptome profiles during pig prenatal development. Here we report on the transcriptome profiles of the genes regulating the expression of the MRF genes and expression patterns of known muscle structural genes.

Materials and Methods

Animal samples

Duroc and Pietrain prenatal samples were isolated from slaughtering pregnant sows at 14, 21, 35, 49, 63, 77, and 91 days of gestation. Fourteen days embryos were recovered by flushing the uterus horns, and thus consist of a pool of all embryos in a single uterus horn. Although 21 day embryos could be collected individually they were too small to isolate the area where myogenesis took place. For 35 days embryos this area was isolated but muscle tissue was not morphologically recognizable. For all

older foetuses longissimus muscle tissue was collected. Samples were frozen in liquid nitrogen and stored at -80^{0}C.

Microarray construction
Two cDNA libraries were constructed from postnatal pig muscle tissue and placed on a microarray (DAVOLI et al., 2002). A literature survey was done to find the genes known to be involved in the regulation of the MRF genes. These pig genes were cloned from a mixture of RNA isolates from all prenatal stages described above and added to the microarray.

Microarray hybridisation and analyses
The microarrays were hybridised with pools of RNA isolated from the prenatal pig samples. For each breed and prenatal stage RNA was isolated from six samples and pooled. Two microgram of RNA pools was labelled with either Cy3 or Cy5. Microarray hybridisations were done in two different experimental designs. Experiment 1 aims to compare the transcriptome of Duroc and Pietrain breeds at seven prenatal age stages related to muscle tissue formation, and experiment 2 aims to highlight the changes in the transcriptomes during prenatal development related to muscle tissue formation (in Duroc pigs).
Experiment 1
Pools of RNA samples derived from Duroc and Pietrain pigs of equal prenatal age were hybridised to the microarrays. Thus, at seven prenatal ages Duroc-Pietrain breed-comparisons were made.
Experiment 2
Pools of RNA samples of Duroc were hybridised to the microarray in a prenatal age-comparison experiment. The following comparisons of transcriptome expression patterns were done: 14 vs 21, 21 vs 35, 35 vs 49, 49 vs 63, 63 vs 77, and 77 vs 91 days of age. Thus, a total of six different age comparisons were made for Duroc.
All hybridisations were done in duplicate and in dye swap duplicate. Thus, each hybridisation analysis contained four independent hybridisations. Hybridisations were first normalised according to a LOWESS fit protocol followed by analyses using the spotfire software. Shortly, differential expression of genes was recorded, and clustering analysis was used to indicate myogenic pathways. Up or down regulation of a number of genes was verified using real time PCR (data not shown).

Results

Experiment 1
Genes were grouped in two functional groups: Myogenesis related and Energy metabolism (Table). The energy metabolism group was added to the microarray since a literature survey suggested that the level of energy supply was related to myogenesis. Each group was divided into subgroups according to their reported role in myogenesis or energy metabolism. The results describe the expression profile of (the majority of) the genes within each subgroup. Individual genes may differ from this pattern, but these differences are not described in detail here.
Comparison of the differential expression profiles between Duroc and Pietrain pigs revealed that the expression of the genes related to proliferation and differentiation of myoblasts in young embryos (i.e. 14-49 days prenatal age) were higher expressed in

Duroc than in Pietrain embryos, while the opposite was found in older foetuses. This transcriptome profile is supported by the expression profile of the muscle structural genes. These results suggest that myogenesis start earlier in Duroc embryos than in Pietrain embryos. However, at later stage Pietrain foetuses catch up. Alternatively, the results may suggest that myogenesis start at a higher rate in Duroc embryos while in older embryos myogenesis is slowing down. The opposite is for Pietrain where myogenesis start-up slowly but accelerate toward the end of gestation.
The energy metabolism genes show a different profile. With the exception of fatty acid metabolism the energy metabolism genes are expressed to a higher level in Pietrain compared to Duroc. Only at day 35 the situation is reversed. The fatty acid metabolism genes show that fatty acid metabolism start early in Duroc at a higher level than in Pietrain. After day 49 of gestation the expression level in Pietrain foetuses is increased to a higher level than the expression level in Duroc foetuses.

Table
Differential expression between two pig breeds, Duroc and Pietrain, at seven prenatal ages. The genes are grouped according to their known role in myogenesis or energy metabolism. The expression differences are indicated as the ratio of the expression in Duroc and in Pietrain for (the majority of) the genes in the group.

		Duroc : Pietrain ratio						
	N	14d	21d	35d	49d	63d	77d	91d
Myogenesis affecting	**175**							
Differentiation stimulating		~	>	>	>	<	<	<
Differentiation inhibiting		>	>	>	~	>	>	>
Proliferation stimulating		~	>	>	~	~	<	~
Proliferation inhibiting		not enough information *						
Diff / Prol affecting		~	<	<	~	<	>	>
Migration regulating		>	>	>	~	~	~	>
Muscle structural		>	>	>	>	<	<	<
Energy metabolism genes	**61**							
ATP metabolism		<	<	>	<	<	<	<
Oxidative phosphorylation		<	<	>	<	<	<	<
Glycolysis		<	<	>	<	<	<	<
Fatty acid metabolism		>	>	>	>	<	<	<
Miscellaneous		<	<	>	<	<	<	<

~: The genes within the group show similar expression levels in Duroc and Pietrain; >: The mean expression of the genes within the group is higher in Duroc than in Pietrain samples; <: The mean expression of the genes within the group is lower than in Duroc in Pietrain samples; *: The number of genes showing reliable (differential) expression is too low to enable a reliable conclusion.

Experiment 2

The genes were grouped in the same groups as described in Experiment 1. The results were analysed for (1) profile of changes of expression of groups of genes, and (2) activation and silencing of groups of genes.

Profile of the changes of expression of groups of genes

The expression levels of the myogenesis related genes shows that differentiation related genes and muscle structural genes peak around day 35 of gestation (Fig. 1). The myoblast proliferation stimulating genes peak just slightly earlier. While the energy metabolism subgroups - represented by the glycolysis genes - have a much more complicated pattern there seems to be a tendency to have a reduced expression level when differentiation affecting genes are at a peak level.

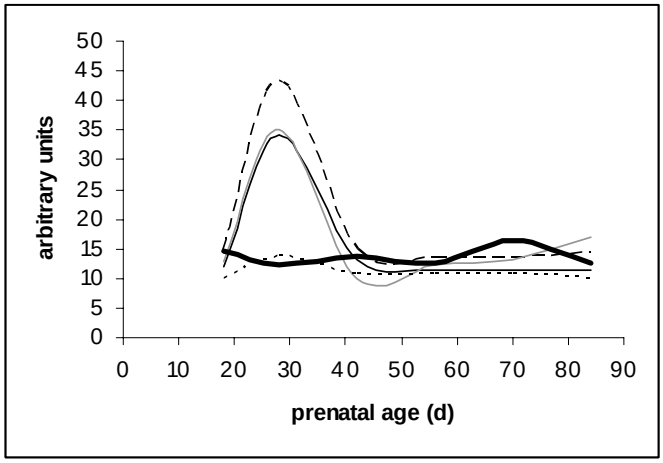

Fig. 1: Profile of mean expression levels per group of genes each developmental age. Black line: differentiation stimulating genes; gray line: differentiation inhibiting genes; broken line: Structural genes; dotted line: proliferation stimulating genes; thick line: glycolysis genes.

Activation and silencing of groups of genes

As already suggested by the results of experiment 1 some of the muscle structural genes are activated early – starting already from day 14 of gestation - and both the number of genes activated and the expression level of the individual genes are increasing until the end of the experiment at gestation time 91 days with a sharp increase in the period 35-49 days of gestation (data not shown). Also many of the myogenesis affecting genes are already activated at day 14 of gestation. The numbers of active genes vary with time, e.g., while differentiation stimulating genes are most activated around day 35 and day 64 of gestation the differentiation inhibiting genes are at a nadir at those times (Fig. 2). The energy metabolism genes represented by the glycolyses genes at a nadir at the time when the number of differentiation stimulating genes is at a peak level.

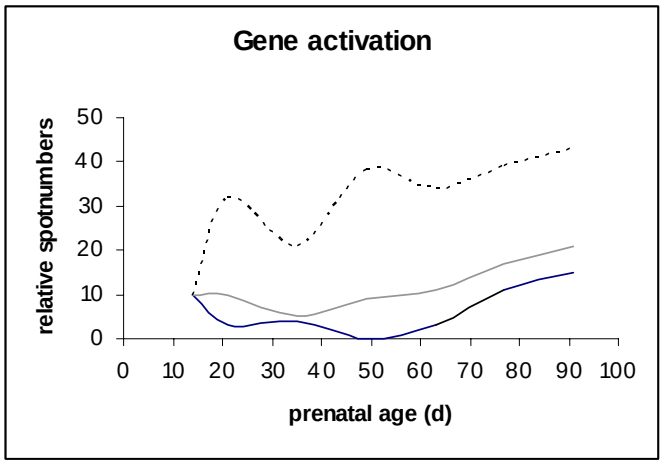

Fig. 2: Activation of genes: analysis of numbers of spots with differential expression of each prenatal developmental age. Black line: differentiation stimulating genes; gray line: differentiation inhibiting genes; broken line: glycolysis genes.

Discussion

Western pigs are selected for high muscle mass during the last decades (MERKS, 2000). Different pig breeds differ in muscle mass and muscle fibre composition. The

Pietrain pig breed with its extremely high muscularity shows predominantly white muscle fibre type, while the Duroc breed is les extreme in muscularity and has more red muscle fibres (SELLIER, 1998). The molecular background of these selection differences remains largely unknown. Muscle mass relates to muscle fibre type, muscle hypertrophy, and to a large extend to muscle fibre numbers. Since muscle fibre formation is an exclusive prenatal event in mammals the study of the transcriptome differences and transcriptome profiles may elucidate the mechanism(s) of past selection response, and may highlight possible future breeding directions. Therefore we studied these processes using microarray technology. The results indicate that indeed there exist remarkable transcriptome differences between Duroc and Pietrain muscle fibre formation profiles. The results suggest that the muscle fibre formation either starts late or begins at a slow speed in Pietrain compared to Duroc. It has been suggested that a characteristic of muscle fibre hyperplasia in selection lines of quails is delayed muscle fibre formation due to an enlarged myoblast proliferation period (COUTINHO et al., 1993). Our results may indicate that a similar mechanism is active in the pig too.

Changes in the level of expression and number of activated genes were studied during myogenesis. The results indicate that the number of activated genes appears to be crucial for the processes taking place. When the number of active genes involved in differentiation induction increases and simultaneously the number of active genes involved in differentiation inhibition decreases differentiation seems to take place. The number of genes involved may suggest that activation of differentiation requires the activation of several pathways. Since it is known that prenatal tissue formation is a highly regulated (spatial and temporal) event this is not surprising.

Energy levels have been related to muscle fibre formation as well (RIERA et al., 2003). Our results in both experiments indicate that the moment of differentiation seems to be associated with a dramatic change in the expression of energy metabolism genes. In experiment 1 the ratio of the expression of energy metabolism genes between Duroc and Pietrain changes abruptly at day 35 of gestation. In experiment 2 we found at days 35 and 63 decreased expressions of the energy metabolism genes. Thus, our results agree with this and indicate that differentiation is associated with a low energy metabolism gene expression. The importance of this remains unknown and further investigation is required on this point.

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