PREFACE

European meat and fish producers are continuously challenged to improve 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 fibres 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 form 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. Results from this meeting were published in a special issue of Archives of Animal Breeding, Volume 48, 2005.

The second work group meeting was held at University of Thessaly, Central Building, Argonauton & Filellinon, 38221 Volos, Greece.

The purpose of the meeting was to fulfil the second milestone *Comparative aspects of prenatal events on growth and meat/fish quality "* and was carried out in serial sessions covering the 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 the contributions of the participants to the sessions of the two working groups either as abstracts or short papers, that report on current scientific activities and results, and some of which also review the current status of knowledge and served as introductory statements of three serial workshops.

We acknowledge and thank all the authors for their contributions to the sessions at the meeting and to this special issue. Furthermore, we appreciate COST for making this work possible. Finally, we thank the editor-in-Chief and the editorial board of Archives of Animal Breeding for the opportunity to publish the contributions of this Work group meeting under COST Action 925.

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Myofibre typing and its relationships to growth performance and meat quality

Abstract

A dramatic increase of growth performance has been achieved through selection for muscle accretion and improvement of nutrition and breeding conditions in meat animals. However, evidence suggests that this may have resulted in altered meat quality. Skeletal muscle is a highly heterogeneous tissue containing myofibres, connective tissue, adipocytes, vascular and neural tissues. Muscle fibres can be characterized by their total number, size and contractile and metabolic properties, and are thought to influence meat quality, even though such a direct effect has not been clearly demonstrated. After a presentation of recent knowledge underlying conventional myofibre typing, the review will focus on relationships between fibre type composition and intramuscular fat content, heritability of myofibre traits, and implications of myofibre traits for growth performance and meat quality. Thus, the presence of four adult myosin heavy chain genes (I, IIa, IIx and IIb) shows that conventional myofibre classifications in three types should be revised, at least in species where IIx and IIb isoforms are expressed, such as pigs. Beyond the difference in the amount and distribution of mitochondria between fibre types, recent studies show that mitochondria are also intrinsically different between fibre types. Interestingly, no relationship seems to exist between fibre type composition and total intramuscular fat content, suggesting that both traits can be manipulated independently. On the opposite, the content and nature of phospholipids located in membranes is closely related to fibre type composition. The high heritability and genetic variability of myofibre traits show that selection can be used to influence muscle biological properties. Data on relationships between myofibre traits and growth performance or meat quality show that identifying the best fibre type to improve meat production and meat quality traits remains a difficult task. It is suggested that selection experiments directly based on myofibre traits and the study of the correlated responses of growth and meat quality may provide better tools to study these relationships. Finally, possible reasons which could explain the difficulty of establishing the specific influence of myofiber traits on meat production and quality traits are discussed.

Key Words: muscle fibre, intramuscular fat, heritability, growth, meat quality

Zusammenfassung

Titel der Arbeit: **Muskelfaser-Typisierung und deren Beziehung zu Wachstumsleistung und Fleischqualität**

Eine drastische Zunahme der Wachstumsleistung ist bei Nutztieren durch Selektion auf Muskelansatz und die Verbesserung der Tierernährung und Haltungsbedingungen erzielt worden. Es gibt jedoch Hinweise, dass dies mit einer Veränderung der Fleischqualität einhergegangen ist. Skelettmuskulatur ist ein heterogenes Gewebe bestehend aus Muskelfasern, Bindegewebe, Fett, Blutgefäßen und Nerven. Muskelfasern können durch ihre Gesamtzahl, Größe und kontraktilen und metabolischen Eigenschaften charakterisiert werden und es wird davon ausgegangen, dass sie die Fleischqualität beeinflussen, obwohl ein solcher Effekt nie direkt gezeigt werden konnte. Neben einer Darstellung des aktuellen Kenntnisstandes zur Muskelfasertypisierung fokussiert diese Übersicht auf das Verhältnis zwischen Fasertyp und intramuskulärem Fettgehalt, Erblichkeit der Muskelfasermerkmale und ihre Bedeutung für Wachstumsleistung und Fleischqualität. Die Existenz von vier Genen für die schweren Ketten des adulten Myosins (myosin heavy chain genes (I, IIa, IIx und IIb), belegt, dass die herkömmlichen Muskelfaserklassifikationen in drei Typen verbessert werden sollten, mindestens bei den Spezies, bei denen die Isoformen IIx und IIb exprimiert werden, wie bei Schweinen. Über den Unterschied bezüglich der Menge und der Verteilung von Mitochondrien zwischen Faserarten hinaus, zeigen neue Studien, dass Mitochondrien auch zwischen Faserarten unterschiedlich sind. Interessanterweise scheint kein Zusammenhang zwischen Fasertypen und intramuskulärem Fettgehalt zu bestehen und beide Merkmale scheinen unabhängig voneinander züchterisch bearbeitet werden zu können. Im Gegensatz dazu besteht ein enger Zusammenhang zwischen Gehalt und Art der Phospholipide in den Membranen und der Muskelfasertypverteilung. Die hohe Erblichkeit und genetische Variabilität der Muskelfasermerkmale zeigen, dass Selektion auf biologische Eigenschaften des Muskels möglich ist. Daten zur Beziehung zwischen Muskelfasertypen und Wachstumsleistung und Fleischqualität zeigen, dass es nach wie vor schwierig ist die hinsichtlich dieser Merkmale vorteilhaften Muskelfasern zu benennen. Experimente mit direkter Selektion auf Muskelfasermerkmale und Erfassung von Wachstums- und Fleischqualitätsmerkmalen sollten weiteren Aufschluss über diesen Zusammenhang geben.

Schließlich werden mögliche Gründe für die Schwierigkeit, den Zusammenhang zwischen Muskelfaser- und Fleischproduktionsmerkmalen herzustellen, diskutiert.

Schlüsselwörter: Muskelfasern, intramuskuläres Fett, Erblichkeit, Wachstum, Fleischqualität

Introduction

To improve efficiency and profitability of meat animal production, genetic selection, nutrition and breeding conditions have led to a dramatic increase in lean tissue content while decreasing fat deposition. However, there have been some concerns that this would have led to a deterioration of meat quality (CAMERON et al., 1999), but the underlying biological mechanisms are not clearly identified. The highly heterogeneous composition of skeletal muscle makes it difficulty to identify the muscle components specifically involved in the variability of meat quality. Skeletal muscle contains about 75% water, 19% protein, 0.5 to about 10% lipid and 1% glycogen, and is mainly composed of different myofibres, along with intramuscular adipocytes and connective, vascular and nervous tissues. It is widely accepted that myofibre type composition is an important source of variation in meat quality. However, such a direct effect has not been clearly demonstrated and an indirect influence through associations with changes in other muscle components, such as intramuscular fat and/or connective tissue may occur. Myofibres can be characterized by their total number, cross-sectional area (CSA), length and contractile and metabolic types. The total number of fibres (TNF) is reported to be definitely fixed before birth in species such as cattle, pig and chicken (ASHTON et al., 2005). In contrast, myofibre CSA remains quite constant during gestation and dramatically increases postnatally, all the more the fibre is becoming glycolytic (REHFELDT, 2005). After a review of recent data underlying myofibre typing (1), the paper will focus on (2) the relationships between fibre typing and intramuscular fat (IMF) content, (3) genetic data on myofibre traits, and implications of myofibre traits for (4) growth performance and (5) meat quality.

1. Myofibre typing

Conventionally, differences in the sensitivity of acto-myosin ATPase activity to pH preincubation has been used to distinguish types I, IIA and IIB fibres by histochemistry (BROOKE and KAISER, 1970). The existence of four adult skeletal myosin heavy chains (MyHC), i.e. types I, IIa, IIx and IIb, was first documented in mice, rats, guinea pigs and rabbits (BÄR and PETTE, 1988; SCHIAFFINO et al., 1989) and led to revise the conventional fibre classification. Each MyHC is encoded by a separate gene (WEISS et al., 1999). Despite the presence of the IIb gene, the type IIb MyHC isoform was initially reported to be unexpressed in skeletal muscles of large mammals such as human, cattle, horse, goat and dog (SMERDU et al., 1994; ENNION et al., 1995; TANABE et al., 1998; RIVERO et al., 1999; ARGUELLO et al., 2001; SMERDU et al., 2005). However, recent data using immunocytochemistry, in situ hybridization (Figure 1) and real time RT-PCR demonstrated that IIb MyHC was highly expressed in glycolytic pig and llama skeletal muscles (LEFAUCHEUR et al., 1998; GRAZIOTTI et al., 2001; DA COSTA et al., 2002; LEFAUCHEUR et al., 2002). Interestingly, conventional type IIB fibres exhibit either a moderate or weak oxidative metabolism (Figure 1B) which actually correspond to real types IIx and IIb MyHC expressing fibres, respectively (Figure 1C,D). Therefore, the conventional histochemical fibre typing in types I, IIA and IIB (BROOKE and KAISER, 1970) is

not well adapted for pig skeletal muscles where four fibre types are present based on MyHC polymorphism , i.e. types I, IIa, IIx and IIb. The limitation of the conventional fiber typing is clearly illustrated when comparing the longissimus muscle fibre type composition between the Large White (LW) and Meishan (MS) breeds (Figure 2). Indeed, no difference in the proportion of conventional types I, IIA and IIB fibres was observed between the two breeds (LEFAUCHEUR et al., 2004), in contrast, the MyHC IIb was the prominent isoform in LW pigs, whereas it was IIx in MS pigs. Individual fibre types exhibit different contractile, metabolic, physiological, chemical and morphological characteristics as presented in Table 1.

Table 1
Biological characteristics of individual fibre types (+, very low; ++, low; +++, medium; ++++, high; +++++, very high)

	I	IIa	IIx	IIb
Contraction speed	+	+++	++++	+++++
Myofibrillar ATPase	+	+++	++++	+++++
Oxidative metabolism	+++++	++++	++	+
Glycolytic metabolism	+	++++	++++	+++++
Mitochondrial ADP affinity	+	+++	+++++	+++++
CK and AK2 coupling	+++++	+++	+	+
Fatigue resistance	+++++	+++	++	+
Myoglobine	+++++	++++	++	+
Triglycerides	+++++	++	+	+
Glycogene	+	+++++	++++	+++++
Buffering capacity	+	++++	+++++	+++++
Collagene	+++++	+	+	+
Diameter	+	+	++++	+++++

Muscle fibres use a large amount of ATP for contraction through activation of the acto-myosin ATPase, and a harmonious functioning needs a balance between ATP consumption and production. The relative importance of the two main metabolic pathways of energy production, i.e. glycolysis and mitochondrial oxidative phosphorylation, varies between fibre types depending on their contractile patterns. Thus, the fast type IIb fibres are adapted to brief and intense contractions fuelled by the glycolytic pathway and immediate availability of phosphocreatine. Type IIx fibres are close to IIb ones, except that they are slightly more oxidative. On the opposite, the slow type I fibres can sustain prolonged low power work in association with a well-developed oxidative metabolism. Type IIa fibres are oxido-glycolytic and exhibit an intermediate contractile function between types I and IIx fibres. Interestingly, the few mitochondria of glycolytic fibres are homogeneously distributed throughout the fibre, whereas the numerous mitochondria of oxidative fibres exhibit a radial gradient with a concentration under the plasma membrane (SWATLAND, 1984). Beyond the well documented difference in the amount and distribution of mitochondria between fibre types, recent studies carried out on permeabilized fibres showed that mitochondria are also intrinsically different between fibre types (GUEGUEN et al., 2005a; GUEGUEN et al., 2005b). Thus, the stimulation of mitochonrial respiration by ADP was more sensitive to ADP in types IIb and IIx (Km = 8 µM) than IIa (72 µM) and I (212 µM) fibres, suggesting that mitochondrial respiration is strongly regulated by ADP in types IIb and IIx fibres, whereas other mechanisms are involved in type I fibres. Thus, the addition of creatine or AMP strongly stimulated mitochondrial respiration in type I fibres, whereas no effect was observed in types IIb and IIx fibres, showing a strong

coupling between mitochondrial kinases (miCK and AK2) and oxidative phosphorylation in type I fibres, type IIa fibres exhibiting intermediate properties between types I and IIx fibres.

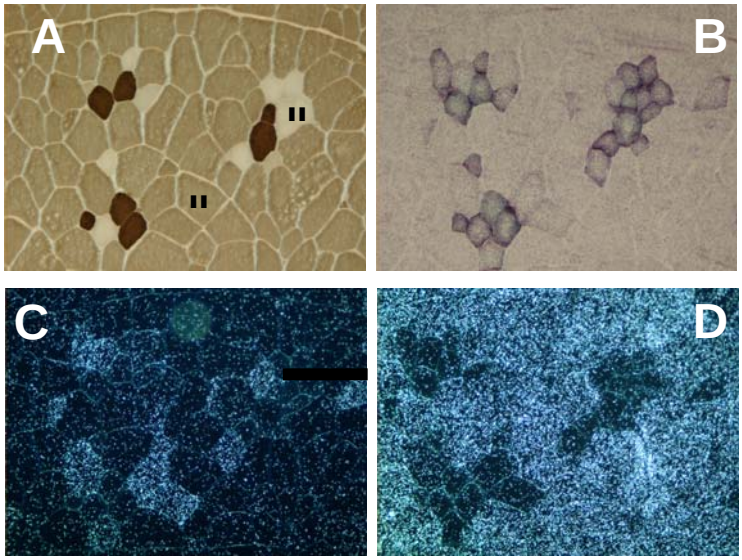

Fig. 1: Fibre typing of M. longissimus of Large White at 62 kg BW (131 d of age). Detection of mATPase after preincubation at pH 4.35 (A) and succino-dehydrogenase activity (B). Fibres types I, IIA and IIB (BROOKE and KAISER, 1970). In situ hybridization with type IIx (C) and IIb (D) MyHC ^{35}S labelled riboprobes. The x letters denote corresponding type IIx fibres on the serial sections. Bar = 100 µm (adapted from LEFAUCHEUR et al., 2002) (Fasertypisierung vom M. longissimus von Large White bei 62 kg KW (131 Tagen). Nachweis von mATPase nach Inkubation bei pH 4.6 (A) und Succinatdehydrogenase (B). Fasertypen I, IIa, IIB (BROOKE and KAISER, 1970). In situ Hybridisierung mit Typ IIx (C) and IIb (D) MyHC ^{35}S markierten Riboproben. Typ IIx-Fasern sind mit x gekennzeichnet. Balken = 100 µm (modifiziert nach LEFAUCHEUR et al., 2002))

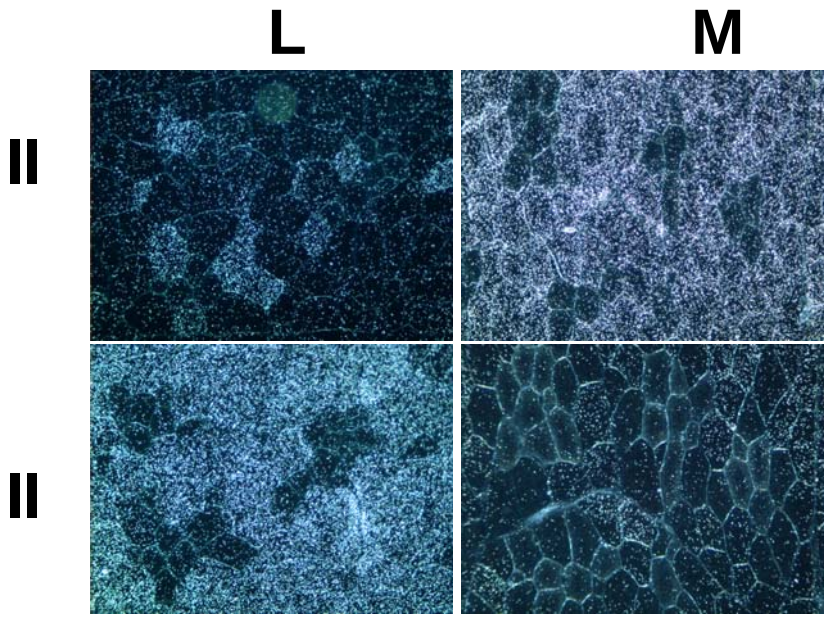

Fig. 2: In situ hybridization using ^{35}S labelled MyHC IIx and IIb riboprobes in longissimus muscle of Large White (LW) and Meishan (MS) pigs at 62 kg BW (adapted from LEFAUCHEUR et al., 2004) (In situ Hybridisierung mit Typ IIx and IIb MyHC ^{35}S markierten Riboproben bei Large White und Meishan Schweinen bei 62 kg Körpergewicht (modifiziert nach LEFAUCHEUR et al., 2004))

Glucose, glycogen and fatty acids are important fuels for muscle bioenegetics. It is well established that glucose uptake (BONEN et al., 1981), incorporation of ^{14}C-glucose into glycogen (BÄR and BLANCHAER, 1965), turnover of glycogen (VILLA-MORUZZI et al., 1979) and the number of insulin receptors (LEFAUCHEUR et al., 1986) are higher in type I than II fibres, in particular glycolytic type IIb fibres. Glycogen distribution is highly variable between fibres and highly dependent on environmental factors, such as exercise, stress and fasting. On average, its level is lower in type I and sometimes type IIA than type IIB fibres, the last ones also exhibit a greater variability of glycogen distribution (KARLSSON et al., 1993; FERNANDEZ et al., 1995). Muscle glycogen content is also influenced by genetic factors. Thus, a single nonsense mutation (R225Q) in the RN gene coding for the γ3 subunit of an AMP dependent serine/threonine kinase (AMPK) has been shown to specifically increase glycogen level by 70% in pig glycolytic muscles, specifically in glycolytic type IIb fibres (MONIN et al., 1987; MARINOVA et al., 1992; MILAN et al., 2000). Intramyocellular triglyceride level is consistently higher in type I fibres than all fast type II fibres (ESSÉN-GUSTAVSSON et al., 1994), with no significant difference between subtype II fibres. Obesity and/or insulin resistance have often been reported to be associated with increased levels of intramyocellular triglycerides (LOON and GOODPASTER, 2005), however, this does not seem to be a functional relationship. Indeed, two factors known to increase intramyocellular triglyceride levels, i.e. type I fibre and endurance training, also increase sensitivity of glucose uptake to insulin and decrease insulin resistance.

2. Fibre typing and intramuscular fat content

Intramuscular fat (IMF) content is generally reported to be positively related with palatability of meat, even though data can be controversial (FERNANDEZ et al., 1999). In pork shops, increasing IMF content up to 2.5% has been shown to improve flavor, juiciness and tenderness (DEVOL et al., 1988). Intramuscular fat is composed of triglycerides and phospholipids which represent 0.5-7% and about 0.5% of fresh pig longissimus muscle, respectively (LESEIGNEUR-MEYNIER and GANDEMER, 1991; FAUCITANO et al., 2004). An overwhelming amount of triglycerides is usually located in intramuscular adipocytes interspersed between myofibres and in the perimysium (ESSÉN-GUSTAVSSON et al., 1994; GONDRET et al., 1998), whereas a small amount is localized as triglyceride droplets specifically within type I myofibers. It is noteworthy that the amount of triglycerides located in intramuscular adipocytes is not related to fibre type composition. This has been confirmed many times in pig in selection experiments (LARZUL et al., 1997), comparisons between genotypes (ESSÉN-GUSTAVSSON and FJELKNER-MODIG, 1985; LEBRET et al., 1999) and different muscles (BEECHER et al., 1965; LESEIGNEUR-MEYNIER and GANDEMER, 1991), and in feeding experiments (ESSÉN-GUSTAVSSON et al., 1994; CANDEK-POTOKAR et al., 1999). All these studies suggest that triglyceride content and fibre type composition can be manipulated independently through genetic and/or nutritional factors. The total triglyceride content of muscle seems to be more related to the general fatness of the animals, even though the genetic correlation only reached 0.30 between both traits (SELLIER, 1998), suggesting that an important part of the genetic variation in IMF is also independent of genetic variation in the overall lipid content of the carcass (SUZUKI et al., 2005). In contrast, a close relationship

exists between fibre type composition and the content and nature of muscle phospholipids, mostly located in membranes (LESEIGNEUR-MEYNIER and GANDEMER, 1991; ALASNIER et al., 1996). Thus, oxidative muscles contain about twice as much phospholipids than glycolytic ones. Because phospholipids are major determinants of cooked meat flavor (MEYNIER and GANDEMER, 1994), muscle fibre type composition is likely to influence flavor through the phospholipids. However, phospholipids also contain more polyunsaturated fatty acids which increase the risk of post-mortem (p.m.) rancidity of meat.

3. Genetic properties of myofibre traits

Several heritability estimates for different production, meat quality and myofibre traits are given in Table 2. Carcass traits are moderately to highly heritable, with an h^2 value of 0.5 for carcass lean content. In contrast, meat quality as a whole is slightly to moderately heritable as h^2 values fall in the range 0.10-0.30, except for IMF ($h^2 = 0.5$). Colour and tenderness seem to be the most heritable ($h^2 = 0.25$-0.30), whereas pH and water holding capacity are slightly heritable ($h^2 = 0.15$-0.20). In comparison, myofibre traits are slightly to highly heritable with h^2 values ranging from 0.2 to 0.5 for TNF, 0.2 to 0.35 for myofibre mean CSA, and around 0.4 for fibre type frequencies. These myofibre traits are also highly variable between individuals with coefficients of variation of 20% for TNF and myofibre mean CSA, and 28% for the proportion of type I fibres in Large White pig longissimus muscle (LARZUL et al., 1997). Both the high heritability and variability suggest that it is possible to select animals directly on myofibre traits.

Table 2
Average values of heritability (h^2) for carcass composition, meat quality and myofibre traits in pig (LARZUL et al., 1997; 1999; SELLIER, 1998; REHFELDT et al., 2000)

Carcass lean %	0.5
Meat quality	0.1 – 0.3
Glycogene	0.3
Intramuscular fat	0.5
Myofibre traits	
Total number	0.2 - 0.5
Diameter	0.2 - 0.3
Fibre types, %	0.4
Muscle enzyme activities	0.2 - 0.4

Comparisons between wild and domesticated animals within different species led to hypothesize that selection for increased growth rate and lean content induced an increase of myofibre CSA and and a shift muscle metabolism towards a more glycolytic and less oxidative type (ASHMORE, 1974; RAHELIC and PUAC, 1981; SOLOMON and WEST, 1985; WEILER et al., 1995). However, such a selection carried out within domesticated animals did not confirm this assumption, in particular in conventional European domesticated pigs (KARLSSON et al., 1993; LARZUL et al., 1997; BROCKS et al., 2000; OKSBJERG et al., 2000; TRIBOUT et al., 2004). Thus, two experiments were been implemented to estimate the realised genetic trends in Danish Landrace pigs from 1976 to 1995 (OKSBJERG et al., 2000), and in French Large White pigs from 1977 to 1998 using frozen semen (TRIBOUT et al., 2004). Besides the expected dramatic improvement in growth rate and carcass leanness, selection either decreased (OKSBJERG et al., 2000) or did not change (TRIBOUT et

al., 2004) mean myofibre CSA in longissimus muscle, suggesting an increase of TNF in both studies. The two experiments also reported an alteration of meat quality as shown by different markers such as an increased cooking loss, lighter and less red meat, a higher glycolytic potential, a lower ultimate pH or a lower tenderness. Surprisingly, no change in IMF content and a decreased activity of the glycolytic enzyme lactate dehydrogenase were observed in longissimus muscle.
A more original approach consists in directly selecting meat animals on myofibre traits and studying the correlated effects on growth performance and meat quality. Unfortunately, very few studies are available. This has been done for myofibre CSA in the pig (WICKE et al., 1991), however, results were biased because of the indirect selection of the mutated RYR1 gene which is known to alter meat quality, but through disturbance of muscular Ca++ ions transfer, and not through myofibre characteristics per se. This stresses that a great attention must be given to the experimental design to avoid misinterpretations of results due to indirect effects. A selection against in vivo glycogen level of longissimus muscle carried out for 6 generations within the Large White pigs free of the mutated RYR1 and RN genes (LARZUL et al., 1999) indicated a positive genetic correlation between in vivo glycogen level and lean meat content and muscle oxidative metabolism, a negative one with technological quality, and no relation with IMF content. It is noteworthy that these results are very similar to the effects of the mutated RN gene, though of weaker intensity.

4. Myofibre traits and growth performance

Muscle mass is related to the TNF, CSA and length of myofibres. Between species variability of TNF is much more higher than that of CSA (PLAGHKI, 1985). A negative phenotypic correlation (rp = -0.3 to -0.8) is frequently reported between TNF and CSA (REHFELDT et al., 2000), and a positive correlation between TNF and carcass lean content is usually reported in pig (LUFF and GOLDSPINK, 1967; HANDEL and STICKLAND, 1988; DWYER et al., 1993). The last correlation is also very obvious when comparing double-muscled with normal cattle (HOLMES and ASHMORE, 1972). In contrast, the relationship between carcass lean content and CSA is highly controversial, which can be explained by the fact that carcass lean content would primarily dependent on TNF. Consequently, one must first take into account TNF before studying relationships between lean content and myofibre CSA. In this case, a strong positive correlation between both traits can be expected at a fixed TNF. The negative correlation sometimes reported between lean content and myofibre CSA is likely due to differences in TNF (HEGARTY and ALLEN, 1978; POWELL and ABERLE, 1981; GONDRET et al., 2005). Thus, animals with a lower TNF usually exhibit larger fibres at a given body weight in association with an advanced age and fatter carcasses, denoting a higher physiological maturity. Myofibre length has been poorly studied, even though it has likely a strong influence on CSA of some muscles such as longissimus where myofibres make a 25° angle to the vertebral axis (DAVIES, 1972). The specific relationships between fibre type composition and growth performance is still highly controversial. Thus, a positive phenotypic correlation was observed between muscle gain and both the activity of the oxidative enzyme citrate synthase and the number of capillaries per fibre in longissimus muscle of Danish Landrace and Large White pigs (HENCKEL et al., 1997), whereas no significant correlations were found in other experiments (LARZUL et al., 1997).

5. Myofibre traits and meat quality

To date, research has mostly been focussed on understanding and controlling genetic factors and changes associated with slaughtering conditions and p.m. meat processing that influence meat quality. Despite intensive research, meat quality still remains highly variable. Based on their individual characteristics (Table 1), myofibres are thought to be important factors influencing meat quality but there is no clear indication as to what extent they explain the residual variation of meat quality. The difficulty to establish their specific involvement could be due to the variation of other muscle components such as connective tissue or IMF in relation to genetic, nutritional and environmental factors in interaction with slaughtering conditions and p.m. processing. It is generally reported that increasing myofibre CSA is detrimental for meat quality, in particular water holding capacity and tenderness (REHFELDT et al., 2000). Among fibre types, several studies identified hypertrophy of oxido-glycolytic fibres as being particularly prone to causing meat deficiencies (LENGERKEN et al., 1994; LARZUL et al., 1997; MALTIN et al., 1997). Several genotypes, such as RYR1 in pig (ESSÉN-GUSTAVSSON et al., 1992) and Callipyge in sheep (CARPENTER et al., 1996), or treatments with β-agonists (DUNSHEA et al., 2005) are all associated with increased myofibres CSA and altered meat quality, however it must be emphasized that this kind of observation does not allow concluding that increasing myofibre CSA deteriorates meat quality. Indeed, the causal mechanisms leading to the deterioration of meat quality in these models are well identified (punctual mutations), and myofibre types per se are not causal factors. Several studies do not support the negative influence of myofibre CSA on meat quality. In Large White pigs, a strong positive genetic correlation (rg = 0.68) was found in longissimus muscle between myofibre CSA and IMF content (LARZUL et al., 1997), a characteristic sometimes associated with improved tenderness and flavour of pork. In chicken, a study carried out in broiler breast muscle showed a positive influence of myofibre CSA on meat quality trough a decreased muscle glycogen content, higher p.m. pH, and improved water holding capacity and tenderness (BERRI et al., 2004). Therefore, a specific relationship between myofibre CSA and meat quality still remains rather controversial. Increasing the TNF is often speculated to be a good strategy to improve both lean meat content and meat quality traits. However, the direct influence of TNF on meat quality remains to be clearly demonstrated. Once more, it is not because doubled-muscled cattle exhibit both almost double the TNF and higher tenderness than normal animals that increasing TNF is responsible of the improved tenderness.

Consistent significant relationships between meat quality and fibre type composition within-breed are scarcely presented. Thus, no correlation between fibre characteristics and meat quality traits were found within normal cattle breeds (WEGNER et al., 2000). In Large White pigs, increasing the proportion of IIB glycolytic fibres (IIBW) in longissimus muscle was shown to increase the rate and extent of p.m. pH decline, reflectance and cooking loss (LARZUL et al., 1997). On the opposite, a study using Danish Large White and Landrace pigs showed a positive relationship between activity of lactate dehydrogenase, a glycolytic enzyme, and water holding capacity of cooked meat, tenderness, juiciness and flavor (HENCKEL et al., 1997). A comparison between pig muscles showed that the rate of p.m. pH decline was much faster in the mixed psoas major than both the slow oxidative semispinalis and fast glycolytic

longissimus (Figure 3), showing that rate of p.m. pH decline is far from being strictly related to fibre type composition. In contrast, the relationships between the extent of p.m. decline and fibre type composition seem to be stronger, with a decreased ultimate pH as the proportion of fast glycolytic fibres increases. Interestingly, a positive relationships between the proportion of fast glycolytic fibres and both p.m. maturation rate and colour stability of meat has been reported, which could be advantageous in species exhibiting slow p.m. meat maturation and colour instability, such as cattle (RENERRE, 1984; OUALI, 1990). Thus, a positive correlation between the relative area of fast glycolytic fibres and tenderness has been reported in cattle (SEIDEMAN et al., 1986), but it was not confirmed in other studies (SOLOMON and LYNCH, 1988). On the opposite, increasing the proportion of type I fibres has also been reported to improve juiciness and flavor in sheep (VALIN et al., 1982), and tenderness and juiciness in cattle (MALTIN et al., 1998).

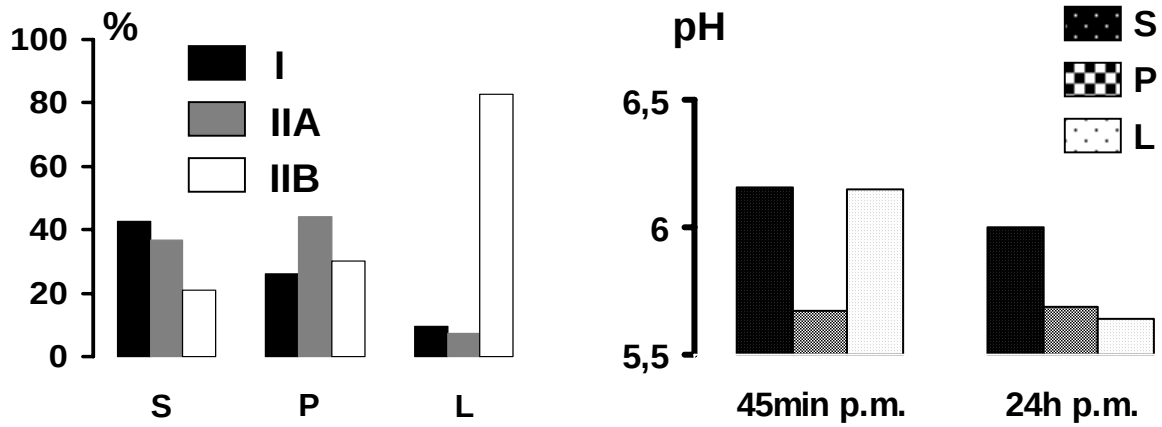

Fig. 3: Fibre type composition (left) and post-mortem pH (right) in Mm. semispinalis (S), psoas major (P) and longissimus (L) in the pig (adapted from LEFAUCHEUR et al., 1992 and MELODY et al., 2004) (Muskelfasertypen (links) und post-mortem pH (rechts) in Mm. semispinalis (S), psoas major (P) and longissimus (L) beim Schwein (modifiziert nach LEFAUCHEUR et al., 1992 and MELODY et al., 2004))

Conclusion

Despite the fact that myofiber type composition is speculated to be an important source of variation in meat quality, identifying a superior fibre type for meat production remains a difficult task. Multiple reasons can be put forward, including muscle tissular heterogeneity, accuracy of fibre typing, choice of predictor muscle, sampling location, pertinence of biological markers, accuracy of meat quality evaluation, animal experimental designs. First, specific influence of myofibres can be masked by variations in other muscle components such as connective tissue and IMF, even though total IMF content seems to be unrelated to fibre type composition. The presence of four adult MyHC, i.e. I, IIa, IIx and IIb shows that the conventional enzyme histochemical classification in three types, e.g. I, IIA and IIB must be revised, at least in species where IIb MyHC is highly expressed such as pig. This could be achieved using monoclonal antibodies specific of each MyHC by immunocytochemistry, western blots and/or ELISA. Unfortunately, these antibodies are not always available and usable with the different techniques in the different species. Other technical limitations reside in defining adequate predictor muscles, optimal sampling locations and acceptable and reliable biopsy techniques. Even

though TNF is widely accepted to be an important factor influencing muscle growth potential and meat quality, its reproducible and accurate measurement remains a challenge. More studies are also required to develop more rapid, quantitative and less expensive methods of muscle fibre type determination. In the future, conventional markers of fibre typing will likely have to be completed by new more pertinent markers resulting from the QTL, transcriptomic and proteomic approaches. Besides these technical limitations, an inadequacy of the experimental designs could also be incriminated. Thus, feeding and rearing conditions often vary between experiments and their interactions with genotypes, slaughtering conditions and p.m. meat processing are not always well controlled. Also, the simple comparison between breeds seems to be insufficient because they differ by many other traits than fibre type composition. Most selection experiments used to study the relationships between myofibre traits, growth performance and meat quality were based on selecting animals on growth performance and studying the correlated changes in fibre type composition and meat quality. However, such experiments did not lead to universal conclusive results. Because of the high genetic variability and heritability of myofibre traits, selection experiments based directly on myofibre traits and the study of the correlated responses of growth and meat quality may be more adapted to study these relationships.

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STEPHANIE A. BAYOL, BIGBOY H. SIMBI and NEIL C. STICKLAND

Eating “junk food” during pregnancy and lactation impairs skeletal muscle development and metabolism in rat offspring at weaning

(Fütterung von Diäten mit geringem Nährwert ("junk food“) an Ratten während der Schwangerschaft und Laktation beeinflusst die Skelett-muskelentwicklung und den Stoffwechsel der Nachkommen beim Absetzen negativ)

The influence of a maternal cafeteria diet on the development of skeletal muscle and adipose tissue was examined in rat offspring at weaning (21 day post-partum). Pregnant rats were divided into three groups and were either fed a cafeteria diet (high fat, high sugar, high calorie) during both gestation and lactation or the cafeteria diet during gestation alone followed by a balanced chow diet during lactation or the chow diet during gestation and lactation. Pups from mothers fed the cafeteria diet during gestation and lactation exhibited a 25% reduction in muscle cross-sectional area with around 20% fewer fibers at weaning when compared with pups born to mothers fed the balanced chow diet during both gestation and lactation. These pups also exhibited increased adiposity i.e. increased fat pad weights as well as elevated intramuscular lipid content accompanied by increased muscle IGF-1, IGF-1 receptor, and PPARγ mRNA levels which could indicate an attempt to maintain normal insulin sensitivity. Pups fed the cafeteria diet during gestation and rehabilitated to the balanced chow diet during lactation did not exhibit the increased adiposity and elevated IGF-1, IGF-1 receptor and PPARγ mRNAs. However, they exhibited reduced muscle cell proliferation (PCNA), reduced insulin receptor mRNA and a trend towards reduced glucose transporter (GLUT)-4 mRNA when compared with pups fed chow throughout the experiment; this may indicate a possible reduction in glucose up-take by muscle tissue. In conclusion, a maternal cafeteria diet either during gestation alone or during both gestation and lactation affected skeletal muscle development and induced metabolic disorders normally associated with insulin resistance in rat offspring at weaning.

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BAYOL, S.A.; SIMBI, B.H.; STICKLAND, N.C.:
A maternal cafeteria diet during gestation and lactation promotes adiposity and impairs skeletal muscle development and metabolism in rat offspring at weaning. Journal of Physiology. **567**(2005), 951-961

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Prenatal undernutrition increases fat deposition and collagen content within skeletal muscle in the porcine fetus.

(Pränatale Unterernährung erhöht den Fettansatz und den Kollagenanteil von Skelettmuskulatur bei Schweinefeten)

Connective tissue plays a key role in the scaffolding and development of skeletal muscle. Pilot studies carried out in our laboratory have shown the smallest porcine littermate has a higher content of connective tissue within skeletal muscle compared to its largest littermate. The present study investigated the prenatal development of intralitter variation in terms of collagen content within connective tissue, intra-muscular fat and myosin heavy chain content of muscle fibres of the *M. semitendinosus*.

Twenty-three pairs of porcine fetuses from a Large White-Landrace origin were used aged from 36 to 86 days gestation. The largest and smallest littermates were chosen by weight and the *M. semitendinosus* was removed from each. Complete muscle transverse sections were stained with Oil Red O (detection of lipids) and immunocytochemistry was performed using an antibody to Collagen I and an antibody to myosin (embryonic).

Paired t-Tests revealed the smallest littermate contained significantly more fat deposition and Collagen I content compared to the largest littermate. The smallest littermate also however contained significantly lower expression of myosin (embryonic) compared to its largest sibling. Recent postnatal studies show elevated levels of intramuscular lipids and low scores for meat tenderness in the smallest littermate. These postnatal studies corroborate our prenatal investigations.

In conclusion, the present study has shown a prenatal nutritional influence on the connective tissue and fat components of muscle and, from other work, this may continue postnatally.

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Changes of the histochemical properties and meat quality traits of porcine muscles during growth. I) Effect of feed restriction in pigs slaughtered at the same age and varying body weight

Abstract

Twenty-four Swiss Large White barrows from six litters were given either ad libitum (A) or restrictive (R) access to a grower diet from 21 to 60 kg body weight (BW). At d 113 of age six pigs of the A- (BW = 62 kg) and six siblings of the R-group (BW = 51 kg) were slaughtered. The remaining 12 barrows were fed a finisher diet from 60 kg BW until slaughter. The day of slaughter barrows were 154 d old and the BW of the A- and R-group averaged 100 and 87 kg, respectively. The histochemical properties of myofibers as well as meat quality traits of the longissimus muscle (LM) and light portion of the semitendinosus (STL) were assessed. Muscle fibers were stained and classified based on the stain reaction as slow oxidative (SO), fast oxidative-glycolytic (FOG), and fast glycolytic (FG), and fiber area and distribution were determined. Regardless of the age at slaughter, pigs of the R-group had smaller ($P \leq 0.04$) SO and FG fibers in the LM and STL, and smaller ($P < 0.01$) FOG fibers in the STL than their siblings of the A-group. The STL had more ($P = 0.03$) FOG and fewer ($P = 0.04$) FG fibers in pigs of the R- than pigs of the A-group. The muscles of pigs in the R-group were less ($P \leq 0.03$) tender and percentages of cooking loss were higher ($P \leq 0.08$) than of pigs in the A-group. In conclusion, hypertrophy as well as differentiation of muscle fibers was affected by the restricted nutrient supply when pigs had the same age at slaughter. However, the extent of the dietary impact varied among muscles.

Key Words: Age at slaughter, Meat Quality, Muscle Fibers, Pig

Zusammenfassung

Titel der Arbeit: **Veränderung histochemischer Eigenschaften und der Fleischqualität von Muskeln des Schweins während des Wachstums. I) Einfluss restriktiver Fütterung bei Schweinen, geschlachtet mit gleichem Alter aber unterschiedlichem Lebendgewicht**

In der vorliegenden Untersuchung wurden 24 Kastraten (je vier Tiere aus dem gleichen Wurf) der Rasse Schweizerisches Edelschwein eingesetzt. Die Schweine hatten von 21 bis 60 kg Lebendgewicht (LG) entweder ad libitum (A) oder rationierten (R) Zugang zum gleichen Vormastfutter. Je sechs Tiere der A- und R-Gruppe wurden am 113. Lebenstag bei einem durchschnittlichen LG von 62 bzw. 51 kg geschlachtet. Die restlichen 12 Tiere wurden von 60 kg LG bis zur Schlachtung mit einem Ausmastfutter gefüttert. Am Tag der Schlachtung waren die Tiere 154 Tage alt und wogen 100 (A) bzw. 87 kg (R). Im langen Rückenmuskel (LM) und im hellen Teil des Semitendinosus Muskels (STL) wurden Größe und Verteilung der einzelnen Muskelfasertypen sowie Fleischqualitätsparameter untersucht. Die Muskelfasern wurden mit der histochemischen Färbungsreaktion in langsam oxidative (SO), schnell oxidativ-glykolytische (FOG), and schnelle glykolytische (FG) Muskelfasern eingeteilt. Unabhängig vom Alter bei der Schlachtung waren im LM und STL der Schweine der Gruppe R die SO und FG sowie im STL außerdem die FOG Muskelfasern kleiner ($P \leq 0.04$) als in der Gruppe A. Schweine der R-Gruppe hatten im STL einen höheren ($P = 0.03$) Anteil an FOG bzw. einen niedrigeren ($P = 0.04$) Anteil an FG Muskelfasern als diejenigen der A-Gruppe. Bei den Schweinen der R-Gruppe waren der LM und STL zäher ($P \leq 0.03$) und die Kochverluste größer ($P \leq 0.08$) als bei den Schweinen der A-Gruppe. Zusammenfassend kann gesagt werden, dass sowohl die Hypertrophie als auch die Differenzierung der Muskelfasern durch die rationierte Nährstoffzufuhr beeinflusst wird, wenn die Schweine bei gleichem Alter geschlachtet wurden. Das Ausmaß des Einflusses ist jedoch zwischen den untersuchten Muskeln unterschiedlich.

Schlüsselwörter: Alter bei der Schlachtung, Fleischqualität, Muskelfasern, Schwein

Introduction

Quantitative and qualitative aspects of postnatal nutrition have a major effect on muscle development through their effect on growth rate and body composition. The dietary impact can vary depending on the stage of development and age of the pig. HARRISON et al. (1996) found that severe feed restriction for 4 weeks post-weaning

significantly impaired myofiber hypertrophy. Concomitantly they found higher percentages of type I fiber in the red rhomboideus but not in the white longissimus (LM) muscle. CANDEK-POTOKAR et al. (1999) suggested that the aforementioned dietary effect on myofiber size was the result of the markedly lower BW because in heavier pigs (100 and 130 kg BW) slaughtered at the same BW feed restriction neither affected the size nor the distribution of the myofibers in the LM. However, in pigs subjected to mild feed restriction and slaughtered at 55 kg BW SOLOMON et al. (1988) reported smaller slow oxidative (**SO**) and fast glycolytic (**FG**) fibers as well as fewer FG and more fast oxidative-glycolytic (**FOG**) fibers in the LM. Thus, it is unclear whether the effect of restricted nutrient supply differs at different developmental stages and depends on the duration of nutrient restriction. Because metabolic differentiation and myofiber hypertrophy is most intense up to four months of age (60 kg BW) (LEFAUCHEUR and VIGNERON, 1986; ONO et al., 1995) the effect of ad libitum versus restricted (80% of ad libitum) feed allowance on contractile and metabolic differentiation of myofibers after the growing and after the finishing period at a given age (different BW) were examined. In order to assess the dietary impact on myofiber development, two porcine muscles were examined, which are known to have a similar myofiber composition but to differ in their allometric growth ratios.

Material and Methods

Swiss Large White barrows (n = 24) originating from six litters were blocked by litters and assigned from within litter to the four treatment groups. The pigs had either ad libitum (**A**) or restricted (**R**) access to a standard growing-finishing diet for the total experimental period. Feed restriction was aiming to achieve an ADG of 750 g from 20 to 100 kg BW. From 20.7 ± 0.67 (start of the experiment) to 60 kg BW, barrows were offered a grower diet (crude protein: 201 g, DE: 15.5 MJ/kg dry matter). At 113 ± 2.4 d of age six pigs (one per litter) of the ad libitum (**A-113)** and six of their siblings of the restricted group (**R-113**) were slaughtered. The BW at slaughter of the A-113- and R-113-pigs averaged 62.1 ± 1.82 and 51.0 ± 1.81 kg, respectively. The remaining 12 barrows were offered a finisher diet (crude protein: 166 g, DE: 15.4 MJ/kg dry matter) from 60 kg BW until slaughter. The day of slaughter barrows were 154 ± 2.6 d old and the BW of the ad libitum (**A-154)** and restricted group (**R-154**) averaged 99.5 ± 2.45 kg and 86.6 ± 2.48 kg, respectively.

The pigs were reared in individual pens (2.6 m^2/pig) on a concrete floor in environmentally controlled buildings (22 °C and 60 to 70% relative humidity). Barrows from the R-group were fed individually twice a day and had free access to water. The total daily feed allowance was adjusted weekly according to the BW (BOLTSHAUSER et al., 1993). Pigs of the A-group were weighed and feed disappearance was recorded weekly. The day prior to slaughter, feed was withheld from pigs 12 h before transportation to the research station abattoir. Slaughter and dissection were carried out according to the Swiss Pig Performance Testing Station (MLP, Sempach, Switzerland) meat cutting standards as previously described by BEE et al. (2004).

Within 40 min after exsanguination, the longissimus (LM) and semitendinosus muscle were removed from the right side of each carcass. Muscle samples for histochemical analyses were excised from the light (STL) portions of the semitendinosus and anterior

to the 10th rib location of the LM. One piece (approximately 1 × 1 × 3 cm) of each muscle was immediately fixed on a labeled flat stick, rolled in talcum powder, and immediately frozen in liquid nitrogen and stored at –80 °C. Histochemical analysis were performed as described by BEE (2004).
From the same samples used for histochemical analyses, two 1.5-cm thick LM chops were cut at the 12th rib level and two slices (approximately 70 g each) were obtained from the STL. From the muscle samples initial and ultimate pH, drip loss percentages after 72 h, thaw and cooking losses, and shear force values were determined as described by BEE et al. (2004).
Data were analyzed with the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). The model used for the analyses of muscle fiber characteristics and meat quality traits included feeding regimen and age at slaughter and the respective interactions as fixed effects and litter as the random effect. When interactions were statistically significant at $P < 0.05$, least squares means were separated using the PDIFF option. Pearson correlation coefficients between histological properties and meat quality traits for each muscle were calculated at 113 and 154 d of age.

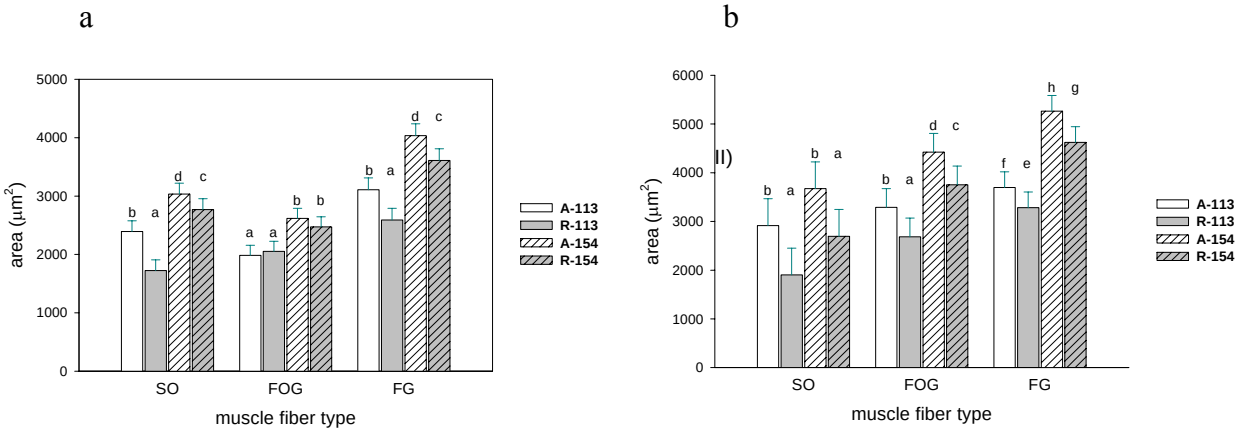

Figure 1: Cross-sectional area of the muscle fiber types (SO = slow oxidative; FOG = fast-oxidative glycolytic; and FG = fast-glycolytic) in the longissimus (a) and light portion of the semitendinosus muscle (b) of pigs with ad libitum (A) or restricted (R) access to the diet and slaughtered at 113 (A-113 and R-113) or 154 d of age (A-154 and R-154). Bars within myofiber type lacking common letters differ ($^{a,b,c,d}P < 0.05$; $^{e,f,g,h}P = 0.07$). (Querschnittsflächen der Muskelfasertypen (SO = slow oxidative; FOG = fast-oxidative glycolytic; and FG = fast-glycolytic) im Longissimus (a) und hellem Anteil des Semitendinosus (b) bei Tieren mit ad libitum (A) oder restriktiver (R) Fütterung nach Schlachtung am Tag 113 oder 154. Unterschiedliche Buchstaben bezeichnen signifikante Differenzen innerhalb Muskelfasertyp ($^{a,b,c,d}P < 0.05$; $^{e,f,g,h}P = 0.07$))

Results and Discussion

Muscle Fiber Area and Distribution

In the present study, myofiber hypertrophy was delayed ($P \leq 0.05$ for each; Figure 1) in both muscles at 113 and 154 d of age in pigs of the R- compared with those of the A-group. The smaller myofiber size was paralleled by smaller LM area and smaller ST girth (data not shown). Except for the FOG fibers in the LM, these findings are in agreement with the general observations that undernutrition delay the hypertrophic growth rate of all myofiber types when comparison are made at the same age (HARRISON et al., 1996; LEFAUCHEUR et al., 2003). The size of SO in the LM and the size of FOG and FG fibers in the LM and STL were larger ($P < 0.01$) in the older and heavier pigs (A-154 and R-154) than in their younger and lighter siblings (A-113

and R-113). The lacking interactions between feeding regime and age at slaughter indicated that for A- and R-pigs changes in myofiber size were not ($P \leq 0.31$) different from 113 to 154 d of age. Neither the dietary nutrient supply nor the age at slaughter affected the fiber type distribution of the LM. By contrast, the STL of R-pigs had fewer ($P = 0.04$) FG (60 vs. 70%) and more ($P = 0.03$) FOG (32 vs. 26%) fibers than the STL of A-pigs. The latter findings are in agreement with results reported by HARRISON et al. (1996) who found larger amounts of oxidative fibers in the rhomboideus muscle after a period of reduced energy availability. Regardless of the dietary treatments, myofiber type distribution in both muscles did not change from 113 to 154 d of age, which confirms results reported by ONO et al. (1995) who showed that the most intense changes in myofiber type distribution occurred from 20 to 60 kg BW.

Meat Quality Traits

Regardless of the age at slaughter, shear force values were higher ($P < 0.05$) in the LM (4.5 vs. 3.7 kg) and STL (4.0 vs. 3.3 kg), and cooking losses of the LM were higher (18.3 vs. 13.7%; $P < 0.01$) in R- than A-pigs. In agreement with results of previous studies (HENCKEL et al., 1997; CANDEK-POTOKAR et al., 1999) the differences in the shear force values were related to neither the size nor the distribution of the myofibers. This is not surprising because pork tenderness depends primarily on the extent of postmortem proteolysis (MELODY et al., 2004). Recent results showed that restricted feeding reduced in vivo protein turn-over at the time of slaughter, which then negatively affected tenderization processes during the conversion of muscle to meat (KRISTENSEN et al., 2002). Regardless of the feeding regime, pH at 30 min (6.3 vs. 6.2) and 24 h postmortem (5.6 vs. 5.4) in the LM and at 24 h postmortem (5.7 vs. 5.6) in the STL were higher ($P = 0.01$) in younger than in older pigs. A determinant factor affecting initial and ultimate pH is the amount of stored glycogen in the muscle at slaughter (BENDALL and SWATLAND, 1988). The latter might partly depend on the size and type of myofibers (FERNANDEZ et al., 1995). In the LM the size of FOG fibers was positively correlated with initial and ultimate pH ($r = 0.63$ for each; $P < 0.03$) at 113 d of age and the size of SO ($r = 0.61$; $P = 0.05$) and FOG ($r = 0.58$; $P = 0.06$) fibers were positively correlated with pH 24 h postmortem at 154 d of age. In the STL pH values 30 min postmortem were positively correlated with higher percentages of SO ($r = 0.63$; $P = 0.04$) and FOG ($r = 0.61$; $P = 0.05$) fibers and negatively correlated with the percentages of FG (-0.70; $P = 0.02$) fibers at 113 d of age. Because within experimental treatments myofiber type distribution was unaffected by the age at slaughter the larger increase in size of the FG (+36%) compared with the SO (+27%) and FOG (+32%) fibers might partly explain the observed drop in the muscle pH from younger to older pigs. Although in both muscles the FG fibers were larger and in the STL the percentages of FG fibers were higher in pigs of the A- compared to the R-group these differences seemed not to be sufficient to affect the pH values between the treatments.

In conclusion for pigs at the same age, when slaughter weight was lower (feed restriction) the cross-sectional areas of the myofibers were smaller except for the FOG fibers in the LM. Furthermore, the STL of pigs subjected to feed restriction was more oxidative. These findings indicated that the impact of feed restriction on hypertrophic growth and differentiation vary among myofiber types and muscles.

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Effect of maternal undernutrition on ruminant carcass and meat quality

(Auswirkungen maternaler Unterernährung auf Schlachtkörper- und Fleischqualität bei Wiederkäuern)

Increasing evidence suggests that maternal nutrient intake at specific periods during pregnancy can influence the subsequent development of the offspring, even when they are provided with an adequate diet. Sheep and cattle, especially those reared extensively, are subjected to variation in their nutrient supply and this project aims to examine the effect of the diet during pregnancy on the fat to lean ratio of the resulting offspring. The number of muscle fibres in the adult is set *in utero* and an increase in the number of these fibres tends to be associated with increased growth rates and reduced adiposity. Our hypothesis is that if nutrient supply to the foetus is restricted at critical periods during pregnancy then there will be a reduction in the number and type of muscle fibres formed. Subsequently when the offspring are grown up for meat production there will be impaired muscle growth and in addition more of the nutrients in its diet will be diverted to fat resulting in a fatty carcass.

Work with sheep conducted in these laboratories, including the use of tissue culture, has identified the time during development that the number of muscle fibres is set and that it is sensitive to maternal nutrition. We identified the critical timing for muscle development by monitoring changes in gene expression for myogenin and various growth factors in developing sheep muscle (FAHEY et al., 2005a). This indicated a peak for myogenin expression (and myogenesis) around d85 gestation. In a second study, maternal nutrition was reduced to 50% of controls during three periods of gestation – before (d30-70), during (d55-95) and after (d85-115) this peak for myogenesis. Nutrient restriction (d30-70) resulted in an increased proportion of slow fibres (predominantly primary fibres) and a decreased proportion of fast fibres (predominantly secondary fibres) in 14 d old lambs. The reduced numbers of fast fibres was associated with increased fast fibre diameters (FAHEY et al., 2005b). Together the data indicate a reduction in the numbers of fast (secondary) fibres, which is a consistent with other literature. Whether such effects persist later in life in the sheep or what the consequences are of having a reduced number of muscle fibres are not clear. Currently we are investigating whether the changes in muscle fibre characteristics observed in lambs following maternal nutrient restriction persist during growth and what consequences there are for growth rates and carcass composition of fattened lambs.

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Metabolic and contractile characteristics of muscles *longissimus thoracis* and *Semimembranosus* from two porcine lines

Abstract

Metabolic and contractile characteristics of *longissimus thoracis* (LT) and *Semimembranosus* (SM) muscles were studied using gilts from two porcine lines (n = 35 per line). One line was based on the Large White breed and the other on a Meishan/Large White crossbred. Pigs from the two lines were slaughtered at approximately 140 days of fattening. The two lines did not differ significantly in carcass weight; the Large White line presented fat depths significantly ($p<0.001$) lower than the Meishan line (13.64 ± 0.66 vs 21.34 ± 0.67 mm). The activity of isocitrate dehydrogenase (ICDH) and lactate dehydrogenase (LDH), and the myosin heavy chain 1 (MHCI) and haem pigments content were determined in both muscles. The two lines showed significant differences ($p<0.05$) in ICDH-SM activity (1.67 ± 0.11 and 1.56 ± 0.11 $nmol.min^{-1}.g^{-1}$) and MHCI-SM percentage (5.6 ± 0.4 and 4.2 ± 0.4 %), both parameters presenting higher values in the Large White line, thus indicating higher oxidative traits in the SM from this line with respect to the same muscle from the Meishan line. Several significant correlations ($p<0.05$) were found between the variables studied. Positive correlations between fat depth and ICDH in LT and LDH in SM were found in the Large White line. In both lines, LDH and ICDH activities were positively correlated in the two muscles. In the Meishan line, MHCI was correlated with ICDH (0.51) and with the glycolytic ratio (-0.46) in the SM muscle. The comparison between the two muscles showed that SM had higher oxidative traits than LT in the two lines: higher ICDH activity and lower glycolytic ratio (LDH/ICDH). The lower MHCI % found in SM would suggest a higher content of myosin heavy chain 2a in this muscle than in LT, as the oxidative characteristics were higher in SM. The results confirmed the importance of breed and muscle type on the metabolic and contractile traits of fibres.

Key Words: Breed, muscle biochemical traits, pig.

Zusammenfassung

Titel der Arbeit: **Metabolische und kontraktile Eigenschaften des Musculus longissimus thoracis und des Musculus semimembranosus (SM) bei zwei Schweinelinien**

Es wurden metabolische und kontraktile Eigenschaften des Musculus longissimus thoracis (LT) und des Musculus semimembranosus (SM) an Jungsauen zweier Linien (n= 35 per Linie) untersucht. Grundlage der einen Linie war die Herkunft Large White, die andere war eine Meishan/Large White Kreuzung. Schweine beider Linie wurden mit einem Alter von ca. 140 Tagen geschlachtet. Zwischen den Linien bestanden keine signifikanten Unterschiede im Schlachtgewicht. Die Speckdicke der Large White Linien war signifikant ($p<0.001$) geringer als bei der Meishan Linie (13.64 ± 0.66 zu 21.34 ± 0.67 mm). Die Aktivität der Isocitratdehydrogenase (ICDH) und Laktatdehydrogenase (LDH), und die Myosin heavy chain 1- (MHCI) und die Hämpigmentgehalte wurden in beiden Muskeln untersucht. Die beiden Linien unterschieden sich signifikant ($p<0.05$) in den Merkmalen ICDH-SM Aktivität (1.67 ± 0.11 und 1.56 ± 0.11 $nmol.min^{-1}.g^{-1}$) und MHCI-SM Anteil (5.6 ± 0.4 und 4.2 ± 0.4 %), beide Parameter zeigten für die Linie Large White höhere Werte, was für einen höheren oxidativen Wert im SM für diese Linie im Vergleich zum gleichen Muskel der Meishan Linie spricht. Mehrere signifikante Korrelationen ($p<0.05$) wurden zwischen den untersuchten Variablen gefunden. Eine positive Beziehung wurde zwischen Fettdicke und ICDH im LT und LDH im SM bei der Linie Large White ermittelt. In beiden Linien waren die LDH und ICDH Aktivitäten in den zwei Muskeln positiv korreliert. In der Meishan Linie war MHCI positiv mit ICDH (0.51) korreliert und mit dem glycolytischem Verhältnis mit (-0.46) im SM-Muskel. Ein Vergleich zwischen den zwei Muskeln zeigte, dass der SM in beiden Linien ein höheres oxidatives Potential hatte als der LT-Muskel: höhere ICDH-Aktivität und und geringeres glycolytische Verhältnis (LDH/ICDH). Der geringere MHCI-Gehalt im SM lässt auf einen höheren Gehalt an Myosin heavy chain 2a in diesem Muskel als im LT schliessen, wie auch die oxidative Charakteristik im SM stärker ausgeprägt ist. Die Ergebnisse bestätigen die Bedeutung der Züchtung und Muskeltypen auf metabolische und kontraktile Merkmale von Muskelfasern.

Schlüsselwörter: Zucht, Muskelbiologiemerkmale, Schwein

Introduction

Muscle fibre characteristics are known to depend on several intrinsic and extrinsic factors as species, breed, muscle type, individual, exercise, nutrition etc. (LEFAUCHEUR and GERRARD, 1998). Fibre types are often defined by the isoforms of myosin heavy chain (MHC) present. In postnatal pig there are four major fibre types characterized by the expression of slow /I, and fast 2a, 2x and 2b MHC gene isoforms (CHANG et al., 2003).
The aim of this study was to evaluate the influence of the breed and of the muscle type on the metabolic and contractile traits of fibres.

Materials and methods

Animals
Gilts of a Large White based line and a Meishan/Large White based line were used in the study (n = 35 per line). Animals were reared under the same environment and production regime and submitted to the same *antemortem* conditions of minimum stress. The average live weight was 109.56 $\pm$ 7.81 kg. Carcass weight was recorded at one h *post-mortem* (pm) and fat depth measured using a Fat-O-Meater at 60 mm from the mid-line at the last rib level.

Sampling
Sampling was performed in the left side of the carcass at 24 h pm. *Longissimus thoracis* (LT) was sampled from the core of the muscle at the last rib level, and *Semimembranosus* (SM) from the internal part at the centre of the muscle. Samples were frozen in liquid nitrogen and then stored at -80ºC until analysis.

Enzymatic analysis
The metabolic traits of the muscles were determined by measuring the lactate dehydrogenase (LDH) and the isocitrate dehydrogenase (ICDH) activities according to ANSAY (1974) and to BRIAND et al. (1981), respectively. Enzyme activities are expressed as µmol NADH per minute per gram of muscle (LDH), and as nmol NADPH per minute per gram of muscle (ICDH).

Slow myosin heavy chain (MHC I)
The percentage of MHC I in the muscle was determined by enzyme-linked immunosorbent assay (ELISA) using a specific MHC I monoclonal antibody and by means of a standard curve prepared from bovine *Masseter* muscle (100% MHC I) and bovine serum albumin (PICARD et al., 1994).

Muscle Pigments
The content of haem pigment in the two muscles was determines according to HORNSEY (1956). Results are given in µg of acid haematin per muscle wet weight.

Statistical Analysis
A least square analysis was performed using the General Linear Model (GLM) Procedure program of the SAS statistical package (SAS, 1999). The effect of genetic line was included in the model as a fixed effect, and the covariate carcass weight if it was significant. The effect of the muscle was evaluated using a T-Test with the PROC ANOVA. Pearson correlations were carried out.

Results and Discussion

Least squares means and standard errors of carcass and muscle characteristics of LT and SM from the two porcine lines are presented in Table 1. Fat depth differed significantly between the two breeds, with the Meishan crossbred showing the highest value. The Meishan breed is characterised by a high fat depth and a low lean content. (PLASTOW et al., 2005). The only differences found in the biochemical variables were in the SM, which presented higher oxidative traits (ICDH activity and content of MHCI) in the Large White line than in the Meishan crossbred. The SM glycolytic ratio (R-SM) did not differ between lines.

Table 1
Least squares means and standard error of carcass and biochemical parameters in the muscle (*longissimus thoracis* and *semimembranosus)* characteristics in two porcine lines

	Genetic line		
Traits	Large White	Meishan/Large White	Significance Level
Carcass weight (kg)	90.43 ± 1.09	88.14 ± 1.10	ns
Fat depth (mm)	13.64 ± 0.66	21.34 ± 0.67	***
ICDH-LT(nmol·min-^{1}·g^{-1})	1.31 ± 0.07	1.26 ± 0.07	ns
MHCI-LT (%)	7.4 ± 0.5	7.0 ± 0.5	ns
LDH-LT (µmol·min^{-1}·g^{-1})	3060.3±121.6	3170.8± 123.3	ns
R-LT(µmol·nmol^{-1}·10^{-3})	2.48 ± 0.16	2.76 ± 0.16	ns
Pigm.LT(µg haematin·g^{-1})	36.35 ± 1.57	39.76 ± 1.60	ns
ICDH-SM(nmol·min-^{1}·g^{-1})	1.67 ± 0.11	1.56 ± 0.11	*
MHCI-SM (%)	5.6 ± 0.4	4.2 ± 0.4	*
LDH-SM (µmol·min^{-1}·g^{-1})	3208.6± 116.4	2860.8± 118.9	ns
R-SM(µmol·nmol^{-1}·10^{-3})	2.04 ± 0.11	2.02 ± 0.12	ns
Pigm.SM(µg haematin·g^{-1})	43.08 ± 1.44	43.18 ± 1.46	ns

LT: *longissimus thoracis*; SM: *Semimembranosus*; ICDH: isocytrate dehydrogenase activity; LDH: lactate dehydrogenase activity; MHCI: myosin heavy chain I; R: ratio LDH/ICDH; Pigm.: Pigment.
ns: non significant (p>0.05); *, p<0.05.

Table 2
Significant correlations (p<0.05) between carcass variables and muscle biochemical traits in *longissimus thoracis* (LT) and *semimembranosus* (SM) from two porcine lines

Genetics	Large White	Meishan/Large White
Fat depth with		
ICDH -LT	0.38	-
LDH -SM	0.47	-
ICDH-LT with		
LDH-LT	0.67	0.39
Ratio LT	-0.72	-0.72
ICDH-SM	-	0.62
LDH-SM	0.42	-
Pigments SM	-	0.38
Ratio SM	-	-0.69
ICDH-SM with		
LDH-SM	0.42	0.74
MHCI-SM	-	0.51
Ratio SM	-0.83	-0.82
Ratio LT	-	-0.5
Ratio SM with		
MHCI-SM	-	-0.46
Ratio LT	-	0.71

LT: *longissimus thoracis*; SM: *Semimembranosus*; ICDH: isocytrate dehydrogenase activity; LDH: lactate dehydrogenase activity; MHCI: myosin heavy chain I; Ratio: ratio LDH/ICDH.

The main significant correlations (p<0.05) obtained between the fat depth and the muscle variables are shown in Table 2. The fat depth was positively correlated with

the enzymatic activities in the Large White line. ICDH activity was positively correlated with LDH activity and negatively with the ratio in both muscles and in the two lines. This is in accordance with the results obtained by GIL et al. (2003) in pig. Conversely, the correlations between these two activities found in the LT from cattle are negative (GIL et al., 2001). This could be explained by the different maturity of these two species at birth. In pigs, most changes occur after birth and the muscle metabolism, as a whole, becomes more glycolytic with age. In bovine muscle the metabolic differentiation starts earlier during the last third of gestation (GAGNIÈRE et al., 1998). In the SM from the Meishan line, the content of MHC I was positively correlated with ICDH and negatively with the glycolytic ratio.
The comparison between LT and SM showed that SM had higher oxidative traits than LT in both lines: higher ICDH activity and lower glycolytic ratio. The MHC I % was lower in SM than in LT, which would suggest that SM could have a higher content of MHC 2a (type IIA fibres) than LT. This was found in the Meishan line, but not in the Large White (results not shown). Pigment content was higher in SM than in LT in the Large White but not in the Meishan line.
In conclusion, the results confirmed the importance of the breed and the muscle type on the metabolic and contractile properties of fibres.

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Growth and differentiation of the chicken *Pectoralis major* muscle: Effect of genotype and early nutrition

(Wachstum und Differenzierung des Pectoralis major Muskels beim Huhn: Effekte des Genotyps und der frühen Fütterung)

We observed that selection for growth and improved breast muscle yield in chickens is associated with muscle hypertrophy (GUERNEC et al., 2003) and lower muscle glycogen content with consequences in term of meat quality (BERRI et al., 2001, 2004). This points out to a need for better understanding the links between muscle growth, differentiation and metabolism. We conducted the present study to test whether variations in growth rate could alter the expression of genes involved in muscle cell growth and differentiation.

We compared chickens from two experimental lines divergently selected for high or low growth rate (HG and LG), which were either fed (F) as of hatch or 2 days later (DF for delayed feeding). The HG birds have a greater muscular mass at the same age because of a higher number and a larger size of muscle fibres (RÉMIGNON et al., 1995). We sampled the *Pectoralis major* (P. major) muscle on days 15 and 18 *in ovo* (E15, E18), at hatch and on days 2, 4, 7 and 43 post-hatch. We chose PCNA and PAX7 as markers of proliferation and satellite cell number, respectively and IGF-I as a candidate paracrine regulator of muscle growth. We estimated the differentiation of the muscle fibres by the measure of the three developmental isoforms of fast MyHC, embryonic 3, neonatal and adult. We quantified the corresponding mRNAs by real time RTPCR as already described (GUERNEC et al., 2003). Absolute mRNA levels of genes under investigation were corrected for 18S rRNA levels to give a relative mRNA level.

The HG chickens exhibited higher body and P. major muscle weight than the LG chickens throughout the experimental period (E15 to 43 day post-hatch). In HG chickens, delayed feeding (DF) induced a delay in growth and muscle development which lasted until day 43. In LG chickens, DF did not alter overall body growth, but decreased P. major muscle weight and yield at 2 and 4 days post-hatch.

The expression of PCNA and IGF-I decreased from E15 to hatch and then increased to reach a peak around day 4. Thereafter their expression decreased until day 43. The expression of PAX7 decreased regularly with age. At day 7 post-hatch, it was lower in HG than in LG and in F than in DF birds. At day 7, the expression of PCNA and IGF-1 was also lower in F than in DF birds, while that of PCNA was lower in HG than in LG at day 43.

The expression of the embryonic fast MyHC isoform increased between E15 and E18, and then decreased progressively to become negligible after day 7. The expression of the neonatal isoform increased from hatch to day 7, and decreased between days 7 and 43. The expression of the adult form remained low until day 7, and then increased strongly. The transition between the different fast MyHC isoforms varied with the genotype and the nutritional status. From day 7 after hatch, there was a trend for

higher levels of the adult isoform mRNA in HG birds, and at day 43 they expressed lower levels of the neonatal MyHC than LG birds. Delayed feeding led to a lower expression of the neonatal MyHC isoform and higher embryonic to neonatal MyHC ratio at day 2.

Our data show that variations in growth rate alter the expression of markers of muscle development, and therefore suggest that they alter the cellular processes of muscle growth and differentiation and their regulation. Assuming that PAX7 expression measures satellite cell number and PCNA, proliferation, the data suggest that satellite cell activity or more generally cell proliferation is delayed in LG compared to HG, and in DF compared to F. The data also suggest that IGF-I could be involved during the period of compensatory growth which follows delayed feeding. The measure of fast MyHC isoform transitions also shows that delayed growth is linked with delayed contractile differentiation of the muscle fibres.

Key Words: Muscle, Growth, differentiation, Myosin Heavy Chain, satellite cell, proliferation

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Role of insulin and IGFs in fish muscle development and quality

Abstract

In order to identify possible relationships between insulin, Insulin-like Growth Factors (IGFs) and the various transcription factors involved in cell proliferation and muscle growth (such as MyoD, myogenin, myostatin), we combined *in vivo* and *in vitro* experiments.
Changes in food regimen in rainbow trout (*Onchorhynchus mykiss*) altered the IGF system at peptide and receptor level. The expression of myogenin and myostatin was also altered. IGF expression and muscle growth were partially restored by refeeding.
In preliminary experiments in zebrafish (*Danio rerio*) muscle, ectopic overexpression of IGF-I increased MyoD and myogenin expression.
By Western Blot we studied the effects of insulin, IGF-I and IGF-II on the two main signalling pathways in muscle using primary cultures of rainbow trout and seabream (*Sparus aurata*) muscle cells. IGFs activated both pathways and their efficiency depended on the culture stage, and human and fish peptides had similar effect. Incubation with specific inhibitors showed that wortmannin decreased Akt phosphorylation stimulated by hIGF-II, while treatment with PD98059 reduced the activation of MAPK.
The role of insulin and IGFs in metabolic processes was studied in seabream myocytes and compared with results in trout. Both IGFs showed a similar effect on glucose uptake stimulation, which decreased when the cells were incubated with wortmannin, confirming that the PI3K/Akt pathway is important for this process in muscle. Insulin and IGFs also stimulated alanine uptake.
To find the best markers for fish growth and quality we did preliminary experiments using c-met. Its expression was high during the first stages of development and decreased as differentiation progressed.

Key Words: insulin, IGFs, signalling pathways, rainbow trout, zebrafish, seabream, growth, muscle

Zusammenfassung

Titel der Arbeit: **Die Einflüsse von Insulin und IGFs auf Muskelwachstum und Qualität beim Fisch**
Um Beziehungen zwischen Insulin, Insulin-ähnlichen Wachstumfaktoren (IGFs, insulin like growth factors) und verschiedenen an Zellproliferation und Muskelwachstum beteiligten Transkriptionsfaktoren (wie MyoD, Myogenin, Myostatin) zu untersuchen, wurden *in vivo* und *in vitro* Experimente durchgeführt.
Bei der Regenbogenforelle (*Onchorhynchus mykiss*) beeinflussen unterschiedliche Fütterungsregime das IGF-System sowohl hinsichtlich der Liganden als auch der Rezeptoren. Die Expression von Myogenin und Myostatin wurde ebenfalls beeinflusst. Die Expression von IGF und das Muskelwachstum erreichten nach erneuter Wiederaufnahme des normalen Fütterungsregimes die ursprünglichen Werte.
Vorherige Experimente am Muskel des Zebrafisch (Dario rerio) zeigten einen Anstieg von MyoD und Myogenin nach ektopischer IGF-I Überexpression.
Die Effekte von Insulin, IGF-I und IGF-II auf die zwei wesentlichen Signalkaskaden wurden mittels Western Blot in primären Zellkulturen von Muskelzellen der Regenbogenforelle und der Goldbrasse (Sparus aurata) untersucht. IGFs aktivieren bei den Signalkaskaden abhängig vom Kulturstadium und humane und Fisch-Proteine zeigen ähnliche Effekte. Inkubation mit spezifischen Inhibitoren zeigte, dass Wortmanin die Dephosphorylierung von Akt stimuliert und durch hIGF-II senkt, während bei Behandlung mit PD98059 die Aktivierung von MAPK reduziert wird.
Die Rolle von Insulin und IGFs auf metabolische Prozesse wurde in Myozyten der Seebrasse untersucht und mit Ergebnissen der Forelle verglichen. Beide IGFs zeigten einen ähnlichen Effekt auf die Stimulation der Glukoseaufnahme, die durch Wortmanin gesenkt wurde. Dies bestätigt die Bedeutung des PI3K/Akt-Signalweges für diesen Prozess im Muskel. Insulin und IGFs stimulieren ebenso die Alaninaufnahme.
Um Marker für Fischwachstum und -Qualität zu identifizieren, wurden erste Experimente mit c-met durchgeführt. Dessen Expression war hoch während der ersten Entwicklungsstadien und nahm mit der Differenzierung ab.

Schüsselwörter: Insulin, IGFs, Signalkaskaden, Regenbogenforelle, Zebrafisch, Goldbrasse, Wachstum, Muskel

Introduction

Insulin-like growth factors (IGFs) have many functions (both metabolic and mitogenic) in several tissues (FLORINI et al., 1996). In fish, as in mammals, muscle is a target tissue for these factors. Recent reports describe the effect of insulin and IGF-I on cell proliferation and glucose and alanine uptake in rainbow trout (*Onchorynchus mykiss*) muscle cells in culture (CASTILLO et al., 2004). Their effects on the main signalling pathways through the insulin and IGF-I receptors in muscle have also been reported (CASTILLO et al., 2006).

The MAPK pathway is involved in cell proliferation and its activation induces mechanisms of cell division. In contrast, the PI3K/Akt pathway is related to metabolic pathways as well as cell survival and differentiation (COOLICAN et al., 1997).

During muscle-cell proliferation and differentiation, there is also sequential activation of several skeletal muscle transcription factors like myf5, MyoD, myogenin and mrf4, which play distinct roles in the control of myogenesis (RESCAN et al., 1994; RESCAN et al., 1995). Members of the FGF family (OLWIN et al., 1994) and myostatin (McPHERRON et al., 1997) also influence this process. The homologues of these proteins have been found in several fish species, like trout, zebrafish and carp, but the effects of IGFs on these factors remain to be established..

Material and methods

Effects of fasting and refeeding on IGF system and muscle growth in rainbow trout.

Juveniles (23.05±1.7g) of rainbow trout (*Oncorhynchus mykiss*) were randomly distributed into experimental groups. Fish were fasted for two weeks. Thereafter, they were fed to satiation twice a day for 4 weeks. At the end of fasting and refeeding, fish from all groups were sampled.

Blood samples were used to determine plasma levels of glucose, insulin. Muscle and liver samples were used to obtain RNA and perform RT-PCR to determine expression of different molecules (IGF-I in liver and myogenin and myostatin in muscle).

Effects of intramuscular injection of IGF-I DNA on the expression of MRFs in zebrafish.

Adult zebrafish (*Danio rerio*) were injected with 50µl of solution containing the copolymer PE-6400 (PITARD, 2002) and the expression vector pCMV with lacZ or IGF-I, and returned to the water. After three days fish were killed and muscle samples were taken. RNA levels of myogenin, myoD and other Myogenic Regulatory Factors were determined by Northern Blot and Real Time RT-PCR.

Effects of insulin and IGFs on rainbow trout and seabream muscle cells in culture.

White muscle was taken from rainbow trout or seabream (*Sparus aurata*) weighing 3-5g. Satellite cells were isolated and cultured following the protocol described by FAUCONNEAU and PABOEUF (2000), with some modifications for the seabream myocytes. Cells were maintained in DMEM 10%FBS until the day of experiment. The effects of insulin, IGF-I and IGF-II were tested at different stages of cell development (myoblast, myocyte and large myotubes). Western Blot assays were performed to study the stimulatory effects of these peptides on the MAPK and PI3K/Akt pathways after 30 minutes of incubation and cell lysis (CASTILLO et al., 2006), and also on the c-met protein level. The metabolic role of insulin and IGFs was studied with glucose and alanine uptake assays (CASTILLO et al., 2004).

Results and discussion

Effects of fasting and refeeding on IGF system and muscle growth in rainbow trout (O. mykiss)

Food restriction affected IGF-I (Fig. 1), but not IGF-II mRNA levels of IGF-I in both liver and muscle decreased after two weeks of fasting and recovered after refeeding. These results are consistent with the findings of others (CHAUVIGNÉ et al., 2003).

Fasting and refeeding also altered myogenin and myostatin mRNA levels. During refeeding myogenin showed an increase in comparison to the fasting period. This tendency is parallel to that of IGF-I mRNA levels. Myostatin levels show an inverse pattern, which agree with the fact that both molecules have inverse effect on growth control (McPHERRON et al., 1997).

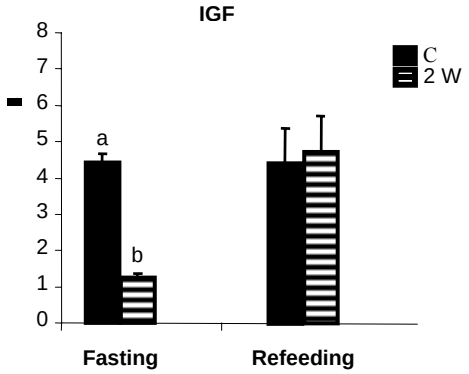

Figure 1: IGF-I mRNA quantification in liver of rainbow trout from control (C) or 2 weeks (2W) fasting, during fasting or refeeding trials. The means (±SEM) of seven animals in each group are shown. Differences were determined by the non-parametric Mann–Whitney *U* test. Differences between letters indicate significantly different means at $P<0.05$. (IGF-I mRNA Quantifizierung in der Leber von Regenbogenforellen in der Kontrolle (C), nach 2 Wochen (2W) Fasten, in Fasten/Füttern-Versuchen. Durchschnittswerte (±SEM) von sieben Tieren je Gruppe werden gezeigt. Unterschiede wurden mit dem nicht-parametrischen Mann-Whitney U-Test bestimmt. Unterschiedliche Buchstaben bezeichnen signifikante Differenzen bei $P<0.05$.)

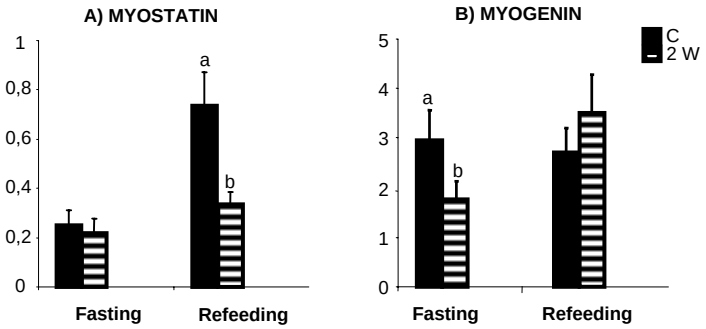

Figure 2: Myostatin (A) and myogenin (B) mRNA quantification in fast myotomal muscle of rainbow trout from control from control (C) and 2 (2W) weeks fasting trout during fasting or refeeding trials. The means (±SEM) of seven animals in each group are shown. Differences were determined by the non-parametric Mann–Whitney *U* test. Differences between letters indicate significantly different means at $P<0.05$. (Myostatin (A) und Myogenin (B) mRNA Quantifizierung in schnellen myotomalen Muskel von Regenbogenforellen in der Kontrolle (C), nach 2 Wochen (2W) Fasten, in Fasten/Füttern-Versuchen.. Durchschnittswerte (±SEM) von sieben Tieren je Gruppe wurden ermittelt. Unterschiede wurden mit dem nicht-parametrischen Mann-Whitney U-Test bestimmt. Unterschiedliche Buchstaben bezeichnen signifikante Differenzen bei $P<0.05$.)

Effects of intramuscular injection of IGF-I DNA on the expression of MRFs in zebrafish (D. rerio)

We have demonstrated here that IGF-I can be overexpressed following direct injection in the zebrafish myotomal muscle of a cDNA conjugated with a polymer. However, the half life of the plasmid in the cells is short and the increase of IGF-I is lower than expected, which is inconvenient.

High variability between animals was seen. Both lacZ- and IGF1-injected fish showed different injection efficiency, as demonstrated by the β-gal activity observed and also by the determination of CMV/IGF-1 mRNA. The degree of IGF-I expression shown by different animals was also highly variable.

Nevertheless, it has been possible to detect differences in the levels of IGF-I, MyoD and myogenin, but no effects in myostatin (Table). This indicates a transitory effect of the overexpression of IGF-I that would lead to variations in these MRFs levels.

Table
Summarized effect of IGF-I overexpression on the mRNA levels of myoD, myogenin and myostatin (Effekte der IGF-I Überexpression auf mRNA Level von MyoD, Myogenin und Myostatin)

	IGF-I	myoD	myogenin	myostatin
control	+	+	+	+
igf1 cDNA injected	+++	+++	++	-

Effects of insulin and IGFs on rainbow trout (O. mykiss) and seabream (S. aurata) muscle cells in culture

For the first time seabream myocytes have been cultured and metabolic studies have been performed. We have demonstrated that insulin, IGF-I and IGF-II stimulate glucose and the alanine uptake, as was seen before in rainbow trout (CASTILLO et al., 2004). The inhibitory effects of wortmannin (specific inhibitor of PI3K/Akt) and PD98059 (inhibitor of MAPK) were tested, showing that the main pathway in muscle involved in glucose uptake is the PI3K/Akt.

Experiments with rainbow trout primary culture show that IGFs stimulate the MAPK pathway more strongly in the early stages of cell development, whereas their effects on the PI3K/Akt pathway increase as the culture progresses (Figure 3). This is consistent with the theory that the two pathways exert opposite effects in muscle, and depending on the differentiation stage of the cells, one or the other is activated (JONES et al., 2001). In some cases, the effect of IGF-II is slightly lower than the effect of IGF-I. This can be explained by the fact that IGF-II can act through the IGF-IR, as seen in primary cultures of rat muscle cells (GALVIN et al., 2003), but it also interacts with its own receptor, which is thought to be in peptide degradation. Fish and mammal peptides exert similar stimulatory effects on Akt-P and MAPK-P, which is consistent with the high conservation of these molecules throughout the evolution (SHAMBLOTT and CHEN, 1992).

C-met in trout and sea bream myocytes was also studied and it was found that it is mostly expressed when the myocytes are proliferating at early stages of the culture (3-5days), decreasing once myotubes are already differentiated (10-11 days).

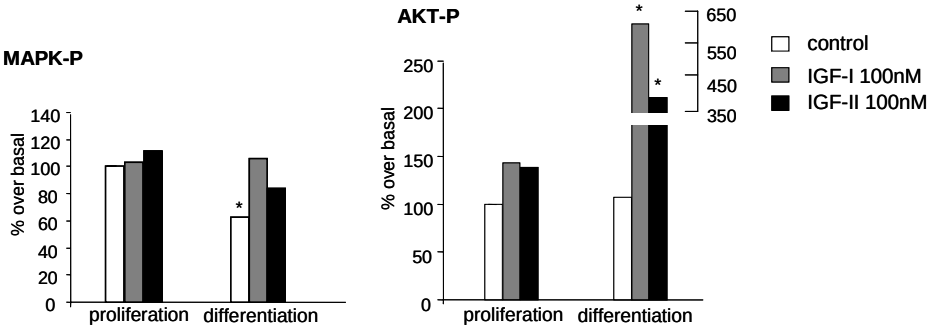

Figure 3: Western Blot for MAPK-P and Akt-P in cell lysates from primary cultures of rainbow trout muscle cells at different stages of development. Effects of IGF-I and IGF-II. Results given as mean values and were analyzed by one factor ANOVA. Asteriscs indicate significantly different means at $P<0.05$ (Western Blot von MAPK-P und AKT-P in Zelllysaten von primäen Kulturen von Muskelzellen der Regenbogenforelle zu verschiedenen Entwicklungsstadien. Effekte von IGF-I und IGF-II. Mittelwerte wurden mit einfaktorieller ANOVA bestimmt. Sterne bezeichnen signifikante Differenzen bei $P<0.05$.)

In conclusion, the regulation of muscle growth is a complex process in fish. However, the role of IGF-I, IGF-II, MyoD, myogenin and myostatin and other myogenic factors is now better understood. The transduction pathways of IGFs, MAPK and AKT have been characterized and, together with c-met, they can be used to recognize proliferative and differentiated stages in fish muscle. These molecules may be used as indicators of fish muscle growth and quality

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The influence of two temperatures maintained constant during incubation and larval life on the early myotomal red muscle growth and activity of European sea bass (*Dicentrarchus labrax* L.)

(Einfluss zweier konstanter Umgebungstemperaturen während der Brut und Larvenentwicklung auf das frühe Wachstum von Myotomen roter Muskulatur und die Aktivität beim Europäischen Seebarsch (*Dicentrarchus labrax* L.)

A study was conducted in order to define the influence of two temperatures maintained constant during incubation and larval life on the early myotomal red muscle growth and activity of European sea bass. Egg incubation (from the stage of epiboly onset) and larval rearing were performed in four tanks supplied in duplicates with seawater at a constant temperature of 15°C or 20°C, all other environmental conditions being similar. Larvae were fed in excess with enriched live preys during 40 days, from mouth opening onwards. Survival was higher in European sea bass incubated/reared at 20°C than in those incubated/reared at 15°C. Morphological and densitometrical studies were performed on transverse sections of sea bass larvae at two larval developmental stages : that of the first exogenous feeding and that of the completion of fin ray counts, this later stage corresponding, according to Kendall *et al.* (1984), to the end of fish larval life. Incubation at 15°C increased significantly larval total length, total cross-sectional area of red muscle, total number of red muscle fibres and maximal diameter of red muscle fibres in sea bass larvae at the stage of first exogenous feeding. After this stage, the larvae incubated/reared at 15°C exhibited the lower total length increase; they reached the stage of completion of fin ray counts with a similar total number of red muscle fibres and a significantly lower number of small diameter red muscle fibres than those incubated/reared at 20°C. The two temperatures tested had thus differently affected the process of red muscle growth, in a developmental-related pattern. Temperature affected also the optical succinate deshydrogenase density of red muscle, witch was significantly higher in 15°C larvae when they completed their fin ray counts. Whether these differences in red muscle phenotype (cellularity and activity) affect fish swimming ability remained to be solved.

Key words: fish, skeletal muscle, hyperplasia, hypertrophy, enzyme activity, temperature

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Effect of water temperature on GH receptor expression during embryonic and post-larval growth of rainbow trout (*Oncorhynchus mykiss*)

(Der Effekt der Wassertemperatur auf die Expression des Wachstumshormon-rezeptors während des embryonalen und post-larvalen Wachstums der Regenbogenforelle (*Oncorhynchus mykiss)*)

In contrast to mammals, water temperature influences the growth of the embryo and the juvenile. The GH receptor (GHR) is a key component of the GH/IGF system which promotes embryonic and post-larval growth. Therefore, the aim of this study was to determine whether variations of growth in response to temperature could be associated with change of GHR gene expression in embryo and juvenile.
Based on the ESTs published homologous to salmon GH receptor, we cloned two cDNA (GHR1 and GHR2) coding for the rainbow trout GH receptor (85% amino acid identities).
During embryonic development, using real time PCR, we showed that incubation temperature (4, 8 or 12°C) increased the amounts of GHR1 transcript until the hatching stage. Conversely, whatever the stage studied, levels of GHR2 mRNA were unaffected by the incubation temperature.
For juvenile fish, to determine if temperature regulates GHR gene expression independently of nutritional state, fish were reared at 8, 12, or 16°C and either fed ad libitum or with the same ration (1.2%/body weight). In muscle of fish fed ad libitum, rearing temperature increased GHR1 ($P<0.001$) but not the GHR2 mRNA levels. When the fish were restricted, temperature did not affect any more the levels of GHR1 and GHR2 transcript. In liver of fish fed ad libitum, rearing temperature increased both GHR1 and GHR2 mRNA levels ($P<0.001$) while in restricted fish no difference was seen.
In conclusion, our results showed that temperature influenced GHR expression differently according to the stage, the tissue and the GHR isoforms. During embryonic development, we observed that high temperature increased the gene expression of GHR1 what could be related to the enhancement of embryonic growth rate. In juvenile (muscle and liver), the temperature stimulated the expression of GHR1 gene expression only under optimal nutritional state. Thus, the GH receptor, by integrating the specific effect of temperature on plasma GH and the changes of nutritional state, would play a key role in growth-promoting effect of temperature.

Key words: GH receptor, temperature, growth rate, rainbow trout

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Sodium ascorbate (ASC) and ascorbic acid phosphate (ASC-P) differently modulate glucocorticoid-dependent metabolic effects in growing rats

Abstract

It is well known that dexamethasone treatment in certain doses induces oxidative stress, insulin resistance, diabetes, and muscle cachexia. This survey was carried out to investigate the effect of ascorbate derivatives on dexamethasone-induced metabolic disturbances. Experiment was performed on 6 weeks old male rats. Oral dose of sodium ascorbate (600 mg/kg b.w., ASC) or ascorbic acid phosphate (785 mg/kg b.w., ASC-P) was given separately (BID) or as co-treatment with dexamethasone phosphate (daily dose of 2 mg/kg b.w., DEX). Rats were randomly divided into control and experimental groups and the effect of 5-day treatment without (CTRL) or with ASC, ASC-P, DEX, or DEX combined with ASC or with ASC-P (Treatment) was compared with respect to indices of animal growth, somatic indices and results of glucose tolerance test. The effects of 5-day treatment and 5-day recovery period (when none of the experimental factors was used) were compared. Administration of DEX caused significant decline of serum ascorbate and dehydroascorbate (-87%, $P<0.001$). This was in part corrected by ASC or ASC-P co-treatment with DEX (-68%, $P<0.001$). ASC and ASC-P were of minor importance to limit DEX-induced growth retardation although they enhanced sensitivity to insulin at least at the level of glucose utilization ($P<0.001$). ASC-P was superior to ASC in sensitization to insulin ($P<0.05$). DEX significantly raised somatic indices (SI) of liver (+52%), kidneys (+44%), heart (+52%) and soleus muscles (+44%) but not gastrocnemius muscles ($P>0.05$). In contrast, spleen SI dropped significantly upon DEX treatment (-57%). After 5-day recovery period DEX-altered SI-s did not return to control values ($P<0.05$). Neither ASC nor ASC-P affected SI-s nor they could reverse DEX-induced changes in SI-s except ASC-P which confined the rise of renal SI ($P<0.05$). ASC in contrast to ASC-P even augmented DEX-dependent hepatomegaly ($P<0.05$). Interestingly, both ascorbate derivatives efficiently inhibited DEX-induced muscle cachexia at least with respect to gastrocnemius muscle ($P<0.05$). Summing up, these results suggest differences between the fast and slow effects evoked by ascorbate in the experimental model of growth retardation and muscle cachexia induced by DEX and accompanied by glucocorticoid-dependent diabetes.

Key Words: ascorbate, ascorbic acid phosphate, glucocorticoids, growth, muscle cachexia, insulin resistance

Zusammenfassung

Titel der Arbeit: **Natriumascorbat (ASC) and Ascorbinsäurephosphat (ASC-P) beeinflussen Glukokortikoid-abhängige metabolische Prozesse bei wachsenden Ratten unterschiedlich**

Es ist etabliert, dass Dexamethasonbehandlung Dosis-abhängig oxidativen Stress, Insulinresistenz, Diabetes und Muskelkachexie verursacht. Hier wurde der Effekt von Ascorbatderivaten auf Dexamethason-ausgelöste Stoffwechselauslenkungen bei 6 Wochen alten männlichen Ratten untersucht. Natriumascorbat (600 mg/kg, ASC) and Ascorbinsäurephosphat (785 mg/kg, ASC-P) wurden oral einzeln oder in Kombination mit Dexamethasonphosphat (2 mg/kg, DEX) verabreicht. Ratten wurden zufällig der Kontroll- bzw. den Versuchsgruppen zugewiesen und hinsichtlich Wachstum, Glukosetoleranz und somatischen Indizes nach fünftägiger Behandlung und nach weiteren fünf Tagen Rekonsolidierung verglichen. DEX verursachte eine signifikante Abnahme des Serumascorbats und des Dehydroascorbats (-87%, $P<0.001$). Die Wirkung wurde teilweise durch Ko-Behandlung mit ASC oder ASC-P (-68%, $P<0.001$) behoben. ASC und ASC-P begrenzten die DEX-verursachte Wachstumsverlangsamung nur geringfügig, obgleich sie die Empfindlichkeit zum Insulin mindestens auf dem Niveau der Glukoseanwendung ($P<0.001$) erhöhten. ASC-P war ASC in der Sensibilisierung für Insulin ($P<0.05$) überlegen. DEX erhöht signifikant somatische Indizes (SI) der Leber (+52%), der Nieren (+44%), des Herzens (+52%) und des Soleusmuskels (+44%) aber nicht des Gastrocnemiusmuskels ($P>0.05$). Demgegenüber fiel der Milz SI erheblich nach DEX-Behandlung (-57%). Nach 5tägiger Rekonsolidierung gingen DEX-geänderte SI-s nicht auf die Ausgangswerte zurück ($P<0.05$). Weder ASC noch ASC-P beeinflussten SI-s, noch

konnten sie DEX-verursachte Änderungen der SI-s ausgleichen, ausgenommen dem Anstieg von Nieren-SI durch ASC-P($P<0.05$) begrenzte. ASC, im Gegensatz zu ASC-P, vergrößerte sogar DEX-abhängige Hepatomegalie ($P<0.05$). Interessanterweise hemmten beide Ascorbatderivate DEX-verursachte Muskelkachexie, zumindestens in Bezug auf den Gastrocnemiusmuskel ($P<0.05$). Zusammenfassend zeigen die Ergebnisse Unterschiede zwischen den schnellen und langsamen Effekten in den experimentellen Modellen der DEX-induzierten Wachstumsverlangsamung, Muskelkachexie begleitet von Glukocorticoid-abhängiger Diabetes.

Schlüsselwörter: Ascorbat, Ascorbinsäurephosphat, Glucokortikoid, Wachstum, Muskelkachexie, Insulinresistenz

Introduction

It is well documented that excess of glucocorticoids leads to growth retardation and muscle cachexia (DARDEVET et al., 1995; HASSELGREN, 1999). Dexamethasone, a synthetic glucocorticoid is thus frequently used to attenuate growth and to trigger muscle wasting (SAVARY et al., 1998; ORZECHOWSKI et al., 2000; ORZECHOWSKI, 2002; MA et al., 2003). The molecular basis for glucocorticoid-dependent muscle atrophy is complex and includes oxidative stress (ORZECHOWSKI et al., 2000), resistance to insulin (DARDEVET et al., 1998), activation of proteasomal system (ATTAIX et al., 1998) and higher activity of myostatin (MA et al., 2001; 2003). In contrast, insulin plays important role in the maintenance of whole-body anabolism. Previously, we reported positive effect of sodium ascorbate on insulin-mediated mitogenicity and cell viability in mononuclear L6 muscle cells (ORZECHOWSKI et al., 2002). Similarly ascorbate derivative, namely ascorbic acid 2-phosphate was shown to accelerate molecular mechanism of terminal differentiation of L6 myoblasts and muscle formation in cell culture model (MITSUMOTO et al., 1994). It was also demonstrated that sodium ascorbate raised phosphorylation of Akt and c-Jun in the same undifferentiated muscle cells (ORZECHOWSKI et al., 2005).

On one hand, several studies indicate that either glucocorticoids excess, or lack of insulin (insulin dependent diabetes mellitus, IDDM) or insulin resistance (non-insulin dependent diabetes mellitus, NIDDM) all trigger oxidative stress (ERIKSSON and KOHVAKKA, 1995; BAKER et al., 1996; PEREIRA et al., 1999; ORZECHOWSKI et al., 2000; CHOI et al., 2003). On the other hand, vitamin C supplementation often results in strenghthening the antioxidant defenses (ARRIGONI and TULLIO, 2002; EGUCHI et al., 2003). Therefore, the purpose of this study was to establish if ascorbate derivatives could prevent disturbances induced experimentally by a high dose of dexamethasone in growing rats. We assumed that at least some of dexamethasone-induced effects (growth retardation, insulin resistance or muscle cachexia) were in causal relationship with oxidative stress. Furthermore, our own observations and available literature data indicate that ascorbate modulates regulatory processes where signals from insulin and insulin-like growth factors (IGF-s) are activated and transduced to target genes (PETERKOFSKY et al., 1991; GOSIEWSKA et al., 1994; MAHMOODIAN and PETERKOFSKY, 1999; ORZECHOWSKI et al., 2005). IGF-binding proteins (IGFBP-s) are often indicated to antagonize IGF-s-dependent growth promoting effects. There is evidence that glucocorticoids upregulate *igfbp-1* gene, whereas insulin acts in opposite way to stop dexamethasone-dependent stimulation of *igfbp-1* gene promoter activity (SUH et al., 1994; SUH and RECHLER, 1997). Thus, in theory insulin action could be augmented by ascorbate so that it could inhibit glucocorticoids in negative control of animal growth. It seems necessary to determine the way of action, effective concentrations and whether vitamin C can

shelter insulin activity impaired by glucocorticoids (secondary glucocorticoid-dependent diabetes). As above-mentioned it was assumed, that insulin plays considerable anabolic function, therefore with regard to developmental processes the positive effect of vitamin C might be associated with the positive relationship between vitamin C and biological effects of insulin.

Various derivatives of ascorbic acid are commercially used as dietary supplements to prevent foods oxidation. Ascorbic acid phosphate (ASC-P) is preferred to fortify feeds since ascorbate (ASC) is released from ASC-P in a relatively slow and constant rate by the action of alkaline phosphatase (AP) present in gut mucosa. In contrast, ascorbate is absorbed almost instantly from the GI tract and within minutes is excreted with urine. By the individual use of equimollar quantities of ASC and ASC-P as feed supplements or intragastric boli it is easy to differentiate between long-term versus short-term effects of ascorbate. In this experiment, we decided to find out how some vitamin C derivatives (sodium ascorbate and ascorbic acid phosphate) would slow down the catabolic effect of dexamethasone and how they can amplify insulin action in a dexamethasone-induced insulin-resistant state.

Material and methods

Animals

Polish Ministry of Agriculture rules for animal welfare were followed during these experiments. All experimental procedures on animals were approved by the Local Ethic and Animal Welfare Commission of the Warsaw Agricultural University. Four-week of age (young) Wistar male rats (n=120) were purchased from Institute of Animal Physiology and Animal Feeding, Polish Academy of Sciences (Jabłonna near Warsaw, Poland). Standard laboratory rodent chow (Wytwórnia Pasz, Andrzej Morawski, Kcynia, Poland) containing 13 MJ kg^{-1} metabolizable energy and 21.2% w/w crude protein was provided twice a day. Any remaining uneaten food was weighed and feed intake was calculated daily. Water was provided *ad libitum*. Each animal was housed individually in controlled environmental conditions (22^{o}C, 75% humidity, 12:12-h light-dark cycle period started 8:00 AM). After a 2-week acclimatization period, the Dex treatment was begun. At this point the rats were 6 weeks of age (180-200 g). Because Dex alters food intake, control animals were pair-fed the average daily amount consumed by the corresponding DEX-treated group. Two 5 days experimental periods - Treatment and Recovery were investigated. From our preliminary experiments (ORZECHOWSKI et al., 2000, 2002) we found that dexamethasone treatment led to muscle cachexia and insulin resistant state in growing rats. To achieve these, DEX-treated rats received 1 mL of dexamethasone disodium phosphate (Sigma, St. Louis, MO, USA) dissolved in saline (0.85% w/v NaCl) given twice a day at 8.00 AM and 4.00 PM (1.0 mL) by intragastric tube in a daily dose of 2 mg/kg b.w. • day^{-1} during 5 consecutive days. After 5 days of treatment one set of rats (Treatment, DEX) was killed and dissected, and another set of rats (Recovery, DEX/REC) was killed after 5 days of the recovery period. Afterwards, control animals (Treatment, CTRL plus Recovery, CTRL/REC) receiving 1 mL of saline (vehicle, 0.85% NaCl) were fed the same amount of food as was consumed by its dexamethasone-treated pair mate during previous day. Similarly, animals from other groups were given the average daily amount of food being eaten by DEX-treated animals. Sodium ascorbate (ASC) and ascorbic acid phosphate (ASC-P) were

purchased from Sigma (St. Louis, MO, USA). ASC and ASC-P were given individually or as co-treatment with DEX at dose of 600 mg/kg b.w • day^{-1} and 785 mg/kg b.w • day^{-1}, respectively. Either factor was dissolved in distilled-deionized water (Aqua pro injectione, Polpharma S.A., Poland) and introduced to rats by the intragastric tube in a volume of 1 mL. Time-schedule was the same as for DEX. Twelve randomized groups of animals (CTRL, CTRL/REC, DEX, DEX/REC, ASC, ASC/REC, P-ASC, P-ASC/REC, ASC-DEX, ASC-DEX/REC, P-ASC-DEX, P-ASC-DEX/REC) were formed (n=10).
Food intake, body weight and wet organ weight data were used to calculate somatic indices: body weight gain [final body weight - initial body weight, BWG in grams], relative body weight gain [(BWG/initial body weight)•100, RBWG in percentage], specific growth rate [(ln final body weight – ln initial body weight)•100, SGR in percentage], protein efficiency ratio [daily protein intake/daily BWG, PER], instantaneous food intake [(daily dry food intake/body weight)•100, IFI in percentage] and somatic indices [(organ wet weight•100/body weight), SI in percentage]. Water intake was also calculated and confronted with PCV to check for dehydration hazard.

Biochemical analyses
Animals were anaesthetised by intraperitoneal injection of 0.3 mL pentobarbital sodium salt (Pentobarbitalum 26.7 mg/ml; Pentobarbitalum Natrium 133/ml, Morbital, Biowet, Puławy, Poland). Abdominal cavity was opened and blood samples were collected into heparinized syringes directly from the abdominal aorta of each animal. Immediately afterwards, selected organs were dissected, washed with ice-cold saline, blotted on absorbent paper, weighed, covered with aluminium foil and placed into liquid nitrogen. The blood samples kept on ice were divided into separate tubes and whole blood, blood plasma and blood cells (after centrifugation at 800 ***g***, 10 min) were each frozen at -80^{o}C. Packed Cell Volume (PCV) was determined routinely with a microcapillar method. Ascorbate and oxidized form of ascorbate (dehydroascorbate) were assayed in blood plasma by HPLC method described by WANG et al. (1995) and RUMELIN et al. (1999).

Determination of glucosuria
To check whether animals are diabetic (*diabetes mellitus*) every morning urine samples from each animal (from treatment and recovery periods) treated individually with DEX or DEX-co-treated were collected on reagent strips (Keto-Diastix, Bayer B. V. Division Diagnostics, Brussel, Belgium) and glucose presence was easily recognized by color indicator.

Glucose tolerance test
One day prior to the end of Treatment or Recovery period all animals (12 groups, 120 animals in total) were individually tested for insulin resistance by the use of glucose tolerance test. Initially, fasting glucose concentration in the whole blood was determined. Five minutes later 1 mL of 40% (w/v) glucose water solution (Pliva, Poland) was given intragastrically („0" time) and 15, 30, 60, 90 and 120 minutes later glucose level was determined in the microliter volumes of blood collected from tail vein. The measurement was performed with Glucocard II GT-1620 apparatus (KDU Corporation, Kyoto, Japan).

Statistical evaluation

Results were statistically evaluated using one way ANOVA and Tukey's multiple range test or two-way ANOVA with Benferroni post test by GraphPad Prism™ version 3.03 software (GraphPad Software Inc., San Diego, CA, USA). Results are expressed as mean ± SEM and a value of $P<0.05$ was determined to be significant, $P<0.01$ as highly significant and $P<0.001$ as very highly significant.

Results

Effect of DEX treatment on glucosuria.

It should be pointed out that glucose was detected in urine of growing rats 2 days after the initiation of DEX treatment (data not shown). When DEX was administered with ASC or ASC-P glucosuria was diagnosed one day later (data not shown). Glucose disappeared from urine one day following DEX treatment (data not shown). Anyway, urine tests confirmed that DEX at the dose of 2 mg/kg b.w. • day^{-1} causes secondary diabetes. Diabetic state recesses immediately after DEX withdrawal from the treatment.

Effect of 5-day DEX and ASC or ASC-P co-treatments on growth indices.

Growth indices decreased markedly in animals, particularly in the 3rd day of dexamethasone treatment. IFI dropped markedly upon DEX action from 13.4% ± 1.14 at day "0" to 10.67% ± 1.0 at day 3 of treatment (-20%, $P<0.05$). At the same time points (day 1 vs. day 3 of DEX-treatment) other dynamic growth indices were impaired as follows: SGR dropped by 210% ($P<0.01$), RWBG by 168% ($P<0.01$), while PER by 358% ($P<0.001$). In contrast to SI-s, after additional 5-day recovery period IFI, SGR, RWBG and PER returned to control levels ($P>0.05$, data not shown) pointing toward transient character of DEX-induced growth retardation. Neither ASC, nor ASC-P influenced feed intake confined by DEX ($P>0.05$, data not shown). In contrast to ASC, however, ASC-P was shown to diminish IFI in average by 10% in comparison to CTRL during recovery period ($P<0.01$, data not shown). DEX administration led to the progressive loss of body weight illustrated by decreased RBWG. The latter became negative (-4.68% ± 0.59, $P<0.05$) starting from the 3rd day of DEX treatment but almost returned to control value (3.02% ± 1.2, $P>0.05$) the day after the end of DEX treatment (1st day of recovery period). Again, neither of ASC derivatives could affect DEX-induced negative effect on RBWG ($P>0.05$, data not shown). When data were plotted on XY graphs the slopes of RBWG and SGR curves fit almost identical. Again, similarly to RWBG SGR became negative (-4.81% ± 0.61, $P<0.05$) at 3rd day and became positive at 1st day of recovery (2.91% ± 1.17, $P>0.05$). These findings were additionally reflected by the significant fall of protein efficiency ratio (from +2.18 ± 1.67 at day "0" to -2.33 ± 0.4 at 3rd day of DEX treatment, $P<0.05$). Again, next day after DEX removal PER raised to +1.06 ± 0.62. There was absolute lack of ASC or ASC-P effect on DEX-induced lower PER ($P>0.05$). From these studies it is clear that in growing rats ASC and ASC-P were of minor importance to limit DEX-induced growth retardation. In contrast to ASC-P which significantly elevated PER at the end of recovery period, ASC significantly diminished PER all along the recovery period ($P<0.05$). These outcomes occur irrespective to the enhanced sensitivity to insulin caused by either of the ascorbate derivative (see glucose tolerance test).

Effect of 5-day DEX and ASC or ASC-P co-treatments on metabolic indices. At 5th day of treatment, total endogenous plasma ascorbate and dehydroascorbate dropped significantly from 65.54 μmol/L found in control to 8.19 μmol/L in DEX-treated rats (-87%, Fig. 1C, P<0.001). Anyway, we did not observe any increase in dehydroascorbate even though we assumed that ascorbate underwent oxidation during DEX-induced insulin resistant state (Fig. 1B). More likely ascorbate/dehydroascorbate were degraded and straight away excreted with urine. Addition of ASC or ASC-P incompletely but significantly made higher serum levels of ascorbate/dehydroascorbate at 5th day of DEX co-treatment (-68%, Fig. 1C, P<0.05).

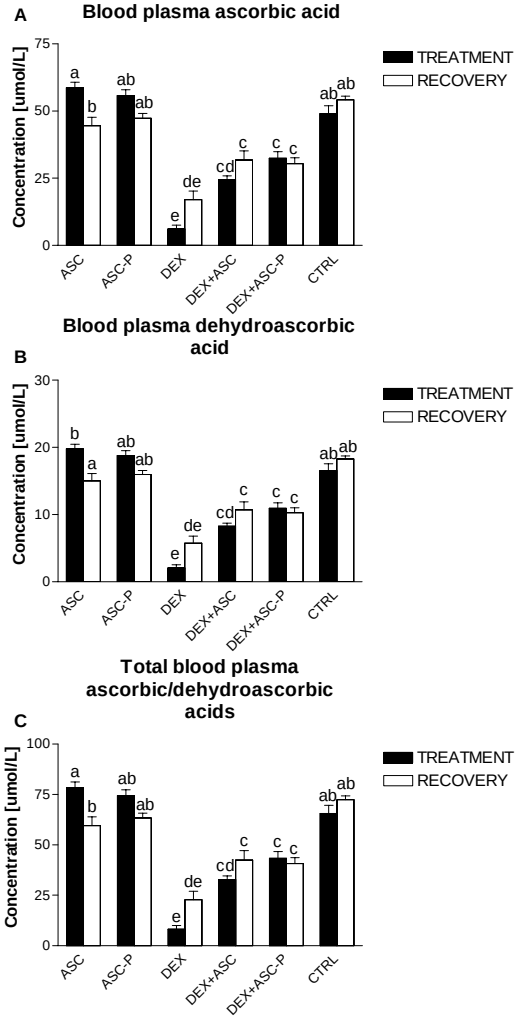

Fig. 1: Bar charts illustrating changes in blood plasma concentrations of (A) ascorbic acid; (B) dehydro-ascorbic acid; (C) total ascorbic/dehydroascorbic acids. Bars represent average concentration ± SEM determined in blood plasma collected after treatment (black) or after recovery period (white). Different letters indicate significant differences (P<0.05). Treatments indicated with abbreviation under abscissa. (Balkendiagramme zu Blutplasmakonzentrationen von (A) Ascorbinsäure, (B) Dehydroascorbinsäure und (C) Gesamtascorbin-/dehydroascorbinsäuren. Balken geben die durchschnittliche Konzentration ± SEM im Blutplasma nach Behandlung (schwarz) oder nach Rekonsolidierung (weiß) an. Unterschiedliche Buchstaben kennzeichnen signifikante Differenzen (P<0.05). Behandlungen sind an der Abzisse mit Abkürzungen spezifiziert)

Whenever given individually, either ASC or ASC-P increased ascorbate/ dehydroascorbate serum concentrations but these effects were statistically insignificant (Fig. 1A). Interestingly, although ASC and ASC-P were of minor importance to limit DEX-induced growth retardation they enhanced sensitivity to insulin (Fig. 2). It was shown that at 90th and 120th min of test the blood glucose concentration descended significantly after combined ASC and DEX co-treatment (Fig. 2A, P<0.001). Apparently, in this regard ASC-P was capable to reduce significantly blood glucose level - starting at 15th min, and was maintained through the test (Fig. 2B, P<0.05; P<0.01; P<0.001). Without DEX co-treatment hypoglycemic effects of either ASC or ASC-P were less evident although remained statistically highly significant (Fig. 2C, Fig. 2D, P<0.01, P<0.001).

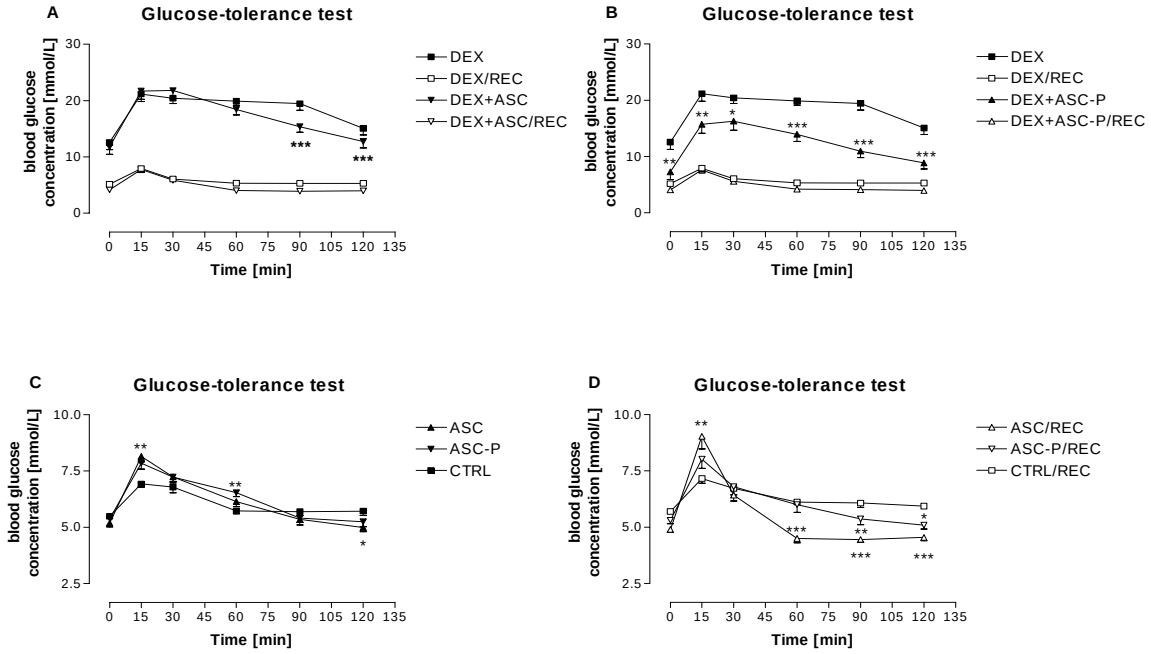

Fig. 2: Figures illustrating time course of changes in whole-blood glucose during glucose tolerance tests performed in rats during treatment and recovery periods that have been given (A) DEX or DEX plus ASC; (B) DEX or DEX plus ASC-P; (C and D) ASC or ASC-P. Average values ± SEM marked with asterisks differ significantly (*, $P<0.05$), highly significantly (**, $P<0.01$), or very highly significantly (***, $P<0.001$) from the respective reference values different for A (DEX), B (DEX), C (CTRL) and D (CTRL) (Verlauf der Blutglukosekonzentration im Glukosetoleranztest bei Ratten nach Behandlung und Rekonstitution mit (A) DEX oder DEX plus ASC; (B) DEX oder DEX plus Asc-p-p; (C und D) Asc oder Asc-p-p. Durchschnittswerte ± SEM. Mit Sternchen gekennzeichnet signifikante Unterschiede zu DEX in A und B sowie zur Kontrolle in C und D)

Effect of 5-day DEX and ASC or ASC-P co-treatments on metabolic indices. Similarly to dynamic indices of growth, where ASC and ASC-P could hardly attenuate DEX-induced alterations, some somatic indices were efficiently modulated by ascorbate derivatives. At the 5th day of treatment, DEX significantly raised SI-s of liver (+52%, $P<0.001$), kidneys (+44%, $P<0.001$)), heart (+52%, $P<0.001$)) and soleus muscles (+44%, $P<0.01$) but not gastrocnemius muscles ($P>0.05$). In contrast, spleen-somatic index dropped significantly upon DEX treatment (-57%, $P<0.001$). After 5-day recovery period DEX-mediated changes in SI-s did not come back to control values ($P<0.05$). Overall, when given individually, neither ASC nor ASC-P affected SI-s, nor they could reverse DEX-induced changes in SI-s with the exception of ASC that deepened DEX-induced hepatomegaly ($P<0.05$). This was not the case for ASC-P, which locked up the rise in renal-somatic index ($P<0.05$). Particularly distinct effect was evoked by both ASC and ASC-P on DEX-induced muscle cachexia at least with respect to gastrocnemius muscle (Fig. 3E). Interestingly, in contrast to soleus muscle, both ascorbate derivatives efficiently inhibited DEX-induced fall in relative weight of gastrocnemius muscles ($P<0.05$). In conclusion, we would like to emphasize that dissociation exists between the fast and slow effects of ascorbate on dynamic indices of growth, somatic indices, and DEX-induced insulin desensitization associated with the secondary diabetes in growing rats.

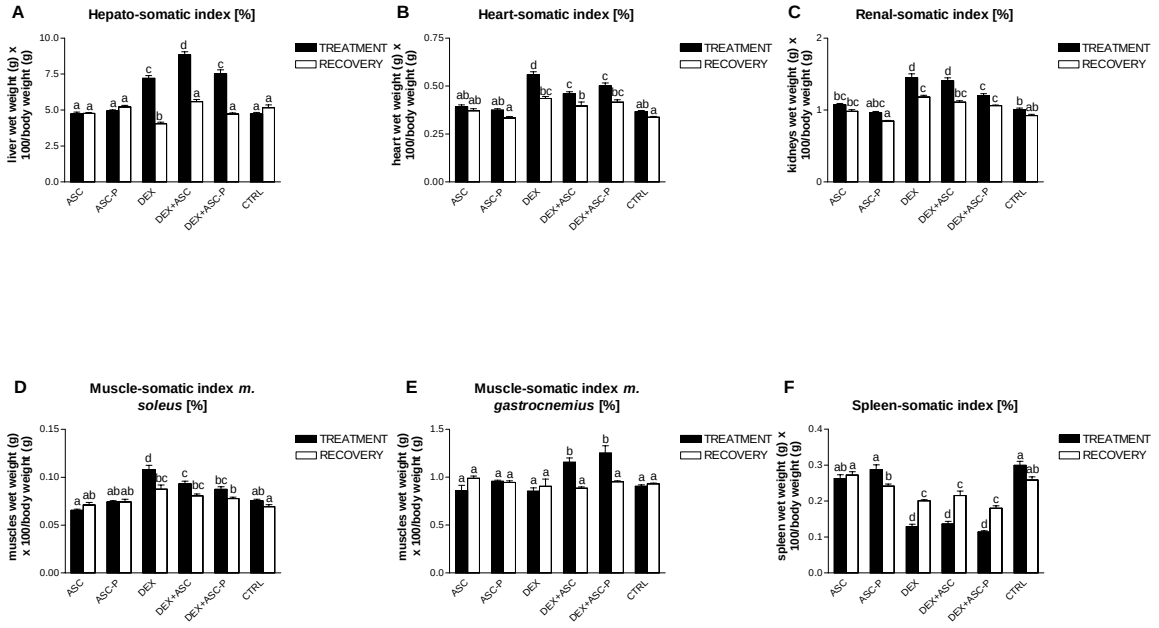

Fig. 3: Bar charts illustrating changes in somatic indices calculated according to the formula described in Material and methods. Bars represent average values ± SEM obtained from *post mortem* measurements after treatment (filled black) or after recovery period (empty white), respectively. Bars marked with different lower case letters differ at least significantly ($P<0.05$). Each treatment is indicated with abbreviation located under abscissa. (Balkendiagramme zur Änderungen metabolischer Indizes, errechnet entsprechend der Formel in Material und Methoden. Balken geben die Durchschnittswerte ± SEM der postmortem Messungen nach Behandlung (schwarz) oder nach Rekonsolidierung (weiß). Unterschiedliche Buchstaben kennzeichnen signifikante Differenzen ($P<0.05$). Behandlungen sind an der Abzisse mit Abkürzungen spezifiziert)

Discussion

5-day administration of DEX led to a progressive significant and severe fall of growth indices in 6-weeks old rats. These changes were caused by both loss of apetite and most likely accelerated whole-body catabolism. Growth retarded by DEX was reversible, since the day after the withdrawal of DEX the dynamic indices of growth increased steadily up to 5th day of recovery period. This was not the case when changes in somatic indices were monitored at 5th day of treatment and recovery period. Relative weights of liver, heart, kidneys and soleus muscles rose during DEX treatment whereas they declined when DEX was removed from treatment. Even then, however, in the 5th day of recovery period the somatic indices were statistically different from control, untreated rats (Fig. 3). One should bear in mind that DEX evoked secondary diabetes and water balance ought to be seriously disturbed. To check dehydration hazard we manage PCV and found no indications that animals were dehydrated as we assume due to the compensatory water intake that raised considerably (data not shown). The losses in IFI, RBWG, SGR and PER found in growing rats were accompanied by a deep fall in the average concentrations of plasma ascorbate and dehydroascorbate (Fig. 3). The latter confirms indirectly that DEX induces oxidative stress. The reducing size of spleen upon DEX treatment is consistent with the general awareness how efficiently glucocorticoids suppress limphoid system. Anyway, the most intriguing observation is linked to gastrocnemius muscle. ASC and ASC-P apparently inhibited DEX-induced changes in the relative weight of the

muscle. It is not clear, why we could not noticed similar effect in soleus muscle. To comprehend the consequences of DEX administration one has to know, that a bulk of data indicates that glucocorticoids in abundance slow down prenatal and postnatal growth (AIN et al., 2005; ORZECHOWSKI et al., 2002). Glucocorticoids and catecholamines are released from adrenals in response to environmental stress that often accompany animals kept indoors in high producing farms. The side-effect of excess glucocorticoids and catecholamines is the developing oxidative stress. The molecular mechanisms of glucocorticoid/catecholamines-associated oxidative stress result from both the impaired antioxidant defenses (PEREIRA et al., 1999; ORZECHOWSKI et al., 2000) and upregulated oxidative metabolism (MANOLI et al., 2005). In our studies we did not measure the indices of oxidative stress (it is currently determined) but we did similar evaluation in the past with similar results (ORZECHOWSKI et al. 2000, 2002). Moreover, short- and long-term administration of exogenous corticosterone caused oxidative stress in broiler chickens (LIN et al., 2004a, 2004b). Interestingly, in broilers and lying hens dietary ascorbate decreased endogenous plasma corticosterone and heat shock protein 70 elevated by cyclic heat stress (MAHMOUD et al., 2003, 2004). Some of the dexamethasone-induced negative affects on growth are associated with the elevated IGFBP-s (SUH et al., 1994) and impaired action of IGF-s whereas other are believed to be a consequence of higher level of myostatin protein (MA et al., 2003). Regardless of the underlying mechanism of DEX action ascorbate derivatives were of minor importance to correct DEX-induced growth restriction. The crucial factor for improvement was apparently the recovery period ($P<0.05$). However, ASC and ASC-P moderately modulated some of the somatic indices; particularly they lowered DEX-induced resistance to insulin.

In conclusion, we demonstrated that oral DEX loading induced metabolic changes such as growth retardation, peripheral blood hyperglycemia, and relative increase in liver, heart, kidneys and soleus muscles wet weight. The above-mentioned alterations in growth indices resulted from the progressive loss of body weight and muscle cachexia. This was not the case for tigh muscles (particularly soleus muscle was not affected). To explain the observed fluctuations in organ wet weight in relation to body weight it is important to stress that other muscles have to be severely affected by DEX. Total ascorbate plasma concentration markedly dropped in DEX-treated animals (Fig. 2, $P<0.001$). Efforts to correct DEX-mediated catabolic effect by the co-treatment with ASC or ASC-P led to slight but not spectacular improvement. This could be a consequence of a high, sub-toxic dose of DEX and/or other not oxidant dependent mechanisms of DEX action in growing rats. Anyway, we observed that ASC and ASC-P significantly advanced sensitivity to insulin, while ASC-P additionally has an advantage to lower DEX-induced average blood glucose level. The latter and other beneficial effects of ASC-P as we suppose were consequence of slow and relatively continuous release of this water soluble antioxidant from GI, since released ascorbate was reported to additionally modulate the activity of phosphatases (EGUCHI et al., 2003). Further studies are needed to evaluate whether other antioxidants (vitamin E, α-lipoic acid, taurine etc.) posses anticatabolic properties to control or prevent DEX- or stress-induced growth constraints. Otherwise, we admit that at least a part of the catabolic activity of DEX originated from alternative reasons other than oxidative stress.

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ARKADIUSZ ORZECHOWSKI

Antioxidants protect mitogenicity of undifferentiated muscle cells

(Antioxidantien schützen die Teilungsfähigkeit undifferenzierter Muskelzellen)

Myotubes and muscle fibers are distinguished by their antiapoptotic phenotype. In contrast, mononuclear muscle precursor cells (MPC-s) differ considerably being at risk of the oxidative stress-induced growth retardation and programmed cell death. In a series of experiments (ORZECHOWSKI and GRZELKOWSKA, 2000a, b; ORZECHOWSKI et al., 2002a) we showed that donors of reactive oxygen/nitrogen species at micromolar concentrations inhibited mitogenesis (DNA synthesis) in MPC-s, whereas at millimolar concentrations they induced apoptosis. Interestingly, after preincubation with antioxidants (ascorbate, catalase, *N*-acetylcysteine) mononuclear muscle cells escaped from the oxidative stress-mediated effects. Similarly, when oxidative stress was provoked by dexamethasone treatment (ORZECHOWSKI et al., 2000c) co-incubation with the above-mentioned antioxidants prevented mitogenicity and extended lifespan of muscle cells (ORZECHOWSKI et al., 2003). The molecular mechanism of ascorbate-dependent protection of insulin-induced mitogenicity in muscle cells challenged with oxidative stress (ORZECHOWSKI et al., 2002b) was associated with the activation of PKB/Akt and c-Jun as illustrated by the elevated levels of phosphorylated forms of these proteins (ORZECHOWSKI et al., 2005). Other antioxidants (quercetin, dimethylsulfoxide) also stimulated mitogenesis but at higher concentrations they blocked myogenesis resulted in muscle cell death from premature mitosis. At the same time phosphorylation of PKB/Akt was diminished (ORZECHOWSKI et al., 2001). Overall, it should be pointed out that the resistance of mononuclear cells to growth retardation and cell death is supported by antioxidant status mediated by activation of PKB/Akt. Similarly, antiapoptosis of myotubes and muscle fibers is associated with the elevated levels of Bcl-2 and NF-κB proteins which directly or indirectly maintain antioxidant status (KWIECINSKA et al., 2005). Furthermore, histochemistry of muscle samples collected from calf fetuses showed higher expression of typical catalase and bFGF in cattle breeds with excessive musculature (ORZECHOWSKI et al., 2002c). Summing up, these results suggest positive relationship between antioxidant status and the effectiveness to grow of skeletal muscles.

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BRIGITTA ESSÉN-GUSTAVSSON[1], K. KARLSTRÖM[1] and E. ÅGREN[2]

Replacement feeding of reindeer cows in late pregnancy and its influence on calf skeletal muscle characteristics

(Fütterung von Rentierkühen während der Hochträchtigkeit und ihr Einfluss auf Muskelmerkmale der Kälber)

Earlier studies on different species have shown that maternal environmental factors, for instance food intake, may influence skeletal muscle development in the offspring. The aim with this study was to compare skeletal muscle characteristics from reindeer calves whose mothers belonged to the same reindeer village but had been handled differently during the calving period. One group of reindeer cows had been free-range grazing in a traditional way while the other group of cows had been kept within a large fenced area and fed full rations of pelleted reindeer feed for a period of about 8 weeks before and during calving. After the calving period from June and during the rest of the year, all the animals were kept under similar circumstances free-range grazing on natural pastures.

When the calves were 6-7 months old, they were slaughtered. The whole small M. extensor indicis were cut out from 39 calves whose mothers had been traditionally handled (T-calves) and from 49 calves whose mothers had been fed pelleted reindeer feed (P-calves). Carcass weight was greater ($p<0.05$) in P-calves (21.1 kg) than in T-calves (19.4 kg). Histochemical analyses were made on whole cross-sections from M.extensor indicis. The weight of this muscle was greater ($p<0.05$) in P-calves (1.34 g) than in T-calves (1.19 g). Muscle fibre number and the % of type I (P-calves = 52, T-calves = 53) and type II (P-calves = 48, T-calves = 47) fibres did not differ between calves. Mean fibre areas, and areas (um^2) of both type I (P-calves =1251, T-calves = 1136) and type II (P-calves = 1916, T-calves = 1717) fibres were greater ($p<0.05$) in P-calves than in T-calves.

The results indicate that not fibre number but the growth of type I and type II fibres in skeletal muscle of calves may be influenced by the handling and feed given to the reindeer cows just before and during the calving period.

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The influence of maternal low and high protein nutrition during pregnancy on postnatal growth, body composition, muscle development and energy metabolism in the offspring of German Landrace sows - Project presentation

(Einfluss einer Niedrig- und Hochproteinernährung während der Trächtigkeit auf postnatales Wachstum, Körperzusammensetzung, Muskelentwicklung und Energiestoffwechsel in Nachkommen von Deutsche Landrasse Sauen – Projektvorstellung)

In various studies it has been shown that a low protein intake throughout pregnancy in rodents results in lower birth weights, altered growth characteristics, and age-related loss of glucose tolerance and insulin resistance (METGES and HAMMON, 2005). We have shown that also maternal high protein (MHP) diet (twice the recommendations) was followed by reduced body weight at day 2 of life but by higher weight at wk 6 and higher body fatness and reduced total energy expenditure at wk 9 in the offspring as compared to control rats (DAENZER et al., 2002). In pigs, severe maternal protein restriction has been shown to have detrimental effects on foetal growth, when either applied transitionally or throughout gestation, but little is known on the effects of gestational protein levels below or above sows' requirements on performance traits in later life. In this context it is of interest that low birth weight piglets (< 1.2 kg) from otherwise normal litters exhibited lower daily gains, higher body fat contents, lower muscle mass, less myofibre numbers, and higher drip losses (REHFELDT, 2005). Recently, we started a long-term evaluation of pregnancy diet effects on growth performance, body composition, muscle characteristics, energy and glucose metabolism and various other traits in the offspring of German Landrace sows. Starting on the day of insemination primiparous sows are fed isoenergetic diets with a control (12%), low (6%), and high (30%) protein level throughout pregnancy. All other nutrients meet or exceed the recommended levels. Offspring is cross-fostered to foster mothers of litter number 2-5 previously fed control diets. Pig progeny is evaluated postnatally at days 1, 28, 80, and 180 of age. Preliminary data indicate that sows receiving the low protein level show lower pregnancy weight gain than control and high protein sows.

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Feeding and management of sows during gestation and its effect on, muscle fibre, fat level, growth rate and carcass quality of progeny

(Effekte von Fütterung und Management von Sauen während der Trächtigkeit auf Muskelfasern, Fettanteil, Wachstumsrate und Schlachtkörperqualität der Nachkommen)

Muscle fibres occur predominantly as primary and secondary fibres. The primary fibres are genetically determined but the secondary fibres can be influenced by factors such as nutrition during gestation but not post-partum. For this reason the lean content in pigs is largely determined prior to birth. There is evidence that nutritional influences during gestation can also affect the development of obesity in offspring and the risk of associated disease (cardiovascular, diabetes) later in life.

In the first study five windows for additional feeding during gestation will be examined (N = 40 sows / treatment). Productivity, weight and backfat level of sows will be monitored throughout the study. From each of 12 sows / treatment, 3 same-sex pigs (light, medium and heavy) will be selected at birth. One hundred and eighty pigs will then be individually penned at weaning and followed through to slaughter. From the remaining sows, groups of same sex pigs will be monitored to slaughter and their growth and carcass traits measured.

In the second study, under nutrition during gestation will be examined on sows of two states of body fatness (fat/obese and thin) at the beginning of the study. Three dietary treatments of restricted feeding will be examined. Sow productivity, weight and backfat level will be monitored throughout the study. Three individual pigs from each litter will be monitored through to slaughter as in study 1.

In both studies 5 sows from each treatment will be sacrificed at day 90 of gestation and muscle fibres in the foetuses analysed. This will also be repeated on the muscle samples of slaughter weight pigs. To identify both novel and previously suspected gene targets whose expression may be affected by these feeding regimes, global and targeted gene expression in muscle cells of progeny will be assessed through microarray and reverse transcriptase (RT)-PCR analyses, respectively.

Meat quality measures such as pH, colour, drip loss, inter-muscular fat, intra-muscular fat and shear force will be performed on meat from both studies following slaughter.

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Effect of maternal feed intake during mid-gestation on pig performance and meat quality at slaughter

Abstract

A study was conducted to evaluate, under commercial conditions, the effect of a high feed intake during mid-gestation on postnatal growth performance and meat quality of the progeny. Sows from 1 to 7 parities were divided into two treatments, control (C) and experimental (E). C sows received 3.0 kg/day (12 MJ of ME/kg) througout all gestation and E sows (E) received +50% and +75% extra feed from 45 to 85 days of gestation for first-parity and multiparous sows, respectively. This treatment (T) was applied thoughout two reproductive cycles (2 replicates; n=103 sows in replicate 1 and n=96 in replicate 2). The offspring (barrows) were divided in 5 weight groups (WG) and reared conventionally throughout nursery (n=958) and growing-finishing (n=636) periods. During the nursery period, E pigs exhibited higher growth rates (ADG) than C group (333 g/d *vs* 316 g/d, $p<0.05$) in replicate 1 and a higher feed efficiency (G:F) than C group (0.48 *vs* 0.47, $p<0.05$) in replicate 2. However, this differences disappeared in the growing-finishing period. The pigs of the lightest weight groups seemed to be the most benefited by the additional maternal feed allowance. At slaughter, E pigs in replicate 1 showed a higher carcass and ham weight. These pigs also showed a higher pH at 24 hours postmortem in the *semimembranosus* muscle and lower lightness values in the *longissimus* muscle than C pigs, and this fact was consistent in both replicates. Overall, increasing feed allowance from 45 to 85 days of gestation had slight effects on growth performance and lead to differences on meat quality traits at market weight.

Key Words: maternal feed intake, growth performance, meat quality

Zusammenfassung

Titel der Arbeit: **Effekt der maternalen Futteraufnahme in der mittleren Trächtigkeit auf Leistung und Fleischqualität der Nachkommen beim Schwein**

Der Effekt einer erhöhten Futteraufnahme in der mittleren Trächtigkeit wurde unter kommerziellen Bedingungen untersucht. Die Sauen (1. bis 7. Trächtigkeit) wurden auf zwei Gruppen aufgeteilt: Kontrollgruppe (C), Versuchsgruppe (E). Die C-Sauen erhielten 3.0 kg/ Tag (12 MJ ME umsetzbare Energie)/kg) in der gesamten Trächtigkeit, und die E-Sauen erhielten +50% (1. Trächtigkeit) bzw. +75% (2. Trächtigkeit) extra Futter vom 45. bis zum 85. Trächtigkeitstag. Diese Behandlung erfolgte für die Dauer von zwei Reproduktionszyklen (n=103 in Wiederholung 1; n=96 in Wiederholung 2). Die Nachkommen (männliche Kastrate) wurden in 5 Gewichtsgruppen eingeteilt und während der Säugezeit (n=958) und Mast (n=636) konventionell aufgezogen. Während der Säugezeit zeigten die Schweine der Versuchsgruppe (E) höhere Wachstumsraten (ADG) als die der Kontrollgruppe (333 g/d vs 316 g/d, $p<0.05$) in Wiederholung 1 und eine bessere Futterverwertung (G:F) in Wiederholung 2 (0.48 vs 0.47, $p<0.05$). Diese Differenzen waren in der Mastperiode jedoch nicht mehr zu beobachten. Die Schweine der leichtesten Gewichtsgruppen schienen am meisten von der zusätzlichen maternalen Fütterung zu profitieren. Die Schlachtschweine der Versuchsgruppe hatten ein höheres Schlachtkörper- und Keulengewicht in der Wiederholung 1. Diese Gruppe zeigte auch in beiden Wiederholungen einen höheren pH Wert 24 h *post mortem* im *M. semimembranosus* und einen geringeren Helligkeitswert im *M. longissimus*. Insgesamt hatte die höhere verfügbare Futtermenge vom 45. bis zum 85. Tag der Trächtigkeit geringe Effekte auf die Wachstumsleistung und führte zu Unterschieden in der Fleischqualität.

Schlüsselwörter: Maternale Futteraufnahme, Wachstumsleistung, Fleischqualität

Introduction

Maternal nutrition and other factors affecting the fetal environment (as hormonal treatments) may cause changes in postnatal growth (REHFELDT et al., 2004). In pigs, muscle mass is largely determined by the number of muscular fibers that, in turn, is positively correlated with postnatal growth (DWYER et al., 1993; GONDRET et al., 2005). Also, prenatal muscle fibre development is though to influence meat quality

(LARZUL et al., 1997), since contractile and metabolic properties of skeletal muscle may affect the pattern of energy metabolism in live animal, as well as during the post mortem conversion of muscle to meat (KARLSSON et al., 1999).

Myogenesis is a biphasic phenomenon with the sequential formation of two generations of muscle fibres. A primary generation forms from 25 to 50 days of gestation, followed by a second generation which form between 50 and 80 days of gestation (WIGMORE and STICKLAND, 1983). A possible positive effect was found on the number of muscle fibres when feed allowance during the first period of the myogenesis was increased (GATFORD et al., 2003). On the contrary, no effects or even opposite effects have been observed in other studies (NISSEN et al., 2003; BEE, 2004). However, this disadvantage overruled when the period of increased maternal feeding was prolonged from day 25 to day 70 of gestation (NISSEN et al., 2003), suggesting that increasing feed allowance during the period of the secondary fiber development may be more advantageous for the offspring. Thus, the present experiment was conducted to study, under commercial conditions, the implications of providing a higher feed intake to the sows during mid-gestation (from d 45 to d 85 of gestation) on growth performance and meat quality of the offspring.

Materials and Methods

The experiment was conducted involving two reproductive cycles (2 replicates). LD x LW PIC sows from 1 to 7 parities were divided randomly into two treatments, Control (C, n=49 and n=46 in replicate 1 and 2, respectively) and Experimental (E , n=54 and 50 in replicate 1 and 2, respectively). C group received 3.0 kg/d (12 MJ of ME/kg feed and 6 g lysine/kg) throughout gestation (level routinely used on the farm) and E group received adiditionally + 50% and +75% of the control diet, for first-parity and multiparous sows, respectively, from d 45 to d 85 of gestation. After weaning, at 22 ± 2 days of age, piglets were reared conventionally throughout the nursery and the growing-finishing period.

Only barrows were used for the study of growth performance throughout the nursery (n=958) and the growing-finishing period (n=636). Pigs were divided into 5 weight groups (WG, being group 1 the heaviest and group 5 the lightest) per treatment and weighed weekly in the nursery and every three weeks in the growing-finishing phase. Feed consumption (ADFI) was also obtained in the nursery for all pens and in the growing-finishing period only for the middle and light WG. Carcass measurements [carcass weight, percentage of lean meat (Fat-O-Meat'er, SFK, Denmark), mid-line fat thickness at the gluteus medium (GM) and ham, *longissimus* (L) and *semimembranosus* (SM) muscle weight] and technological meat quality measurements [pH, drip loss and meat colour (Minolta CR300)] were registered in pigs from the lightests groups of weight (n=90), that were slaughtered at an average weight of 104.1±1.16 kg in replicate 1 and 120.9±2.65 kg in replicate 2.

Data was analysed using SAS (SAS Inst., Inc., Cary, NC, 2001). In all cases, gestation feeding level served as treatment and main factor. Statistical differences between treatments were analysed using the GLM procedure. In growth performance analysis, pen was the experimental unit and WG was added as a main factor and thus, interaction T*WG was also studied. In carcass and meat quality data analyses pig was the experimental unit. In all cases, the results are expressed as LS-means ± SE.

Results

During the nursery period, E pigs showed higher ADFI and ADG in replicate 1 and a higher feed efficiency (G:F) in replicate 2 than C pigs (Table 1). These parameters were studied in detail within the nursery and the growing-finishing periods (data not shown) and it was observed that in both replicates, differences became statistically significant by days 53-58 of age. At this point, the interaction T*WG was significant for the lightests groups of pigs in test in replicate 1, being E pigs heavier than C. In spite of the differences found in the nursery period, these were not mantained during the growing-finishing period neither in replicate 1 nor in replicate 2 (Table 1).

Table 1
Nursery and growing-finishing growth performance (Wachstumleistung in der Aufzucht und Mast)

Replicate	1			2		
	Maternal treatment[1]			**Maternal treatment**[1]		
Growth period	**C**	**E**	***P-value***	**C**	**E**	***P-value***
Nursery [2]						
Pigs, No	230	231	-	246	251	-
ADFI, g/d	430 ± 0.004	448 ± 0.004	0.008	455 ± 0.006	455 ± 0.006	0.962
ADG, g/d	316 ± 0.004	333 ± 0.004	0.013	327 ± 0.005	333 ± 0.005	0.316
G:F	0.73 ± 0.005	0.74 ± 0.005	0.322	0.72 ± 0.005	0.74 ± 0.005	0.038
Growing-Finishing[3]						
Pigs, No	188	189	-	129	130	-
ADFI, g/d	1670 ± 0.041	1630 ± 0.041	0.518	1970 ± 0.038	2010 ± 0.038	0.491
ADG, g/d	789 ± 0.012	774 ± 0.012	0.390	808 ± 0.011	797 ± 0.011	0.442
G:F	0.47± 0.005	0.48 ± 0.005	0.627	0.40 ± 0.009	0.38 ± 0.009	0.164

[1] Maternal treatment, C: 3 kg/d and E: +50% or +75% amount of feed than C from 45 to 85 days of gestation,; [2] Nursery period: from 21 to 62 d on average; [3] Growing-Finishing period: from 62 to 184 d on average.

Table 2
Carcass and meat quality traits (Schlachtkörper- und Fleischqualitätsmerkmale)

Replicates	1			2		
	Maternal treatment[1]			**Maternal treatment**[1]		
Variable	**C**	**E**	***P-value***	**C**	**E**	***P-value***
Pigs, No	25	25	-	20	20	-
Carcass wt (kg)	72.4 ± 2.020	77.9 ± 1.978	0.056	87.43 ± 2.360	86.7 ± 2.360	0.835
Lean meat (%)	54.23 ± 0.738	52.68 ± 0.738	0.146	54.11 ± 0.920	53.35 ± 0.920	0.563
GM[2] (mm)	17.52 ± 1.018	19.24 ± 1.018	0.238	22.6 ± 1.033	21.65 ± 1.033	0.520
pH_{45}[3]						
SM[4]	6.18 ± 0.047	6.22 ± 0.047	0.546	6.24 ± 0.048	6.14 ± 0.048	0.145
L[5]	6.17 ± 0.051	6.30 ± 0.051	0.079	6.19 ± 0.035	6.12 ± 0.035	0.124
Main cuts weight (kg)						
SM[4]	0.972 ± 0.026	1.059 ± 0.028	0.030	1.177 ± 0.033	1.165 ± 0.033	0.720
L[5]	2.65 ± 0.111	2.67 ± 0.223	0.695	3.22 ± 0.099	3.21 ± 0.099	0.920
Ham	10.56 ± 0.265	11.38 ± 0.271	0.036	13.17 ± 0.324	13.27 ± 0.324	0.840
pH_{24}[6]						
SM[4]	5.53 ± 0.027	5.62 ± 0.029	0.045	5.60 ± 0.031	5.71 ± 0.031	0.013
L[5]	5.53 ± 0.019	5.53 ± 0.019	0.918	5.59 ± 0.016	5.62 ± 0.016	0.175
Meat colour[7]						
L	53.89 ± 0.567	52.38 ± 0.567	0.066	51.88 ± 0.755	49.39 ± 0.755	0.025
a	5.36 ± 0.242	5.43 ± 0.242	0.852	5.61 ± 0.213	6.13 ± 0.213	0.090
b	3.86 ± 0.271	4.32 ± 0.271	0.238	4.47 ± 0.227	3.98 ± 0.227	0.135
Drip loss (%)	1.84 ± 0.294	1.87 ± 0.294	0.939	0.87 ± 0.178	1.22 ± 0.178	0.171

[1] Maternal treatment, C: 3 kg/d and E: +50% or +75% amount of feed than C from 45 to 85 days of gestation,; [2] GM: Mid-line fat thickness at gluteus medium; [3] pH_{45}: pH at 45 minutes postmortem; [4] SM: *Semimembranosus* muscle; [5] L: *Longissimus* muscle; [6] pH_{24}: pH at 24 hours postmortem; [7] Meat colour (L: lightness; a: redness; b: yellowness) was measured in LD muscle.

Increased maternal feed intake during mid-gestation did not lead to differences on lean meat content, GM, pH_{45} and drip loss measurements in the 90 selected pigs (Table 2). Regarding to the main cuts weight, in replicate 1, E pigs showed higher SM muscle and ham weights, according to the tendency to a higher carcass weight observed for this group. At 24 h post-mortem, pH_{24} in the SM muscle was significantly higher and lightness in the L muscle tended to be lower in the E group compared to the C group, in both replicates.

Discussion

Feed restriction during pregnancy has negative effects on the postnatal growth of the offspring (POND et al., 1985). However, the effects of feed supplementation during gestation are not so clear in the literature. Contradictory results have been obtained on muscle fiber development and postnatal growth (DWYER et al., 1994; GATFORD et al., 2003; NISSEN et al., 2003; BEE et al., 2004; HEYER et al., 2004). In the present experiment, the supplementation took place later than in most of the studies previously mentioned (from day 45 to 85 of gestation) and slight differences in growth performance and growth efficiency between treatments were observed at the near end of the nursery period (50 to 60 days of age). It has been shown that latter in the nursery phase, growth is more correlated to the number of muscle fibres (potential for growth) than to other factors such as birth weight (DWYER et at., 1993; GONDRET et al., 2005). When differences in growth performance appeared, the higher response was observed in the lightest WG, as it has been previously reported (DWYER et at., 1994; REHFELDT et al., 2001). For this reason, we select this range of weight for carcass and meat quality. Later, in the growing-finishing phase, the differences in growth performance disappeared. However, in replicate 1, we observed that the E pigs tended to show a higher carcass weight, suggesting that feed supplementation during mid-gestation could lead to a higher growth rates in the lightest groups of pigs, also during the growing-finishing phase.

On the other hand, low pH values have been related with an increased lightness and drip loss parameters (SCHAFER et al., 2002). In the present study, contrary to NISSEN et al. (2003) and HEYER et al. (2004), C pigs showed a lower pH_{24} in the SM muscle and higher lightness values in the L muscle than E pigs, and these results were consistent between replicates. However, values of pH_{24} and lightness in both treatments were within the normal for meat pork.

From our results we conclude that increasing feed allowance during the time of the secondary muscle development (from 45 to 85 days of gestation) has slight effects on growth performance. In addition, changes in meat quality parameters have been observed. Wether these differences are due to changes in muscle fiber characteristics is still unknown. Within the present work, further studies are being conducted to establish the effect of this feeding strategy on sow performance and muscle fiber development.

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Changes of the histochemical properties and meat quality traits of porcine muscles during growth. II) Effect of feed restriction in pigs slaughtered at the same body weight and varying age

Abstract

Twenty-four Swiss Large White barrows from six litters (four pigs per litter) had either ad libitum (A) or restrictive (R) access to a grower and finisher diet from 20 to 60 and from 60 to 100 kg BW, respectively. Two littermates from both feeding regime were slaughtered at 61 (A60 and R60) or 101 kg BW (A100 and R100). The histochemical properties of myofibers as well as meat quality traits of the longissimus muscle (LM) and light portion of the semitendinosus (STL) were assessed. Muscle fibers were stained and classified based on the stain reaction as slow oxidative (SO), fast oxidative-glycolytic (FOG), and fast glycolytic (FG), and fiber area and distribution were determined. Myofiber size was not ($P \geq 0.11$) affected by the dietary treatments. Regardless of the BW at slaughter, in the LM of R-pigs the relative amount of SO fibers was lower ($P = 0.06$) and that of FG fibers was higher ($P < 0.01$) compared with the LM of A-pigs. The SO fibres were more abundant in the STL of R60- than A60-, A100-, and R100-pigs (feed restriction × BW interaction; $P = 0.02$). Compared to pigs of the A-group, the STL of R-pigs was less tender ($P < 0.01$) and percentages of cooking loss were higher ($P = 0.02$). In conclusion, at the same BW at slaughter, feed restriction did not impair the myofiber size. By contrast myofibers of the LM were more glycolytic in R- compared with A-pigs suggesting a dietary impact on myofiber maturation. Although several quality traits were negatively affected by the feed restriction, they were unrelated to the myofiber characteristics as evidenced by the lacking significant correlation coefficients between meat quality traits and myofiber characteristics.

Key Words: BW at slaughter, Meat Quality, Muscle Fibers

Zusammenfassung

Titel der Arbeit: **Veränderung histochemischer Eigenschaften und der Fleischqualität von Muskeln des Schweins während des Wachstums. II) Einfluss restriktiver Fütterung bei Schweinen, geschlachtet bei gleichem Lebendgewicht aber unterschiedlichem Alter**

In der vorliegenden Untersuchung wurden 24 Kastraten (je vier Tiere aus dem gleichen Wurf) der Rasse Schweizerisches Edelschwein eingesetzt. Die Schweine hatten von 20 bis 60 kg und von 60 bis 100 kg Lebendgewicht (LG) entweder ad libitum (A) oder rationierten (R) Zugang zum gleichen Vor- bzw. Endmastfutter. Je sechs Tiere der A- und R-Gruppe wurden bei einem durchschnittlichen LG von 61 (A60 und R60) und die restlichen 12 Tiere bei einem durchschnittlichen LG von 101 kg (A100 und R100) geschlachtet. Im langen Rückenmuskel (LM) und im hellen Teil des Semitendinosus Muskels (STL) wurden Größe und Verteilung der einzelnen Muskelfasertypen sowie Fleischqualitätsparameter untersucht. Die Muskelfasern wurden mit der histochemischen Färbungsreaktion in langsam oxidative (SO), schnell oxidativ-glykolytische (FOG), and schnelle glykolytische (FG) Muskelfasern eingeteilt. Weder bei 60 noch bei 100 kg LG bestand zwischen der A- und R-Gruppe ein Unterschied in der Muskelfasergröße ($P \geq 0.11$). Unabhängig vom LG bei der Schlachtung hatten die Schweine der R-Gruppe im LM einen tieferen Anteil an SO ($P = 0.06$) und einen höheren Anteil an FG ($P < 0.01$) Muskelfasern als diejenigen der A-Gruppe. Im STL war der Anteil an SO Muskelfasern bei den Schweinen der R60-Gruppe größer als bei denjenigen der A-60, A-100 und R100-Gruppe (Versuchsverfahren × LG Interaktion; $P = 0.02$). Bei den Schweinen der R-Gruppe war der STL zäher ($P < 0.01$) und die Kochverluste größer ($P < 0.02$) als bei denjenigen der A-Gruppe. Zusammenfassend kann gesagt werden, dass die Hypertrophie der Muskelfasern durch die rationierte Nährstoffzufuhr nicht beeinflusst wird, wenn die Tiere bei gleichem LG geschlachtet wurden. Hingegen war der Anteil an glycolytischen Muskelfasern im LM größer bei den Schweinen der R- als bei denjenigen der A-Gruppe, was auf eine fütterungsbedingte Beeinflussung der Muskelfaserdifferenzierung hinweist. Obwohl die rationierte Nährstoffzufuhr verschiedene Fleischqualitätsmerkmale negativ beeinflusste, bestand keine Beziehung zu der Größe oder der relativen Verteilung der Muskelfasertypen.

Schlüsselwörter: Fleischqualität, Lebendgewicht bei der Schlachtung, Muskelfasern

Introduction

The impact of the feeding intensity on contractile and metabolic development of myofibers is controversial. In weaned piglets slaughtered at the same age HARRISON et al. (1996) reported smaller cross-sectional areas of all myofiber types and greater proportions of SO fibers in piglets subjected to restricted compared with ad libitum feeding. In accordance to the results obtained with piglets we observed that feed restriction during the growing as well as during the growing and finishing period resulted in smaller myofibers in the longissimus (LM) and light portion of the semitendinosus (STL) and in increased oxidative capacity of the STL myofibers when pigs were slaughtered at the same age but at a lower BW (BEE et al., 2006). The differences in the metabolic properties suggested that nutrient restriction affected maturation of a fast developing muscle (DAVIES, 1974). However, the impact of nutrient restriction on myofiber size might be confounded by the overall effect on BW as suggested by CANDEK-POTOKAR et al. (1999a) who reported no effects of nutrient supply on the size and distribution of myofibers in pigs slaughtered at the same BW (100 and 130 kg BW). However, at an earlier developmental stage SOLOMON et al. (1988) determined larger slow oxidative (SO) and smaller fast glycolytic (FG) fibers as well as more fast oxidative-glycolytic (FOG) and fewer FG fibers in the longissimus muscle (LM) of pigs with restricted compared to ad libitum access to the diet and slaughtered at 55 kg BW. In the aforementioned studies different breeds as well as different feeding strategies at different developmental stages were used, which makes a comparison of the dietary effects difficult. Following a similar experimental design as applied in the previous study (BEE et al., 2006), the aim of the present experiment was to establish the effects of restricted nutrient supply at the end of the growing and after the growing-finishing period on histochemical properties of myofibers and meat quality traits of the LM and STL in pigs slaughtered at the same BW but different age.

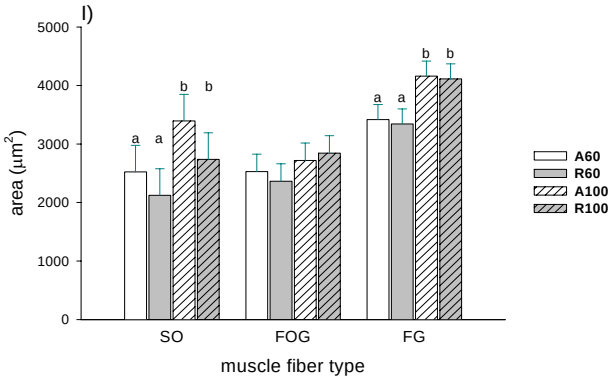
Material and Methods

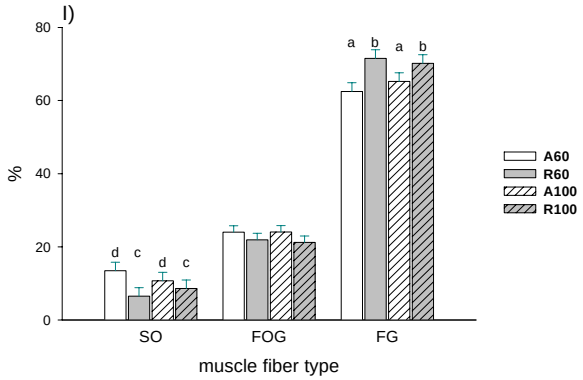
Swiss Large White barrows (n = 24) originating from six litters were blocked by litters and assigned from within litter to the four treatment groups. The pigs had either ad libitum (**A**) or restricted (**R**) access to a standard growing-finishing diet for the total experimental period. Feed restriction was aiming to achieve an ADG of 750 g from 20 to 100 kg BW. Two littermates from either treatment were slaughtered at 61.3 ± 0.99 kg (**A60** and **R60**) or 101.3 ± 1.48 kg BW (**A100** and **R100**). The age at slaughter of the A60-, R60-, A100-, and R100-pigs averaged 104 ± 3.9, 119 ± 4.2, 145 ± 4.5, and 167 ± 4.1 d.

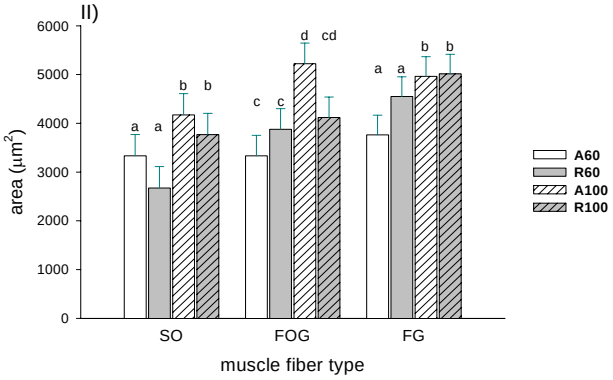
The housing condition during the growing-finishing period, the slaughtering procedure, the tissue collection at slaughter, and the performed measurements were the same as previously described (BEE et al., 2006).

Data were analyzed with the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). The model used for the analyses of muscle fiber characteristics and meat quality traits included feeding regime (F) and BW at slaughter (W) and the respective interactions (F × W) as fixed effects and litter as the random effect. When interactions were statistically significant at $P < 0.05$, least squares means were separated using the PDIFF option. Pearson correlation coefficients between histological properties and

meat quality traits for each muscle were calculated at 60 and 100 kg BW as well as within feeding regime.







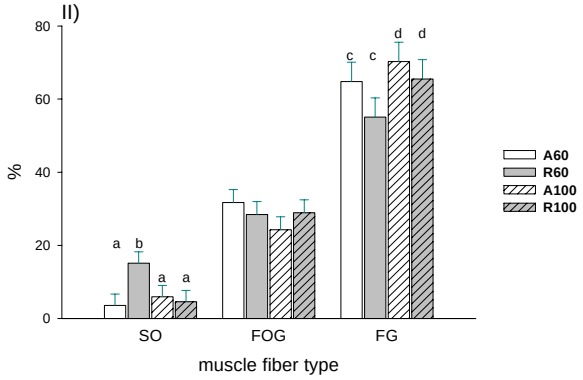


Fig. 1: Cross-sectional area of the muscle fiber types (SO = slow oxidative; FOG = fast-oxidative glycolytic; and FG = fast-glycolytic) in the longissimus (I) and light portion of the semitendinosus muscle (II) of pigs with ad libitum (A) or restricted (R) access to the diet and slaughtered at 60 (A60 and R60) and 100 kg BW (A100 and R100). Bars within myofiber type lacking common letters differ (^{a,b} $P < 0.05$; ^{c,d} $P = 0.06$) (Querschnittsflächen der Muskelfasertypen (SO = slow oxidative; FOG = fast-oxidative glycolytic; and FG = fast-glycolytic) im Longissimus (a) und hellem Anteil des Semitendinosus (b) bei Tieren mit ad libitum (A) oder restriktiver (R) Fütterung nach Schlachtung bei 60 und 100 kg Lebendgewicht. Unterschiedliche Buchstaben bezeichnen signifikante Differenzen innerhalb Muskelfasertyp (^{a,b,c,d},$P < 0.05$; ^{e,f,g,h},$P = 0.07$))

Fig. 2: Distribution of the muscle fiber types (SO = slow oxidative; FOG = fast-oxidative glycolytic; and FG = fast-glycolytic) in the longissimus (I) and light portion of the semitendinosus muscle (II) of pigs with ad libitum (A) or restricted (R) access to the diet and slaughtered at 60 (A60 and R60) and 100 kg BW (A100 and R100). Bars within myofiber type lacking common letters differ (^{a,b} $P < 0.05$; ^{c,d} $P = 0.06$) (Anteil an Muskelfasertypen (SO = slow oxidative; FOG = fast-oxidative glycolytic; and FG = fast-glycolytic) im Longissimus (a) und hellem Anteil des Semitendinosus (b) bei Tieren mit ad libitum (A) oder restriktiver (R) Fütterung nach Schlachtung bei 60 und 100 kg Lebendgewicht. Unterschiedliche Buchstaben bezeich-nen signifikante Differenzen innerhalb Muskelfasertyp (^{a,b,c,d},$P < 0.05$; ^{e,f,g,h},$P = 0.07$))

Results and Discussion

Muscle Fiber Area and Distribution

The cross-sectional areas of the myofibers in the LM and STL of pigs slaughtered at 60 and 100 kg BW were not ($P \geq 0.11$) affected by the feeding regime (Figure 1). Within the dietary treatments, pigs slaughtered at 100 kg BW had in both muscles larger ($P \leq 0.05$) SO and FG fibers than pigs slaughtered at 60 kg BW. By contrast, the size of the FOG fibers did not ($P = 0.57$) change in the LM from 60 to 100 kg BW and in the STL only FOG fibers in A100-pigs tended to be larger than in A60-pigs (F × W interaction; $P \leq 0.06$). In agreement with results reported by CANDEK-POTOKAR et al. (1999a), the present findings showed that the impact of nutrient restriction vanished

when pigs were slaughtered at the same BW. Based on previous results (BEE et al., 2006), myofiber hypertrophy seemed not to be limited by the daily but rather the total amount of supplied nutrients. In R60-pigs the STL had more SO fibers than the A60-pigs (F × W interaction; $P = 0.02$), whereas fiber type distribution did not differ at 100 kg BW (Figure 2). These findings partly confirmed our previous observation (BEE et al., 2006) that in this portion of the muscle the oxidative capacity was higher due to restricted nutrient supply. Surprisingly, the LM had more ($P < 0.01$) FG and tended to have fewer ($P < 0.06$) SO fibers in R- compared with A-pigs (Figure 2), which is in contrast to results presented by SOLOMON et al. (1988) and CANDEK-POTOKAR et al. (1999a).

Meat Quality Traits

While meat quality traits of the LM were not ($P \geq 0.15$) impaired by feed restriction, cooking losses (23.4 vs. 19.1%) and shear force values (4.31 vs. 3.29 kg) were higher ($P \leq 0.02$) in the STL of R- compared with A-pigs. Because the dietary impact on muscle fiber characteristics was small (Figure 1 and 2) it is unlikely that the observed changes in the quality traits were related to the size or distribution of the myofiber types confirming results of previous studies (CANDEK-POTOKAR et al., 1999a; BEE et al., 2006). Within feeding regime the LM of pigs slaughtered at 60 kg BW had higher ($P \leq 0.05$) percentages of thaw (11.8 vs. 8.1%) and cooking losses (20.2 vs. 17.9%) than the LM of pigs slaughtered at 100 kg BW. Both traits were correlated with neither the size nor the distribution of one of the three muscle fiber types ($P > 0.05$). The LM of R100-pigs was less tender than the LM of R60-pigs (5.37 vs. 4.21 kg; R × W interaction; $P = 0.06$), whereas shear force values were similar in the LM of A60- and A100-pigs (4.32 vs. 4.36 kg), Similarly, CANDEK-POTOKAR et al. (1998b) found tougher meat in older and heavier pigs but these differences were not related to the feed restriction.

In conclusion for pigs slaughtered at the same BW, the effect of feed restriction on the size of the three myofiber types was of small importance. Combined with the findings of our previous study (BEE et al., 2006) these results suggested that hypertrophy of myofibers during the growing finishing period paralleled the increase in BW and therefore depended less on the daily amount of ingested nutrients. By contrast, myofiber type distribution differed between the dietary groups, which suggested that the daily amount of supplied nutrients influenced myofiber maturation. Several important quality traits were affected by the feed restriction but they were unrelated to the myofiber characteristics as evidenced by the lacking correlations.

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The influence of postnatal growth rate on meat quality within litters of pigs reared and slaughtered under commercial production conditions

(Einfluss von postnataler Wachstumsrate auf Fleischqualität innerhalb Würfen von Schweinen, die unter kommerziellen Produktions-bedingungen aufgezogen und geschlachtet werden)

The objective of this study was to examine the influence of postnatal growth rate on meat quality traits within litters of pigs reared under commercial conditions and slaughtered at a commercial slaughter plant. All pigs within 16 litters (total of 149 pigs) were used in this study. The average litter size at slaughter was 9.3. Pigs were delivered to the slaughter plant once a week according to their weight. Pigs within a litter were slaughtered over a period of up to 5 weeks (5 delivery days). By this approach, the aim was to slaughter pigs within litter at approximately the same live weight but varying in days to slaughter according to their growth rate. As expected, average daily gain (ADG) differed significantly ($P < 0.001$) among the five different delivery days (1^{st} delivery: 791 g/d (n = 40); 2^{nd} delivery: 652 g/d (n = 47); 3^{rd} delivery: 628 g/d (n = 38); 4^{th} delivery: 607 g/d (n = 18); 5^{th} delivery: 567 g/d (n = 5)). The attempt to deliver pigs at approximately the same weight did not fully succeed, as the slaughter weight varied significantly ($P < 0.05$) among the five different delivery days (1^{st} delivery: 84.0 kg; 2^{nd} delivery: 81.4 kg; 3^{rd} delivery: 81.4 kg; 4^{th} delivery: 80.3 kg; 5^{th} delivery: 76.1 kg). Especially pigs at the 1^{st} and 5^{th} delivery differed from the others. The meat percentage did not differ among the 5 delivery days. Several meat quality parameters were measured post mortem: pH at 45 min and 24 h, drip loss, Minolta colour (lightness, redness and yellowness) and Warner-Bratzler shear force. All of these quality parameters did not differ significantly among various delivery days. Thus, in this study, the measured meat quality parameters did not differ between littermates with different postnatal growth rate.

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The influence of calf birth weight on selected beef quality characteristics

Abstract

While cattle nutrition and type have been the focus of much attention, little information is available on the impact of pre-natal growth on beef quality. The objective of this preliminary study was to determine the effect of birth-weight of single-born calves on meat quality. Data were available from two studies. In Experiment 1, 36 heifers of similar breed, were reared according to a 20-month beef production system. In Experiment 2, 30 bulls, the progeny of one of two Charolais sires were reared similarly and slaughtered at 16 months of age. Within each experiment, all animals were slaughtered on the same day. Post-slaughter, carcass weight and fat grade were recorded. Two days post-mortem, pH, colour and drip loss of *longissimus dorsi* (LD) was measured and after vacuum storage for an additional 12 days, tenderness was measured instrumentally and organoleptic characteristics were assessed by a trained sensory panel. Within each treatment in each experiment, animals were categorised as having low (L) or high (H) birth-weight. Data were subjected to ANOVA using a model that had birth-weight, experiment and treatment (within production system) as main effects. For L and H, birth-weight averaged 41.4, and 54.1 kg ($P<0.001$), respectively. The corresponding carcass weight was 315 and 336 kg ($P<0.05$), and carcass fat score was 3.83 and 3.54 ($P<0.1$). The fat concentration in LD was 28 and 20 g/kg ($P<0.05$) for L and H, respectively. It is concluded that within the limitations of this study, high birth-weight resulted in heavier carcasses which tended to be leaner but birth-weight did not influence beef quality.

Key Words: calf, birth-weight, beef quality

Zusammenfassung

Titel der Arbeit: **Der Einfluss des Geburtsgewichtes von Kälbern auf Merkmale der Rindfleischqualität**

Während die Fütterung von Rindern Fokus vieler Untersuchungen ist, sind wenig Informationen über die Auswirkung des prenatalen Wachstums auf Rindfleischqualität vorhanden. Die Zielsetzung dieser Studie war, den Effekt des Geburtsgewichts von Kälbern auf die Fleischqualität zu untersuchen. Daten waren von zwei Studien vorhanden. In Experiment 1, wurden 6 Färsen ähnlicher Rassen, entsprechend einem 20-Monate Rindfleisch-Produktion System gehalten. In Experiment 2, wurden 30 Bullen, die Nachkommen von einem von zwei Charolaisbullen waren, ähnlich gehalten und im Alter voni 16 Monaten geschlachtet. Innerhalb jedes Experimentes wurden alle Tiere am gleichen Tag geschlachtet. Schlachtkörpergewicht und Fettmerkmale wurden unmittelbar nach der Schlachtung erfasst. Zwei Tage post mortem wurden pH, Farbe und Tropfsaftverlust am Longissimus Dorsi (LD) erfasst und nach weiteren zwölf Tagen Vakuumlagerung wurde die Zartheit instrumentell gemessen und organoleptische Eigenschaften durch trainierte Personen sensorisch beurteilt. Innerhalb jeder Behandlung in jedem Experiment, wurden die Tiere Gruppen mit niedrigem (L) oder hohem (H) Geburtsgewicht zugeordnet. Daten wurden mittels ANOVA mit einem Modell mit den Effekten Geburtsgewicht, Experiment und Behandlung (innerhalb des Produktion Systems) evaluiert. Für L und H betrugen die durchschnittlichen Geburtsgewichte 41.4 und 54.1 kg ($P<0.001$). Die Schlachkörpergewichte waren 315 und 336 kg ($P<0.05$), die Fettmaße waren 3.83 und 3.54 ($P<0.1$). Der Fettgehalt im LD war 28 und 20 g/kg ($P<0.05$) für L und H. In dieser Untersuchung stehen hohe Geburtsgewichte folglich im Zusammenhang mit hohem Schlachtkörpergewicht und mageren Schlachtkörpern. Ein Einfluss auf die Fleischqualität wurde nicht beobachtet.

Schlüsselwörter: Kalb, Geburtsgewicht, Rindfleischqualität

Introduction

The appearance and eating quality of meat are influenced by many pre and post-slaughter variables within a beef production system. While cattle nutrition and type of animal have been the focus of much attention, little information is available on the impact of pre-natal growth on beef quality. HOUGHTON et al. (1990) reported that energy restriction during gestation decreased birth weight of calves while REHFELDT

(2005) reported that within a litter of piglets, low birth-weight results in meat with inferior quality traits. The objective of this preliminary study was to determine the effect of birth-weight of single-born calves, presumed to reflect maternal nutrient consumption, on meat quality

Material and methods

Data were available from two studies in which the calves were born at Grange Research Centre. In Experiment 1, 36 heifers of similar breed, were reared according to a 20-month beef production system and for the final 69 days were offered either grass silage *ad libitum* and 3kg concentrates or a restricted amount of concentrates and straw to ensure similar growth for both treatment groups. In Experiment 2, 30 bulls, the progeny of one of two Charolais sires were reared similarly and slaughtered at 16 months of age. Within each experiment, all animals were slaughtered on the same day. Post-slaughter, carcass weight and fat grade were recorded. Two days post-mortem, pH, colour and drip loss of *longissimus dorsi* (LD) was measured and after vacuum storage for an additional 12 days, tenderness (as shear force) was measured instrumentally and organoleptic characteristics were assessed by a trained sensory panel. Procedures employed were described by French *et al.* (2001). Within each treatment in each experiment, animals were sorted on birth-weight and the lower and upper tertiles were categorised as having low (L) or high (H) birth-weight. Data were subjected to ANOVA using a model that had birth-weight, experiment and treatment (within production system) as main effects and associated interactions. As the interactions were not significant, they were removed from the final reduced model.

Results and discussion

Data relating to animal growth and carcass characteristics are summarised in Table 1. Calves categorised as having low birth-weight were lighter ($P<0.05$) at birth, grew more slowly during life ($P<0.05$), were lighter ($P<0.05$) at slaughter and had lower ($P<0.05$) carcass weight, compared to those categorised as having high birth-weight. There was also some evidence (higher carcass fatness score and numerically greater internal fat deposition) that light birth-weight calves were fatter at slaughter.

There was no difference between birth-weight categories in date of birth or age at slaughter.

The difference in birth-weight in this study may reflect differences in maternal energy consumption during gestation (HOUGHTON et al. 1990). It may also reflect some variation in gestation length since calves that were lighter at birth were born on average 9.4 days earlier than heavier calves. Though the difference was not statistically significant, it does merit further investigation. The predisposition of calves with lower birth-weight towards increased adipose tissue accretion is consistent with observations on pigs (REHFELDT, 2005) and sheep (GREENWOOD et al., 1998). It should be noted that these studies were carried out within litters of >2 animals, in contrast to the single-born calves in the present study. The present data suggest that a decrease in the variation in calf birth-weight would decrease the variation in subsequent carcass fatness. However, dystocia caused by excessive calf weight sets a limit on the extent to which calf birth-weight can be increased *per se*.

Table 1
Animal and carcass characteristics in cattle with low (L) or high (H) birthweight (Tierzahl und Schlachtkörpermerkmale bei Rindern mit niedrigem (L) und hohem (H) Geburtsgewicht)

	L	H	Sed	Significance
Number of animals	22	22	-	-
Birth-weight (kg)	41.4	54.1	1.16	***
Birth date[1]	70.7	80.1	6.27	NS
Growth rate (g/d)	989	1077	23.7	***
Slaughter age (d)	528.4	519.0	6.30	NS
Slaughter weight (kg)	539.3	590.1	12.58	***
Carcass weight (kg)	314.7	336.3	7.36	**
Fat score[2]	3.83	3.54	0.156	0.07
Kidney/channel fat (kg)	8.43	8.08	0.510	NS
(g/kg carcass)	27.0	24.7	1.53	NS

[1]Relative to January 1. [2]1 =little fat, 5=very fat

Data relating to beef quality are summarised in Table 2. The intramuscular fat concentration in muscle from animals with low birth-weight was higher ($P<0.05$) than in muscle from animals with high birth-weight. There was no difference between groups in any other muscle characteristics examined.

The difference in muscle fat concentration was consistent with other indices of fat deposition and with observations in pigs (REHFELDT, 2005). The lack of effect of calf birth-weight on the measured meat quality variables contrasts with REHFELDT (2005) who reported higher drip loss for low birth-weight pigs. GONDRET et al. (2005) similarly found no difference in pH in muscle for low and high birth-weight pigs but muscle from low birth pigs was less tender. Differences between studies likely reflect different muscle structure, the absolute difference in birth-weight and differences in the production system used to rear the animals.

Table 2
Longissimus dorsi characteristics in cattle with low (L) or high (H) birthweight (Eigenschaften des Longissimus dorsi bei Rindern mit niedrigem (L) und hohem (H) Geburtsgewicht)

	L	H	Sed	Significance
pH	5.53	5.54	0.051	NS
Composition (g/kg)				
Lipid	28	20	3.7	*
Moisture	738	744	3.9	NS
Protein	226	223	2.3	NS
Colour				
L	37.5	37.2	0.54	NS
a	13.5	13.6	0.41	NS
b	8.9	8.9	0.24	NS
Drip loss (g/kg)	28.2	27.5	1.56	NS
Shear force (N)	50.6	47.3	4.05	NS
Sensory[1]				
Tenderness	5.7	5.5	0.22	NS
Texture	3.7	3.6	0.16	NS
Juiciness	5.1	4.8	0.22	NS
Chewiness	3.4	3.4	0.16	NS
Flavour	4.3	4.3	0.08	NS
Firmness	5.2	5.3	0.14	NS
Acceptability	4.0	3.9	0.15	NS

[1]Where 1 =extremely tough, very poor texture, extremely dry, not chewy, very poor flavour, not firm and not acceptable, respectively: 8= extremely tender, 6= very good texture, 8= extremely juicy, 6= extremely chewy, 6= very good flavour, 8= very firm and 6= extremely acceptable, respectively

It is concluded that within the limitations of this study, high birth-weight resulted in heavier carcasses which tended to be leaner but birth-weight did not influence the appearance or sensory characteristics of beef.

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Environmental influences on the development of lordosis and musculo-skeletal tissues in sea bass (*Dicentrarchus labrax*): the ORCIS project

(Umwelteinflüsse auf die Entwicklung von Lordosen und des muskulo-skelettalen Systems beim Seebarsch (*Dicentrarchus labrax)*: Das Projekt ORCIS)

The aim of this EU ORCIS project was to optimise the rearing conditions of larval and juvenile sea bass (Dicentrarchus labrax) such that the incidence of lordosis was significantly reduced and co-ordinated musculo-skeletal growth was improved. This was achieved through an integrated, multidisciplinary approach where an understanding of the underlying science was addressed in consort with the aquaculture industry.

The specific objectives of this project were (1) to assess the influence of rearing temperature and current velocities and their interactions on lordosis incidence and (2) to assess, for the same factors and groups of fish, the influence on musculo-skeletal development and growth at tissue, cell, protein, and molecular level, as an informed basis for reducing the problem of lordosis and for optimising musculo-skeletal growth.

Sea bass were reared at 15°C and 20°C at egg and larval (first feeding, notochord flexion, and metamorphosis) stages. At metamorphosis, fish reared at 15°C and 20°C were kept separately and placed in tanks (18.5°C) for two weeks, before swim currents (0, 25, 50, and 75% of the species relative critical swimming speed, RUcrit) were applied in the phase of 20-45mm mean TL. Larval and juvenile samples were analysed at the tissue, cell, protein, and molecular levels to determine the effects of temperature and current velocities on musculo-skeletal development and growth. Lordosis development was documented using x-ray analysis and histology.

Results showed that the occurrence of lordosis was greater at 20°C rearing compared to 15°C and that higher current velocities also exacerbated the production of this deformity. Additionally, temperature was shown to affect myogenesis (by altering myogenic factor and insulin-like growth factor (IGF) expression), vertebrae and myomere allometry and, thus, possibly causing a “buckling” effect. Aerobic capacity of red muscle and swimming performance was also enhanced in 15°C reared fish.

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Preservation of mitochondrial function in muscle samples – a prerequisite for the extension of physiological experiments

Abstract

Physiological experiments using the musculature of farm animals are limited by the reduced temporal stability of specific cellular functions in the tissue. To expand the amount of samples for investigations about pre- and postnatal muscle growth the evaluation of appropriate freezing and thawing conditions are necessary. Due to this we investigated how the mitochondrial respiration – a very sensible cellular parameter – could be preserved in muscle samples of pigs.

Diaphragmatic muscle samples of pigs were collected at a local abattoir. Considering different storage, freezing and thawing conditions the mitochondrial respiration of permeabilised fibers was determined using the substrates pyruvate and succinate. Base of these preservations experiments were the results of KUZNETSOV et al. (2003) who investigated human and murine muscle probes.

Chilled diaphragmatic samples conserved without the storage solution showed a reduced mitochondrial respiration 24 h after slaughter, whereas the storage medium could preserve the pyruvate- and succinate-dependent respiration up to 48 h. However, it is interesting to note that in most experiments the pyruvate-dependent respiration increased during storage up to 48 h after slaughter. In the long storage experiments it could be shown, that freezing of muscle probes in 40 % DMSO resulted in the best respiration rates after thawing in comparison to the other used DMSO concentrations. However, the pyruvate-dependent respiration was generally lower than those of the fresh muscle probes. Varying the freezing and thawing conditions showed that freezing of dissected samples and thawing at 37°C for up to 2´ improved the pyruvate-dependent respiration rates. However, since until now no comparable pyruvate-dependent respiration rates between fresh and freeze-thawed muscle samples had been determined further experiments are necessary especially considering the process of freezing and thawing.

Key Words: musculature, pig, storage, physiological function, mitochondria

Zusammenfassung

Titel der Arbeit: **Konservierung der mitochondrialen Funktion in Muskelproben – eine Voraussetzung für die Erweiterung von physiologischen Untersuchungen**

Physiologische Experimente, in denen die Muskulatur von Haustieren verwendet wird, werden durch die reduzierte zeitliche Stabilität von spezifischen zellulären Funktionen des Gewebes limitiert. Zur Erweiterung der Anzahl von Proben, die für die Untersuchung des prä- und postmortalen Muskelwachstums verwendet werden können, müssen adäquate Gefrier- und Auftaubedingungen bestimmt werden. Deswegen untersuchten wir, wie die mitochondriale Atmung – ein sehr sensibler zellulärer Parameter – in Muskelproben vom Schwein konserviert werden kann.

Muskelproben vom Zwerchfell des Schweins wurden an einer nahegelegenen Schlachtstätte gesammelt. Unter Berücksichtigung verschiedener Lagerungs-, Gefrier- und Auftaubedingungen wurde die mitochondriale Atmung von permeabilisierten Fasern unter Verwendung der Substrate Pyruvat und Succinat bestimmt. Grundlage waren die Ergebnisse von KUZNETSOV et al. (2003), der humane und murine Muskelproben untersucht hatte.

In gekühlten Zwerchfellproben, gelagert ohne den Lagerpuffer, konnte bereits 24 h nach der Schlachtung eine Reduktion der mitochondrialen Atmung erkannt werden, wohingegen nach Kühlung im Lagerpuffer die Pyruvat- und Succinat-abhängige Atmung bis zu 48 h erhalten bleibt. Es ist interessant zu berücksichtigen, dass in den dem meisten Experimenten die Pyruvat-abhängige Atmung bis zu 48 h nach der Schlachtung ansteigt. In den langfristigen Lagerungsexperimenten konnte gezeigt werden, dass das Einfrieren der Muskelproben in 40 % DMSO in den besten Atmungsraten nach dem Auftauen im Vergleich zu den anderen verwendeten DMSO-Konzentrationen resultierte. Allerdings war die Pyruvat-abhängige Atmung im Allgemeinen niedriger als die in frischen Muskelproben. Die Variation der Gefrier- und Auftaubedingungen zeigte, dass das Einfrieren von zerfaserten Proben und das Auftauen bei 37°C für bis zu 2` die Pyruvat-abhängige Atmungsraten verbesserte. Bis jetzt konnten allerdings noch keine vergleichbaren Pyruvat-abhängigen Atmungsraten zwischen frischen und gefrorenen-aufgetauten Muskelproben bestimmt werden. Deswegen sind weitere Experimente notwendig, die besonders den Ablauf des Gefrierens und Auftauens berücksichtigen.

Schlüsselwörter: Muskulatur, Schwein, Lagerung, physiologische Funktion, Mitochondrien

Introduction

Mitochondria are organelles not only involved in the oxidative energy metabolism of the cells, but also in other important cellular processes like the induction of apoptosis, the production of reactive oxygen species (ROS) or the regulation of the calcium metabolism (GUNTER et al., 2004; GIZATULLINA et al., 2005). Mitochondria are usually analyzed in frozen samples using histochemical, enzymatical and molecular biological methods. However, the direct assessment of the mitochondrial function e.g. by measuring coupled respiration is also an important method to elucidate alterations in the cells that are strongly related to the mitochondria. A major limitation of the determination of the mitochondrial respiration – especially in muscle samples of farm animals - is that the experiments have to be performed immediately after collection of the probes. Since respiration experiments are time-consuming and serial investigations of samples are necessary, it is important to reduce the progressive, storage-dependent deterioration of mitochondrial function. Temperature reduction is an important factor minimizing the negative alterations during storage of cells. However, long-time conservation of the cellular function by freeze-thawing is quite problematic. Since KUZNETSOV et al. (2003) demonstrated the successful cryopreservation of human and murine muscle samples the objective of the presented experiments was to investigate the effects of chilling and freeze-thawing on storage-dependent changes by determining the mitochondrial respiration – a sensible indicator of the cellular function - in porcine muscle samples.

Material and Methods

Samples of the diaphragm were collected from slaughter pigs at a local abattoir about 20 min after slaughter and were transported on ice to the laboratory in Vechta (15 min).

In the laboratory the muscular parts of the diaphragm samples were extracted, transferred to tubes with or without a specific storage solution (49 mM potassium morpholino ethanesulfonic acid (PMES, pH 7,1), 10 mM Ca^{2+}-EGTA (0,1 µM free Ca^{2+}), 9,5 mM $MgCl_2$, 3 mM KH_2PO_4, 20 mM taurine, 5,2 mm ATP, 15 phosphocreatine, 20 mM imidazole, 0,5 mM DTT) and chilled at 4°C for up to 48 h. At different times during the storage period small pieces were removed from the muscle probes, transferred to the storage solution and prepared for the investigation of mitochondrial respiration.

In the cryopreservation experiments fresh muscle samples (ca. 1 h after slaughter) of the muscular part of the diaphragm were prepared for the investigation of the mitochondrial respiration and transferred to a cryotube. After addition of the storage solution (+ 10 mg/ml BSA fatty acid free) containing different concentrations of DMSO or glycerol (cryoprotectant solution) and short equilibration (5 sec) the muscle samples were frozen in liquid nitrogen and stored at -70°C. After thawing the muscle samples were immediately transferred to storage solution and washed for several times before subsequent permeabilisation.

The muscular part of the diaphragm was carefully dissected with two small needles and mechanically permeabilised in incubation buffer (20 mM Tris-HCl (pH 7,4), 75 mM mannitol, 25 mM sucrose, 0,1 M KCl, 10 mM KH_2PO_4, 0,5 mM EDTA, 5 mM $MgCl_2$, 0,5 mM DTT, 1 mg/ml BSA).

The skinned-fiber bundles (5-10 mg) were analysed on respiratory activity in an OROBOROS® oxygraph (Innsbruck, Austria) at 30°C. Changes in the oxygen

consumption rates were determined with the substrates pyruvate/ malate before and after the addition of ADP followed by the addition of rotenone and subsequently of succinate.
The data were statistically analyzed with the software Statistica 6.0 (StatSoft, Hamburg) using the one-way ANOVA. Significance was determined with the Fischer LSD test considering a probability of error P of 0.05.

Results

Storage of the muscle samples at 4°C without the storage solution resulted in a decrease of the succinate-dependent respiration within 24 h to approximately 70 % of the beginning respiration rates (ca. 1 h after slaughter) with no further decrease within the following 24 h. The mitochondrial respiration with the substrates pyruvate/ malate after addition of ADP (state-3-respiration) decreased only slightly from ca. 1 to 0.8 nmol oxygen/ min*mg. However, the difference was not statistically significant ($P<0.05$) after 24 h and 48 h of storage without storage solution (Figure 1).

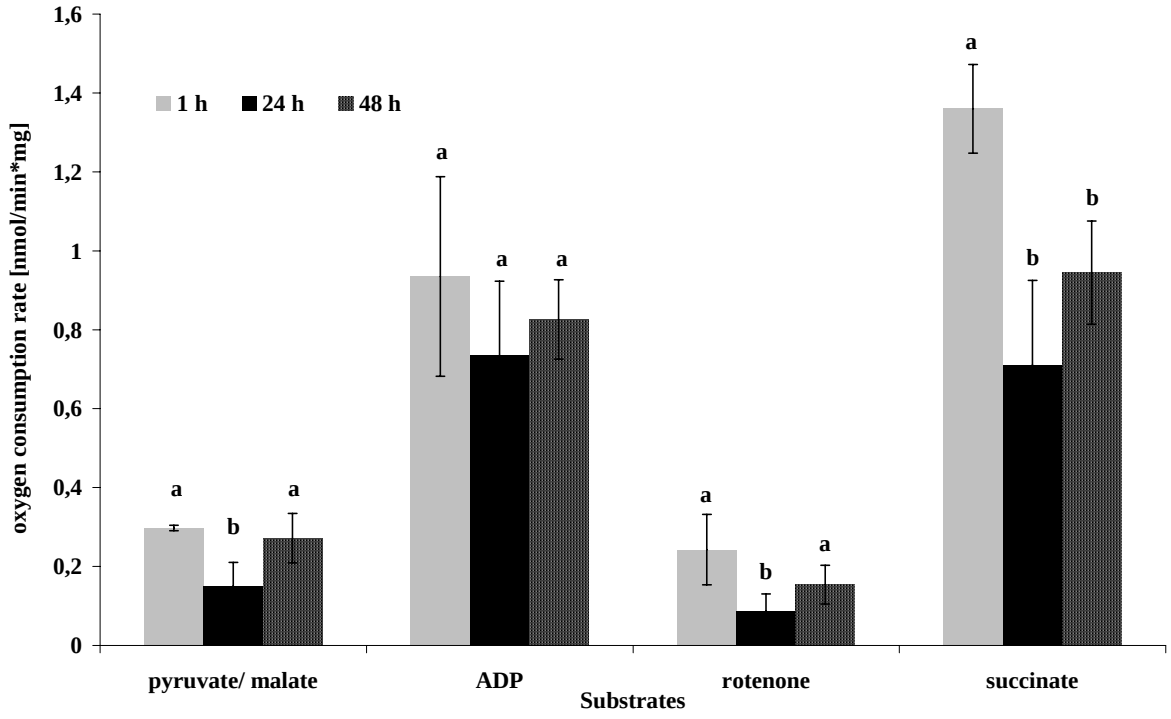

Fig. 1: Mitochondrial respiration of porcine diaphragmatic muscle samples with different substrates after storage at 4 °C for 1 h, 24 h and 48 h without storage solution. Presented are the mean values (± S.D.) of at least four independent experiments. Columns with different letters (a,b) between the storage times differ significantly ($P< 0.05$) (Mitochondriale Respiration unterschiedlicher Substrate in Zwerchfellmuskelproben vom Schwein nach Lagerung bei 4 °C für 1 h, 24 h und 48 h ohne Lagerlösung. Durchschnittswerte von mindestens vier unabhängigen Messungen. Balken mit unterschiedlichen Buchstaben (a,b) zwischen Lagerzeiten unterscheiden sich signifikant ($P< 0.05$))

If the diaphragmatic muscle samples were stored with the storage solution at 4°C no reduction in the pyruvate- and succinate-dependent respiration rates could be determined up to 48 h after beginning of the storage. It is interesting to note, that with the substrates pyruvate/malate after addition of ADP the respiration in the muscle samples stored for 24 h and 48 h increased significantly ($P<0.05$) in comparison to the results of the fresh muscle samples (Figure 2).

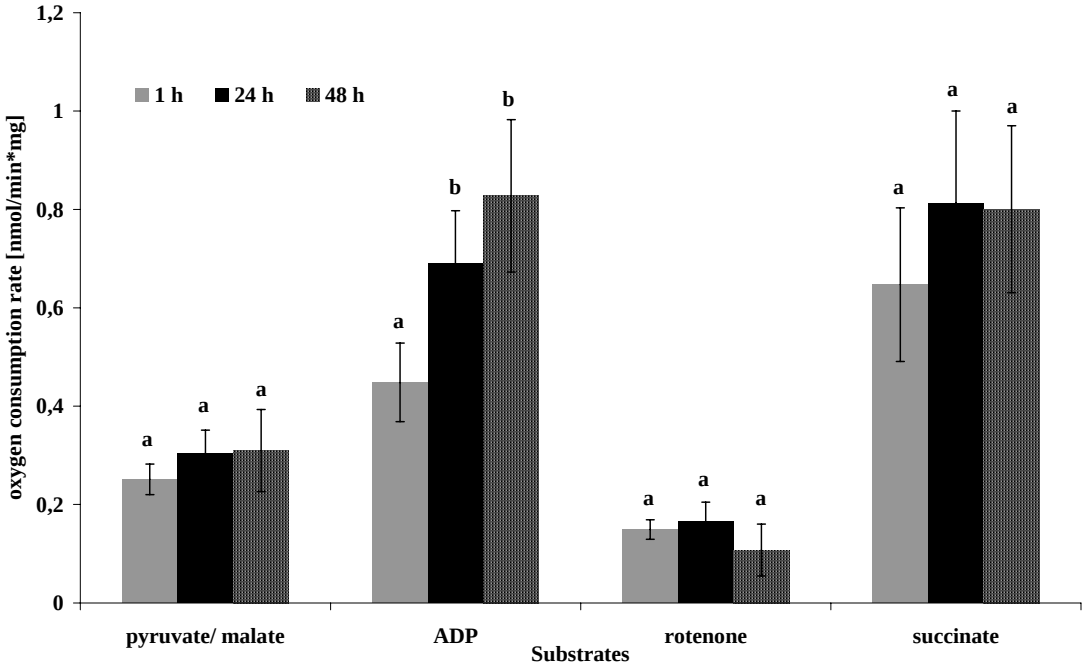

Fig. 2: Mitochondrial respiration of porcine diaphragmatic muscle samples with different substrates after storage at 4 °C for 1 h, 24 h and 48 h with buffer solution. Presented are the mean values (± S.D.) of at least four independent experiments. Columns with different letters (a,b) between the storage times differ significantly (P< 0.05) (Mitochondriale Respiration unterschiedlicher Substrate in Zwerchfellmuskelproben vom Schwein nach Lagerung bei 4 °C für 1 h, 24 h und 48 h mit Lagerlösung. Durchschnittswerte von mindestens vier unabhängigen Messungen. Balken mit unterschiedlichen Buchstaben (a,b) zwischen Lagerzeiten unterscheiden sich signifikant (P< 0.05))

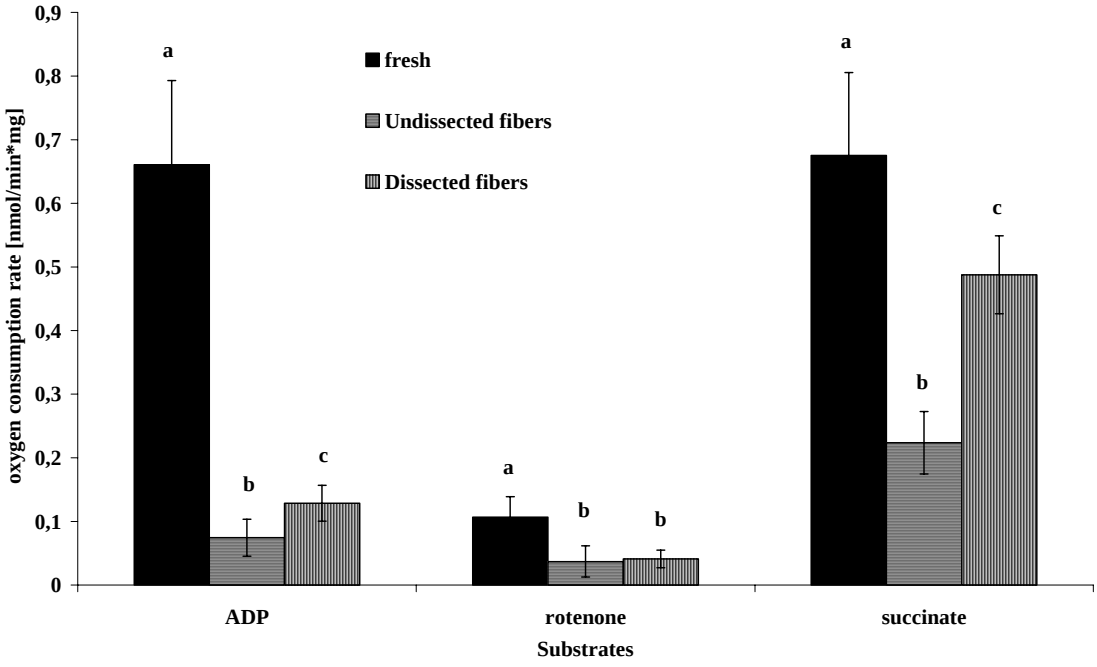

Fig. 3: Mitochondrial respiration of porcine diaphragmatic muscle samples after freezing in 30 % DMSO either undissected, or dissected in comparison to fresh muscle probes measured immediately after muscle collection (1 h after slaughter). Presented are the mean values (± S.D.) of at least four independent experiments. Columns with different letters (a,b,c) between the freeze-thawed and fresh probes differ significantly (P< 0.05) (Mitochondriale Respiration unterschiedlicher Substrate in Zwerchfellmuskelproben vom Schwein nach Einfrieren mit 30 % DMSO mit und ohne Dissektion im Vergleich zum frischen Muskel (1 h nach Schlachtung). Durchschnittswerte von mindestens vier unabhängigen Messungen. Balken mit unterschiedlichen Buchstaben (a,b) zwischen Lagerzeiten unterscheiden sich signifikant (P< 0.05))

After determination of the mitochondrial respiration in short-time stored porcine muscle samples the stability of the mitochondrial function after long-time storage in liquid nitrogen was analysed. Base of the experiments was the study of KUZNETSOV et al. (2003) who showed that freeze-thawing of human and murine muscle probes in 30 % DMSO resulted in the optimal preservation of the mitochondrial respiration rates. However, it was not possible to reproduce the described freeze-thaw conditions to the porcine muscle samples. For this reason in several tests the long-time storage conditions were varied in order to find the best freeze-thaw-procedure for porcine muscle samples. It could be shown that freeze-thawing of dissected fibers resulted in higher respiration rates (Figure 3), that the thawing of the frozen material at low temperatures (37°C) for up to 2´ was better in comparison to thawing at higher temperatures (60°C) for a shorter period of time (data not shown) and that the dissected fibers have to be diluted 1: 5 in the cryopreservation solution prior to freezing (data not shown).
Despite the improvement of the respiration rates after varying the freeze-thaw conditions the cryopreserved muscle samples could not be fully reactivated after thawing. In further experiments the type and the concentration of the cryoprotectant was varied. It could be shown that freezing of the muscle probes in glycerol resulted in lower respiration rates after thawing in comparison to the results of DMSO-treated muscle samples (data not shown). Varying the DMSO concentration in the cryoprotectant solution from 20 to 40 % resulted in an increase of the succinate-dependent respiration with rising DMSO content to the level of the fresh muscle probes. However, this reactivation could not be found with regard to the pyruvate-dependent mitochondrial respiration (Figure 4).

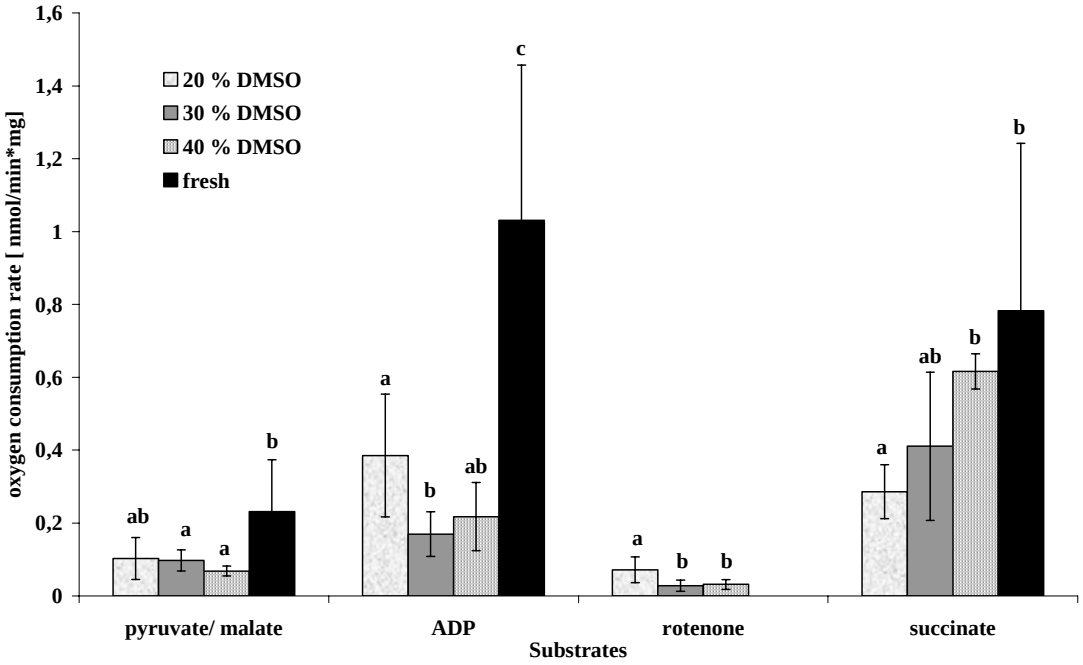

Fig. 4: Mitochondrial respiration of porcine diaphragmatic muscle samples after freezing in different DMSO concentrations (20, 30, 40 %) in comparison to fresh muscle probes measured immediately after muscle collection (1 h after slaughter). Presented are the mean values (± S.D.) of at least four independent experiments. Columns with different letters (a,b,c) between the freeze-thawed and fresh probes differ significantly (P< 0.05) (Mitochondriale Respiration unterschiedlicher Substrate in Zwerchfellmuskelproben vom Schwein nach Einfrieren mit unterschiedlichen DMSO Konzentrationen (20, 30, 40 %) im Vergleich zum frischen Muskel (1 h nach Schlachtung). Durchschnittswerte von mindestens vier unabhängigen Messungen. Balken mit unterschiedlichen Buchstaben (a,b) zwischen Lagerzeiten unterscheiden sich signifikant (P< 0.05))

Discussion

Physiological experiments using skeletal muscle tissue are limited by the reduced temporal stability of the cellular functions. Since many of these investigations are very time-consuming and for proper statistical analysis serial experiments are necessary the preservation of the functionality of the different metabolic pathways of the cells for a sufficient time is very important.

In the present study it was shown that for a short-time storage the muscle samples have to be stored in a specific storage solution, suggesting that the substances in the buffer reduce the deteriorating processes in the cells (e.g. by acidosis, proteases). In this solution mitochondrial function could be preserved up to 48 h, interestingly accompanied with an increase of the pyruvate-dependent respiration. The possibility to store muscle probes for short-time was also demonstrated in samples of rabbit and human musculature (KRAFT et al. 1995; TRUMBECKAITE et al., 2001). Interestingly, TRUMBECKAITE et al. (2001) also found that in rabbit muscle probes the pyruvate-dependent respiration rates increased during storage up to 34 h. A reason of this result could be an uncoupling of the mitochondria during storage accompanied with the disturbance of the electron transport chain and the ATP synthesis regulation.

In the long-time preservation experiments it could be shown that especially the pyruvate-dependent respiration is affected by the freeze-thaw-procedure. These results are in contrast to KUZNETSOV et al. (2003) who found a full preservation of the mitochondrial function in human and murine muscle samples after freeze-thawing in 30 % DMSO. A species specific difference in the sensibility to cryopreservation could be assumed. This thesis is supported by the results that e.g. a successful freeze-thawing of sperm cells, which are mainly composed of mitochondria in the motility system of the tail, differs also between the species. Human semen shows a good and porcine a quite bad motility outcome after thawing (CLARKE et al., 2003; GOOLSBY et al. 2004).

A possible reason for the reduction of the pyruvate-dependent respiration after freeze-thawing in the porcine muscle samples could be an alteration of the complex I - also called NADH-ubiquinone-oxidoreductase - of the electron transport chain. Complex I is related to pyruvate because the NADH-molecules produced in the citric acid cycle after addition of pyruvate are introduced into the electron transport chain via the complex I protein. The assumption that an alteration of the NADH-ubiquinone-oxidoreductase mainly affects the reduced mitochondrial function after thawing is supported by the presented result that the respiration after addition of succinate – a substrate that is introduced via the following protein (complex II) of the electron transport chain - is not affected by the freeze-thawing procedure. One reason of this reduction in the activity could be the higher sensitivity of the enzyme to changes in the microenvironment (e.g. acidosis, proteases) due to the complex structure of the protein which consisted of at least 45 subunits (VOGEL et al., 2005). Another reason might be the influence of other cellular systems like the calcium metabolism or the ROS generating processes on the complex I activity as it has been shown in several ischemia/reperfusion experiments (SADEK et al., 2004).

The presented investigation shows that the cryopreservation of porcine muscle samples is more problematic then the long-time conservation of muscles of other species like human or mouse. The main negative effect on the mitochondrial function is related to the pyruvate-dependent complex I activity of the electron transport chain. Further

experiments are necessary to elucidate if the alteration of complex I is caused by structural, regulatory or structural/regulatory changes in the cell in order to find appropriate freeze-thaw conditions and storage buffer systems that could possibly prevent these negative alterations.

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Preliminary study of octopus arm muscle: general arrangement and fibre types

(Erste Untersuchungen der Muskulatur von Octopusarmen: Grundsätzlicher Aufbau und Fasertypen)

Abstract

Octopuses are new serious candidates for aquaculture due to their short-life cycle, high reproductive rate, easy adaptation to captivity and high growth rate and market price. However, information about muscle development is scarce. It is then necessary to characterize muscle fiber types, and to distinguish between hypertrophic and hyperplasic processes before any factors that regulate myogenesis and muscle development can be considered.

A first histological approach of octopus muscle was carried out in arm muscle sections (8-10 μm) of a sub-adult octopus *Eledone cirrhosa*. Routine histological stains: haematoxylin, Red Syrius, and periodic acid Schiff's (PAS), and histochemistry staining for both succinic dehydrogenase (SDHase) and myofibrillar ATPase (mATPase) activities were used. Immunohistochemistry was also performed using antibodies against pufferfish (*Takifugu rubripes*) MyoD factor, and against mouse α-actinin and laminin.

Transverse and longitudinal sections of octopus arm revealed circular, longitudinal, radial and oblique muscle layers. The first layer comprises external circular fibres just beneath the connective tissue, followed by oblique fibres. Longitudinal fibres could be observed next, sandwiched between the first oblique muscle and a second layer of deeper oblique fibres, with an opposite orientation than the former. The following layer largely consisted of longitudinal fibres running parallel to the main axis of the arm. Radial bundles of fibres were observed crossing over all the bulk of longitudinal fibres, and seemed to leave from the oblique layer, ending in a thin inner circular layer of muscle, near to the axial nerve cord.

Sections from the tip of the arm revealed that the superficial circular layer, and possibly the first oblique layer, are presumptive analogues of slow "red muscle" since they were stained positive for SDHase activity. PAS staining also suggested the presence of glycogen reserves in these peripheral fibres, and/or the presence of abundant glycoproteins.

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MARCUS MAU, TORSTEN VIERGUTZ and CHARLOTTE REHFELDT

Influence of estrogens and isoflavones on porcine muscle satellite cell growth

Abstract

The role of estrogens and estrogen-like compounds, such as dietary phytoestrogens, in pig skeletal muscle growth is largely unknown. The aim of this study was therefore to investigate the *in vitro* effects of estrogens and isoflavones on porcine muscle satellite cell growth. Myogenic cells were derived from *M. semimembranosus* of newborn piglets, typified for muscle cell specific proteins (e.g. desmin) and grown in culture. The effects of different concentrations of 17ß-estradiol (0.1 nM; 1 nM; 1µM), estrone (1 nM; 1µM), and of genistein and daidzein (0.1; 1; 10; 100 µM) on DNA synthesis measured as 6 h-[^{3}H]thymidine incorporation were determined in serum-free growth medium. After 7 h (1+6 h) exposure both 17ß-estradiol and estrone slightly decreased DNA synthesis (-4 to -7%). Slight decreases were also observed in response to 1 and 10 µM genistein (-5; -10%) and to 1, 10, and 100 µM daidzein (-3 to -13%), whereas 100 µM genistein (-74%) substantially lowered DNA synthesis. Decreases in cell number (DNA) up to 8% were observed with genistein (0.1; 1; 100 µM) and daidzein (0.1; 100 µM). Long-term exposure (26 h) to 100 µM genistein caused an arrest in G_2/M and S phase of the cell cycle. In addition, DNA synthesis substantially decreased with 100 µM genistein (-89%), whereas it was significantly increased by 10 µM genistein (+106%), 10 µM daidzein (+31%) and 100 µM daidzein (+79%). Decreases in cell number (DNA) were observed with 100 and 10 µM genistein as well as with 100 µM daidzein. Furthermore, 100 µM of genistein and daidzein showed negative effects on protein-synthesis measured as [^{3}H]phenylalanine incorporation. Measurement of lactate-dehydrogenase (LDH) activity as indicator of cell damage in cell culture supernatants revealed increased values in response to 100 µM genistein and daidzein. The results suggest that both estrogens and isoflavonic phytoestrogens may directly affect porcine muscle cell growth with the effects being dose- and time-dependent.

Key Words: estrogens, phytoestrogens, porcine satellite cells, genistein, daidzein

Zusammenfassung

Titel der Arbeit: **Einfluss von Östrogenen und isoflavonen Phytoöstrogenen auf das Wachstum porciner Muskel-Satellitenzellen**

Über die Bedeutung der Östrogene und Phytoöstrogene für das Wachstum der Skelettmuskulatur von Schweinen ist bisher noch sehr wenig bekannt. Das Ziel dieser Arbeit war es daher, *in vitro* Effekte von Östrogenen und Isoflavonen auf das porcine Muskelzellwachstum zu untersuchen. Die Satellitenzellen wurden aus dem *M. semimembranosus* neugeborener Ferkel isoliert, bezüglich muskelzellspezifischer Proteinen (z.B. Desmin) typisiert und als Primärkultur etabliert. Es wurden die Effekte verschiedener Konzentrationen von 17β-Östradiol (0.1 nM; 1 nM; 1µM), Östron (1 nM; 1µM), sowie Genistein and Daidzein (0.1; 1; 10; 100 µM) auf die DNA-Synthese als 6 h-[^{3}H]Thymidin-Einbau in serumfreiem Wachstumsmedium gemessen. Nach 7 Stunden (1+6) wurde die DNA-Synthese unter der Einwirkung von 17β-Östradiol und Östron leicht reduziert (-4% bis –7%). Geringere DNA-Syntheseraten wurden auch bei 1 und 10 µM Genistein (-5; -10%) sowie bei 1, 10, und 100 µM Daidzein (-3 to -13%) gemessen, während 100 µM Genistein (-74%) die DNA-Synthese deutlich herabsetzten. Eine Reduzierung der Zellzahl (DNA) um bis zu 8% wurde unter dem Einfluss von 100 µM Genistein (0.1; 1; 100 µM) und Daidzein (0.1; 100 µM) beobachtet. Die Langzeit-Behandlung (26 h) mit 100 µM Genistein verursachte einen Block in der G_2/M- und S-Phase des Zellzyklus. Außerdem nahm die DNA-Synthese bei 100 µM Genistein (-89%) sehr stark ab, während sie bei 10 µM Genistein (+106%), 10 µM Daidzein (+31%) und 100 µM Daidzein (+79%) deutlich erhöht war. Eine Abnahme der Zellzahl (DNA) konnte mit 100 und 10 µM Genistein ebenso beobachtet werden wie mit 100 µM Daidzein. Darüber hinaus zeigte die als [^{3}H]Phenylalanin-Einbau gemessene Protein-Syntheserate einen stark negativen Einfluss von 100 µM Genistein sowie 100 mM Daidzein. Die Messung der Laktatdehydrogenase (LDH)-Aktivität im Kulturüberstand als Indikator der Zellschädigung ergab erhöhte Werte für 100 µM Genistein und Daidzein. Die Ergebnisse zeigen, dass sowohl die Östrogene als auch die isoflavonen Phytoöstrogene Schweinemuskelzellen direkt in ihrem Wachstum beeinflussen können, wobei Differenzen in Abhängigkeit von der Dosis und der Einwirkdauer auftreten.

Schlüsselwörter: Östrogene, Phytoöstrogene, Schweinemuskelzellen, Genistein, Daidzein

Introduction

Biologically active compounds in the maternal circulatory system influence both the maternal organism and the foetus by crossing the placenta. In this context the phytoestrogens are of great interest. Phytoestrogens are plant-derived steroid-like dietary compounds with weak estrogenic effects (BARRET, 1996). The four major classes of phytoestrogens are the lignans, coumestanes, flavones and isoflavones (KURZER & XU, 1997). Isoflavones reach highest concentrations in soy, clover and other legumes (REINLI & BLOCH, 1996). They have been shown to exhibit multi-biological properties including estrogenic and anti-estrogenic effects. Estrogens like 17-β-estradiol bind to membrane estrogen receptors as well as to cytoplasmatic estrogen receptors (MONJE & BOLAND, 1998; ROLLEROVA et al., 2000). The activated cytoplasmatic receptors float into the nucleus, bind to special HREs (hormone response elements) and subsequently influence cell cycle processes. The isoflavones can interfere with the cellular metabolism via the same mechanism of action. They can function as agonists of the estrogens by activating estrogen receptors, or they can act as antagonists by competing for the ER binding sites (SETCHELL et al., 1998). Furthermore, isoflavonic phytoestrogens are indicated to have inhibitory effects on protein tyrosine kinases (REN et al., 2001). Especially genistein has been reported to directly interact with tyrosine kinases (AKIYAMA et al., 1987) and in this way it could inhibit receptors of growth factors (e.g. IGF, insulin, EGF) by inhibition of tyrosine kinase phosphorylation. However, the role of estrogens and estrogen-like compounds, such as dietary phytoestrogens, in pig skeletal muscle growth is still largely unknown. There are indications of positive effects on foetal and postnatal growth and development after a daidzein supplement to the diet of sows (LIU et al., 1999). Furthermore, *in vitro* experiments with genistein and daidzein on muscle cell lines of rat (L_6) and mice (C_2C_{12}) revealed that genistein inhibits myotube formation and myogenin expression in a dose-dependent manner (PAN et al., 2001; HASHIMOTO et al., 1995; JI et al., 1999). Results on the influence of isoflavones on the growth and differentiation of porcine satellite cells are not yet available. Therefore, the aim of this study was to investigate short- and long-term effects of different dosages of estrogens and the isoflavones genistein and daidzein on the *in vitro* growth of porcine muscle satellite cells.

Material and methods

Tissue of *M. semimembranosus* was collected from newborn German Landrace piglets. Satellite cells were enriched by using a Percoll gradient. Prior to starting various experiments a pool was established from all cells obtained from 9 piglets (5 male, 4 female). Cells were typified for muscle cell specific proteins (desmin, N-CAM) with 95% positive satellite cells. For the experiments cells were seeded in gelatine -coated 96-well microplates at about $5x10^3$ cells per well. The cells were grown for 1 day in MEMα plus 10% FBS and 10% HS. In each of 2-4 replicates, a total of 8 wells spread over two plates was used for each concentration of 17ß-estradiol (E2) (0.1 nM; 1 nM; 1µM), estrone (E1) (1 nM; 1µM), and of genistein (G) and daidzein (D) (0.1; 1; 10; 100 µM) in serum-free growth medium (DOUMIT et al., 1996). The cells were incubated with E2, E1, G, and D for 7 h and 26 h, respectively, and DNA synthesis was measured during the last 6 hours as [^{3}H]thymidine incorporation (dpm/µg DNA) (REHFELDT & WALTHER, 1997). Accordingly, protein synthesis was analysed as 6

hours – [³H]phenylalanine incorporation (dpm/µg protein). In order to determine the rate of cell death (LEGRAND et al., 1992), lactate-dehydrogenase (LDH) activity was measured in cell culture supernatants according to the kit instructions formerly provided by Sigma Aldrich, Deisenhofen, Germany. Cell cycle analysis was performed by flow cytometry of propidium-iodide-labelled cells (LÖHRKE et al., 1998) seeded at 10^5 cells in 35 mm dishes. Data were subjected to analyses of variance by using the GLM or mixed procedure of SAS with treatment and replicate as fixed factors and plate as random factor, if applicable.

Results

After 7 h incubation DNA synthesis was slightly decreased by 1 nM and 1 µM 17ß-estradiol, 1 nM and 1 µM estrone (-4 to –7%), by 1 and 10 µM genistein (-5 to –10%) and by 1, 10 and 100 µM daidzein (-3 to –13%). Furthermore, DNA synthesis was substantially lowered with 100 µM genistein (-74%). Decreases in cell number (DNA) were observed with both genistein (0.1; 1; 100 µM) and daidzein (0.1; 100 µM). After 26 h exposure, DNA synthesis remained slightly lowered with 1 µM estrone (-6%). The effect of 100 µM genistein on DNA synthesis was again negative (-89%), increases were observed with 10 µM genistein (+106%) and 10 as well as 100 µM daidzein (+31%; +79%). Decreases in cell number (DNA) were observed with genistein (0.1; 10; 100 µM) and 100 µM daidzein.

Table
Summary of the effects of long-term exposure (26h) of porcine muscle satellite cells to the isoflavones genistein and daidzein (100 and 10 µM) (Effekte der Langzeitbehandlung (26 h) porziner Satellitenzellen mit den Isoflavonen Genistein und Daidzein (100 und 10 µM))

Effector	DNA	DNA synthesis	Protein synthesis	Cell cycle	Cell death
100 µM genistein	↓↓	↓↓	↓↓	G2/M block	↑↑
100 µM daidzein	↓	↑↑	↓↓	G2/M block (tendency)	↑↑
10 µM genistein	↓	↑↑	=	S-phase ↓	=
10 µM daidzein	=	↑	=	=	=

In order to further elucidate growth related changes, analyses of cell cycle, protein synthesis and cell death were carried out. Preliminary results including the most effective doses of genistein and daidzein are summarized in the Table. Cell cycle of the adherent cells was analyzed after 26 h exposure to different concentrations of daidzein and genistein. At the highest concentration (100 µM), genistein caused an G_2/M-phase block in cell cycle. DNA synthesis rate, DNA amount and protein synthesis rate dropped dramatically with 100 µM genistein. Additionally, the rate of cell death measured after exposure to 100µM genistein by LDH activity in cell culture supernatants was approximately doubled. From these findings it is concluded that

genistein at 100 µM inhibits cell proliferation and acts severely toxic on porcine satellite cells. Similar changes with respect to cell death and protein synthesis rate were seen with 100 µM daidzein, whereas only a tendency for the G_2/M block was seen. The increase of the DNA synthesis rate with 100 µM daidzein is rather due to DNA-repair than to *de novo* synthesis, as both the DNA-amount and the protein synthesis rate were even decreased. Altogether, 100 µM daidzein are also clearly toxic and slightly inhibit cell proliferation.
DNA synthesis rate was also found to be increased with 10 µM genistein. However, the number of S-phase cells was reduced from 14 to 11% after 26 h exposure. Moreover, there was no raise in DNA-amount or protein synthesis rate, which should be increased too, if a higher DNA synthesis rate really would reflect increased cell proliferation. The rate of cell death remained unchanged compared to untreated control. Altogether, 10 µM genistein slightly inhibit cell proliferation with increased DNA synthesis probably indicating DNA repair. Finally, 10 µM daidzein slightly raised the DNA synthesis rate, whereas it had no effect on the DNA-amount, protein synthesis and cell cycle suggesting that there are almost no effects on satellite cell viability and growth at this concentration. Again, the reasons for increased [^{3}H]thymidine incorporation are probably due to repair mechanisms like at 100 µM daidzein and will be further investigated.

Conclusions

From our findings we conclude that high non-physiological concentrations of estrogens are weak inhibitors of porcine satellite cell growth. At high concentrations, the isoflavones genistein and daidzein act as toxins and as inhibitors of porcine satellite cell growth. Long-term exposure to 100 µM genistein causes a cell cycle arrest in G_2/M and S phase, and 100 µM daidzein tend to cause similar changes preventing cell growth. Furthermore, the increased [^{3}H]thymidine incorporation at 10 µM genistein, 10 µM and 100 µM daidzein is supposed to rather result from DNA repair of impaired cells than from true *de novo* DNA synthesis of remaining viable cells. Altogether, the results suggest that both estrogens and isoflavonic phytoestrogens may directly affect porcine muscle cell growth with the effects being dose- and time-dependent. However, the mechanisms and signal pathways behind those effects remain to be investigated.

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Egg quality in Arctic charr *(Salvelinus alpinus)*

Abstract

Fatty acids are important parameters of egg quality in teleost species. The n-3/n-6 polyunsaturated fatty acid ratio in the natural diet of all limnic organisms differs between fresh and marine environment, being much lower in fresh-water compared to marine water. In the development of Arctic charr farming, the feed used in other salmonid species, mostly rainbow trout, is assumed to be suitable. It has been discussed that Arctic charr fatty acid requirements differ from other salmonid species, caused by different abilities of desaturation and elongation of fatty acids. The quality difference and hatching results comparisons between wild and farmed broodstock fish gametes support this idea. Therefore we have analysed the fatty acids and aim to develop a suitable broodstock feed with an evolutionary well balanced fatty acid composition.

Key Words: Artic charr, egg quality, fatty acids, hatching, selection program

Zusammenfassung

Titel der Arbeit: **Eiqualität beim Seesaibling (Salvelinus alpinus)**

Fettsäuren sind wichtige Parameter der Eiqualität bei Knochenfischspezies. Das Verhältnis mehrfach ungesättigter n-3/n-6 Fettsäuren in der natürlichen Diät aller Wasserorganismen unterscheidet sich zwischen Süß- und Salzwasser und ist in Frischwasser verglichen mit Meerwasser viel niedriger. In der Entwicklung der Aquakultur des Seesaiblings wird eine Fütterung entsprechend anderen Salmoniden, vor allem der Regenbogenforelle, als geeignet betrachtet. Es wird aber diskutiert, dass die Anforderung an die Versorgung mit Fettsäuren beim Seesaibling wegen unterschiedlicher Fähigkeit zur Fettsäure-Desaturierung und -Elongation, abweichend sind. Unterschiede in der Qualität und dem Bruterfolg von Gameten von Fischen aus wilden Beständen bzw. Aquakultur stützen diese Annahme. Daher haben wir die Fettsäuren analysiert mit dem Ziel für die Aquakultivierung geeignete Diäten mit ausgeglichener Fettsäurezusammensetzung zu entwickeln.

Schlüsselwörter: Seesaibling, Eiqualität, Fettsäuren, Bruterfolg, Selektionsprogramm

Introduction

The interest for Arctic char in the aquaculture industry is increasing, especially in Northern Europe but an increase in the Central European market is expected. The advantage of the species is its preferences for cold clear water where an increased nutritional load is expected to be of minor importance for the water quality (a governmental investigation: Swedish aquaculture- a future industry, Department of Agriculture, Ds 200:42).

A selection program on Arctic char started 1984 in Sweden and is an example of a successful selection program leading to enhanced growth and postponed sexual maturation (NILSSON, 1992). A drawback in the demand of eggs for fish farmers is a high and unpredicted variation in hatching success of the eggs. The selection program as such can not be the cause as a high variation in egg survival also occurs in other unselected stocks where the parent fish have been raised in farms and fed commercial salmonid feed. An increased mortality can to some extent be caused by increasing water temperatures in summer and early autumn but also farms that are supplied with groundwater never exceeding 8 ºC have a high variation in mortality.

The n-3/n-6 polyunsaturated fatty acid ratio in the natural diet of all limnic orga-nisms differs between fresh and marine environment, being much lower in fresh-water compared to marine water (ARTS, 2001; HENDERSON & TOCHER, 1987;

KAITARANTA and LINKO, 1984; PICKOVA et al., 1997). This has been mirrored in the composition of fatty acids in lipids of fish eggs. The lipid rich char eggs contain high proportion of storage lipids, which is a requirement in eggs with long incubation times (WIEGAND, 1996). Landlocked Atlantic salmon (*Salmo salar*) eggs have been investigated by PICKOVA et al. (1999) with regard to lipid composition in eggs of farmed and wild females. A significant difference was found between the fatty acid composition and hatching rate between the egg production from females of different origin. The egg lipids from farmed female salmon were richer in n-3 fatty acids, which was not in line with the findings from the lipid composition of free living females in the Lake Vänern. The hatching success was much greater in the wild offspring (approx. 90%). The results from that study indicated that fatty acid composition of the broodstock diet has to be optimized. In the present study Arctic char (*Salvelinus alpinus*) have been investigated. Char have been reared over the past few decades, allowing a comparison between the eggs from both wild females feeding on a diet based on lipids of limnic (natural food chain) origin and eggs from females fed an artificial diet (in farms) of marine origin. In a review by JOBLING et al. (1998) variability in egg quality was mentioned as one of the main problems that have arisen. This problem has not been solved since. JOBLING et al. (1995) investigated the importance of temperature on gonad development and the fatty acid composition. Unfortunately that study did not compare fish feeding different diets and the main fatty acid of interest, the arachidonic acid was not identified. The aim of the present study is to explore if a change in dietary fatty acid composition will influence the hatching percentage and survival at early life stages of Arctic char.

Material and methods

Wild fish were caught in one freshwater lake and farmed broodstock fish from two farms were sampled and all were stripped, the gametes were collected and eggs were fertilized. The eggs from individual females were incubated separately in incubation boxes in flow through systems. The eggs were hatched and the hatching rates were recorded. Eggs from all females of different origin were sampled (1-2 g/female) and lipids were extracted and fatty acids of phospholipid and triacylglycerol fractions were analysed (PICKOVA et al., 1997). Feed used in the farmed fish was analysed for fatty acid composition of total lipids.

Results

Hatching rates varied greatly between the wild and farmed origin eggs. Lipid analyses showed significant difference in composition of fatty acids. The content of 20:5n-3 in phospholipid fraction was higher in eggs from reared females compared with eggs from natural population. Further, in PL fraction, 20:4n-6 levels in these eggs were significantly lower (1.5% versus 8.9%). Also, the content in the triacyl-glycerol fraction differed greatly between wild and eggs from reared females, whereas this fraction showed almost no corresponding difference in EPA content. In addition, the level of 22:6n-3 differed greatly between the two groups, 31% in the farmed fish eggs vs 22.5% in the wild (Table). The fatty acid composition of feed based on marine lipids had a ratio between arachidonic and eicosapentaenoic acids totally in disagreement with the natural conditions if compared with freshwater data published (ARTS, 2001; KAITARANTA and LINKO, 1987; PICKOVA et al., 1999), see the

Table. The hatching rates of farmed char were significantly lower, with a large variation, when compared to the stripped wild collected char eggs.

Table
Fatty acid composition in % of total identified fatty acids in phospholipid fraction of wild and farmed broodstock char and total lipid of feeds (Fettsäurezusammensetzung in % der Phospholipidfraktion wilder und gezüchteter Seesaiblinge sowie des Futters)

	Wild	Farmed 1	Feed
14:0	2.6	1.2	7.5
16:0	20.8	18.0	17.8
18:0	8.1	6.4	3.1
18:1 n-9	9.9	11.3	9.9
18:1 n-7	7.4	4.6	2.6
18:2 n-6	1.6	1.4	4.5
18:3 n-3	0.9	0.3	1.2
20:1	2.0	6.6	5.6
20:4 n-6	8.0	1.3	0.5
20:5 n-3	9.6	7.2	10.4
22:5 n-3	3.5	2.1	1.1
22:6 n-3	19.1	31.4	11.3
AA/EPA	0.83	0.18	0.05
PUFA n-3	33.1	41.0	24.0
PUFA n-6	9.6	2.7	5.0
PUFAn-3/ PUFAn-6	3.45	15.2	4.8

Abbreviations: AA: arachidonic acid, EPA: eicosapentaenoic acid, PUFA: polyunsaturated fatty acids, LCPUFA: long chain polyunsaturated fatty acids

Conclusions

Arachidonic acid is more than 15 times higher in the naturally produced eggs of Arctic char compared to the fed broodstock char. In addition, the ratio between long chain polyunsaturated fatty acids of n-3 and n-6 groups is significantly more altered that the ratio of n-3 and n-6 polyunsaturates, which gives an indication that the elongation and desaturation ability in this salmonid species is very limited. These results indicate that the natural balance of essential fatty acids in terms of eicosanoid precursors and thereby membrane properties are greatly altered, plausibly being the one cause of the low hatching success in the farmed eggs. The changed balance of essential fatty acid is probably not the only cause behind high mortality of eggs from farmed females but may lower the resistance to environmental constrains such as high water temperature, considering the bioactivity of fatty acid derived eicosanoids in the immunity, inflammation, reproduction and other mechanisms (CALDER, 2001). The effect of global warming is expected to change the geographical limit of Arctic char both as wild population but in particular limiting the suitable location of fish farms specialized in Arctic char. We conclude that an economically feasible farming protocol requires an improved diet with higher content of n-6 fatty acids, especially arachidonic acid, to achieve a better egg quality in char.

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Use of casein zymography to measure the activities of µ- and mM calcium-dependent calpains during myogenesis in primary porcine satellite cell cultures (preliminary data)

(Anwendung von Casein-Zymographie zur Messung von den Aktivitäten der µ- und mM Kalzium-abhängigen Kalpaine während der Myogenese in primären Satellitenzellkulturen von Schweinen (vorläufige Daten))

Muscle cells in cultures cease to proliferate and start to differentiate morphologically (fusion of cells) and biochemically (expression of muscle specific proteins) following a reduction of growth factors in the medium. Several pieces of evidence suggest that the calpain system (µ- and mM calpains, their inbibitor calpastatin, and muscle specific calpain p94), especially the mM calpain, is involved in the fusion processes. Measuring the activity of µ- and mM calpains by purification requires large amounts of cells. Measurement of calpain activities by use of casein zymography, which is more sensitive, may be an alternative. Consequently, we have studied µ- and mM calpain activities by use of casein zymography during myogenesis of porcine satellite cells. Porcine satellite cells were isolated from 12-week old pigs and seeded in 24-well plates. Cells were grown in growth medium until 80% confluence after which the medium was replaced with a differentiation medium. Cells were harvested at 50% and 80% confluence and the days after changing the medium were designated d1, and d2, d3, and d 4. Following homogenisation zymography was carried out on none-denaturated 12.5% separating gels with 0.5% casein incorporated. The activities were quantified as density of bands. Results:

Developmental stages	50%	80%	d 1	d 2	d 3	d 4
mM-Calpain	345	369	835	827	868	659
µM Calpain	nt	nt	326	329	524	574
CK, mU/well	1.71	3.04	3.90	9,79	15,9	14,4

The data show that mM calpain activity was low in proliferating cells but increased to high levels preceding the fusion of cells (creatine kinase activity) and stayed high in differentiated cultures. The activity of µM calpain was not detectable in proliferating cells but increased steadily during differentiation cultures. In conclusion, the increased activity preceding fusion indicates that at least mM calpain is required in the fusion of muscle cells during myogenesis.

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Is the myosin heavy chain IIb isoform expressed in bovine muscles?

(Wird die Isoform IIb der schweren Kette des Myosin im bovinen Muskel exprimiert?)

According to CHIKUNI et al. (2004), the myosin heavy chain (MyHC) IIb isoform would not be expressed in bovine muscles. However, MACCATROZO et al. (2004) have recently reported its presence in an extraocular muscle. The purpose of this study was to check the presence of this isoform in the muscles of Blonde Aquitaine (BA) bulls.

We have analysed samples of *semitendinosus* (ST) and *longissimus thoracis* (LT) muscles of eleven 15-month-old bulls at shaughter. Samples of ST muscle were also collected on group of 11 BA bulls by biopsy at 3 weeks, at 5, 9 months of age and at slaughter at 15 months of age. MyHC isoforms were separated by SDS-PAGE and were quantified by densitometry. Western-blot analyses were carried out using antibodies raised against the different fast MyHC. Total RNA was extracted, digested by DNAse I and reverse-transcripted into cDNA.

In 2 of the 11 bulls, we revealed the presence of an additional MyHC isoform representing on average 42% (ST) and 26% (LT) of total MyHC. These bulls have a common ancestor, which was characterized by good index of muscular development, of conformation and of meat yield. This isoform was also found in the biopsies of some animals at all the studied ages.

Western-blot analyses confirmed the presence of a fast MyHC but did not allow us to reveal its identity. Using specific primers allowing the amplification in the 5' -UTR of the pig MyHC IIb, we amplified a cDNA fragment whose sequence corresponds to a myosin IIb type. Altogether, these results indicate that a MyHC IIb isoform is expressed in the ST muscle and, in smaller amount, in the LT muscle.

Amplification, cloning and sequencing of the "loop2" encoding domain, specific of the different fast MyHC in mammals, are in progress to develop a real time RT-PCR assays in the bovine muscles.

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The two isoforms of myosin light chain 2 in gilthead sea bream (*Sparus aurata*); alternative polyadenylation site selection and tissue expression

Abstract

Myosin light chain 2 (MLC2) is an essential component of the myosin molecule, with a regulatory role in binding Ca^{2+}. In gilthead sea bream, a MLC2 clone has been isolated and characterized, that encodes for a 170 aa peptide and contains three potential polyadenylation signals in the 3' UTR. In this study, the isolation of three alternative transcripts of the already known MLC2 (isoform A) is reported, along with the isolation and characterization of a second MLC2 isoform (B). All three isoform A transcripts encode the same peptide but differ in the length of their 3' UTRs (284bp, 788bp and 876bp respectively) and are generated by alternative polyadenylation site selection. Transcripts of isoform A were detected both in white and red muscle. MLC2 isoform B encodes also for a 170 aa protein. Isoform B was detected in all tissues examined (red, white, smooth and cardiac muscle, kidney, liver, spleen, brain, gills, epidermis). The differential expression of the two isoforms and of the alternative transcripts of isoform A during development is currently under study, in order to investigate the functional significance and regulation of 3' UTR length in transcription and mRNA turnover rate.

Key Words: myosin light chain 2, gilthead sea bream, alternative polyadenylation, isoforms, tissue expression

Zusammenfassung

Titel der Arbeit: **Die zwei Isoformen der leichten Kette des Myosin 2 in der Goldbrasse (*Sparus aurata*); Auswahl von alternativen Polyadenylationstellen und Gewebeexpression**

Die leichte Myosin-Kette 2 (MLC2) ist eine essentielle Komponente der Myosin-Moleküle mit regulierender Funktion bei der Bindung von Calcium. Bei der Goldbrasse wurde ein MLC2-Klon isoliert und charakterisiert, der für ein 170 Aminosäuren langes Peptid kodiert und drei potentielle Polyadenylierungssignale am 3'UTR enthält. In dieser Studie berichten wir über die Isolierung von drei alternativen Transkripten des schon bekannten MLC2 (Isoform A), sowie über die Isolierung und Charakterisierung einer zweiten MLC2 Isoform (B). Die drei Transkripte der Isoform A kodieren für das gleiche Peptid und unterscheiden sich nur in der Länge ihrer 3'UTR (jeweils 284bp, 788bp und 876 bp), die von der Auswahl alternativer Polyadenlyationstellen abhängt. Die Transkripte der Isoform A wurden sowohl im weißen als auch im roten Muskel erfasst. Die MLC2 Isoform B kodiert ebenso für ein 170 Aminosäuren langes Peptid. Die Isoform B wurde in allen untersuchten Geweben erfasst (rote, weiße, glatte und Herzmuskulatur, Nieren, Leber, Milz, Gehirn, Kiemen und Epidermis). Weitere Studien der unterschiedlichen Expression der zwei Isoformen und der Expression der alternativen Transkripte der Isoform A während der Entwicklung der Goldbrasse werden noch durchgeführt, um die funktionelle Signifikanz sowie die Regulation der Länge des 3'UTR während der Transkription und die turnover Rate der mRNA zu klären.

Schlüsselwörter: Leichte Myosin-Kette 2, Goldbrasse, Isoformen, alternativen Polyadenlyationstelle, Gewebe-expression

Introduction

Myosin, the major component of striated muscle, is a complex molecule of heavy and light chains, which appear in different isoforms and are under continuous replacement to meet developmental and environmental demands. Myosin light chain 2 (MLC2) is an essential component of the myosin molecule, with a regulatory role in binding Ca^{2+} (WEEDS and LOWEY, 1971).

In gilthead sea bream (*Sparus aurata*), a MLC2 clone has been isolated and characterized (accession number AF150904; MOUTOU et al., 2001), that encodes for

a 170 aa peptide and contains three alternative polyadenylation signals in the 3' UTR. MLC2 transcripts 1.56 kb long were detectable from the beginning of somitogenesis (20 hours post-fertlisation), whereas a second MLC2 transcript of 0.89 kb was present 7 hours later (MOUTOU et al., 2001). *In situ* hybridisation studies showed that MLC2 expression marks the germinal zones of skeletal muscle and the newly formed white fibers, while in adults, its expression is restricted to myogenic cells interspersed in the fast white muscle (MOUTOU et al., 2005).
The present study reports the isolation and characterization of a second isoform of MLC2 (isoform B) in sea bream, along with the isolation of three alternative transcripts of the previously identified MLC2 (isoform A) generated by alternative polyadenylation site selection. The tissue expression pattern of the two isoforms was studied by RT-PCR.

Materials and Methods

Isolation of MLC2 cDNA clones

Gilthead sea bream cDNA encoding different MLC2 isoforms were retrieved from a cDNA library constructed with mixed embryonic and larval stages during an EST project (SARROPOULOU et al. 2005) Briefly, an embryonic/larval cDNA library was created using 5μg of mRNA extracted from a mix of five embryonic/larval stages (gastrula, neurula, pectoral fin budding, newly hatched and brown eye) according to the instructions provided with the Lambda Uni-ZAP XR cloning kit (Stratagene, La Jolla, USA). Colonies were isolated after mass excision and plasmid extraction was performed with a robotic system (BIOMEK 2000) using the QIAprep 96 Turbo BioRobot Kit. Sequencing was performed using the primers T7 and T3 of the pBK-CMV vector. Each of the clones was fully sequenced to five fold coverage.

MLC2 tissue expression pattern by reverse transcription-polymerase chain reaction (RT-PCR)

Juvenile sea bream maintained in through-flow seawater tanks at 21±2^{o}C under natural photoperiod for winter in Volos, Greece, was killed by stunning and decapitation. Red and white muscle, stomach, pyloric caeca, anterior and posterior intestine, heart, kidney, liver, spleen, brain, gills and epidermis were immediately dissected out, frozen in liquid nitrogen and stored at –80^{o}C.
Total RNA was extracted using TRI Reagent (Sigma Co). cDNA was synthesised from 1 μg of total RNA and PCR was carried out in a reaction (50 μl) containing 2 μl of cDNA, 1X PCR buffer B (Promega), 2.5mM $MgCl_2$, 0.2mM each of deoxynucleotide triphosphate, 100 pmol of forward and reverse primer respectively and Taq DNA polymerase (5 units; Promega). The cDNA template was amplified after an initial denaturing step at 94^{o}C for 2 min, using 30 cycles of the following PCR protocol: 94^{o}C for 1 min, 59^{o}C for 2 min and 72^{o}C for 1 min. Isoform specific primers were designed and PCR products were verified by sequencing.

Results and Discussion

Four (4) different MLC2 transcripts were isolated. Three (3) of the transcripts corresponded to the previously identified MLC2, isoform A; they shared a common coding region but they possessed 3'UTRs of different length, 284bp, 788bp and 876bp respectively. Sequence alignment of the different 3'UTRs of isoform A (Fig. 1)

indicated that the three transcripts were generated by the use of the three alternative polyadenylation signals. Transcript 2 (3'UTR–788bp) corresponds to the previously isolated MLC2 clone. Polyadenylation is a post-transcriptional modification that regulates the transcription termination by RNA polymerase II, the transport of the mature mRNA from the nucleus to the cytoplasm via nuclear pores, as well as the stability and translatability of mRNA (EDWARDS-GILBERT et al., 1997). The physiological importance of this mechanism has been studied in 95 genes with alternative poly(A) site selection within a single 3'UTR, in which the mRNAs formed are differentially processed from the primary transcript during the cell cycle or in a tissue-specific or developmentally specific pattern (EDWARDS-GILBERT et al., 1997). The existence of alternative polyadenylation of the MLC2 gene may correspond to a regulation point with high physiological significance for the regulation of MLC2 transcript turnover and translation rate by hormonal and environmental factors.

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Transcript 1  TAAATCCCCCTCTCTTTCAAGATCCTTACCTCCGCTCAAACCCAATACTCGACGCAACATCTACTCTACTCACTC
Transcript 2  TAAATCCCCCTCTCTTTCAAGATCCTTACCTCCGCTCAAACCCAATACTCGACGCAACATCTACTCTACTCACTC
Transcript 3  TAAATCCCCCTCTCTTTCAAGATCCTTACCTCCGCTCAAACCCAATACTCGACGCAACATCTACTCTACTCACTC
              ********************************************************************************

Transcript 1  TTCTCCGATGCCGTGGCTCCCTCGCACACTCTCGCGCCCTCGGCCCGCTCTGTCCGCTTGCCAGCTCACTACAAA
Transcript 2  TTCTCCGATGCCGTGGCTCCCTCGCACACTCTCGCGCCCTCGGCCCGCTCTGTCCGCTTGCCAGCTCACTACAAA
Transcript 3  TTCTCCGATGCCGTGGCTCCCTCGCACACTCTCGCGCCCTCGGCCCGCTCTGTCCGCTTGCCAGCTCACTACAAA
              ********************************************************************************

Transcript 1  AAGACTTGTCTCCTGTTCTTGAGATACTCAGTGAGAGGACTGGGGGCTGTGGGGTTGTTTGTGTGTGAGTACCAA
Transcript 2  AAGACTTGTCTCCTGTTCTTGAGATACTCAGTGAGAGGACTGGGGGCTGTGGGGTTGTTTGTGTGTGAGTACCAA
Transcript 3  AAGACTTGTCTCCTGTTCTTGAGATACTCAGTGAGAGGACTGGGGGCTGTGGGGTTGTTTGTGTGTGAGTACCAA
              ********************************************************************************

Transcript 1  CAGGTGAACATGGGATTATTTTCAATAAAAATAATCTTGTGGCACTGAAACTCTCTCTCCATCTCTGTCCCTGCC
Transcript 2  CAGGTGAACATGGGATTATTTTCAATAAAAATAATCTTGTGGCACTGAAACTCTCTCTCCATCTCTGTCCCTGCC
Transcript 3  CAGGTGAACATGGGATTATTTTCAATAAAATAATCTTGTGGCTCTGAAAAAAAAAAAAAAAAAAAAAAAAAA
              ********************************************** ******

Transcript 1  TCTTGTTCCCCCTGCTTTTCCTCCCATCACTCATTCTGTCCTTCTGCGTTGAGGCCAACAGTGCATGCATCATGC
Transcript 2  TCTTGTTCCCCCTGCTTTTCCTCCCATCACTCATTCTGTCCTTCTGCGTTGAGGCCAACAGTGCATGCATCATGC

Transcript 1  CTATGTACAGCGCGTATGCATATGCAGTCCAGTGTATACAGTGGCCAGTCAGACATATCTCTTGGGTGCTGTGGT
Transcript 2  CTATGTACAGCGCGTATGCATATGCAGTCCAGTGTATACAGTGGCCAGTCAGACATATCTCTTGGGTGCTGTGGT

Transcript 1  GCGTTGAGGCCAACAGTGCATGCAGCAAGCACAGCCGCTCACTTGAAACAAGTGAGCGGCCTGACCCGAGTGGTC
Transcript 2  GCGTTGAGGCCAACAGTGCATGCAGCAAGCACAGCCGCTCACTTGAAACAAGTAAGCGGCCTGACCCGAGTGGTC

Transcript 1  TGTTAGTCTCAACCTGACACAGAGTGTTTTATGGACTCGTCCCTTTGTTTGTATCAGGGAGGATAGCACAGTGAA
Transcript 2  TGTTAGTCTCAACCTGACACAGAGTGTTTTATGGACTCGTCCCTTTGTTTGTATCAGGGAGGATAGCACAGTGAA

Transcript 1  GAGTGGGAGTACCGTACTATAATAGATTGCCTACTCCTTCTCTTTAATCTGTCTCTCCTTCTCTTAAACACAGGC
Transcript 2  GAGTGGGAGTACCGTACTATAATAGATTGCCTACTCCTTCTCTTTAATCTGTCTCTCCTTCTCTTAAACACAGGC

Transcript 1  ATGACAGGAAAAGTTGCAGTGAAAATGGGAAAGCATGATTTGGTTCAAATCTTGTAATTGGAGAAAGAGATGGTG
Transcript 2  ATGACAGGAAAAGTTGCAGTGAAAATGGGAAAGCATGATTTGGTTCAAATCTTGTAATTGGAGAAAGAGATGGTG

Transcript 1  AAAGATGGTGAGTGGGAGGGAGAGATGAAATAAACGAAAGTGAAATGTCAAAAAAAAAAAAAAAAAAA
Transcript 2  AAAGATGGTGAGTGGGAGGGAGAGATGAAATAAACGAAAGTGAAATGTCTTGTTTTGGTCTCTTCTATCTCCGAC

Transcript 2  TCACTGCTGTTTCTCTCCTGTTTTCATGACTGTACCAAATAAAGAAGTACAAATAAAATCCACTATCTTTCGTAA
              AAAAAAAAAAAAA
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Fig. 1: Alignment of the 3'UTRs of MLC2 isoform A (accession number AF150904) alternative transcripts. The stop codon is shaded and polyadenylation signals are underlined (Vergleich der 3´UTR der alternativen Transkripte der MLC2 Isoform A (Zugangsnummer AF150904). Das Stopkodon ist schattiert, die Polyadenylierungssignale sind unterstrichen)

In addition to multiple MLC2A transcripts a second MLC2 isoform, isoform B was isolated. The cDNA for isoform B encoded a putative protein of 170 aa with 92% identity to isoform A (Fig. 2). The gilthead sea bream is the second teleost in which

two MLC2 isoforms exist, as two forms were previously reported in the Atlantic halibut (*Hippoglossus hippoglossus*, Accession numbers AJ488287. AJ488286). Comparison of the putative amino acid sequence of isoform B MLC2 in gilthead sea bream and halibut reveals they share 98% identity. Gilthead sea bream isoform A and B differed by thirteen (13) amino acids, eleven (11) of which appeared to be characteristic of isoform B (Fig.2).

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                                                          Ca++-binding domain
S.aurata       A   MAPKKAKRRQQQGEGGSSNVFSMFEQSQIQEYKEAFTIIDQNRDGIISKDDLRDVLATMG
S.aurata       B   MAPKKAKRRQAAGDGGSSNVFSMFEQSQIQEYKEAFTIIDQNRDGIISKDDLRDVLASMG
H.hippoglossus B   MAPKKAKRRQAAGDSGSSNVFSMFEQSQIQEYKEAFTIIDQNRDGIISKDDLRDVLASMG
                   ********** *  ****************************************:**

S.aurata       A   QLNVKNEELEAMVKEASGPINFTVFLTMFGEKLKGADPEDVIVSAFKVLDPEATGAIKK
S.aurata       B   QLNVKNEELEAMIKEASGPINFTVFLTMFGEKLKGADPEDVILSAFKVLDPEGTGSIKK
H.hippoglossus B   QLNVKNEELEAMIKEASGPINFTVFLTMFGEKLKGADPEDVILTAFKVLDPEATGSIKK
                   ************:****************************: ********.** ***

S.aurata       A   EFLEELLTTQCDRFTAEEMTNLWAAFPPDVAGNVDYKNICYVITHGEEKEE
S.aurata       B   EFLEELLTTQCDRFTKDEIKNMWAAFPPDVAGNVDYKNICYVITHGEEKEE
H.hippoglossus B   EFLEELLTTQCDRFTKDEIKNMWSAFPPDVAGNVDYKNICYVITHGEEKEE
                   *************** :*:.*:*:**************************
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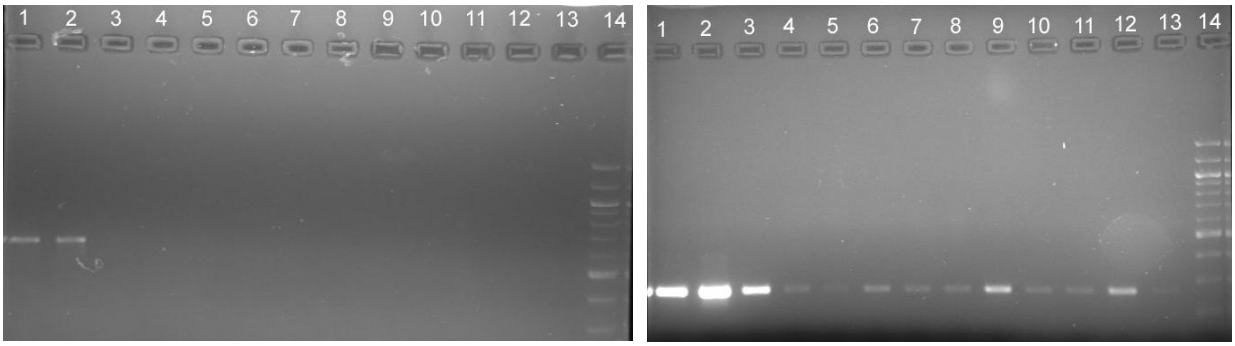
Fig. 2: Alignment of the deduced amino acid sequences of gilthead sea bream MLC2 isoforms and halibut (*Hippoglossus hippoglossus*) MLC2 isoform B (accession number AJ488286). Shaded amino acids distinguished isoforms B from isoform A (Vergleich der abgeleiteten Aminosäurensequenz der MLC2 Isoformen der Goldbrasse sowie der MLC2 Isoform B (Zugangsnummer AJ488286) des Heilbutts (Hippoglossus hippoglossus). In den schattierten Aminosäuren unterscheiden sich die Isoformen A und B)

The existence of two different skeletal MLC2 genes in rainbow trout (KRASNOV et al., 2003) is supportive of the idea that isoforms A and B are products of different genes rather than alternative transcripts of the same gene. It remains to be established if this is the case for the isoforms of MLC2 found in gilthead seabream. However, the dispersion of amino acid substitution throughout the encoded protein makes it likely that two genes encode the two isoforms of MLC2 identified.



MLC2 isoform A. MLC2 isoform B

Fig. 3: Tissue-specific expression of MLC2 isoforms A and B in gilthead sea bream by RT-PCR. 1: red muscle; 2: white muscle; 3: stomach; 4: pyloric caeca; 5: anterior intestine; 6: posterior intestine; 7: heart; 8: kidney; 9: liver; 10: spleen; 11: brain; 12: gills; 13: epidermis; 14: DNA ladder (Gewebsspezifische Expression der MLC2 Isoformen A und B bei der Goldbrasse nach Rt-PCR. 1: roter Muskel; 2: weißer Muskel; 3: Magen; 4: Pyloruscaecum; 5: vorderer Darmabschnitt; 6: hinterer Darmabschnitt; 7: Herz; 8: Niere; 9: Leber; 10: Milz; 11: Gehirn; 12: Kiemen; 13: Epidermis; 14: DNA Leiter)

The two MLC2 isoforms in gilthead sea bream exhibited a differential tissue expression pattern. Isoform A expression was restricted to skeletal muscle, white and

red, whereas isoform B was widely expressed, with transcripts present in all tissues tested (Fig. 3). The different contractile properties of the two isoforms, their presence in different tissues and their expression pattern during development constitute important information for the understanding of muscle formation and function in early development and for the study of the effects of prenatal events on postnatal muscle growth.

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Expression patterns of MLC isoforms during halibut (*Hippoglossus hippoglossus* L.) metamorphosis

Abstract

Atlantic halibut is an important commercial fish in the countries of the North Atlantic and is emerging as a promising species for marine cold-water aquaculture. The axial musculature of the developing larvae is the largest and most rapidly growing tissue and during the transition from larval to adult muscle fibre types significant changes in fibre morphology and gene transcription occur. In fact the change in myotome height correlates well with different larval halibut stages. In the present study the spatial and temporal expression of myosin light chain 1 (MLC1), 2 (MLC2) and 3 (MLC3) was studied in metamorphosing halibut by *in situ* hybridization. As a first step to establishing a role for the thyroid axis in halibut muscle development whole body thyroid hormone (TH) concentrations were also determined. In first feeding larvae MLC1, MLC2A and MLC3 transcripts had a similar distribution and were confined to the muscle fibres of the germinal zones. In pre-metamorphic larvae transcripts were highly expressed throughout the epaxial and hypaxial musculature and expression levels reached a maximum in larvae starting metamorphosis, this change coincided with a significant increase in the concentration of thyroid hormones. By the time larvae reached the metamorphic climax, MLC1, MLC2A and MLC3 expression was still high throughout the musculature but expression was confined to fibres adjacent to the myosepts and to small cells scattered in the musculature, possibly satellite cells. MLC2A was also expressed in the red muscle fibres; no transition between larval and adult MLC isoforms was detected.

Key Words: Flatfish, in situ localisation, muscle development, thyroid hormones

Zusammenfassung

Titel der Arbeit: **Expressionsmuster von MLC Isoformen während der Metamorphose beim Heilbutt *(Hippoglossus hippoglosus)***

Der Atlantikheilbutt ist ein kommerziell wichtiger Fisch für Länder des Nordatlantiks und entwickelt sich zu einer viel versprechenden Spezies für die Aquakultur. Die axiale Muskulatur sich entwickelnder Larven stellt das größte und am stärksten wachsende Gewebe dar. Während der Transformation von den larvalen zu adulten Muskelfasertypen treten signifikante Änderungen der Fasermorphologie und der Genexpression auf. Die Myotomgröße korreliert gut mit verschiedenen Stadien der Heilbuttlarven. In der vorliegenden Untersuchung wurde die lokale und temporale Expression der Gene der leichten Myosinketten 1, 2 und 3 (MLC1, MLC2, MLC3) während der Metamorphose mittels in situ Hybridisierung dargestellt. Als erster Schritt um die Rolle der Schilddrüsenhormon-Achse in der Muskelentwicklung des Heilbutts zu beleuchten, wurden auch die Gesamtkörpergehalte an Schilddrüsenhormonen (TH) bestimmt. Im frühen Larvenstadium (Stadium 5) haben MLC1, MLC2A und MLC3 Transkripte eine ähnliche Verteilung und sind auf Muskelfasern der Keimzonen begrenzt. In prä-metamorphen Larven werden hohe Transkriptionsraten in der epaxialen und hypaxialen Muskulatur gefunden. Die Expression erreicht ein Maximum in den Larven zu Beginn der Metamorphose; gleichzeitig tritt eine signifikante Zunahme der Konzentration der Schilddrüsenhormone auf. Bis Larven den metamorphen Höhepunkt erreichen, ist die Expression von MLC1, MLC2A und MLC3 in der Muskulatur hoch, aber die Expression ist begrenzt auf Fasern nahe den Myosepten und auf kleine Zellen, die in der Muskulatur verstreut sind, vielleicht Satellitenzellen. MLC“A wird auch in den roten Muskelfasern exprimiert; kein Umschalten von larvalen zu adulten MLC Isoformen wird beobachtet.

Schlüsselwörter: Plattfisch, in situ Lokalisation, Muskelentwicklung, Schilddrüsenhormone

Introduction

The importance of the thyroid hormones (THs); thyroxin (T_4) and triiodothyronine (T_3), in vertebrate development is well established (POWER et al., 2001). In fish, THs are involved in the transition of larvae to juveniles, the most dramatic manifestation of which is flatfish metamorphosis. TH treatment stimulates flatfish metamorphosis and the transformation from larvae to juvenile of a variety of teleost species although the mechanism by which it acts still remains to be clearly established (DE JESUS et al.,

1998; SOLBAKKEN et al., 1999). In developing fish larvae the axial musculature is the largest and most rapidly growing tissue and the transition from larval to adult muscle fibre types occurs gradually but appears to accompany metamorphosis raising questions about the role of THs. In fact THs have been associated with the developmental transition of myosin isoforms during flounder metamorphosis (KEIKUZE et al., 1993). The functional unit of myosin in vertebrate adult fast skeletal muscle is composed of two heavy chains and four associated light chains; two regulatory myosin light chains (MLC2) and two alkali light chains (MLC1 and MLC3) (THIÉBAUD et al., 2001). In the present study, the ontogeny and distribution of regulatory and alkali light chain myosin was studied using *in situ* hybridization and related to whole body TH concentrations and thyroid histology.

Material and methods

The Atlantic halibut larvae, used in this study, were raised at Fiskey, Iceland. Larval rearing was carried out using standard commercial production routines. The larvae were reared at 10-11 ºC, under constant light conditions (24L) and fed live artemia. and were sampled at regular intervals from the age of 260 D° through to the end of metamorphosis 800ºD. The T_4 and T_3 content of whole larvae were assessed by radioimmunoassay (RIA) of larval extracts. Frozen larvae were extracted in methanol, reextracted in 50µl methanol, 200µl chloroform and 100µl barbital buffer, centrifuged (3,000 rpm for 30 min at 4^{o}C). Then, the upper phase removed, lyophilized, reconstituted in assay buffer and assayed. Assays for both T_3 and T_4 were performed using a double-antibody method under equilibrium conditions. Free hormone was separated from the bound hormone using precipitation with a second antibody. Larvae for histology and *in situ* hybridization were fixed in paraformaldehyde (PFA, 4%) overnight at 4^{o}C, decalcified in EDTA pH 8 when necessary, embedded in low melting point paraffin and sagittal and transverse section prepared and mounted on APES treated glass slides. In order to study thyroid gland development, sectioned larvae were stained using the Cleveland-Wolfe trichrome method, after dewaxing and rehydrating the sectioned material. Digoxygenin-labelled riboprobes for MLC1, MLC2A and MLC3 were prepared by *in vitro* transcription of plasmid DNA containing PCR fragments encoding halibut MLC1, MLC2A and MLC3. Riboprobe synthesis was carried out using linearized DNA, 20U of the appropriate RNA Polymerase in transcription buffer (Promega, USA) with 1µl of Digoxygenin-RNA labeling mix (Roche Diagnostics, Germany). Hybridization was carried out overnight at 58ºC using 2 µg µl^{-1} of riboprobe in hybridization solution (50% formamide, 4xSSC, 0.1% torula RNA, 0.01% Heparin, 1× Denhart's, 0.1% Tween 20, 0.04% CHAPS). To remove non-specifically bound probe, high stringency washes were carried out. Detection of hybridized probe was done using anti-digoxigenin-AP Fab fragments (1:600, Roche Diagnostics) and colour detection was carried out using as substrate the chromagens NBT and BCIP. Control experiments were performed by treating samples with RNase prior to hybridization and/or by omitting riboprobe from the reaction. Sections were analyzed using a microscope (Olympus BH2) coupled to a digital camera (Olympus DP11).

Results

Histological studies of the thyroid gland indicate that its activity appears to accompany the change in concentration of whole body THs measured by RIA which accompanies halibut development. In first feeding larvae (Fig. 1A) thyroid follicles are visible in the loose connective tissue of the pharynx at the insertion of the gill bars, they are infrequent, small and composed of a flattened unicellular epithelial cell layer surrounding a colloid-filled center. In halibut larvae following the yolk-sack period, vesicles are evident in the periphery of the colloid, and instances are found where the colloid is absent from follicles, suggesting increased thyroid activity. Furthermore, the number and size of thyroid follicles and their apparent activity continually increases until the start of metamorphosis (Fig. 1B) when follicles are numerous of a large diameter and frequently lack colloid, indicative of high thyroid activity. After the climax of metamorphosis thyroid follicles are still abundant but are less active. The variation in the concentration of T_3 and T_4 measured by RIA of whole halibut larval extracts accompanies the activity cycle of the thyroid follicles. Prior to metamorphosis T_4 (2 pg mg^{-1} wet weight, n=6) and T3 (0.5pg mg^{-1} wet weight, n=6) is fairly constant. Larval T_4 (6 pg mg^{-1} wet tissue) and T_3 (2 pg mg^{-1} wet tissue) content peaks at 610 D° just around the start of metamorphosis. After this, the T_4 and T_3 content of larvae declines.

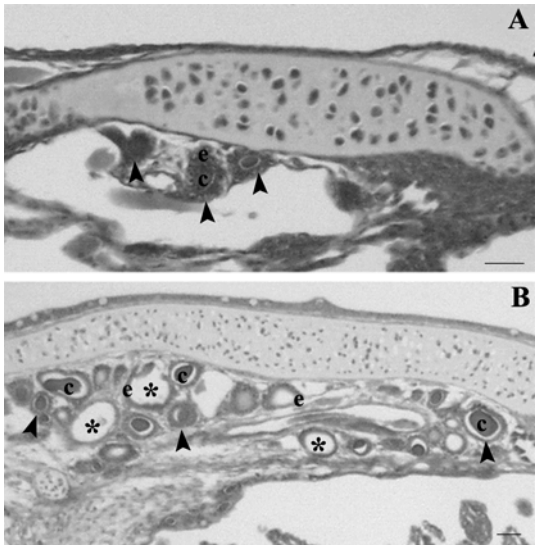

Fig. 1: Cleveland-Wolfe trichrome staining of sagital sections of Atlantic halibut (*Hippoglossus hippoglossus*) larvae showing the development of thyroid follicles (arrowheads). (A) first feeding (stage 5); (B) start of metamorphosis (stage 8); colloid, c; epithelial cell layer, e; follicles lacking colloid, ∗. Scale bars indicates 20µm (Färbung nach Cleveland-Wolfe: Entwicklung der Schilddrüsenfollikel in Sagitalschnitten von Heilbuttlarven (Hippoglossus hippoglossus) (Pfeilspitzen). (A) frühes Stadium (Stadium 5); (B) Start der Metamorphose (Stadium 8); Kolloid c; epitheliale Zellschicht, e; Follikel ohne Kolloid, *. Maßstab 20 µm)

At first feeding (Fig. 2 A) MLC1, MLC2A and MLC3 are expressed and have a similar pattern of distribution, with the hybridization signal being most intense at the periphery, of the dorsal and ventral areas where the germinal zones occur (Fig. 2 A'). The expression levels reached a maximum in white muscle at the beginning of metamorphosis (Fig. 2 B, B') and all three transcripts are highly expressed throughout the epaxial and hypaxial musculature. By the time larvae reached the metamorphic climax (Fig. 2 C), MLC1, MLC2A and MLC3 expression is still high but expression is confined to fibres adjacent to the myosepts and to small cells scattered in the white

muscle. MLC2A is also expressed in the red muscle fibres. A provisional analysis of MLC expression by RT-PCR revealed that there is a gradual increase in the expression up until stage 9 after which it declines. Moreover, there did not appear to be a transition in MLC isoforms during the larval to juvenile transition.

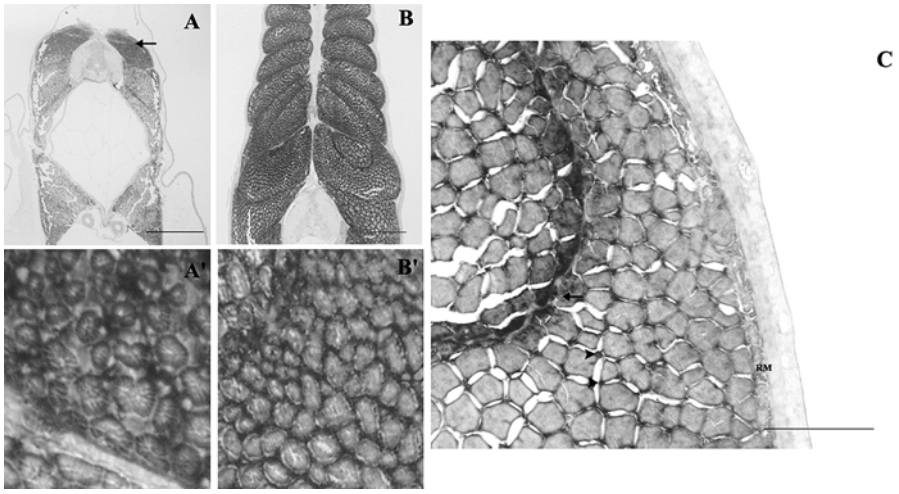

Fig. 2: Determination of temporal and spatial expression of, MLC2a by means of *in situ* hybridization with DIG labeled riboprobe in halibut larvae sections. (A) Stage 5, arrow indicates germinal zones; (A') detail of the germinal zone in (A); (B) stage 8; (B') detail of (B); (C) stage 10, arrow indicates fibers staining adjacent to the myosept; arrowheads indicate positive staining cells; RM, red muscle fibers. Scale bars indicates 200µm (Darstellung der temporalen und lokalen Expression von MLC2a mittels in situ Hybridisierung mit DIG-markierten Riboproben in Schnitten von Heilbuttlarven. (A) Stadium 5, Pfeile zeigen Keimzonen; (A´) Details der Keimzonen aus (A); (B) Stadium 8; (B´) Detail von (B); (C) Stadium10, Pfeil deutet auf Fasern nahe der Myosepten; Pfeilspitzen zeigen positive gefärbte Zellen; RM, rote Muskelfasern, Maßstab 200µm)

Discussion

Associated with the radical change in the volume and organisation of axial muscle which occurs during fish larval development there is a shift in the distribution of myosin isoforms expressed. The ontogeny of thyroid tissue development and activity coincides with the change in muscle which accompanies metamorphosis. The present observations are consistent with the notion that the change in MLC gene expression may be driven by thyroid hormones. Further work will be required to establish if THs act directly or indirectly on gene encoding muscle proteins to change in musculature during metamorphosis. The functional consequence for growth and survival of the changes in musculature which accompany development and metamorphosis requires much more study. As muscle is of key importance for aquaculture production, it will be of importance to establish a better understanding of how muscle development is regulated by hormones.

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Identification and characterization of potential regulatory elements for the porcine MYF5 gene

(Identifizierung und Charakterisierung von regulatorischen Elementen im porcinen MYF5 Gen)

The myogenic regulatory factor *myf5* is integral to the initiation and control of skeletal muscle formation during embryogenesis. *Myf5* expression in adult muscle is restricted to satellite cells. Recent results in mice demonstrated a complex regulation of the prenatal *myf5* expression by a series of genomic elements located as far as 140 kb upstream of the transcription initiation site of the *myf5* gene. The aim of our work was the analysis of the respective genomic region in swine to (I) characterize regulatory elements already described in mice (H1 and H2; Buchberger et al. 2003) and (II) to identify further potential regulatory sequences for *myf5*. Porcine genomic sequences generated by NIH Intramural Sequencing Center were assembled covering 210 kb upstream of the *myf5* transcription initiation site. By comparison of porcine with human and murine sequences conserved elements of varying length were identified. Beside homologues for enhancer elements H1 and H2 we found four additional conserved elements (E1 – E4) in the region -140 to -80 kb of the *myf5* gene. The unique character of these sequences was tested by screening the human genome sequence. The length of these elements varies between 211 and 745 bp with a conservation degree of 83 to 92% between swine and human. All elements contained at least 1 bHLH domain (E-box) among further putative binding motifs. Comparative sequencing of the elements in piglets suffering from splay leg and healthy individuals revealed several polymorphisms, however, no relationship with the disease could be established. In contrast, homology elements H1 and H2 described in mice were monomorphic in all piglets investigated. Our results indicate a similarity of the regulation of *myf5* in mouse and pig. The identified polymorphisms in putative regulatory elements provide a basis for further investigations at the myf5/myf6 loci in swine.

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Variability of myostatin genes in rainbow trout (*Oncorhynchus mykiss*) strains exhibiting distinct hyperplastic growth: preliminary results

Abstract

The distinct growth capacities exhibited by slow- and fast-growing rainbow trout strains were associated with the potential to form new muscle fibres. The structural variability of myostatin genes (myostatin 1 and myostatin 2), negative regulator of muscle growth, was then investigated in trout red muscle. Myostatin cDNAs did not show any missense mutations in its coding sequence, in any of the analysed strains. However, several silent single nucleotide polymorphisms (SNP) were found in both myostatin genes. Other allelic polymorphisms, probably related to the existence of heterozygosity within strains, were also detected in myostatin 1. No sequence variability was found in the myostatin genes, in either of the two strains, which would obviously modify the functional properties of the corresponding proteins.

Key Words: muscle, rainbow trout, hyperplastic muscle growth, Myostatin, polymorphisms

Zusammenfassung

Titel der Arbeit: **Variabilität der Myostatingene bei Regenbogenforellen (*Oncorhynchus mykiss*) - Linien mit unterschiedlichem hyperplastischem Wachstum: Erste Ergebnisse**

Der Zusammenhang zwischen den spezifischen Wachstumseigenschaften langsamer und schnell wachsenden Regenbogenforellen-Linien, mit deren Potential neue Muskelfasern zu bilden, wurde dargestellt. Die strukturelle Variabilität der Myostatingene (Myostatin 1 und Myostatin 2), die das Muskelwachstum negativ regulieren, wurde dann im roten Muskel der Forellen untersucht. Myostatin cDNAs zeigten keine nicht synonymen Mutationen in kodierenden Abschnitten der untersuchten Linien. Jedoch wurden einige stumme Nukleotidpolymorphismen (SNP) in beiden Myostatingenen gefunden. Im Myostatin 1 wurden weitere allele Polymorphismen innnerhalb der Linien gefunden, die auf Heterozygotie hinweisen. Es wurden keine Sequenzvariationen gefunden, die offensichtlich die funktionellen Eigenschaften der Myostatingene beeinflussen.

Schlüsselwörter: Muskel, Regenbogenforelle, hyperplastisches Muskelwachstum, Myostatin, Polymorphismen

Introduction

Genetic selection programs in rainbow trout resulted in the development of strains with distinct growth rates. Recent studies have shown that the growth potential of slow and fast-growing strains, associated with the food ingestion capacity, was a function of the genetic potential for producing new muscle fibres (VALENTE et al., 1998; VALENTE et al., 1999). The fast-growth strain exhibited a higher hyperplastic growth, obtaining a larger body size. Such a difference could be associated with a structural variability of genes, which regulate the proliferation/differentiation of myogenic cells. Since myostatin acts as inhibitory factor of muscle development, limiting muscle fibre formation, a mutation in, myostatin altering the biological activity of the protein, would enhance recruitment of new muscle fibres. Such mutation has been observed in fast-growing cattle strains and recognised as double-muscle phenotype (MCPHERRON and LEE, 1997). The presence of myostatin fish

orthologs has already been reported in various fish species (ROBERTS et al., 2001; RESCAN et al., 2001; RODGERS et al., 2001; MACCATROZZO et al., 2001; OSTBYE et al., 2001). In contrast to mammalian, the expression of Myostatin in fish occurs in a variety of tissues, including skeletal muscle, brain, intestine, gills, ovary and skin (OSTBYE et al., 2001; RADAELLI et al., 2003; ROBERTS and GOETZ, 2001), suggesting that the functional role of fish myostatin may not be restricted to muscle growth regulation, but may have additional functions. Moreover, resulting from recent tetraploidisation of the salmonid genome two myostatin genes have been identified in rainbow trout (RESCAN et al., 2001). Trout myostatin 1 and 2 proteins are highly similar (94%), but exhibit a different expression, being myostatin 2 mRNA predominantly expressed in red muscle (RESCAN et al., 2001) and probably functions to regulate growth. Recent research studies with transgenic zebrafish overexpressing the myostatin prodomain (XU et al., 2003) and transgenic salmon exhibiting decreased myostatin expression (ROBERTS et al., 2004) both showed enhanced hyperplasia, suggesting that myostatin inhibits fish muscle growth, as it does in mammals. However, allelic polymorphisms in the fish myostatin gene have never been described. The key role of Myostatin in muscle growth has prompted us to investigate whether the different growth potential of rainbow trout strains could be associated with significant polymorphism in the myostatin gene.

Materials and Methods

Fast (C) and slow-groing (M) rainbow trout (*Oncorhynchus mykiss*) strains were grown until adult size in the experimental fish farm at INRA, Drennec, France. A sample of red muscle was removed from the lateralis muscle and stored in liquid nitrogen. Total RNA was isolated with Trizol reagent according to the CHOMCZYNSKI and SACCHI (1987) method and quantified by absorbance at 260nm. RNA yield and quality were analysed by electrophoresis in ehtidium bromide-stained gel. The Access RT-PCR system (Promega) was used for cDNA synthesis and amplification. The primer sets used for myostatin 1 and myostatin 2 synthesis were obtained from the coding region (RESCAN et al., 2001). The reverse transcription and PCR reaction conditions were as follows: 1 μg of total RNA, AMV/Tfl5x Reaction buffer, 10 mM of each dNTP, 25 mM MgSO4, 1 μmol l^{-1} upstream primer (ATCATGACAATGGCCACTGAA) and 1 μmol l^{-1} downstream primer (TGCCCGTTCATTCGCTGAGC) to generate myostatin 1, 1 μmol l^{-1} upstream primer (ATCATGACAATGGCCACTGAA) and μmol l^{-1} downstream primer (CCCTGTCCATTCGCTGAGC), 1 μmol l^{-1} and 1 μmol l^{-1} upstream primer (TTCACGCAAATACGTATTCA) and 1 μmol l^{-1} downstream primer (CGGGCACAGCTATGGGTGCAC) to generate myostatin 2, 5 units μl^{-1} of AMV reverse transcriptase and 5 units μl^{-1} of thermostable Tfl DNA Polymerase. The amplification conditions were: denaturation at 94 ºC for 30 s, annealing at 60 ºC for 1 min and extension at 68 ºC, 2 s. The PCR products were analysed on a 1% agarose gel. Amplification products were extracted from agarose gel (Kit Ultrafree-DA Milipore, Amicon) and purified (Microcon, Amicon). Primers and excess nucleotides were eliminated (Kit ExoSAP-IT, Amersham Pharmacia). After purification, amplification products were sequenced using the ABI PRISM Big Dye Terminator kit (Applied

Biosystems) and an automatic sequencer (Applied Biosystems), with the same primers used for cDNA synthesis and amplification. The sequences were submitted to the NCBI database using the BLAST program (http://www.ncbi.nlm.nih.gov/BLAST). Myostatin 1 and 2 sequences exhibited similarities with trout myostatin 1 (Tmyostatin 1, accession number AF273035) and myostatin 2 (Tmyostatin 2, accession number AF273036) respectively. Myostatin gene sequences were then aligned and compared (Autoassembler, Applied Biosystems).

Results

The myostatin 1 and 2 gene sequences were obtained by aligning overlapping sequences (Figure 1 and 2). In both rainbow trout strains analysed no mutations were identified in myostatin 1 gene sequence capable of promoting alterations in the coding sequence of the gene. However, a non-synonymous substitution (transition T→C) was found outside the protein coding region, localized at nucleotide 1136 (Table). In addition, nucleotide substitutions that did not affect the aminoacid sequence were found in both strains.

```
-59    AT GCC AAT ACA TAT TAC ATT TGG GAT TCA ATT TTT ATA GCA AAC TCC GCA CCT TAG ATA     -1
1    ATG CAT CTG ACG CAG GTT CTG ATT TAT CTC AGT TTC ATG GTT GCT TTC GGT CCA GTG    57
     Met His Leu Thr Gln Val Leu Ile Tyr Leu Ser Phe Met Val Ala Phe Gly Pro Val
58   GGT CTT GGT GAT CAA ACC GCG CAC CAC CAG CCC CCT GCC ACG GAT GAC GGC GAG CAG   114
     Gly Leu Gly Asp Gln Thr Ala His His Gln Pro Pro Ala Thr Asp Asp Gly Glu Gln
115  TGC TCA ACA TGC GAG GTC CGA CAG CAG ATC AAA AAC ATG AGA TTA CAC GCC ATC AAG   171
     Cys Ser Thr Cys Glu Val Arg Gln Gln Ile Lys Asn Met Arg Leu His Ala Ile Lys
172  TCC CAA ATT CTT AGC AAA CTG CGA CTC AAG CAA GCT CCC AAT ATC AGC AGA GAT GTT   228
     Ser Gln Ile Leu Ser Lys Leu Arg Leu Lys Gln Ala Pro Asn Ile Ser Arg Asp Val
229  GTC AAG CAG CTC CTG CCT AAG GCA CCA CCT TTG CAG CAA CTT CTT GAC CAG TAC GAT   285
     Val Lys Gln Leu Leu Pro Lys Ala Pro Pro Leu Gln Gln Leu Leu Asp Gln Tyr Asp
286  GTT CTT GGA GAT GAC AAT AAG GAT GGA CTT ATG GAA GAA GAT GAT GAA CAT GCC ATC   342
     Val Leu Gly Asp Asp Asn Lys Asp Gly Leu Met Glu Glu Asp Asp Glu His Ala Ile
343  ACA GAA ACA ATC ATG ACA ATG GCC ACT GAA CCC GAA TCC ATC GTC CAA GTC GAT CGG   399
     Thr Glu Thr Ile Met Thr Met Ala Thr Glu Pro Glu Ser Ile Val Gln Val Asp Arg
400  AAA CCC AAG TGT TGC TTA TTC TCC TTC AGT TCG AAG ATT CAG GTG AAC CGC ATA GTT   456
     Lys Pro Lys Cys Cys Leu Phe Phe Phe Ser Ser Lys Ile Gln Val Asn Arg Ile Val
457  CAT GCG CAG TTA TGG GTG CAC CTT TTG CCA GCT GAC GAA GTC ACC ACC GTG TTT CTG   513
     His Ala Gln Leu Trp Val His Leu Leu Pro Ala Asp Glu Val Thr Thr Val Phe Leu
514  CAA ATC TCC CGC CTG ATG CCT GTC ACG GAC GGG GGC AGG CAC ATA GGT ATC CGG TCT   570
     Gln Ile Ser Arg Leu Met Pro Val Thr Asp Gly Gly Arg His Ile Gly Ile Arg Ser
571  CTA AAG ATC GAC GTG AAT GCA GGA GTC AGC TCT TGG CAA AGT ATC GAC GTG AAA CAA   627
     Leu Lys Ile Asp Val Asn Ala Gly Val Ser Ser Trp Gln Ser Ile Asp Val Lys Gln
628  GTG CTG TCC GTA TGG CTG CGG CAG CCG GAG ACG AAT TGG GG* ATC GAG ATT AAT GCG   684
     Val Leu Ser Val Trp Leu Arg Gln Pro Glu Thr Asn Trp Gly Ile Glu Ile Asn Ala
685  TTC GAC TCG AAG GGA AAT GAT CTG GCG GTT ACC TCA GCA GAA GCG GGA GAA GGA CTG   741
     Phe Asp Ser Lys Gly Asn Asp Leu Ala Val Thr Ser Ala Glu Ala Gly Glu Gly Leu
742  CAA CCC TTC ATG GAG GTG ACG ATT TCA GAG GGC CCA AAG CGC TTC AGG AGA GA* TCG   798
     Gln Pro Phe Met Glu Val Thr Ile Ser Glu Gly Pro Lys Arg Phe Arg Arg Asp Ser
799  GGC CTG GAC TGT GAC GAG AAC TCC CCC GAG TCC CGC TGT TGC CGC TAC CCG CTC ACG   855
     Gly Leu Asp Cys Asp Glu Asn Ser Pro Glu Ser Arg Cys Cys Arg Tyr Pro Leu Thr
856  GTA GAC TTT GAA GAC TTT GGC TGG GAC TGG ATT ATT GCC CCC AAG CGC TAC AAG GCC   912
     Val Asp Phe Glu Asp Phe Gly Trp Asp Trp Ile Ile Ala Pro Lys Arg Tyr Lys Ala
913  AAC TAC TGC TCT GGT GAG TGC GAG TAC ATG CAC CTG CAG AAG TAC CCC CAC ACC CAC   969
     Asn Tyr Cys Ser Gly Glu Cys Glu Tyr Met His Leu Gln Lys Tyr Pro His Thr His
970  CTG GTG AAC AAG GCT AAC CCT CGC GGC ACT GCC GGG CCC TGT TGC ACC CCC ACC AAG  1026
     Leu Val Asn Lys Ala Asn Pro Arg Gly Thr Ala Gly Pro Cys Cys Thr Pro Thr Lys
1027 ATG TCC CCC ATC AAC ATG CTC TAC TTC AAC CGC AAA GAG CAG AT* ATC TAC GGC AAG  1083
     Met Ser Pro Ile Asn Met Leu Tyr Phe Asn Arg Lys Glu Gln Ile Ile Tyr Gly Lys
1084 ATC CCC TCC ATG GTG GTG GAC CGA TGC GGC TGC TCG TGA GCG AGA GCT CTG C*G GTG  1140
     Ile Pro Ser Met Val Val Asp Arg Cys Gly Cys Ser Ter
1141 AGG GGG AGG GGC TCA GCC AGG GTC TCC CCC CTG GAC TTT GGG ACA GAT CCA TCC ACC  1197
1198 ACT ACC AGT GCT TTC TGC AGA ACA CGG TGC AAT AGA GCC AGA ATA GCG   1245

ATG- Initiation codon        TGA- Stop-codon
```

Fig. 1: Red muscle Myostatin 1 cDNA sequences of rainbow trout strains, C and M. The predicted amino-acid sequences are indicated below the corresponding coding region by abbreviations. Numbering starts with the first base of the initiator codon. Codon differences are in *italics* with the single nucleotide polymorphisms (SNPs) indicated by a star. Cystein residues characteristic of myostatin are indicated with grey shading and putative proteolitic processing site is boxed (Myostatin 1 cDNA Sequenz von roten Muskeln der Regenbogenforellen-Linien, C und M. Die abgeleitete Aminosäuresequenz ist unter der kodierenden Sequenz angegeben. Die Numerierung startet am Intitiationskodon. Variable Kodons sind kursiv mit Stern an der variablen Stelle. Cysteine, charakteristisch für Myostatin sind schattiert, putative proteolytische Stellen umrahmt)

As for myostatin 1, no mutation capable of promoting alterations in the coding sequence of this gene that would affect the activity of the resulting protein was detected in the myostatin 2 gene, in the two analysed strains. However, comparison of myostatin 2 sequences enabled us to identify three alterations (Table). Two transitions were identified, G→A and C→T, positioned respectively at nucleotides 111 and 186. However, these transitions did not lead to the substitution of the codified amino acid,

corresponding to silent polymorphisms. A third transition was identified, A→G, positioned at nucleotide 394. This alteration led to a conservative substitution of asparagine, in strain M, by aspartic acid, in strain C. Nevertheless, these amino acids are localized in the non-active domain of the protein, which has to be cleaved to begin its biologic activity.

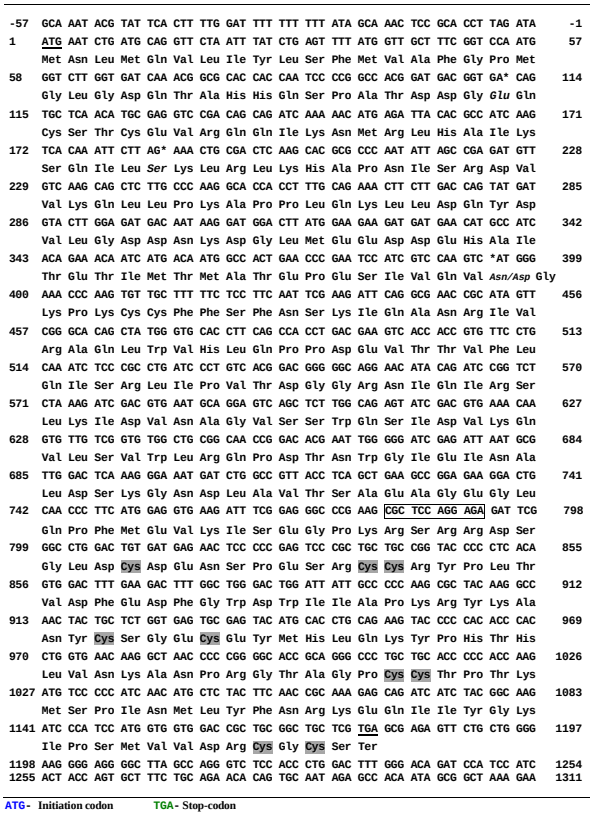

Fig. 2: Red muscle Myostatin 2 cDNA sequences of rainbow trout strains, C and M. The predicted amino-acid sequences are indicated below the corresponding coding region by abbreviations. Numbering starts with the first base of the initiator codon. Codon differences are in *bold* with the single nucleotide polymorphisms (SNPs) indicated by a star. Cystein residues characteristic of myostatine are indicated with grey shading and putative proteolitic processing site is boxed (Myostatin 2 cDNA Sequenz von roten Muskeln der Regenbogenforellen-Linien, C und M. Die abgeleitete Aminosäuresequenz ist unter der kodierenden Sequenz angegeben. Die Numerierung startet am Intitiationskodon. Variable Kodons sind kursiv mit Stern an der variablen Stelle. Cysteine, charakteristisch für Myostatin, sind schattiert, putative proteolytische Stellen umrahmt)

Table
Myostatin 1 and 2 nucleotide localization leading to sequence alteration, in two rainbow trout strains (C e M) (Variable Positionen in Myostatin 1 und 2 der beiden Regenbogenforellenlinien (C und M))

Altered nucleotide position	Codon		Amino acid change
	Strain M	Strain C	
Myostatin 1			
669 (3rd position)	GG***A*** / GG***G***	GG***A*** / GG***G***	null
795 (3rd position)	GA***T*** / GA***C***	GA***T*** / GA***C***	null
1071 (3rd position)	AT***C*** / AT***A***	AT***C*** / AT***A***	null
1136 (2nd position)	C***C***G / C***T***G	C***C***G / C***C***G	null / null
Myostatin 2			
111 (3rd position)	GA***G***	GA***A***	null
186 (3rd position)	AG***C***	AG***T***	null
394 (1st position)	***A***AT	***G***AT	Asparagine ↓ Aspartic acid

Discussion

Myostatin 1 and 2 cDNAS were characterized in two trout strains exhibiting distinct hyperplatic growth. Sequencing reveals several single nucleotide polymorphisms (SNPs) but no significant mutations were detected, however, that would explain the high growth performance of the fast-growth strain (strain C). This is different from the situation in several double-muscled cattle breeds, which have mutations affecting the normal activity of myostatin gene (GROBET et al., 1997; MCPHERRON and LEE, 1997; KAMBADUR et al., 1997; GROBET et al., 1998; SMITH et al., 2000).

Despite the lack of any mutation affecting aminoacid sequence in myostatin 1 and 2 genes sequences from either rainbow trout strain, several SNPs were found in the sequences analysed and some of these changes have led to a non-synonymous substitution. In myostatin 1 gene a non-synonymous substitution was identified, however, this change is localised outside of the protein-coding region. In myostatin 2 genes, besides the identification of two silent nucleotide substitutions, a non-synonymous substitution was observed giving asparagine in strain M and aspartic acid in strain C. However, this change is localised in the non-active prodomain of the protein, which has to be cleaved for biologic activity. XU et al. (2003) have recently reported an increased number of myofibers in skleletal muscles of adult transgenic zebrafish overexpressing the Myostatin prodomain, although no effect on the expression of myogenic regulatory genes was observed (XU et al., 2003). Moreover, HILL et al. (2002) demonstrated that the myostatin prodomain was able to bind to the bioactive Myostatin C-terminal active domain and inhibit its biological activity, presumably preventing Myostatin from binding to a receptor and blocking its function. Further studies are required in order to ascertain whether the observed non-synonymous substitution in the rainbow trout prodomain could have any interference with Myostatin function. The results of this study did not show the existence of variability in myostatin gene sequences of the two rainbow trout strains, which would obviously modify the functional properties of the corresponding proteins. Thus, growth differences observed between both strains cannot be attributed to structural variations in the coding domain of the gene. Nevertheless, we cannot exclude the existence of variations in the promoter or intronic domains of the gene, which could cause differences in myostatin gene transcription and ultimately muscle growth.

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Target genes of myostatin loss-of-function in bovine foetuses

(Zielgene der Myostatin-Mutation bei bovinen Feten)

Myostatin is a highly conserved Transforming Growth Factor beta family member which negatively regulates muscle growth. Double-muscled (DM) cattle have a loss-of-function mutation in their myostatin gene. They display a hypermuscular mass due to an increase in the number and in the aera of muscle fibres. Thus these animals represent a good model to understand the underlying mechanisms at the origin of muscular hypertrophy.

In this study, we have analysed the expression profiles in the *semitendinosus* muscle of 260-day-old DM foetuses compared to that of normal cattle (NM) foetuses (n=3 per group), using high density oligonucleotide arrays. Our aim was to identify the genes that are differentially regulated between DM and NM animals in order to identify individual genes or networks that may be myostatin targets.

A great number of genes was found to be differentially expressed according to the genetic type, some with a fold change higher than 5. They belonged to various functional categories. The genes underexpressed in DM foetuses corresponded mainly to genes encoding extracellular matrix proteins, slow contractile proteins and ribosomal proteins. On the other hand, the genes overexpressed in DM foetuses were involved mainly in the regulation of transcription, cell cycle/apoptosis, translation, DNA metabolism and neurogenesis. These data are in agreement with the so-called characteristics of DM animals. They may also illustrate that a longer proliferation period or a later generation of fibres might occur at the end of foetal life in the DM animals. Interestingly, 63 genes out of the 1083 differentially-declared genes were also differentially expressed in DM cows. Among the latter, C1QTNF3 (adipocyte differentiation marker) and HDAC4 (controlling the transcription) may be important target genes.

Thus, the myostatin loss-of-function mutation affects several physiological processes involved in the development and the determinism of the functional characteristics of the muscle.

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Analysis of the differential transcriptome expression profiles during prenatal muscle tissue development in pigs

Abstract
In this contribution two microarray experiments are reviewed aiming to describe (1) the differences in the expression profiles of Duroc and Pietrain pigs during prenatal muscle tissue development, and (2) The changes in the expression profiles of genes related to myogenesis in Duroc pigs. Furthermore, we describe bioinformatics and pathway analyses methods to extract biological meaningful knowledge.

Key Words: Pig breeds, Myogenesis, Microarray, Bioinformatics, Pathway analysis

Zusammenfassung
Titel der Arbeit: **Analyse der differentiellen Transkript-Expressionsprofile während der pränatalen Muskelentwicklung beim Schwein**
Der Beitrag erläutert zwei Microarray Experimente die darauf abzielen, (1) die Unterschiede in den Expressionsprofilen von Duroc- und Pietrainschweinen während der prenatalen Muskelentwicklung aufzuzeigen und (2) die Änderungen der Expressionsprofile der Gene zu beschreiben, die in der Myogenese bei Durocschweinen relevant sind. Außerdem beschreiben wir Bioinformatik- und Pathway-Analyse-Methoden, um biologisch sinnvolle Erkenntnisse abzuleiten.

Schlüsselwörter: Schweinezucht, Myogenesis, Microarray, Bioinformatik, Pathway-Analyse

Introduction
Mammalian myogenesis, the formation of new multinucleated muscle fibres from mononucleated precursor cells called myoblasts, is an exclusive prenatal process determining muscle characteristics such as fibre numbers, which may be related to muscle strength and function (REHFELDT et al., 2000). Muscle fibre formation takes place in two waves, the primary and secondary muscle fibre formation (WIGMORE and EVANS, 2002). Each wave consists of proliferation of myoblasts and fusion to form new muscle fibres. While primary muscle fibres form de novo, secondary myofibres form using the primary fibres as a template.
Myogenesis is under complex genetic regulation. The Muscle Regulatory Factors (MRF) gene family are known to be transcription factors activating muscle-specific genes during different stages of myogenesis (OLSON, 1990; WEINTRAUB et al., 1991). The expression of the MRF genes is under tight temporal and spatial regulation, and numerous factors affecting MRF expression levels are known. A network of genes affects the expression patterns of the MRF genes (OLSON, 1993; RAWLS and OLSON, 1997; CAPDEVILA and JOHNSON, 2000; DOBOSY and SELKER, 2001; KITZMANN and FERNANDEZ, 2001; LEE et al., 2001; ZHU et al., 2001). By doing so they affect muscle and body growth potential.
Pig breeding has mainly focused during the past decades on improving growth rate and muscularity (MERKS, 2000). Pig breeds differ in muscle traits such as muscularity, muscle fibre type, colour, etc. For example, Duroc are slow growing pigs with a relatively high intramuscular fat content (SELLIER, 1998) and relatively red muscle

fibre types. Pietrain pigs are faster growing pigs with relatively low intramuscular fat content (JONES, 1998; SELLIER, 1998) and whiter muscle fibre types. Also overall fatness of Duroc pigs is greater than Pietrain pigs. These two breeds are considered to represent extremes of modern western pig breeds. It can be expected that differences in myogenesis are a major underlying mechanism for the observed phenotypes.
Using microarray technology we studied the porcine expression of genes known to affect myogenesis in laboratory animals and *in vitro* model systems. Microarray technology can simultaneously measure the differential expression of a large number of genes in a given tissue and may identify the genes involved in different phenotypes. Typically, microarray experiments produce long lists of genes that are differentially expressed between two different situations. This does not necessarily shed light on the underlying complex genomic regulation that creates the different phenotypes. Here we discuss several of our experiments to elucidate the genetic background of the myogenesis in pigs and the differences between pig breeds (TE PAS et al., 2005a,b; CAGNAZZO et al., 2006), and we extend these studies using bioinformatics.

Materials and Methods

Microarrays and analyses:
For details of the experiments see TE PAS et al. (2005a,b) and CAGNAZZO et al. (2006).

Pathway analysis
For pathway analysis we used the KEGG (Kyoto Encyclopaedia of Genes and Genomes) and BioCarta data bases (resp. http://www.genome.ad.jp/kegg/, and http://www.biocarta.com/). The databases were searched with lists of differentially expressed genes using either home made software (http://www.do.asg.wur.nl/research/researchprojects2.asp?projectnr=105) or GoMiner (http://discover.nci.nih.gov/gominer/). Furthermore, the Spotfire software package (http://www.spotfire.com/) was used to investigate for putative pathways that are presently not covered by either of the two databases.

Results

Comparison of the Myogenesis-associated transcriptomes of Duroc and Pietrain (TE PAS et al., 2005; CAGNAZZO et al., 2006)
Genes were grouped into three major groups: myogenesis, energy metabolism, and muscle structural genes. Results were analysed for (1) up / down regulation - i.e. the log ratio between the expression level in Duroc and the expression level in Pietrain (M-value), and (2) for general expression level (average of log intensities or A-value).
Energy metabolism. A major difference between the energy metabolism in Duroc and Pietrain embryos and foetuses was observed at all gestational ages. The energy metabolism in the Pietrain is at a higher level than in the Duroc except 35 d of gestation where a reversed situation was found.
The expression profile of the genes of the fatty acid metabolism indicate that fatty acid metabolism is at a higher level in early Duroc embryos (d 14 - 49 of gestational age) compared to Pietrain embryos while the reverse situation is found in older foetuses from d 63 of gestation and onwards suggesting that the observed overall higher fatness in Duroc compared to Pietrain already develops in the early embryo.

Myogenesis. The transcriptome expression profiles of myogenesis related genes suggests that myogenesis starts up earlier in Duroc than in Pietrain. From 49d and onwards with the exception of the differentiation-inhibiting group of genes Pietrain shows increased myogenesis. The results suggest that early primary myogenesis more relates to Duroc muscle fibre formation while Pietrain muscles are more formed during secondary muscle fibre formation. The expression profiles of the *muscle structural genes* support the results of the myogenesis.

The Duroc-specific Myogenesis-associated transcriptome profiles (TE PAS et al., 2005a, b)

The genes involved in muscle fiber formation, i.e. differentiation-stimulating, differentiation-inhibiting, and muscle fiber structural genes, show a peak expression around day 35. The genes regulating myoblast proliferation show lower peak levels a few days earlier. The gene-activation profiles coincide in general with these profiles. Together the results suggest that the switch from myoblast proliferation to myoblast fusion (differentiation) is regulated by a decreasing number of expressed proliferation regulating genes and an increasing number of expressed differentiation regulating genes.

Furthermore, expression of glycolysis metabolism genes is at a nadir at the two period's central in differentiation: around days 35 and 49-63. ATP metabolism follows that profile later in time while oxidative phosphorylation has less variable expression. Together these results suggest that energy metabolism is coupled to myoblast proliferation and differentiation, but a possible causal relationship remains unclear.

Pathway analysis

Genes with known pathway information

While these results show interesting details associated with the regulation of myogenesis, the biological mechanisms that are active during those processes remain poorly understood. It will be necessary to take the lists of genes that are differentially regulated and compare them with physiological data to find the biological active components of these processes. That is where we started to analyse the active pathways of those genes. Physiological pathway information was extracted from the KEGG and BioCarta databases. The genes that were active in each represented pathway were grouped together and the myogenesis-associated transcriptome profiles in Duroc were compared within each relevant pathway. Over 20 different pathways were found to be active in myogenesis. The number of genes within each pathway varied from only two genes to more than 20 genes. Individual gene expression profiles were recorded over developmental age as the M-values for each two successive prenatal ages. Figure 1 shows two examples. In Figure 1A the TGF-β pathway represented by 12 genes shows that all genes in the pathway have a peak level at 35-49 days of age followed by a decrease in expression to a nadir at days 63-77 and an increase towards the end of the profile at day 91 of prenatal development. The beta-Catenin pathway in Figure 1B represented by 20 genes does not show such an ordered profile. Several genes show peak levels where others show nadirs, and no general profile can be observed. If this occurs we ordered the genes in profiles such that the genes within a profile have a similar expression profile. In the beta-Catenin pathway at least two profiles can be described. One profile with genes showing a peak value around day 40, a nadir around day 60 and a peak at day 91, a second profile shows

exactly the opposite profile. Additionally, a third profile could be assigned with genes showing no differential expression from day 14 to day 50, a nadir around day 60 and a peak level at the end of the profile. This suggests that this pathway could be seen as three independent pathways (related to the expression profiles) each independently regulated.

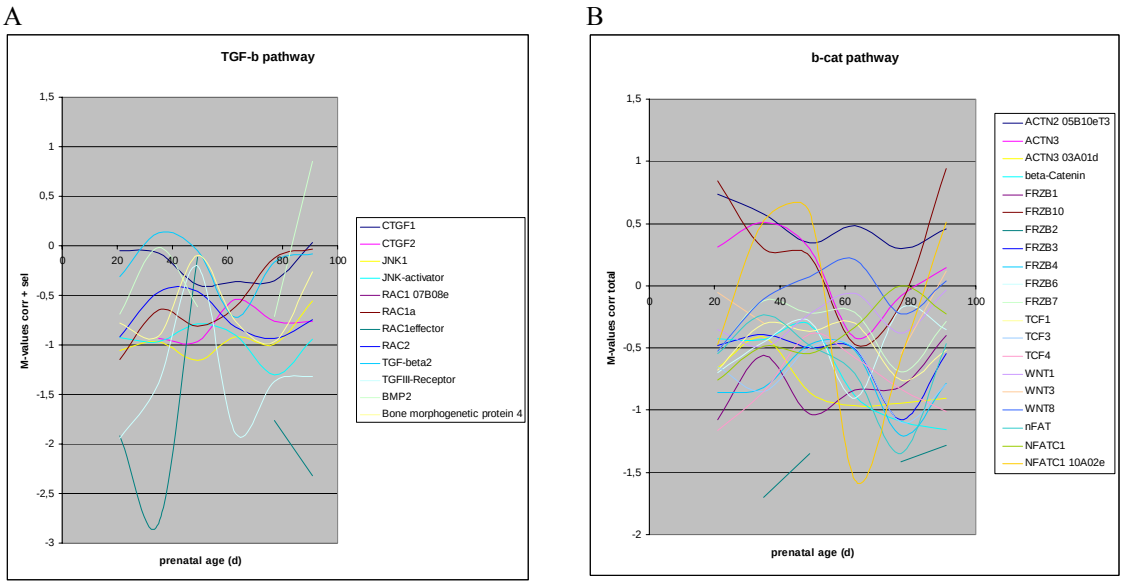

Fig. 1: Expression profiles of genes in two pathways influencing myogenesis: The TGF-β (A) and the beta-Catenin (B) pathways (Expressionsprofile von Genen zweier Reaktionswege der Myogenese: TGF-β (A) und the beta-Catenin (B) Pathways)

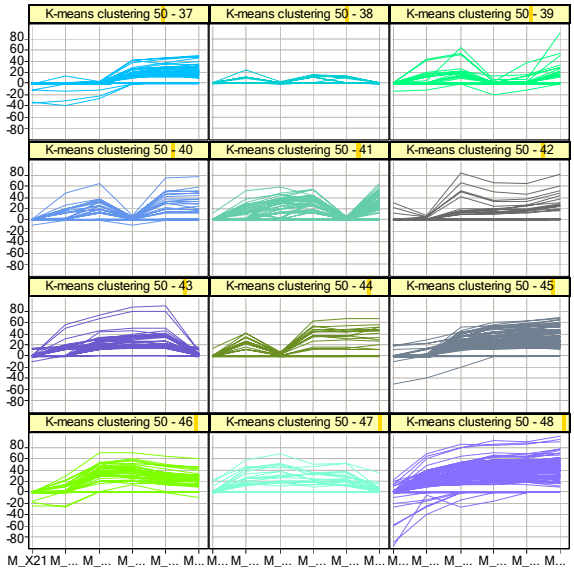

Fig. 2: Cluster analysis using K-means clustering (Klusteranalyse mit dem K-means Algorithmus)

Genes without known pathway information

Many genes on the microarray have no information in pathway databases, either because no information is available, or because the identity of the spot remains unknown. To improve our knowledge about the process it would be interesting to add pathway information of these genes to the myogenesis regulating pathways. Cluster analysis using the K-means clustering method can cluster genes with similar

expression profiles together. It can be assumed that genes with similar expression profiles have similar regulation of expression and can therefore be in common pathways or in pathways that act together. Figure 2 shows an example of part of a clustering analysis. It can be seen that the genes within a cluster have very similar expression profiles while major differences exist between the expression profiles of the genes in different clusters. Each cluster consists of spots of genes with known pathway information and genes without such information. It can be assumed that genes that cluster together act either in the same pathway or act in pathways that function together.

Discussion

With the onset of the "omics" sciences biology moved from studying isolated steps of a process towards integration of the study of live as a whole. However, the information content of the "omics" experiments is huge and it is a major task to extract biological meaningful knowledge. With the integration of the diverse "omics" sciences (transcriptomics, proteomics, metabolomics) this task will only become more complex. In the experiments that we have reported on myogenesis we only studied transcriptomic data, and we are still analysing the results in order to understand the biology behind the data. In this paper we shortly reviewed the previously published experiments and we indicated possible routes that we are working on to understand the biology of the regulation of myogenesis. Pathway information tells us how genes act together in a single or multiple pathways to perform myogenesis. Pathway information may tell us how the expression of all these genes is regulated in a large system. Understanding pathways and interactions between pathways may give us understanding on the functioning of the genome. These analyses are still going on but the first results are promising: we can now see that some pathways are most probably regulated as a single unit while others seem to have more complex regulation.

Although physiology has uncovered many pathways still many other pathways are only poorly known, whilst others remain unknown. Therefore, we are trying to add more genes to known and unknown pathways. Cluster analysis groups genes with similar expression profiles suggestive for similar regulation. Although no proof is given that genes within a cluster belong to the same pathway such results may be the starting point for further research. Adding more pathways of life to our knowledge will help us understand how life functions.

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Linkage and expression analysis to elucidate the genetic background of muscle structure and meat quality in the pig

Abstract

Genome scans are the most general approaches to identify genomic regions exhibiting quantitative trait loci, QTL, without prior hypothesis of the physiology and genetic control of a trait. Function-oriented expression analyses are a complementary approach to derive hypotheses of the physiologic and genetic background of phenotypic variation. The proportion of muscle fibre types and their size affect body composition traits, muscularity and obesity as well as and functional properties of skeletal muscle and meat quality. We detected QTL for microstructural and biophysical muscle properties as well as traits related to muscling and obesity in a porcine experimental population that is based on Duroc and Berlin Miniature Pig. Regions with either significant QTL for muscle fibre traits or significant QTL for meat quality and muscularity or both were detected on chromosomes 1, 2, 3, 4, 5, 13, 14, 15, and 16. Here effects on the complex traits of muscularity, obesity, metabolic type and biophysical muscle characteristics might be the result of genetic variation primarily affecting fibre type distribution traits. In order to complement the QTL approach by displaying trait-associated expression-profiles and detection of eQTL (expression QTL) we evaluated the quantification of transcripts of the myosin heavy chain isoforms, MYHC isoforms, by real time PCR as a new phenotype that was found to be significantly correlated to results of fibre typing by ATPase staining. This new phenotype is probably more suitable to unravel the genetic background of variation in traits related to muscle and meat properties than technological meat quality parameters and conventional fibre typing.

Key Words: QTL, muscle fibre, meat quality, myosin heavy chain isoforms, transcript quantification, pig

Zusammenfassung

Titel der Arbeit: **Kopplungs- und Expressionanalysen zur Klärung der genetischen Grundlagen mikrostruktureller und biophysikalischer Merkmale des Muskels beim Schwein**

Genomscans stellen einen Ansatz zur Identifizierung von QTL, quantitative trait loci, dar, der ohne vorherige Hypothese über die Physiologie und die genetische Steuerung eines Merkmals auskommt. Funktions-orientierte Genexpressionsanalysen stellen einen komplementären Ansatz dar zur Ableitung von Hypothesen über den physiologischen und genetischen Hintergrund phänotypischer Variation. Die Verteilung der Muskelfasertypen und ihre Größe beeinflussen Merkmale der Körperzusammensetzung, des Muskel- und Fettansatzes, der Funktion der Muskulatur und der Fleischqualität. Wir haben QTL für mikrostrukturelle und biophysikalische Muskeleigenschaften sowie Merkmale der Schlachtkörperzusammensetzung in einer porcinen Ressourcen-population basierend auf Duroc und Berliner Miniaturschwein identifiziert. Regionen mit entweder genomweit signifikanten QTL für Muskelfasermerkmale oder genomweit signifikanten QTL für Fleischqualität und Schlachtkörpermerkmale oder beides wurden auf Chromosomen 1, 2, 3, 4, 5, 13, 14, 15 und 16 ermittelt. Hier könnten Effekte auf die komplexen Merkmale der Körperzusammensetzung, des Stoffwechseltyps und der Fleischqualität das Ergebnis genetischer Variation mit direktem Einfluss auf Muskelfasermerkmale sein. Um diese Ergebnisse durch die Darstellung Merkmals-abhängiger Expressionsprofile und die Identifizierung von eQTL (Expressions-QTL) zu ergänzen, haben wir Echtzeit-PCR zur Quantifizierung der Transkripte der Isoformen der schweren Ketten des Myosins (myosin heavy chain MYHC) als neuen Phänotyp evaluiert, der signifikant mit Ergebnissen der ATPase-Muskelfasertypisierung korreliert. Dieser neue Phänotyp ist potentiell besser geeignet den genetischen Hintergrund der Variation in Merkmalen der Muskel- und Fleischeigenschaften zu beleuchten, als technologische Fleischqualitätsparameter und das herkömmliche Verfahren der Muskelfasertypisierung.

Schlüsselwörter: QTL, Muskelfasern, Fleischqualität, Myosin-Isoformen, Transkriptquantifizierung, Schwein

Introduction

Basic principles of QTL analyses

Researchers have used different strategies to detect genes controlling quantitative traits. Genome scans are the most general approaches to identify genomic regions exhibiting quantitative trait loci, QTL, without prior hypothesis of the physiology and genetic control of a trait.

The phenotypic variation, Vp, among individuals in a population such as F2 for any trait can be easily measured. Vp is the result of genetic and environmental components, i.e. Vp=Vg + Ve. The proportion of Vp arising from genetic causes is the heritability of that trait in that population, h^2=Vg/Vp. Vg represents the combined effects of all QTL. By combining the measurement of phenotypic variation and genotypic variation, using a number of unambiguous single site genetic markers, it is possible to study individual QTL. Within pedigrees the co-segregation of trait phenotypes and marker genotypes is observed. Among the number of markers distributed throughout the genome at least some will be linked to QTL for the trait of interest. QTL analysis depends on the fact that where such linkage occurs, the marker locus and the QTL will not segregate independently but linkages disequilibrium exists within the pedigrees examined. Differences in those marker genotypes will be associated with different trait phenotypes (Figure 1).

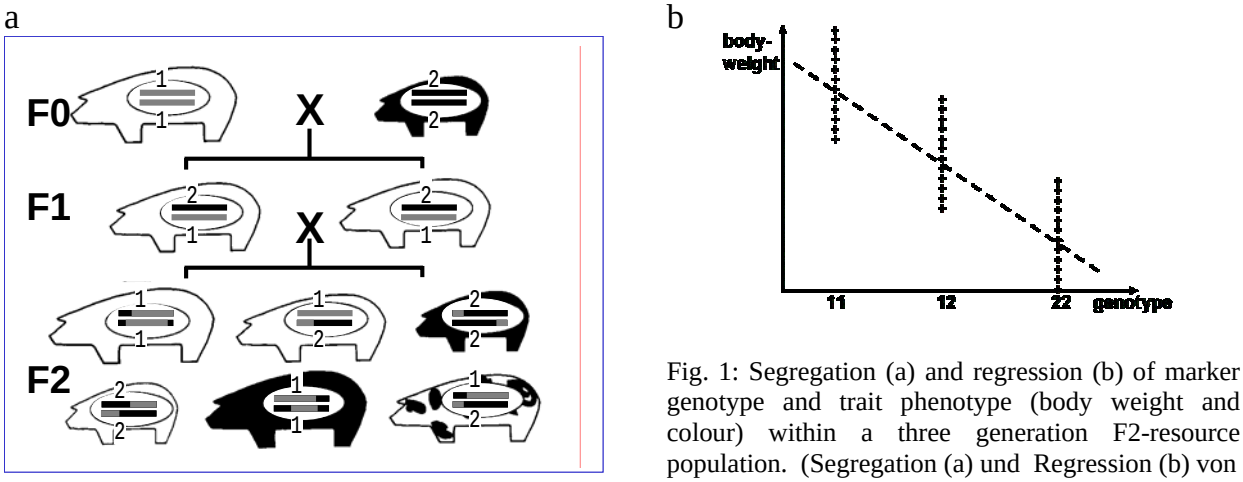

Fig. 1: Segregation (a) and regression (b) of marker genotype and trait phenotype (body weight and colour) within a three generation F2-resource population. (Segregation (a) und Regression (b) von Markergenotyp und Merkmal (Körpergewicht und Farbe) innerhalb einer Drei-Generationen F2-Ressourcen-Population).

F2 animals that inherited the marker genotype 11 are large but either white or black; animals of the genotype 22 are small and also either black or white; animals with the genotype 12 are of intermediate weight and also either black or white. Thus the marker locus investigated is in linkage to a QTL for body weight; the marker allele 1 is in linkage disequilibrium with the QTL+ allele. Interval mapping based on least square regression analysis involves estimation of additive genetic effects as half of the difference of the trait value between homozygous carriers of alternative QTL alleles, i.e. the QTL alleles derived from the divergent founder populations. Dominance effects are estimated as the difference between the trait value of heterozygous individuals and the mean trait value observed for homozygous animals. Subsequently, additive and dominant coefficients at fixed positions in the genome of each F2 animal and their phenotypic values were regressed onto the additive and dominance coefficients in intervals of 1 cM.

In order to estimate the most likely position of a QTL, its effects, and to test its significance several statistical approaches have been developed and implemented in various softwares. The most commonly used analytical approaches explore the interval between pairs of markers for the presence of QTL (interval mapping) (LANDER and

BOTSTEIN, 1989). The likelihood of a QTL at any point between the marker pairs is deduced from the observed trait and genotype information. The test statistic is the likelihood ratio (LOD) of a QTL across all intervals compared to getting this result by chance. Regression analysis provides comparable results (HALEY and KNOTT, 1992). QTL analyses have been conducted successfully for numerous traits in various pig (crossbred-) populations. The current status of QTL studies in the pig can be found at `Pig Quantitative Trait Loci database, PigQTLdb´ (HU et al., 2005).

QTL for muscle and meat traits

The power of QTL analyses depends largely on the size of the experiment in terms of number of animals and markers used and also on the trait analysed. The application of QTL analyses, which are a priori hypothesis-free, for traits of high heritability increases the power of the approach. Disentangling complex traits in their constituent phenotypes might therefore facilitate the identification of QTL and the elucidation of the pleiotropic nature of QTL effects.

Each muscle consists of three main fibre types, slow-twitch oxidative, fast-twitch oxido-glycolytic and fast-twitch glycolytic fibres (PETER et al., 1972), which are characterised by different microstructural, biochemical and metabolic properties. The number and size of the muscle fibres are major factors determining growth and weight of each muscle and post mortem development of muscle to meat and thus meat quality traits in pigs (LENGERKEN et al., 1994; LARZUL et al., 1997; FIEDLER et al., 2004). Hence, with regard to muscle and meat, muscle fibre properties represent some of the phenotypic components that contribute to the complex traits of meat and carcass quality. Consequently, we aimed to identify QTL for for microstructural and biophysical muscle properties as well as traits related to muscling and obesity in a porcine experimental population that is based on Duroc and Berlin Miniature Pig. Moreover, in order to complement the QTL approach by displaying trait-associated expression-profiles and detection of eQTL (expression QTL) we evaluated the quantification of transcripts of the myosin heavy chain isoforms, MyHC isoforms, by real time PCR as a new phenotype.

Material and methods

Animals

Analyses were done in a three-generation porcine F2 population (DUMI population) based on reciprocal crossbreeding of Duroc and Berlin Miniature Pig breeds (HARDGE et al., 1999). Thirty-three full sib-families comprising 469 F2-individuals born from 32 sows and four boars were kept and performance tested on the research farm of the Institute of Animal Science in Berlin Dahlem, Humboldt University of Berlin (up to day 100) and at the performance test station of the federal country Brandenburg (day 100 to day 200). At the research farm Frankenforst of the Institute of Animal Breeding and Genetics, University of Bonn, 436 F2 animals of 21 full sib-families were born from 11 sows and three boars, raised and performance tested. F2 piglets were weaned at about 6 weeks of age and kept in flat decks until day 100 and subsequently in single pens until slaughter at 200 days of age. Performance testing and trait recording was done according to according to the German performance test directives (ZDS, 2003)

Phenotypes

Muscle fibre characteristics of the longissimus muscle were determined by microscopic image analyses after histochemical fibre type differentiation. The samples were taken immediately post mortem at the $13^{th}/14^{th}$ rib, frozen in liquid nitrogen and stored at –70 °C. Serial cross-sections (12 µm) were obtained in a cryostat microtom (–20 ^{o}C) in order to be processed for the following histochemical reactions:

In order to differentiate the three main fibre types "red", "intermediate", "white" and "slow twitch oxidative = STO", "fast twitch oxidative = FTO", "fast twitch glycolytic = FTG", respectively, the samples were stained either by the NADH tetrazolium reductase reaction (NADH-TR) alone or by the combined NADH-TR/ATPase reaction. For the identification of the capillaries the alkaline phosphatase reaction was used detecting this marker enzyme of endothelial cells (JOSZA et al., 1993).

The quantitative microscopic determination of fibre type proportion, fibre size and capillar density was done on 400 fibre cross sections per animal. In total 308 F2 animals were examined for microstructural muscle traits. QTL analysis for these traits involving differentiation of fibre types based on staining intensity was performed separately for the two subsets of the material with phenotypic evaluation using either NADH-TR (n=168) or combined NADH-TR/ATPase reaction (n=140).

Markers and QTL-analysis

Altogether the animals of the DUMI population were genotyped at 88 loci covering the porcine autosomes with mean interval size of 30.7 cM. The set of markers includes 72 microsatellites and 16 biallelic markers. Linkage analysis was performed using the program CRI MAP, version 2.4 (GREEN et al., 1990). The QTL analysis was done with the program QTLexpress (SEATON et al., 2002) that is accessible via internet and developed to perform interval mapping based on least square regression analysis in three generation F2 populations and half-sib families (see Figure 1). Least square regression models used for QTL analysis included along with additive and dominance coefficients for the putative QTL the fixed effects of family, parity and sex as well as slaughter weight as a co-variable, which were found to affect almost all traits analyses in previous analyses of variance ignoring any molecular genetic information. Paternal half-sib analysis was accomplished making no assumptions on the relative frequencies of the QTL alleles in the founder populations. Therefore the F2-animals were treated as paternal half-sib families and the probability for the occurrence of a paternal allele was estimated in intervals of 1 cM. The probabilities of inheritance of distinct paternal gametic phases were regressed onto allele substitution effects at the putative QTL. The regression model included sex and litter as fixed effects and slaughter weight as co variable. Significance thresholds at the 5 and 1% level were determined empirically by permutation for individual chromosomes (CHURCHILL and DOERGE, 1994). Chromosome-wide 1 and 5% significance thresholds became genome-wide significance thresholds after Bonferroni correction for 18 autosomes of the haploid porcine genome.

MYHC isoform quantification

In order to establish the quantification of transcripts of myosin heavy gene (MYHC) isoforms (slow/I, IIa, IIx, and IIb) in M. longissiumus dorsi by SyBR green real time

RT-PCR assays as a new phenotype a comparison of results of these assays and ATPase fibre typing was conducted. RNA and serial cross-sections were obtained from M. longissimus dorsi samples of 30 animals of a Duroc x Pietrain F2 crossbreed population, which represent discordant sibpairs for the trait loin eye area. RNA was reverse transcripted using oligo(d)T and random hexamer primers. Real time PCR using isotype-specific primers (DA COSTA et al., 2002) were performed on an ABI Prism 7000 instrument. Abundance of transcripts of MYHC isoforms were normalized for transcript levels of the 18S gene and expressed as proportion of the total amount of MYHC transcripts. Serial cross-section were stained by ATPase reaction after preincubation at pH4.6 and evaluated visually to obtain the relative proportion of typeI, typeIIb/x, and typeIIa fibres.

Results and discussion

QTL for muscle fibre, meat quality and carcass traits

Results of the QTL analyses are compiled in Tables 1 and 2, in which all QTL exceeding the 5% genome-wide significance threshold are included. Suggestive QTL are detailed elsewhere (Wimmers et al., 2006).

Due to their economic importance, meat quality and body composition traits are recorded in routine in pig breeding. Correspondingly, there are many efforts to identify QTL responsible for the variation in these traits. Genome scans were conducted in many different experimental and commercial populations and revealed QTL effects on all 18 autosomes and chromosome X (for review see the `Pig Quantitative Trait Loci database, PigQTLdb´). Here, we report QTL for meat quality traits with genome-wide significance on SSC3, 5, 13, 15, 16 and 17 and QTL for traits related to lean meat content on SSC2, 4, 6, and 16.

Table 1
Evidence for QTL significant at the 5% genome-wide level for traits related to microstructural muscle properties, to meat quality, and to carcass composition obtained by F2 analysis. Estimated significance levels (F-value), position, % of F2 variance explained by each QTL, and gene effects. (QTL mit Genom-weiter Signifikanz für Muskelstruktur, Fleischqualitäts- und Schlachtkörpermerkmale gemäß F2-Analyse. Geschätzte Signifikanzlevel (F-Werte), Position, % der F2-Varianz erklärt durch den QTL und Geneffekt)

	Trait	SSC	Position [cM]	F-Value	% Variance[1]	*Additive* Effect[2]	S.E.	*Dominance* Effect[2]	S.E.
muscle fibre traits	Dia_{AnF}	1	3	8.4*	11.8	2.64	1.39	-7.04	1.80
	Dia_{FTG}	2	63	9.4*	17.8	-7.40	1.90	-10.07	5.91
	Dia_{mean}	2	66	9.5*	17.9	-7.04	1.78	-8.85	5.58
	Dia_{FTG}	4	96	8.6*	16.3	0.57	2.14	8.82	2.53
	Dia_{w}	14	102	7.9*	12.7	1.60	1.96	-15.65	4.09
meat & carcass traits	MC_{opto}	3	0	13.4**	3.6	-1.74	0.35	0.57	0.51
	FOM	4	80	19.0**	5.1	3.21	0.53	0.06	0.89
	MA_{ML}	4	78	13.8**	3.7	1.27	0.25	0.47	0.42
	MA_{ML}	6	175	11.4*	3.0	-0.99	0.23	0.52	0.35
	MC_{opto}	15	117	8.0*	2.2	-1.27	0.37	-0.89	0.50

*: significant at the 5% genome-wide level, **: significant at the 1% genome-wide level,
[1]the fraction of phenotypic variance in the F2 explained by a QTL; calculated as the proportion of residual variance of the statistical models with and without the QTL effect
[2]positive values of additive genetic and dominance effects imply higher trait values forced by the Duroc allele;

Table 2
Evidence for QTL significant at the 5% genome-wide level for traits related to microstructural muscle properties, to meat quality, and to carcass composition obtained by half-sib analysis. Estimated significance levels (F-value), position, % of F2 variance explained by each QTL, and gene effects. (QTL mit Genom-weiter Signifikanz für Muskelstruktur, Fleischqualitäts- und Schlachtkörpermerkmale gemäß Halbgeschwister-Analyse. Geschätzte Signifikanzlevel (F-Werte), Position, % der F2-Varianz erklärt durch den QTL und Geneffekt)

	Trait	SSC	Position [cM]	F-Value	% Variance[1]
muscle fibre traits	Pro_{FTO}	2	100	5.8*	18.2
	Cap_{STO}	2	123	5.6*	17.5
	Pro_{GiF}	4	132	6.2*	8.8
	Pro_{FTO}	8	55	6.0*	18.9
	Fib/mm²	11	91	5.2*	5.9
	Pro_{GiF}	12	38	10.3*	24.4
	Dia_{GiF}	12	56	7.4*	17.5
	Pro_{GiF}	15	32	12.7**	15.5
meat & carcass traits	FOM	2	20	6.6**	3.8
	MAML	2	20	10.0**	5.8
	MCOpto	3	0	7.6**	5.6
	FOM	4	73	5.7**	3.3
	MCOpto	5	9	6.6*	4.9
	FOM	6	0	4.7*	2.7
	MAML	6	32	5.4**	3.1
	MCOpto	13	43	8.0**	5.9
	pH24ML	15	48	5.4*	3.1
	FOM	16	0	5.2**	3.0
	C1ML	16	20	4.5*	2.3
	C24ML	17	0	5.2*	3.0

*: significant at the 5% genome-wide level, **: significant at the 1% genome-wide level,
[1]the fraction of phenotypic variance in the F2 explained by a QTL; calculated as the proportion of residual variance of the statistical models with and without the QTL effect

For muscle fibre traits we found QTL on SSC1, 2, 4, 8, 11, 12, 14, and 15. In detail, the telomeric region of SSC1 contained a QTL for the diameter of angular fibres (Dia_{AnF}) reaching 5% genome-wide significance under the line-cross model. Estimated additive and dominance effects indicated an overdominant QTL (dominance effect > additive genetic effect) with the Miniature Pig allele causing higher trait values (Table 1). According to line-cross model a QTL for mean fibre diameter (Dia_{mean}) mapped to the intermediate region of SSC2 that explained a considerable high proportion of phenotypic variation (Table 1); distal of these are QTL with genome-wide significance and strong effects on proportion of slow twitch fibres and their capillarisation (Pro_{STO} and Cap_{STO}) (Table 2). Line-cross analysis detected significant QTL for lean meat content, FOM, and eye muscle area, MA_{ML}, as well as for diameter of FTG fibres in the intermediate region of SSC4 (Table 1). According to the half-sib model the more distal region of SSC4 bore QTL for the number and size of giant fibres (Table 2). Chromosomes 5, 6 and 7 did not show any or just suggestive QTL for microstructural muscle properties. Loci controlling lean meat content segregated on SSC6 - proximal according to the half-sib model and distal according to the line-cross model (Tables 1, 2). For SSC8 using the half-sib model a significant QTL for proportion of fast twitch oxidative fibres (Pro_{FTO}) was detected (Table 2). While on SSC9 and 10 there were no significant QTL for muscle fibre traits SSC11 had a significant QTL for fibre number per mm² (Fib/mm²) as detected by half-sib analysis (Table 2). According to the half-sib analysis loci affecting proportion and size of giant fibres (Pro_{GiF}, Dia_{GiF}) segregated on SSC12 (Table 2). No QTL reaching genome-wide significance mapped to SSC13.

The distal part of SSC14 exhibited a significant QTL for fibre diameter (Dia_w). SSC15 showed significant QTL for proportion and diameter of giant fibres (Pro_{GiF}, Dia_{GiF}) and for $ph24_{ML}$ as revealed by half-sib analysis (Table 2). More distal a significant QTL for meat colour was found in the line cross model analysis (Table 1). The telomeric regions of SSC16 and SSC17 contained significant QTL for meat quality ($C1_{ML}$, $C24_{ML}$) and carcass traits (FOM) under the half-sib model (Table 2). SSC 18 carried no QTL reaching the genome-wide significance for the traits analysed.
In contrast to many production traits for which various QTL regions have been identified, information about QTL for muscle fibre traits is scare. Recently, NII and coworkers (2005) reported on QTL for muscle fibre traits and meat quality in a Japanese Wild Boar x Large White intercross on SSC 1, 2, 6, 14, and X. Discrimination of type I, IIA, or IIB fibres was based on myosine ATP method after alkaline preincubation expected to reveal phenotypes corresponding to ours. NII and co-worker (2005) found genome-wide significant QTL for type I fibres on SSC1 and 14 in vicinity of the regions where we found chromosome-wide significant QTL for slow twitch and red fibres, respectively. Moreover, QTL for type IIA, IIB and intermediate, white, and fast twitch fibres on the intermediate region of SSC2 and proximal on SSC14 have been found by us and NII et al. (2005). QTL for proportion of type I fibres were detected on SSC8.

With regard to the final target to develop marker assisted selection tools those genomic regions are of interest that exhibit both (1) QTL for carcass and meat quality traits, which are used to select in breeding routine, and (2) QTL for muscle fibre number and distribution traits, which are more strictly genetically controlled but affect growth, body composition and meat quality to a large extent.
Regions with either genome-wide significant QTL for fibre type traits or genome-wide significant QTL for meat quality or both are on SSC1 ($pH24_{ML}$ and Dia_{AnF}), on SSC2 ($C1_{ML}$ and Dia_{mean} and Dia_{FTG}), on SSC3 (MC_{opto} and Pro_{Gif}, Pro_{im}, Pro_{FTO}), on SSC5 (MC_{opto} and Dia_{red}), on SSC13 (MC_{opto} and Fib/mm²), on SSC14 ($C24_{ML}$ and Dia_w), on SSC15 ($pH24_{ML}$ and Pro_{GiF}), and on SSC16 ($C1_{ML}$ and Pro_{red}). With regard to the relationship between meat quality, lean meat content and fibre type distribution traits p-arm of SSC2 and proximal region of q-arm of SSC4 are of interest. On SSC2 and SSC4 we found genome-wide significant QTL for FOM and area of M longissimus on the one hand and fibre diameter (Dia_{FTG}, Dia_{mean}) on the other hand that might again indicate a common genetic background. On SSC7 and 9 we only found QTL for muscle fibre type distribution traits. SSC6 showed genome-wide significant QTL for FOM and MA_{ML} and for meat quality traits, but no QTL for muscle fibre traits. NII et al. (2005) found QTL for meat colour and hematin content on SSC6 close to QTL for type II fibres.

In summary, least square regression interval mapping revealed five significant and 42 suggestive QTL for traits related to muscle fibre composition under the line-cross model as well as eight significant and 40 suggestive QTL under the half-sib model. For traits related to body composition and biophysical parameters of meat quality five and twelve significant plus nine and 22 suggestive QTL were found under the line-cross and half-sib model, respectively. Microstructural properties of pig muscle and meat quality are governed by genetic variation at many loci distributed throughout the

genome. Estimates of the degree of phenotypic variation explained by the QTL range between some 2% for suggestive QTL for meat quality and carcass traits and 24% for a QTL for proportion of giant fibres on SSC12.
In general, QTL for microstructural properties explained a larger proportion of variance than did QTL for meat quality and body composition. This indicates higher power of the analyses for the fibre type traits. Muscle fibre traits have moderate to high heritabilities (h^2=0.20 – 0.59) compared to meat quality traits (h^2=0.15 – 0.32) (LARZUL et al., 1997). Thus large effects of QTL for fibre traits might reflect the larger impact of genetic variation on these traits than on meat quality. Thus, by looking at the microstructural properties of muscle rather than measuring complex meat quality traits one gets closer to the genes` effects. Moreover, coefficients of variation for fibre size were close to 15%, for proportion of STO and FTO fibres exceeded 30%, while it was up to 3% for meat pH, 10 and 15% for meat colour and conductivity. Thus, the large effects of QTL for fibre traits may also be due to higher variation in fibre type traits than meat quality traits in the DUMI population. Higher phenotypic variation of these traits facilitates the identification of QTL. The application of linkage analyses, which are a priori hypothesis-free, on traits of high heritability, increases the power of the approach. Disentangling complex traits in their constituent phenotypes might facilitate the identification of QTL and the elucidation of the pleiotropic nature of QTL effects.
We found more QTL using the half-sib model than the line-cross model indicating that the founders of the DUMI population are not fixed for different alleles at many of the QTL. QTL analysis under both, the line-cross and half-sib model, allows detecting QTL that are fixed or segregating among the founder populations and thus provide comprehensive insight into the genetic variation of the traits under investigation. The line-cross model assuming that different QTL alleles are fixed in founder populations is very powerful when this assumption corresponds to the true state of nature of the QTL and it is quite robust to limited deviations from this ideal situation. The half-sib model is more general with no assumption about the number and frequency of QTL alleles in founder populations and probably more realistic for many QTL.

Perspectives: MYHC isotype transcript abundance as a new phenotype
The map-based data provided here will facilitate the identification of genes affecting muscle fibre traits and/or meat quality traits especially when combined and complemented by function-driven genomic approaches, including QTL-genotype-dependent and QTL-region-specific expression patterns, trait-associated expression profiles, as well as detection of eQTL, i.e. loci controlling the transcription level of target genes. We previously analysed muscle expression profiles depending on muscle size, breed and/or muscle developmental stage (PONSUKSILI et al., 2000; WIMMERS et al., 2005). By mapping differentially expressed genes we combined functional and positional evidence for the candidacy of these loci. Recently, by microarray analysis, we identified genes that are differentially expressed among discordant sibpairs of a Duroc and Pietrain based F2 crossbreeding population. The discordant sibpairs differed significantly in the trait eye muscle area, but not in body weight. We aimed to address the three-way-relationship between muscle area, muscle fibre type traits as determined by histochemical methods and the abundance of transcripts of the muscle fibre type specific myosine heavy chain isoforms. Animals

with large muscle areas had significant higher numbers of typeIIb fibres and a significant lower number of typeIIa fibres; also typeI and typeIIx fibres tended to be less frequent in large muscle. Interestingly, simultaneous muscle fibre typing by ATPase reaction and MYHC isoform transcript quantification by real time PCR revealed significant correlations ranging between .5 and .7 (typeI -MYHCI: r=0.71 (p=0.004); typeIIb&x - MYHCIIb and IIx: r= 0.52 (p=0.05); typeIIa -MYHCIIa: r= 0.67 (p=0.008). Taking into account (1) existing difficulties to standardised conventional myofibre typing, (2) the fact that the conventional histochemical fibre typing in types I, IIA and IIB is not well adapted for pig skeletal muscles where four fibre types are present based on MYHC polymorphism, i.e. types I, IIa, IIx and IIb (LEFAUCHEUR, 2006) and (3) considerable variation of mRNA expression of each MYHC isoform among muscles even within the same animal, that is likely to reflect differences in the physiological state of individual muscles (DA COSTA et al., 2002) we propose the abundance of transcripts of MYHC isoforms as a new more precise phenotype. This new phenotype is probably more suitable to unravel the genetic background of variation in traits related to muscle and meat properties than technological meat quality parameters and conventional fibre typing in further attempts to display trait-associated expression profiles and to detect eQTL.

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Growth rate, muscle physiology, carcass traits and meat quality in pigs - A collage of studies on pigs at the University of Helsinki

Abstract

Department of Food Technology/ Meat Technology Section, University of Helsinki, Finland, has focused on interactions between pig (and poultry) physiology and meat quality. The special emphasis in muscle physiology has been put on the effects of growth rate, fibre type distribution, fibre sizes in relation to oxidative capacity, especially carbohydrate metabolism. Also the strength of connective tissue and bones has been studied. In collaboration with Veterinary Physiology, University of Helsinki, the activity of monocarboxylate transporters has also been studied.

Key Words: growth rate, carbohydrate metabolism, muscle fibre types, connective tissue, meat quality

Zusammenfassung

Titel der Arbeit: **Wachstumsrate, Muskelphysiologie, Schlachtkörpermerkmale und Fleischqualität beim Schwein – eine Darstellung von Untersuchungen an der Universität von Helsinki**

Im Mittelpunkt der Forschungsarbeit des Institutes für Lebensmitteltechnologie/Sektion Fleischtechnologie, Universität Helsinki, Finnland, stehen die Wechselwirkungen zwischen der Physiologie von Schwein und Geflügel und der Fleischqualität. Schwerpunkt auf dem Gebiet der Muskelphysiologie sind die Effekte der Wachstumsrate, der Verteilung der Muskelfasertypen und der Größe der Muskelfasern in Bezug auf die oxidative Kapazität, insbesondere des Kohlenhydratstoffwechsels. Weitere Forschungsthemen sind die Stärke des Bindegewebes und der Knochen. In Zusammenarbeit mit dem Fachbereich Physiologie, Fakultät für Veterinärmedizin, Universität Helsinki, wurde die Aktivität des Monocarboxylate-Transporters untersucht.

Schlüsselwörter: Wachstumsrate, Kohlenhydratstoffwechsel, Muskelfasertypen, Bindegewebe, Fleischqualität

Average muscle fibre cross sectional area and meat quality traits

Porcine *M. longissimus dorsi* contains about 80 per cent of glycolytic type IIB fibres calculated based on the fibre number and about 90 per cent calculated based on the fibre area (OKSBJERG et al., 1995; RUUSUNEN et al., 1996). Therefore, the size of these fibres has a great effect on the average muscle fibre cross sectional area in this muscle. When a muscle grows, the diameter of type IIB fibres increases faster than that of type I. At the slaughter weight, the size of type IIB fibres are twice as large as the size of type I and type IIA fibres (BADER, 1983).

In a study of RUUSUNEN & PUOLANNE (2005) it was investigated, how much the average muscle fibre cross sectional area ranges in pork loins at the live weight of 95-100 kg. The study consisted of 27 Finnish Landrace and 28 Yorkshire pigs slaughtered at the live weight of 95-100 kg. Average muscle fibre cross sectional area in *M. longissimus dorsi* was measured with image analyzer from sections stained with myosin ATPase method. Pork loin cross sectional area was measured one day after slaughter.

In the study it was found that gilts had a larger loin area ($p<0.05$) and a larger average muscle fibre cross sectional area ($p<0.05$) than the barrows. KARLSSON et al. (1994) and RUUSUNEN et al. (1996) have also shown that both the muscle fibre cross sectional area and the loin area are larger in gilts than in barrows. The differences in

the loin area and in the average fibre cross sectional area between the breeds were not significant ($p>0.05$). According to CHRYSTALL et al. (1969), the growth of muscle fibres in *M. longissimus dorsi* is most rapid early in life declining later to a near stationary level. One reason for this is that the fat starts to accumulate in the carcass at that stage. Carcass fat content affects muscle fibre size so that in fat carcasses the average muscle fibre cross sectional area is smaller than in lean carcasses (SEIDEMAN et al., 1989). Therefore, it is important to take both the live weight of the pig and carcass lean fat/content into account when comparing muscle fibre cross sectional area between the pigs. The feeding influences carcass fat content. According to RUUSUNEN et al. (2005a) the pigs with a low-lysine/low-protein supply had a lower carcass lean meat content ($p<0.01$), but higher carcass fat content ($p<0.01$) resulting in a smaller average cross sectional area of muscle fibres in light muscles compared to pigs with high-lysine/high-protein supply ($p<0.05$).

Muscle fibres are oxidative at the early stages of growth due to the dense capillarization and the small cross sectional area of muscle fibres, but when the fibres grow, they become more anaerobic. Thus, selection based on small fibre cross sectional area could result in pigs with more oxidative muscle.

A high number of muscle fibres with small fibre cross sectional area per loin area permits more growth potential to the muscle without a decrease of the oxidativity (ASHMORE & VIGNERON, 1988). It is, however, not yet known which muscle fibre cross sectional area at a certain live weight leads to (i) a fast growth, (ii) oxidative muscles, (iii) pigs whose carcasses contain a lot of lean meat and (iv) also meat with good technological and sensory quality.

RUUSUNEN et. al. (2005b) have also found that the light muscles of the pigs with a low-lysine/low-protein supply contain a higher glycogen content resulting in lower pH value measured 45 minutes post mortem from *M. longissimus dorsi* ($p<0,05$) than those of the pigs with high-lysine/high-protein supply ($p<0.05$).

C*onclusions:* The muscle fibre cross sectional area can vary considerably in pork loin with the same cross sectional area at the live weight of 95-100 kg regardless of breed or sex. A low lysine/low-protein supply in the diet increases glycogen content and glycolytic potential in porcine light muscles and decreases the pH value measured 45 minutes post mortem from *M. longissimus dorsi*.

Monocarboxylate transporters

Professor Reeta Pösö and MSc Katri Sepponen, Department of Veterinary Physiology, have studied in a collaboration study with us the monocarboxylate transporters (MCT) in porcine muscles (PÖSÖ & PUOLANNE, 2005). MCTs cotransport lactate anions and protons across the cell membranes and thus regulate the muscle pH. They measured MCT1, MCT2 and MCT4 isoforms both in oxidative and highly glycolytic muscles. They found that porcine muscles contain MCT2, which has not been reported before. The results together with measured concentrations of lactate suggest that MCT2 may function as housekeeping lactate and proton transporter, preventing acidification especially in highly glycolytic muscles in which the capacity of oxidizing lactate is low. The results also support the view that, as in other species, MCT4 would

be important at high lactate + proton concentrations that occur in stress (SEPPONEN et al., 2003).

The activity of glycogen debranching enzyme in meat animals

The degradation of glycogen is achieved by cooperation of two enzymes: glycogen phosphorylase (PHOS) and glycogen debranching enzyme (GDE) (BROWN & ILLINGWORTH-BROWN, 1966). The GDE breaks down the branching points of glycogen (so-called limit dextrin state), enabling the further action of PHOS on the linear chains of glycogen (BROWN & ILLINGWORTH-BROWN, 1966; NELSON et al., 1969).

The effect of pH value and temperature on the activity of GDE was studied in porcine *M. longissimus dorsi* and in *M. masseter* (KYLÄ-PUHJU et al., 2005). In both muscles, the pH had only a weak effect on the activity at the pH values found in carcasses post-slaughter. However, the activity of GDE decreased strongly ($p<0.001$) when the temperature decreased from values of 39°C and 42°C found just after slaughter to values of 4°C and 15°C found during cooling. The activity of GDE began to fall at temperatures below 39°C and was almost zero when the temperature decreased to below 15°C. Thus, the activity of GDE may control the rate of glycogenolysis during cooling, but does not block rapid glycolysis and pH decrease when the temperature is high, which may be important in formation of PSE meat.

In pig and cattle, the activity of GDE and the activity of PHOS were higher in the fast-twitch glycolytic muscles (*M. longissimus dorsi, M. semimembranosus*) than in the slow-twitch oxidative muscles (*M. masseter, M. infraspinatus*) (YLÄ-AJOS et al., accepted). Also in chicken the activity of PHOS was very high in fast-twitch glycolytic *M. pectoralis superficialis* and low in slow-twitch oxidative *M. quadriceps femoris* muscle, but in contrast the activity of GDE was lower in the former (YLÄ-AJOS et al., submitted). The activity of GDE was high in porcine muscles, intermediate in bovine muscles and low in chicken muscles, the differences being most significant between the fast-twitch muscles of the animals concerned.

The glycogen content is higher in fast-twitch than in slow-twitch muscles (KARLSSON et al., 1999; MONIN et al., 1987; YLÄ-AJOS et al., accepted; YLÄ-AJOS et al., submitted). Furthermore, the high activity of PHOS in fast-twitch muscles indicates high glycolytic activity. Even though the activities of both GDE and PHOS increased with the fast twitch and glycolytic character of a muscle of a given animal, the increase in the activity of PHOS was more pronounced. Thus, the ratio between the two glycogen degrading enzymes PHOS/GDE was higher in the fast twitch muscles than in the SO muscles and the GDE may restrict the rate of glycolysis in these muscles. The proportionally low activity of GDE in relation to the activity of PHOS in fast twitch muscles may be a protective mechanism against a sudden pH decrease. In strenuous physical stress, a high PHOS/GDE ratio in FG muscles enables a short burst of glycolysis, which leads to a rapid increase in H^+ content. The high buffering capacity of FG muscles (DAVEY, 1960; KYLÄ-PUHJU et al., 2004; PUOLANNE & KIVIKARI, 2000; RAO & GAULT, 1989; TALMANT et al., 1986) protects these muscles against a sudden pH decrease, but the proportionally low activity of GDE compared to the activity of PHOS may be needed to further restrain glycogenolysis.

Connective tissue in normal and loose structured porcine meat

The main roles of connective tissue in muscle are to ensure the passive elastic response of muscle transform the force from muscle fibers into mechanical movement and to mechanically support the vessels and nerves (KJAER, 2004). In meat science research on the thermal stability of connective tissue has been a tool for efforts to understand mechanisms of meat tenderization (GOLL et al., 1963; CARMICHAEL & LAWRIE, 1967). Animal age and muscle type affect thermal stability of collagen (KING, 1987). Defects in the structure (zones of PSE-like meat, loose structure) on the lateral side of porcine *M. semimembranosus* have been observed in few countries. This loose structured meat is also characterised by the ease at which bundles can be pulled away by hand (personal observations on Finnish and Irish samples). These observations on loose structure meat raised a question: Does connective tissue have a role in weakening of the meat structure?

This study was conducted in collaboration with The National Food Centre (Teagasc), Ireland (VOUTILA et al. 2005). We collected 7 loose structured and 7 normal structured porcine *M. semimembranosus* samples (about 100g each) in the boning halls of two commercial Irish abattoirs. Selection was made by visual assessment (overall a very low percentage of loose structured meat was observed). To obtain information on meat quality we analysed rough estimate for drip loss, pHu, colour, conductivity and reflectance. Connective tissue analysis consisted of onset and peak of thermal transition of connective tissue and total and soluble collagen. DFD (pHu>5,9) was observed in the group of normal structured meat so the statistical analyses were performed by including and excluding the DFD samples.

In both cases (including and excluding the DFD samples) the onset ($p<0.001$; 56.94ºC vs. 59.82ºC) and peak ($p<0.001$; 62.59ºC vs. 64.06ºC) of thermal transition temperature were significantly lower in loose structured meat than in normal structured meat. No difference between the two groups was observed in total and soluble collagen when the DFD like samples were excluded. There was a tendency for normal structured meat to have more soluble collagen than loose structured meat ($0.10<p<0.05$; 10.5% vs. 9.4%) when the DFD like samples were included in the statistical analysis. Reflectance% was lower ($p<0.01$; 45.18% vs. 69.17%) and the colour lighter (higher L value; $p<0.01$; 55.05 vs. 45.52) and more yellow (higher b value; $p<0.001$; 18.27 vs. 14.78) in loose structured meat than in normal structured meat when the DFD like samples were excluded. The colour results are in agreement with those of MINVIELLE et al. (1999). It was not feasible in this experiment to obtain early *post mortem* pH data therefore we do not have direct evidence of fast pH fall which could have related the loose structure to PSE defect.

Conclusion: These results suggest that loose structure in pork *semimembranosus* muscle could be attributed in part to connective tissue properties possibly in conjunction with PSE effects.

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The impact of bone development on final carcass weight

Abstract

Proper development and function of the skeleton is crucial for the optimal growth of an organism, with rapid growth rates often resulting in skeletal disorders in farm animals. Yet, despite clear benefits for breed selection and animal welfare (HARRISON et al., 2004), the impact of bone development on final livestock characteristics remains largely undetermined. Male Shropshire lambs, sired by a ram with a high genetic potential for daily live weight gain (n =11), or a ram with a high genetic potential for both daily gain and lean content (n =12), were slaughtered at a mean age of 146 days. The femur was removed and its parameters correlated with carcass weight. Results suggest that both femur length and femur weight act as good predictors of final carcass weight in lambs. However, no effect of paternal genetics, on the femur to carcass correlations, was noted.

Key Words: femur, small ruminants, paternal genetics

Zusammenfassung

Titel der Arbeit: **Der Einfluss der Knochenentwicklung auf das Schlachtkörpergewicht**

Adäquate Entwicklung und Funktion des Skeletts ist für das optimale Wachstum eines Organismus entscheidend, wobei hohe Wachstumsraten bei landwirtschaftlichen Nutztieren häufig Skelettschäden verursachen. Trotz der Tatsache dass klare Vorteile für die Züchtung und den Tierschutz ersichtlich sind (HARRISON et al., 2004), bleibt der Einfluss der Knochenentwicklung auf die Leistungsfähigkeit der Nutztiere bisher weitgehend unberücksichtigt. Männliche Lämmer der Rasse Shropshire, die von einen Bock mit hohem genetischen Potential für tägliche Zunahme (n = 11) bzw. von einem Bock mit hohem genetischen Potential für beides tägliche Zunahme und Magerfleischanteil (n = 12) abstammen, wurden mit durchschnittlich 146 Lebenstagen geschlachtet. Der Femur wurde präpariert und seine Eigenschaften korreliert mit dem Schlachtkörpergewicht. Die Ergebnisse zeigen, dass sowohl die Femurlänge als auch das Femurgewicht mit dem Schlachtkörpergewicht korrelieren. Es wurde kein Effekt der väterlichen Genetik, auf die Korrelation zwischen Femurmaßen und Schlachtkörpergewicht gefunden.

Schlüsselwörter: Femur, kleine Wiederkäuer, väterliche Genetik

Introduction

With an increased demand for meat that is lean, there is a pressure on the producer to slaughter animals at a younger age, yielding cuts with less fat than older and heavier animals (HAMMOND et al., 1983). One consequence of this action has been the selection of animals that have a fast rate of postnatal growth, and this trait in itself has been shown to be associated with improved tenderness of meat (FISCHELL et al., 1985; THERKILDSEN et al., 2004). Whilst gender is known to affect body composition, as female lambs have a greater proportion of fatty tissue *cf.* males (HAMMELL and LAFOREST, 2000), the role of paternal genetics does not seem to have been investigated so intensively.

It is known, however, that 70–80% of peak bone mass (PBM) is genetically determined, the remaining 20–30% being ascribed to environmental factors, of which the most important are exercise and nutrition (EASTELL and LAMBERT, 2002). There is also evidence that carcass composition is correlated to carcass weight, which in turn is determined by the content of muscle, fat and bone (DELFA and TEIXEIRA, 1998). Yet the most striking finding is that sheep breeders in Iceland have been using the traits of

the metacarpal bone (Cannon bone) as a selection criterion for meat quality improvement since the 1980´s (THORSTEINSSON and BJÖRNSSON, 1982).
To this end, the present study has chosen to investigate the relation between carcass weight and the length and weight of a hind limb bone (femur) in two groups of genetically different Shropshire lambs.

Material and methods

The study was performed on 23 Shropshire male lambs. Lambs and ewes were housed indoors in pens at the large animal facilities in Tåstrup, The Royal Veterinary and Agricultural University, Frederiksberg, Denmark, and provided with drinking water *ad libitum*. Ewes were fed silage *ad libitum* (56% Dry Matter (DM), 6.1% ash, 7.9 % Crude Protein (CP), 1% fat) + 400 g supplement (200g barley and 200g commercial protein supplement) per day. Twelve male lambs were sired by Ram 71200-01283 with a high genetic potential for daily growth, whereas 11 male lambs were sired by Ram 85335-00461 with a high genetic potential for increased cross-sectional area of *m. Longissimus dorsi* and minimal back fat thickness. From day 21 *post partum* lambs were fed a commercial concentrate and silage *ad libitum*. Lambs were weaned from the ewes at the age of 8 weeks of life and body weight of approximately 18 kg. Lambs were slaughtered under license at a mean age of 146 days at the experimental abattoir facilities of Foulum Research Centre, Tjele, Denmark.

Statistical analysis

All results were assessed for statistical significance within groups by two-way analysis of variance using the statistics package PROC MIXED in SAS (Version 8). 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 non-significant.

Results

No difference was found in the birth weight of the twelve male lambs sired by Ram 71200-01283 compared with the 11 male lambs sired by Ram 85335-00461. The mean carcass weight of the lambs was 25.0 ± 0.6 kg and a slaughter % of 50.0 ± 0.4 was recorded. The femur was excised from the carcass and found to have a mean weight of 161.3 ± 4.2 g whilst its length was 162.4 ± 1.5 mm. A plot of carcass weight *versus* femur length revealed a positive and significant overall correlation (r^2 = 0.71; P<0.0001: $Y = 1.82X + 117.30$) and a plot of carcass weight *versus* femur weight revealed a slightly better positive correlation (r^2 = 0.74; P<0.0001: $Y = 5.28X + 30.64$) – see the Figure below. However, no difference in paternal genetics, in terms of the femur to carcass correlations, was noted.

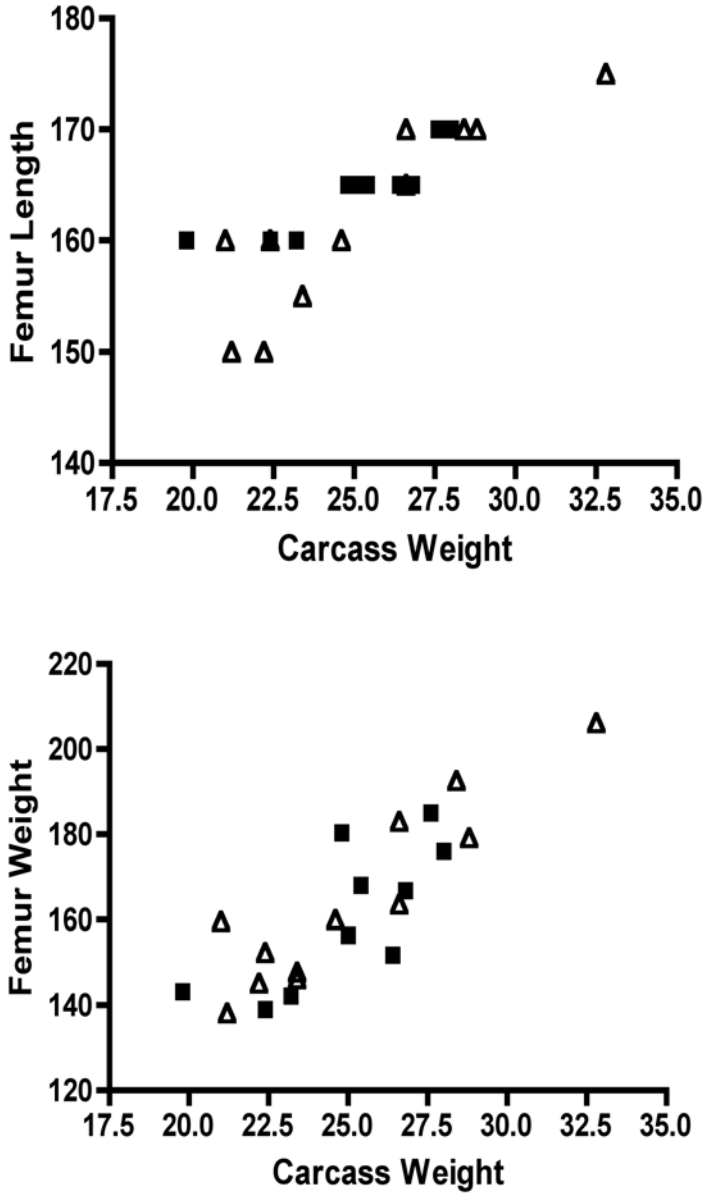

Figure: Upper Panel- a plot of femur length (mm) against carcass weight (kg), and Lower Panel- a plot of femur weight (g) against carcass weight (kg). A total of 23 lambs are represented of which 11 (ν) were sired by a ram with high genetic potential for daily growth, and 12 (△) were sired by a ram with high genetic potential for increased cross-sectional area of m. Longissimus dorsi and minimal back fat thickness.
(Obere Abbildung: Graph von Femurlänge (mm) gegen Schlachtkörpergewicht (kg); untere Abbildung: Graph von Femurgewicht (g) gegen Schlachtkörpergewicht (kg). Insgesamt 23 Lämmer wurden untersucht, von denen 11 (ν) von einem Bock mit hohem genetischen Potential für tägliche Zunahme und 12 (△) von einem Bock mit hohem genetischen Potential für tägliche Zunahme und große M. Longissimus dorsi Muskelfläche bei geringer Rückspeckdicke abstammen)

Discussion

The findings of this study suggest that both femur length and femur weight act as good predictors of final carcass weight in lambs. It has been known for some time that carcass composition can be predicted from the detailed dissection of specific cuts (KEMPSTER et al., 1976). However, if muscle development is determined by the weight of bones as well as their length, considering that the muscular system needs to

be able to bear and move the weight of an animal, then bone development may be a key determinant of carcass composition. Furthermore, with 70–80% of bone mass being genetically determined, perhaps there should be greater focus placed on the selection of animals with heavier and longer bones, based on a hypothesis that with it comes a means of improving carcass weight and composition. Indeed, this hypothesis has formed the basis of selection criteria for sheep meat quality improvement in Iceland for decades, where it has been shown that cannon bone weight correlates favourably with carcass weight ($r^2 = 0.51$) and that cannon bone weight is reasonably heritable ($h^2 = 0.53$) (THORSTEINSSON and BJÖRNSSON, 1982).

Also of interest is the work of ERMIAS and coworkers (2005), who report carcass quality for two breeds of Ethiopian sheep; Menz sheep are indigenous to the central highlands of Ethiopia (2500-3000 m above sea level), while Horro sheep are indigenous to the mid-altitude regions (1400-2000 m above sea level), with both breeds being of medium body size. Upon slaughter, at a similar live weight and age (13 months), carcass traits between the two breeds were assessed. The Menz breed was found to have more fat and less bone than the Horro breed. However, the data from this study was further assessed using an algorithm for variance parameter estimation in order to calculate the heritability of specific slaughter traits. ERMIAS et al., (2005) found that the most heritable trait was carcass bone weight (0.61), followed in succession by carcass fat percentage (0.49), carcass weight (0.48), carcass lean weight (0.35) with the least heritable trait being carcass lean percentage (0.05). Moreover, in further partial support of the hypothesis, a recent study of suckling lambs slaughtered at a weight of 14 kg, found that carcass muscle content was highly correlated with the amount of bone in individual cuts, especially in the leg ($r^2 = 0.88$) and shoulder ($r^2 = 0.84$) (DIAZ et al., 2005).

Thus, assessment of carcass weight in relation to femur length and weight in lambs sired by two genetically different rams, suggests that both bone parameters act similarly in both groups as good predictors of carcass weight. Moreover, this study indicates that femur length and weight correlates more closely, ($r^2 = 0.71$ & $r^2 = 0.74$, respectively) than the correlations of cannon bone length and weight with carcass weight ($r^2 = 0.25$ & $r^2 = 0.51$, respectively) reported by THORSTEINSSON and BJÖRNSSON, (1982).

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Muscle fibre growth and quality in fish

Abstract
Striated muscle in fish is in parity with skeletal muscle in higher vertebrates made up of three major compartments - contractile proteins, lipids, and connective tissue, - all affecting the product and food quality of the muscle as meat. The most striking difference between striated fish muscle and that found in higher vertebrates is the separation of fibre types in to discrete layers in fish, where the high glycolytic and anaerobic type (Fast- White fibres) dominates constituting 90-95% of all muscle in most fish species. Red muscle fibres are commonly confined to a narrow strip along the lateral line and constitute less then 10 % of the myotomal musculature. Intermediate or pink fibres are in accordance to their name not only intermediate in position between red and white muscle fibres but also in many other aspects. Salmonids seem not to have pink fibres. In contrast to other higher vertebrates in the majority of fish species growth continues through out life and the growth of muscle is the combined effect of formation of more muscle cells (fibres) and increase in size of already existing fibres. In spite of the apparent large connective tissue compartment of fish muscle, only 2-3 % of the protein is found here. All the above factors are believed to be in part under genetic control, but a gene x environment interaction seems to be evident. The current state of knowledge is reviewed here and the impact of muscle structure on quality is discussed.

Key Words: muscle fibre, fish, quality, growth, muscle structure

Zusammenfassung
Titel der Arbeit: **Muskelfaserwachstum und Qualität beim Fisch**
Quergestreifte Muskulatur bei Fischen besteht entsprechend der Skelettmuskulatur in höheren Wirbeltieren aus drei Hauptkomponenten, kontraktilen Proteinen, Lipiden und Bindegewebe, die die Produkt- und Nahrungsmittelqualität des Muskels als Fleisch beeinflussen. Der größte Unterschied zwischen Fischmuskel und dem höherer Wirbeltiere besteht in der Anordnung verschiedener Fasertypen in getrennten Schichten, von denen die mit glykolytischen und anaeroben Fasern (schnelle weiße Fasern) mit 90-95% den höchsten Anteil haben. Rote Muskelfasern sind allgemein auf einen schmalen Streifen entlang der seitlichen Linie begrenzt und kleiner und machen 10 % der myotomalen Muskulatur aus. Intermediäre- oder rosafarbene Fasern sind in der Übereinstimmung mit ihrem Namen, in Position zwischen den roten und weißen Muskelfasern angeordnet aber auch in vielen anderen Aspekten intermediär. Salmoniden scheinen rosafarbene Fasern nicht zu haben. Im Gegensatz zu anderen höheren Wirbeltieren setzt sich bei der Mehrheit der Fischspezies Wachstum durch das gesamte Leben fort und Muskelwachstum ist gekennzeichnet durch Hypertrophie und Hyperplasie. Trotz des offensichtlichen großen Bindegewebeanteils des Fischmuskels befinden sich nur 2-3 % des Proteins hier. Die oben genannten Faktoren unterstehen genetischer Steuerung, aber auch Genotyp x Umwelt Interaktion ist von Bedeutung. Der gegenwärtige Wissensstand sowie die Bedeutung der Muskelstruktur auf die Qualität werden hier diskutiert.

Schlüsselwörter: Muskelfasern, Fisch, Qualität, Wachstum, Muskelstruktur

Introduction
Striated muscle in fish is in parity with skeletal muscle in higher vertebrates made up of three major compartments all affecting the product and food quality of the muscle as meat. The contractile protein which is found organised in myofibrils inside the muscle fibres. The lipids which are found as the major component of cell membranes in the form of phospholipids, as storage lipids in adiposities or as lipid droplets in the

cytoplasm of the muscle fibres. And the connective tissue which is made up of collagen and form the cytoskeleton of the muscle.

The main object for fish muscle are movement, unlike the mammals were the muscles also give important support to the skeleton. The most striking difference between striated fish muscle and that found in higher vertebrates is firstly the separation of fibre types in to discrete layers in fish, where the high glycolytic and anaerobic type dominates constituting 90-95% of all muscle in most fish species (Fig.1 and 2). Secondly in the majority of fish species growth continues through out most of the life and the growth of muscle is the combined effect of formation of more muscle cells (fibres) and increase in size of already existing fibres.

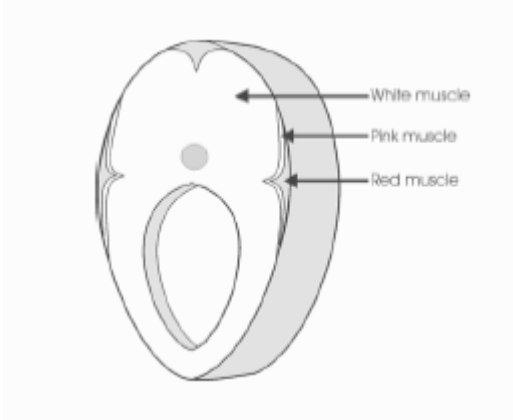

Fig. 1: A cross-section of a fish muscle showing the location of red (high aerobic, slow twitch), pink (high aerobic, high glycolytic and fast twitch) and white (anaerobic, high glycolytic and fast twitch) muscle fibres (Querschnitt durch Fischmuskel mit roter (aerob, langsam), intermediärer (aerob, schnell) und weißer (anaerob, glykolytisch, schnell) Muskulatur)

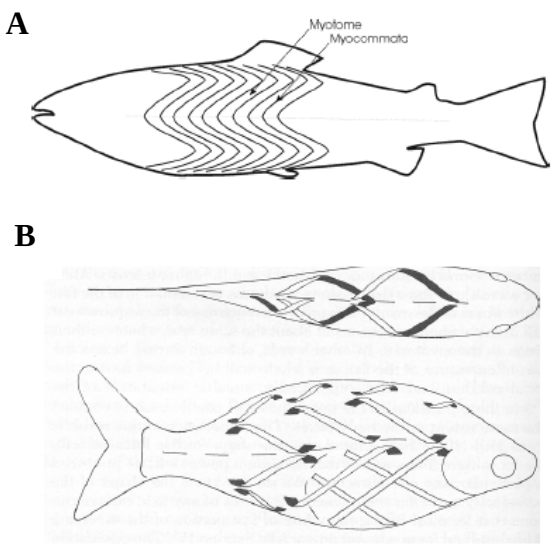

Fig. 3: A) Schematic drawing of somatic muscle in fish showing the arrangement of myotomes (muscle fibers) and myocommata (connective tissue). B) Schematic dorsal and lateral views of a typical teleost showing courses of myotomal muscle fibers in successive myotomes along the body. The helices shown where found by taking the origin of one muscle fiber from the point at which the muscle fiber in the myotome next anterior inserts onto the common myoseptum, and so on along the fish. From Alexander, 1969, *J. Mar. Biol. Assoc. U.K.* (**49**:263-290). (A) Anordnung der Myotome (Muskelfasern) und der Myokommata (Bindegewebe). B) Dorsale und laterale Ansicht eines Teleostfisches mit typischer Anordnung von Muskelfasern)

The striated muscle of teleost fish, which make up the fillet, consists of long sheets of muscles (myotomes) extending on both sides of the body from head to tail (Fig.3). Connective tissue makes up 2% to 5% of the muscle in bony fish and takes the form of fine membranes (myocommata) separating the long muscles into segments that are one cell deep (DUNAJASKI, 1979). Each myotome contains a superficial region lying directly beneath the skin, where the muscle fibres are run parallel to the body axis, and a deeper part where the muscle fibres are arranged in a helical fashion, forming angles of up to 40º (Fig. 3). This typical orientation of muscle fibres is associated with the need for constant amount of sarcomere shortening at different body flexures (SÄN-

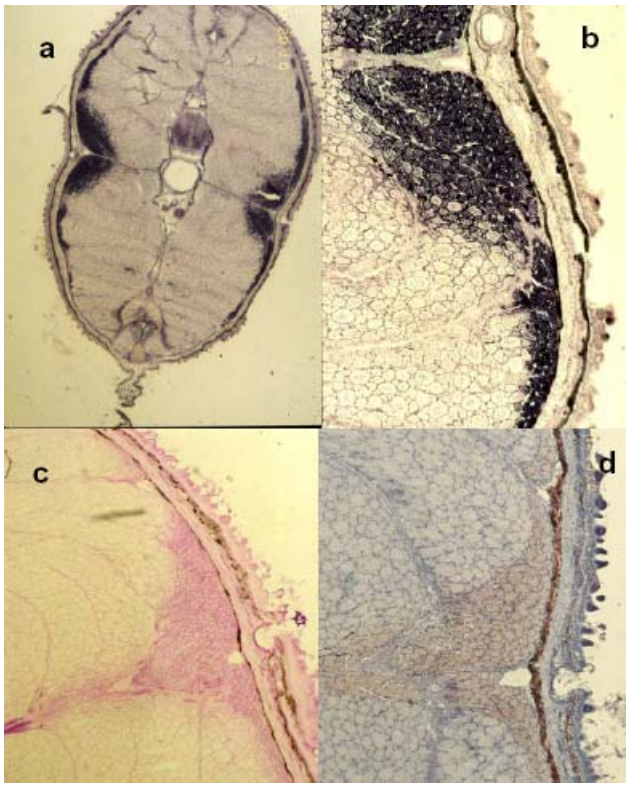

Fig. 2: a) Whole cross section of a fish muscle stained for oxidative capacity demonstrating localisation of red and white muscle. Sub cross sections of red and white muscle at the lateral line, demonstrating the metabolic characteristics of the two fibre types (b-d): stained for succinate dehydrogenase (mitochondria), glycogen (PAS-staining) and triglycerides (oil-o staining), respectively. The sections from a 2 gram Mahi Mahi (*latin)* larva in the study described in Kiessling and Ostrowski, (1997) (Querschnitte durch Fischmuskulatur gefärbt entsprechend oxidativer Kapazität als rote und weiße Muskulatur. Vergrößerungen des lateralen Abschnitts (b-d) mit Färbung der Fasern nach metabolischen Eigenschaften: Succinat-dehydrogenase-Aktivität (Mitochondrien), Glykogen- (PAS), Triglyceridgehalt (Öl-O-Färbung). Gewebe von Mahi Mahi Larve (nach Kiessling and Ostrowski, 1997))

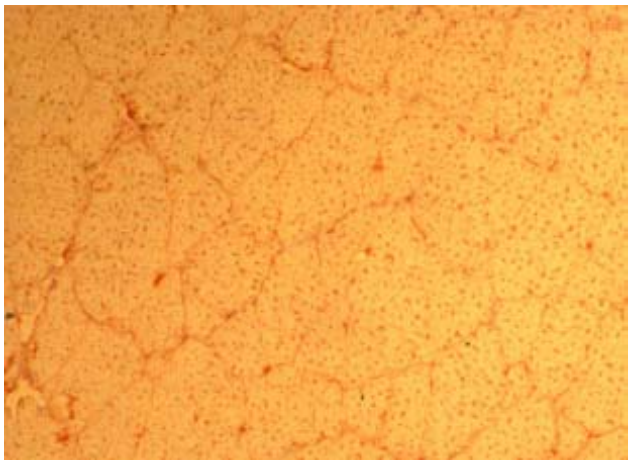

Fig. 4: Histochemical cross section of white adult rainbow trout muscle stained for Oil O (neutral lipids staining red). Photo from the study described in Kiessling et al. 1990 (Weiße Muskulatur einer adulten Regenbogenforelle Öl-O-gefärbt (neutrale Lipide rot) (nach Kiessling et al. 1990))

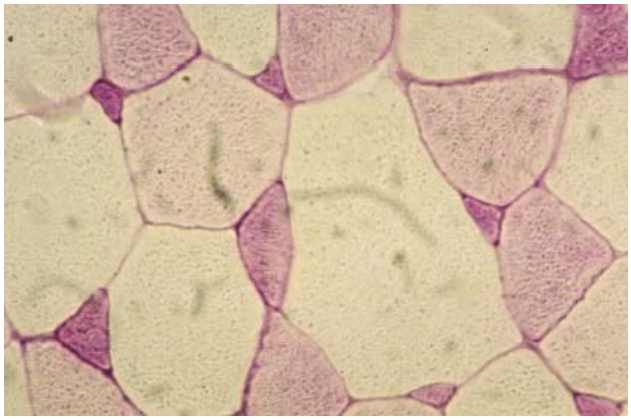

Fig. 5: White muscle in rainbow trout stained for glycogen (PAS-staining) demonstrating the difference in glycogen content between small (young) and large (old) fibres in a spawning migrating sockeye salmon (*Oncorhynchus nerka*). Photo A. Kiessling. (Glykogen-Färbung (PAS) weißer Muskulatur einer Regenbogenforelle zeigt unterschiedliche Glykogengehalte in kleinen (jungen) und großen (alten) Fasern in *Oncorhynchus nerka*. Photo A. Kiessling)

GER and STOIBER, 2001). Length of muscle fibres decreases in length towards the tail end of the fillet (LOVE, 1988); and the muscle fibre cross sectional area is smaller at the tail and head than in between (LOVE, 1988; SIGURGISLADOTTIR, 2001).
All fish have two main types of locomotor muscle fibres, red and white, specialized for either low speed cruising or short bursts of maximum speed, respectively (BONE, 1978). In several fishes there are more than these two fibre types (SÄNGER and STOIBER, 2001). The organization found in teleosts is as follows: the axial muscle consists mainly of fast white fibres, covered by a thin layer of slow-red muscle fibres, with a layer of pink intermediate muscle fibres in between them (Fig. 4). Salmonids lacks this intermediate fibre type (MARTINEZ et al., 1993; KIESSLING et al., 1995)

Slow-Red fibres; Red muscle fibres are commonly confined to a narrow strip along the lateral line. Red muscle fibres usually constitute less then 10 % of the myotomal musculature and are small in diameter (25-45 μm). The red muscle fibres are also called slow fibres and are used mainly for sustained energy efficient swimming. The characteristic of this muscle type are good capillary supply, high amount of mitochondria, lipid droplets and glycogen stores (Fig. 2). Concentration of myoglobin and cytochromes are high. The energy metabolism in red muscle is almost entirely aerobic, based mainly on lipid as fuel complemented with carbohydrates (SÄNGER and STOIBER, 2001).

Fast- White fibres; White muscle fibres compose the major part of the skeletal muscle in fish and constitute never less than 70% (SÄNGER and STOIBER, 2001). The white fibres show the largest fibre diameter ranging between 50 and 100 μm or even more. The proportion of the cross-sectional area of the skeletal muscle that is comprised of white muscle varies along the length of the fish, being greatest in the anterior of the animal and declining caudally. Generally, the white muscle type is used at high swimming speeds e.g., in fast-start burst swimming for prey capture and escape response, though there is an overlap of labour between red and white muscles in most teleosts. White muscle fibres are tightly packed with myofibrils occupying between 75 and 95% of the fibre volume. Organelles such as mitochondria which interrupt the arrays of myofibrils, are few and both lipid droplets and myoglobin are present in very low levels in most species. Salmonids and a few other fatty fish constitutes the exception with significant amounts of intrafibrilar fat (Fig. 4) (KIESSLING et al. 1990; ZHOU et al., 1995). Vascularization in white glycolytic muscle is poor. Glycogen content is also low with granules mainly located between the myofibrils. However, there seems to be a marked heterogeneity in glycogen content between different sized white fibres, with a significantly higher content in the smaller fibres (Figs. 2 and 5, KIESSLING et al. 1990; KIESSLING et al. 1991a; KIESSLING and OSTROWSKI, 1997). The energy for white muscle, operating in a nearly closed system, dominates by anaerobic breakdown of intramuscular glycogen with small contribution from cytosolic phosphocreatine (PCr) and ATP. In addition to this glycolytic based system is energy likely also provided via the slower but more efficient aerobic break down of lipids. Enzymatic activity levels β-oxidation and respiratory chain in the range of 10% of that found in red muscle is reported through out life in white muscle of rainbow trout (KIESSLING et al. 1991b), Atlantic salmon (FRØYLAND et al. 1998) and migrating sockeye salmon (KIESSLING et al., 2004a).

In parity with glycogen levels aerobic activity seems to be concentrated to small rather than large fibres (KIESSLING et al. 1990). It has therefore been speculated if this heterogeneity between fibres of different sizes is related to regeneration of glucose in small fibres from lactate formed during anaerobic glycolysis in the large fibres and/or from an aerobic catabolism of pyrovate from glycolysis to fuel contraction in small fibres during intermediate swimming speeds (KIESSLING et al., 2004a).

Intermediate -Pink fibres; Intermediate or pink fibres is in accordance to their name not only intermediate in position between red and white muscle fibres but also in many other aspects. In juveniles and adults of most teleost species, a zone of intermediate or pink fibres is inserted between red and white fibres. The mean fibre diameter lies between those of red and white. Pink fibres are characterized as fast contracting with intermediate resistance to fatigue and intermediate speed of shortening between red and white muscles. Salmonids on the other hand seems not to have pink fibres, but only white and red (MARTINEZ et al. 1991 re Arctic charr; 1993 re Atlantic salmon; KIESSLING et al. 1995 re rainbow trout).

Muscle growth; Most fishes continue to grow throughout their lives. Growth in fish has been studied intensively because it is a good indicator of health. Rapid growth indicates abundant of food and other favourable conditions, whereas slow growth is likely to indicate just the opposite. Growth is commonly measured as changes in body weight, length or condition factor (i.e. weight/length relationship) over time. Post-embryonic growth of the muscle tissue involves an increase in the number and diameter of the fibres and a contemporary remodeling of the associated connective tissue, nerve and blood supply. Muscle growth can therefore be studied as the contribution of hyperplasia (increase in fibre number) and hypertrophy (increase in fibre size) to muscle growth by various forms of histological methods combined with morphometric analysis (ROWLERSON and VEGETTI, 2001). Muscle fibre morphometric variables most commonly used are diameter or cross-sectional area and number of muscle fibres measured within a representative area of the musculature. From this, size distribution histograms are made or a probability density function (pdf) where the increase in fibre size describes hypertrophic growth and the increase of small fibres denotes hyperplastic growth, i.e. recruitment of new fibres.
Growth is usually positive, in that the fish increase in size over time. The principal factor controlling the growth processes are growth hormones secreted by the pituitary and steroid hormones from the gonads. However, the rate of growth of fish is highly variable because is it greatly dependent on a variety of interacting environmental factors such as water temperature, levels of dissolved oxygen and ammonia, salinity and the photoperiod (MOYLE and CECH, 1982). Such factors interact with each other to influence growth rates, and with others such as the degree of competition, the amount and quality of food ingested, and the age and state of the maturity of the fish.

Hypertrophy; In fish the muscle grows by enlargement of existing fibres (hypertrophy) throughout post-embryonic life until they reach a functional maximum diameter, which is in the range 100-300 μm for white fibres in most fish (ROWLERSON and VEGETTI, 2001). Hypertrophic growth persists long after hyperplastic growth has ceased (e.g. STICKLAND, 1983; WEATHERLEY et al., 1988; KIESSLING et al.,

1991a; rev: ROWLERSON and VEGETTI, 2001). As the fibres increase in size they get packed with myofibrils. Fibres also acquire additional nuclei as they grow (JOHNSTON, 1993; USHER et al., 1994; NATHANILIDES et al., 1996; ALAMI-DURANTE et al., 1997). The new nuclei are supplied by a population of satellite cells (already present in the muscle), which fuse with existing muscle fibres to provide the additional nuclei (Fig. 4) (JOHNSTON, 2001). To supply the number of nuclei required during growth, this population must be capable of proliferation. In fish, a major uncertainty is whether there are separate muscle stem cell populations for fibre recruitment and fibre hypertrophy.

Hyperplasia; Hyperplastic growth of muscle refers to the increase in muscle fibre number due to the formation of new fibres. After the initial two muscle layers have been formed during embryonic life, hyperplastic growth continuous in two successive and distinct phases. The first phase is a continuation of embryonic myogenesis and completes the formation of the definitive muscle layers (slow red, pink and fast white), followed by a second and quite different hyperplastic process resulting in a large increase in the total number of fibres in all muscle layers, especially in the white muscle layer (ROWLERSON and VEGETTI, 2001). New presumptive fast white fibres during embryonic and into larval life, appear in a germinal layer or proliferation zone located just under the superficial monolayer and extends dorsally from the horizontal septum into the apex of the myotome. In many fish species which remain small, this second hyperplastic growth phase is lacking, whereas fast-growing fish generally show greater hyperplasia than slow-growing fish of the same age (WEATHERLEY et al., 1979; WEATHERLEY and GILL, 1984; HIGGINS and THORPE, 1990; KIESSLING et al., 1991a; VEGETTI et al., 1993; VALENTE et al., 1999). In most fish, which grow to a large final size, the majority of muscle fibres are formed in a long-lasting hyperplastic growth process disseminated throughout the entire myotome. This process gives rise to the typical mosaic appearance of muscle cut in transverse section, with fibres of different ages (and therefore diameter) intermingled (Fig. 5). Mosaic hyperplastic muscle growth, which occurs principally during juvenile life, is of great interest in commercial aquaculture because it contributes to the market size of the fish. The intensity of mosaic hyperplastic growth is most pronounced in early juvenile life: later it decreases gradually until the fish reaches a characteristic fraction of body size after which further growth occurs by hypertrophy only (WEATHERLEY et al., 1988; STICKLAND, 1983; ROWLERSON et al., 1995). There is indirect evidence for the existence of a distinct population of myogenic cells supporting mosaic hyperplastic growth (ROWLERSON and VEGETTI, 2001).

Muscle structure and quality

Important quality factors in fish are texture, colour, fillet gaping, taste and flavour. We will look into how the muscle structure affects some important quality traits below.

Texture; Texture is one of the criteria of flesh quality. It is a sensory characteristic for the consumer and an important attribute for the mechanical processing of fillets. Very soft texture is frequently reported and the industry is requesting methods able to measure fish texture, and is also seeking answers to what causes fillet softness.

Textural properties depend on the chemical composition and the structural properties, in particular the myofibrillar and connective tissue proteins. The connective tissue forms a supporting network through the whole fish muscle. The content of connective tissue is lower and more evenly distributed in fish muscle compared with warm-blooded animals, though there is increased firmness along the anterior-posterior axis of the fillet.
The fibre distribution of the muscle has been found to affect the texture in fish (Fig. 6, KIESSLING, RUOHONEN, BJØRNEVIK and ESPE, submitted 2005). Intra-species comparison has shown that muscle fibres measured as average fibre cross-section area, increases with decreasing sensory firmness in cooked fish (HATAE, 1990; HURLING et al., 1996). Also in fresh and smoked Atlantic salmon and in fresh brown trout, studies have shown a weak decrease in flesh firmness as the size of the fibres increases (or the fibre density decreases) (JOHNSTON et al 2000, 2004; BUGEON et al., 2003). On the other hand there are also studies on Atlantic salmon and Atlantic cod that was not able to confirm this finding (SIGURGISLADOTTIR, 2001; BJØRNEVIK et al., 2003). An underlying rational for this discrepancy between studies may firstly be found in the fact that texture varies as a factor on the rostral-caudal location n the fillet (SIGURGISLADOTTIR, 2001; BJØRNEVIK et al., 2003).

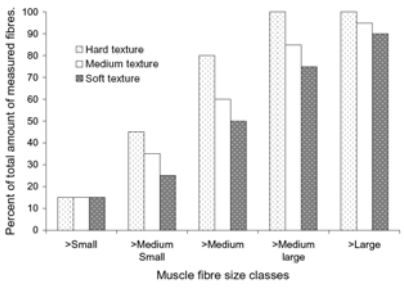

Fig. 6: Percent of fibres accounted for (accumulated, y-axis) in each fibre size class (cross section) in farmed Atlantic salmon (*Salmo salar*) displaying either hard, intermediate or soft texture, as measured by transverse cutting (shear force) with a Warner Bratzler type blade. Based on data from Kiessling, Ruohonen, Bjørnevik and Espe, (In progress 2006).(Anteil an Muskelfasern an der gemessenen Gesamtzahl (akkumuliert, Y-Achse) in jeder Fasergrößen-klasse (Querschnitt) mit weicher, intermediärer oder harter Textur (Scherkraft, Warner Bratzler Messer))

Muscle fibre size may only partly explain the variation in mechanical resistance of the flesh (40% with the mean fibre size diameter is found in brown trout) (BUGEON et al., 2003). Muscle structure is more complex than a physical structure based on muscle fibre, and other factors could explain flesh texture characteristics. An increase in insoluble collagen with increasing texture firmness has been seen indicating that connective tissue also may contribute to the firmness of fish flesh (ESPE et al. 2004).

Gaping; Fillet gaping is a well known quality problem in fish. Gaping in fillets occurs as slits between muscle blocks. The slits can range from slight separation at the cut surface to complete separation right down to the skin of the fillet. This phenomenon is of considerable economic importance to the fish industry, as gaping significantly decreases the technological and market value of the fish, spoils the appearance of fillets and make skinning difficult or impossible.
The relationship between fibre area and occurrence of gaping has been studied, and contradictory results have been reported. JOHNSTON (2001) found a negative relationship between fibre density and occurrence of gaping in Atlantic salmon, i.e. increasing gaping score with larger mean white fibre area, whereas BJØRNEVIK et al. (2004) found a negative relationship between fibre area and gaping, while KIESSLING, RUOHONEN, BJØRNEVIK and ESPE, (submitted 2005) report a lower

number of intermediate fibres in Atlantic salmon displaying severe gaping compared to fish with no or intermediate gaping (compare with Fig. 6 for texture).
Each muscle fibre is surrounded by reticular fibres, the "endomysium", and it has been speculated that a fish with many small fibres would have a relatively larger amount of connective tissue, compared with a fish with larger and fewer muscle fibres. And that this larger amount of connective tissue would prevent the fillet from gaping.

Flesh colour; Visual appearance is a very important property in the food industry. In salmonids the red colour of the flesh is of particular importance, and for white fishes, a delicate white appearance is preferred. It has been argued that a perceived change in flesh colour can be caused by an altered reflection due to the change in surface properties with altered fibre area. Different studies have so far not been able to verify this hypothesis. JOHNSTON et al (2000) reported a positive relationship between Roche colour score and fibre density in Atlantic salmon, whereas no such relationship was found in other studies (BJØRNEVIK et al., 2004; ESPE et al., 2004). A weak positive relationship is found between lightness and fibre density in Atlantic salmon, higher density and more intermediate sized fibres coincides with a darker flesh (KIESSLING, RUOHONEN, BJØRNEVIK and ESPE, submitted 2005, compare with Fig. 6), whereas no such relationship was seen in cod (BJØRNEVIK et al., 2003).

In conclusion; muscle fibre growth in fish consist of two distinct phases. The first during early larvae age, comparable to that seen in higher vertebrates during the embryonic stage, and a second during adult life signified by a combined hyperplasia and hypertrophy. Growth during both these stages are under a combined of genetic and environmental control. Several studies are under way in order to determine the genetic component of this control (e.g. EU project "Progress" as well as the control mechanisms (see ASHTON et al., 2005 for a review). The relationship between final muscle fibre composition and size has long been debated but only recently the target for systematic research. Undoable a relationship exist, however, the predictive power is weak using the fibre component alone. Based on available data the degree of explanation varies from 0-25% depending on species, life stage and variable studied. The majority of variation measured in quality of the fish fillet is probably an effect of the connective tissue, the lipid component and naturally also to handling of the flesh post mortem.

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Cellular aspects of breast muscle development in chickens with high or low growth rate

Abstract

The present study was conducted to describe the cellular aspects of muscle fibre growth, including satellite cells proliferative activity, in chickens divergently selected for High (HG) or Low (LG) growth rate, and the evolution of circulating IGF-I and IGF-II levels during their first week of life. The two genotypes already differed for body size at hatch, and the amplitude of the differences increased with age. The levels of circulating IGF-I and IGF-II were higher in HG than in LG from day 2 to 7. Breast muscle fibres grew in size from 1 to 7 days of age, and were bigger in HG across the first week of age. The cytoplasm to nucleus ratio also increased with age and was higher in HG than in LG from day 2 to 7. By contrast, the proportion of dividing nuclei decreased between day 2 and 4 in both genotypes, then remained low in HG while it increased in LG. As a result of this interaction, it was higher in HG than in LG at day 2 and 4 and lower at day 7.

Key Words : chicken, muscle, growth, growth factors

Zusammenfassung

Titel der Arbeit: **Parameter des Zellwachstums während der Entwicklung des Brustmuskels bei Hühnern mit hoher und niedriger Wachstumsrate**

Das Muskelzellenwachstum, einschließlich Satellitenzellvermehrung, sowie die IGF-I und IGF-II Blutkonzentration während der ersten Lebenswoche wurden bei Hühnerlinien divergent selektiert für hohe (HG) bzw. niedrige (LG) Wachstumsrate untersucht. Bereits die Geburtsgewichte unterscheiden sich zwischen den Linien und die Gewichtsunterschiede steigen mit wachsendem Alter. Die Blutkonzentrationen von IGF-I und IGF-II sind zwischen Tag 2 bis 7 höher bei Hühnern der Linie HG als bei LG. Die Größe der Muskelfasern im Brustmuskel wuchs vom ersten bis zum siebenten Tag. Die Muskelfasern waren während der ersten Lebenswoche bei HG größer als bei LG. Das Verhältnis aus Zytoplasma zu Kernvolumen erhöht sich mit dem Alter und war größer in HG als in LG im Zeitraum von Tag 2 bis zum Tag 7. Im Gegensatz dazu war der Anteil sich teilender Kerne abnehmend zwischen dem zweiten und vierten Tag in beiden Linien und blieb anschließend klein in HG während es sich bei LG erhöht. Daher war der Anteil sich teilender Zellen zwischen Tag 2 und 4 bei HG höher als bei LG, am Tag 7 aber niedriger.

Schlüsselwörter: Hühner, Muskel, Wachstum, Wachstumsfaktoren

Introduction

Experimental lines of chickens divergently selected for High or Low growth rate (HG or LG) are a valuable model for the study of muscle growth and its underlying regulatory mechanisms (BECCAVIN et al., 2001). At hatch, the HG chicks already exhibit a higher body weight and muscle mass than the LG chicks, while they show muscle fibres of similar cross-sectional area (REMIGNON et al., 1995). By one week of age, the muscle fibres are significantly bigger in HG than LG chicks (REMIGNON et al., 1995). It is generally assumed that bigger muscle fibres accumulate more nuclei, as a result of satellite cells activity (MOSS and LEBLOND, 1971). By directly interfering with the activity of satellite cells through irradiation, it has been shown that muscle growth potential in turkey poults was governed mostly by myonuclear accretion and to a lesser extent by an increase in the volume of cytoplasm per nucleus (MOZDZIAK et al., 1997). The difference between HG and LG chicks could therefore result from a higher activity of the muscle satellite cells. Insulin like growth factors,

which stimulate the mitotic activity of cultured chick satellite cells (DUCLOS et al., 1996) circulate at higher levels in HG than in LG chicks between 1 and 6 weeks of age (BECCAVIN et al., 2001). The present study was conducted to describe the cellular aspects of muscle fibre growth, including satellite cells proliferative activity, during the first week of life and to measure the levels of circulating IGF-I and IGF-II in HG and LG chicks.

Material and methods

HG and LG chicks were bred on the floor and fed a conventional starter diet provided ad libitum. Four groups of 10 birds per genotype were chosen for sacrifice at 0, 2, 4 and 7 days of age. At 2, 4 and 7 days of age, five of the ten chicks were injected with bromodeoxyuridine (BrdU) for the evaluation of the mitotic activity of muscle satellite cells following a previously described procedure (MOZDZIAK et al., 1994). Plasma samples were collected, and following sacrifice, Pectoralis major (PM) muscle samples were excised for histology and fixed in Carnoy solution,. In all histological samples, total nuclei were also counted and fibre diameter was measured allowing for the estimation of the cytoplasm volume to nucleus ratio (CNR). IGF-I and IGF-II concentrations were measured by radioimmunoassay as described before (BECCAVIN et al., 2001). All data were analysed by 2 ways ANOVA (age, line, age*line interaction) using the StatView software, and for the main effect of age differences between groups were tested by Student Newman Keuls. In the case of significant interactions, further analyses were done by one way ANOVA (effect of age within line, effect of line at any given age).

Table 1
Body weight, circulating IGF-I and IGF-II levels in HG and LG chicks between hatch (Day 0) and 7 days of age. Values are means + SEM from 10 (BW) or 8 (IGF-I or –II) individuals per genotype and age. The p values for the main effects of Age, Line and the interaction are indicated (Körpergewicht, IGF-I und IGF-II Blutkonzentration bei Hühnern der Linien HG und LG zwischen Schlupf (Tag 0) und Tag 7. Mittelwerte und Standardfehler der Mittelwerte von 10 (BW) und 8 (IGF-I, IGF–II) Individuen pro Genotyp und Zeitpunkt. P-Werte für die Effekte Alter und Linie und deren Interaktion sind angegeben)

	Body weight (g)		IGF-I (ng/ml)		IGF-II (ng/ml)	
Day	HG (+ SEM)	LG (+ SEM)	HG (+ SEM)	LG (+ SEM)	HG (+ SEM)	LG (+ SEM)
0	37 + 1 a, A	31 + 1 b, A	3.94 + 0.36	3.94 + 0.22	34.0 + 2.0 a, A	33.5 + 2.0 a, A
2	46 + 1 a, B	35 + 1 b, B	5.78 + 0.21	4.35 + 0.20	38.6 + 2.5 a, A	26.9 + 1.4 b, B
4	60 + 1 a, C	39 + 1 b, C	6.85 + 0.45	5.12 + 0.32	47.6 + 2.3 a, B	40.2 + 1.7 b, C
7	91 + 14 a, D	52 + 1 b, D	7.56 + 0.48	6.51 + 0.25	44.3 + 1.3 a, B	32.8 + 1.7 b, A
Age	P=0.0001		P=0.0003		P=0.0001	
Line	P=0.0001		P=0.0001		P=0.0001	
I	P=0.0001		P=0.16		P=0.01	

[a, b] Different letters indicate differences between genotypes at the same age for a given parameter. [A, B, C, D] Different letters indicate differences between ages within a genotype.
[a, b] Unterschiedliche Buchstaben kennzeichnen signifikante Unterschiede zwischen Genotypen zu einem Zeitpunkt, [A, B, C, D] Unterschiedliche Buchstaben kennzeichnen signifikante Unterschiede zwischen Zeitpunkten innerhalb Genotyp

Results

The chicks already differed for body size at hatch, and the amplitude of the differences increased with age (Table 1). At hatch, the levels of circulating IGF-I and IGF-II were

similar between genotypes, then they were higher in HG than in LG from day 2 to day 7.

Breast muscle fibres grew in size from 1 to 7 days of age, especially between 4 and 7 days (Table 2). A significant difference between genotypes was observed across the first week of age and increased markedly between day 4 and 7. The cytoplasm to nucleus ratio also increased with age essentially between 4 and 7 days of age, it was higher in HG than in LG from day 2 to day 7. By contrast, the proportion of dividing nuclei decreased between day 2 and 4 in both genotypes, then remained low in HG while they increased in LG at day 7 (Table 2). As a result of this interaction, it was higher in HG than in LG at 2 and 4 days of age and lower at 7 days of age.

Table 2

Cellular parameters of breast muscle growth in HG and LG chicks between hatch (Day 0) and 7 days of age. Values are means + SEM from 5 individuals per genotype and age. The p values for the main effects of Age, Line and the interaction are indicated (Parameter des Zellwachstums bei Hühnern der Linien HG und LG zwischen Schlupf (Tag 0) und Tag 7. Mittelwerte und Standardfehler der Mittelwerte von 5 Individuen pro Genotyp und Zeitpunkt. P-Werte für die Effekte Alter und Linie und deren Interaktion sind angegeben)

	Fibre diameter (µm)		CNR (µm3)		Labelling index (%)	
Day	HG (+SEM)	LG (+SEM)	HG (+SEM)	LG (+SEM)	HG (+SEM)	LG (+SEM)
0	5.69 + 0.13 a, A	5.15 + 0.09 b, A	388 + 15 a, A	389 + 20 a, A	ND	ND
2	7.12 + 0.16 a, B	6.36 + 0.16 b, B	442 + 9 a, A	379 + 17 b, A	9.76 + 0.65 a, A	7.86 + 0.97 a, A
4	7.97 + 0.28 a, B	7.02 + 0.23 b, B	504 + 30 a, A	427 + 18 b, A	2.99 + 0.41 a, B	1.32 + 0.90 b, B
7	13.55 + 0.53 a, C	10.89 + 0.17 b, C	1446 + 90 a, B	1066 + 76 b, B	2.32 + 0.16 a, B	4.51 + 0.30 b, C
Age	P<0.0001		P<0.0001		P<0.0001	
Line	P<0.0001		P<0.001		P=0.29	
I	P<0.001		P<0.001		P<0.001	

[a, b] Different letters indicate differences between genotypes at the same age for a given parameter
[A, B, C, D] Different letters indicate differences between ages within a genotype. ND: Not Determined.
[a, b] Unterschiedliche Buchstaben kennzeichnen signifikante Unterschiede zwischen Genotypen zu einem Zeitpunkt
[A, B, C, D] Unterschiedliche Buchstaben kennzeichnen signifikante Unterschiede zwischen Zeitpunkten innerhalb Genotyp; ND=nicht bestimmt

Discussion

The present study extends our previous observations on HG and LG chicks, by showing that differences in overall development (body weight) already existed from the day of hatch and that their amplitude increased quickly. The IGF peptides which stimulate growth by activating cellular growth and metabolism already circulated at higher levels in HG than in LG chicks, from day 2. This observation further points to them as possible candidates to explain those differences.

Our data show that differences in muscle fibre size between HG and LG chicks were observed from the day of hatch and increased strongly between day 4 and 7. The CNR was identical at hatch, indicating a similar nuclear density. It increased with age in both genotypes. This observation is consistent with a study conducted on turkey chicks showing that the CNR increases with age from 3 to 26 weeks of age (MOZDZIAK et al., 1994). Therefore a similar mechanism occurs in both chicken and turkey and the present study shows that it starts very early in the chicken. The CNR increased more rapidly in the HG than in the LG genotype and therefore it was higher in the former than in the latter from day 2 to 7 (+17 to +35 %). This lower nuclear density implies that the HG should present only about 39% more nuclei in their Pectoralis major

muscle, which from the differences in body weight is expected to be about 75% heavier.

The BrdU labelling index decreased with age as previously observed in the turkey using a similar procedure applied to older birds aged 3 to 26 weeks (MOZDZIAK et al., 1994). The values of the present study are much higher than those observed at 3 weeks of age (around 1.4 %). The BrdU labelling index differed between the two genotypes, but with a strong age by genotype interaction. It was about double in HG than in LG at day 4, while it was lower in HG than in LG at day 7. This suggests that satellite cell activity is first delayed, and then prolonged in this slower growing genotype.

The growth curves of HG and LG chicks are somehow reminiscent of those obtained when comparing chicks from a standard genotype either fed from hatch or starved for their first 48h of life (HALEVY et al., 2000). Starved chickens showed a reduced mitotic activity of their satellite cells in vitro (HALEVY et al., 2000), consistent with a lower BrdU and PCNA (a marker of DNA synthetic activity) labelling indexes on muscle sections. During catch-up growth following delayed feeding from day 2, the starved chicks showed a higher PCNA labelling index at day 3 and 4. In a comparable study conducted on turkey chicks similar differences in BrdU labelling index were observed between fed and starved chicks 3 days post-hatch (MOZDZIAK et al., 2002). In this study, a reduced fibre size and a higher nuclei density were recorded in starved turkeys. However, no evidence for catch-up proliferative activity was noted during catch up growth between 2 and 9 days of age (MOZDZIAK et al., 2002).

Assuming that the feed intake of LG chicks is much lower than that of HG chicks, the results of our study are consistent with those obtained when comparing delayed versus early fed chicks (HALEVY et al., 2000) or turkeys (MOZDZIAK et al., 2002). Furthermore, it was recently observed that circulating IGF-I levels were lower in delayed than in early fed chicks (GUERNEC et al., 2004). Accordingly, the activity of the P70S6 kinase, a substrate for IGF action on proliferation and protein synthesis, was lower in the former than in the later (BIGOT et al., 2003). Therefore, a network of data is consistent with the hypothesis that a difference in feed intake between HG and LG chicks, during the first week post hatch, could induce differences in IGF levels, with an impact on satellite cell mitotic activity as well as on muscle protein synthesis and therefore the CNR. The primary cause remains to be identified.

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