Chapter 15
Importance of muscle development and growth for animal production and meat quality

N. Oksbjerg & M. Therkildsen

This chapter deals with:

- The basic principles of muscle development and growth
- Myogenesis: Factors of importance for regulating myogenesis
- Intrauterine growth restriction and implications for livestock animal performance
- Postnatal growth (the growth of the individual fibre in length and girth)
- Protein turnover (protein synthesis and protein degradation)

1. Introduction

Muscle growth rate is the most important economic trait in meat production, because muscle growth rate is highly and positively related to daily gain, highly negatively correlated with feed conversion ratio (kg feed/kg gain) and highly positively correlated with meat content of the carcass (Figure 15.1). In other words, the higher the muscle growth rate, the higher the daily gain, the higher the meat content, and the lower the feed conversion ratio. The relationships are true both when pigs have free access to feed and when they are fed restrictedly. Daily gain, feed conversion ratio and meat content in the carcass are crucial traits in meat production, because feed costs constitute around 70-80% of the total expenses for meat production, and weight and lean meat percentage determine the price of the carcass at the slaughterhouse. The main purpose of this chapter is to describe the principles of muscle growth and development, and discuss factors of significance influencing muscle development, growth and meat quality.

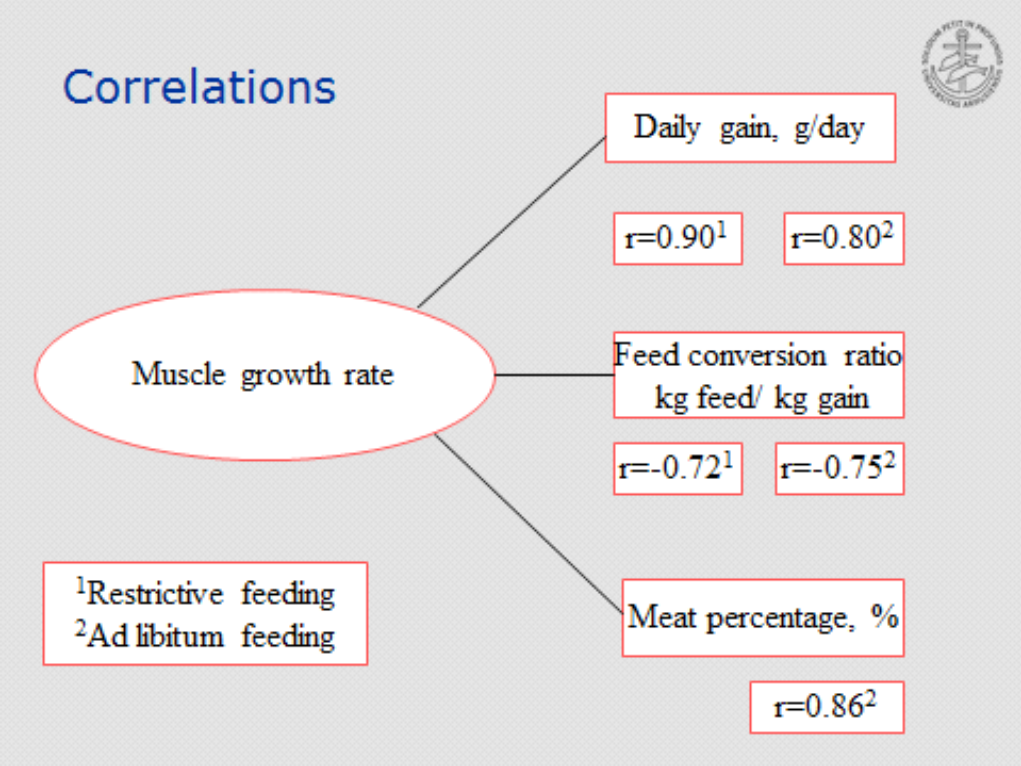


Figure 15.1.Relationship between muscle growth rate and performance.

2. Basic principles of muscle development and growth

2.1. Prenatal events

Muscle tissue develops from the mesoderm and all events leading from mono-nucleated mesodermal cells to highly differentiated muscle fibres are called myogenesis (formation of muscle). Mesodermal cells are specified to become myoblasts (muscle cells), which proliferate several times. They align and eventually fuse forming multinucleated myotubes (a primitive muscle fibre), which express muscle-specific proteins (myosin, actin, troponin etc.) (Figure 15.2). Finally, they become innervated by the nervous system and mature. Two populations of muscle fibres are formed. The first population is formed from day 25 to day 55 of gestation and is termed Primary fibres (P-fibres). This population is few in number, but with a large cross-sectional area. From day 50 to day 90 of gestation, a second population of muscle fibres forms, termed Secondary muscle fibres (S-fibres). These fibres use the surface of the P-fibres as a template. In the pig, the ratio between S:P fibres is 22-24:1. It is in general accepted that the number of muscle fibres is constant at or shortly after birth. However, recent studies have identified muscle fibres with small cross-sectional areas neonatal, and it has been speculated that these fibres may constitute a third population of muscle fibres. In fact, it has been shown that in pigs the number of muscle fibres increases from birth until day 35 of age. Thereafter, no changes in muscle fibre number occur at least until day 210 of age. Several studies have shown that the muscle fibre number is positively correlated to daily gain and negatively correlates to the feed conversion ratio (Figure 15.3). Thus, the higher the muscle fibre number, the higher the daily gain and the lower the amount of kg feed per kg gain. Because of these relationships it is important to study the mechanisms behind the genetic and environmental variations in muscle fibre number in order to develop tools for increased muscle fibre number in our meat producing animals in harmony with the animals. Factors of importance are maternal feeding, selection, hormone and growth factors, and proteolytic enzymes.

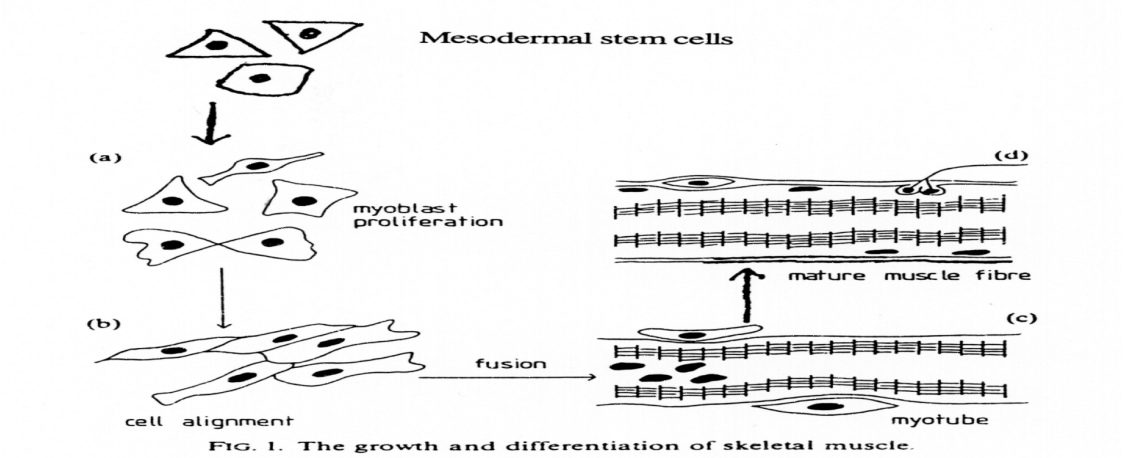


FIG. 1. The growth and differentiation of skeletal muscle.

Figure 15.2.The various steps in the myogenesis (data from Harper & Buttery (1988)).

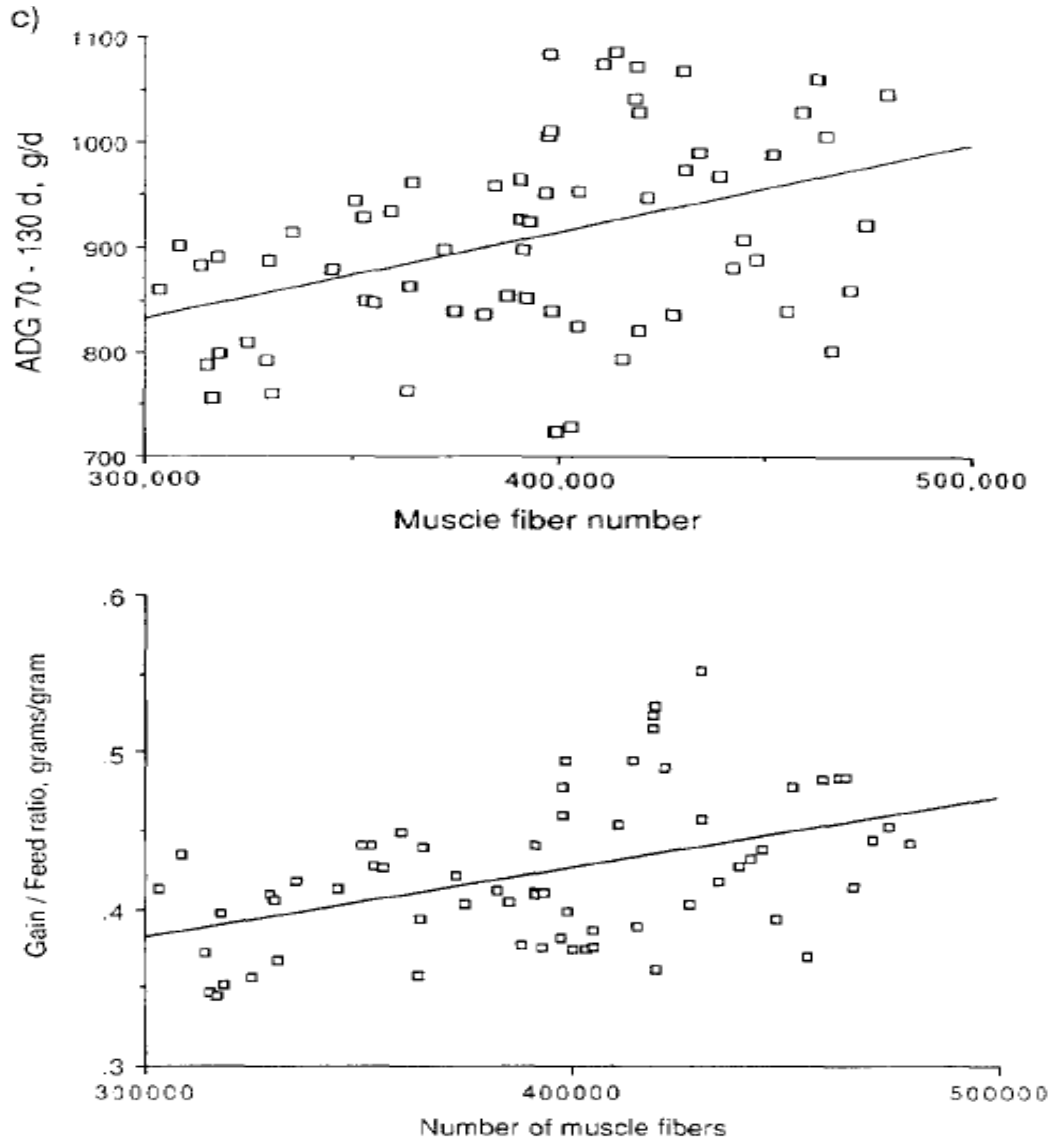


Figure 15.3. Postnatal daily gain (top) and kg gain/kg feed (bottom) in relation to total muscle fibre number of M. semimembranosus [3].

2.2. Postnatal events

Besides the number of muscle fibres, postnatal growth is related to i) satellite cell proliferation and ii) increased cross-sectional area and increased length of the individual muscle fibres. During growth, the number of myonuclei or content of DNA increases in muscles. This happens although

the myonuclei are mitotic inactive. However, between the sarcolemma (plasma membrane) and the basement membrane of the fibres is where mononucleated cells, called satellite cells, are situated. The satellite cell was shown to divide (proliferate), and one or both daughter cells may fuse and add additional DNA to existing fibres. This is an important event because DNA provides the machinery (gene codes) for protein synthesis. The increase in cross-sectional area and length of muscle fibres is due to two dynamic processes, i.e. the rates of muscle protein synthesis and protein degradation. In growing animals, the rate of protein synthesis exceeds the rate of protein degradation. In fully outgrown (or mature) animals, protein synthesis is similar to that of protein degradation, whereas in aging muscle the rate of protein degradation exceeds the rate of protein synthesis causing smaller muscle fibres. Mechanistic studies on factors regulating satellite cell proliferation and the rate of protein turnover is therefore important in order to obtain optimal growth and efficiency of growth. Many factors like fibre types, nutrition, selection, hormones and growth factors affect postnatal muscle growth.

3. Myogenesis: Factors of importance for regulating myogenesis

3.1. Relationship between muscle fibre number and performance

Muscle fibre number is calculated by measuring the cross-sectional area of the muscle divided by the mean cross-sectional area of the fibres (Figure 15.4).

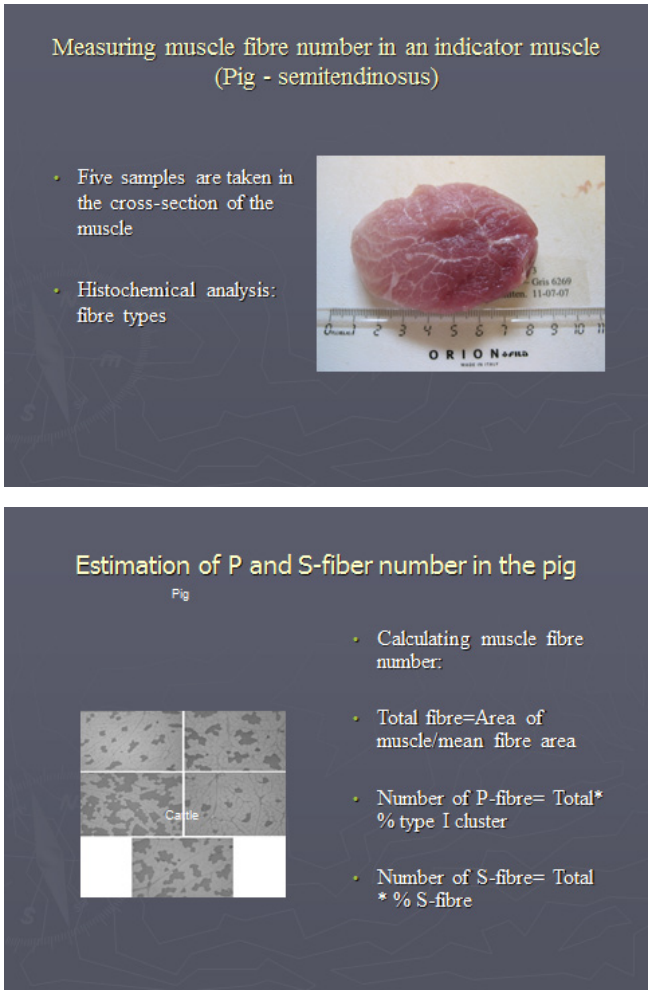


Figure 15. 4. Estimating the total fibre number and the total number of Primary and Secondary fibres.

M. Semitendinosus is used as an indicator muscle in that this muscle is cylindrical and the muscle fibres run parallel with the muscle from one end to the other. In pigs, as well as in other mammals, two major fibre types are present based on myosin ATPase isoforms: Type I fibres are slow-contracting and energy used for contraction is released from aerobic processes. Type II fibres are fast-contracting and energy for contraction is obtained mainly from anaerobic glycolytic processes. Type II can be subdivided into Type IIa and Type IIb. The metabolic difference is that Type IIa is more oxidative than Type IIb. In pig muscles, the Type I fibres are organised in clusters (Figure 15.4) and it has been shown that one of these fibres within the cluster was originally formed as a Primary fibre. Thus, it is possible, as shown in Figure 15.4, to estimate the number of Primaries formed during myogenesis. Small sections of a muscle piece are then cut in a cryostat and subsequently immune-stained for Type I fibres. By image analysis, the frequency of Primaries (= frequency of clusters) is then estimated and the mean cross-sectional area of muscle fibres is determined. The number of Primaries and the total number of muscle fibres formed during foetal development can then be calculated as shown in Figure 15.4. If a fast method existed for measuring the muscle fibre number, it would be obvious to use this in selection, not least because improved performance by increased muscle fibre number is associated with less impact on meat quality traits, whereas increased mean fibre area may result in some deterioration of some meat quality traits. However, such a method or marker for muscle fibre number does currently not exist.

3.2. Changes in myogenesis by selection for performance

In the mid-nineties, we examined the effect of 20 years of selection for performance on muscle and meat quality traits. This was possible because the former Institute of Animal Sciences at the research station (Tylstrup) had maintained a genotype representing Danish Landrace anno 1976. This genotype was compared to a modern Danish Landrace bought from certified breeders and raised under identical environmental conditions. This study showed that the modern genotype had an almost 300 g/day higher daily gain (958 vs. 670 g/day); a 13 % higher feed intake (2.68 vs. 2.37 kg/day) and a dramatically reduced feed conversion ration (2.75 vs. 3.64 kg/day) when pigs were grown from 40 kg to approx. 95 kg live weight. It was estimated that the number of muscle fibres in the longissimus dorsi and biceps femoris had increased by 20%. This strongly indicates that selection for performance is causing correlated increases in muscle fibre number supporting the increased performance. This presumption is supported by a German study that showed that muscle fibre number was genetically correlated with daily gain and meat lean content. These data, thus, support that the number of muscle fibre is directly related to the performance of pigs.

3.3. Regulation of myogenesis by hormones and growth factors

Myogenesis is regulated by transcription factors, hormones and growth factors. The main transcription factors are the MyoD family consisting of four transcriptions factors: MyoD, Myf-5, myogenin and Myf-6. These factors regulate the expression of muscle specific proteins like the contractile proteins. Transcription factors are nuclear proteins that can bind DNA and thereby regulate the activity of DNA. During specification, mesodermal cells start to express MyoD and Myf-5. Just before fusion of myoblasts, myogenin is upregulated, while Myf-6 is upregulated later during differentiation when the muscle fibres are maturing. The importance of transcription factors has been evaluated in knock-out studies. Thus, it has been found that silencing MyoD and Myf-5 in mice prevents the formation of muscle. On the other hand, silencing myogenin and Myf-6 result in muscle tissue, but without muscle fibres.

The rate of proliferation of myoblasts and differentiation of myoblasts into myotubes are regulated by several growth factors. These growth factors may inhibit or stimulate these processes. Insulin-like growth factor I (IGF-I) and II (IGF-II) both stimulate proliferation of myoblasts. Although proliferation and differentiation are mutually opposite processes, IGF-I and IGF-II also stimulate

differentiation. These growth factors are very important in regulating muscle fibre development and are regulated by growth hormone. Other examples stimulating proliferation are fibroblast growth factor (FGF) and transforming growth factor-β. Myostatin is a growth factor that inhibits proliferation and differentiation and thus has a dramatic effect on muscle development. The extreme muscularity of the double-muscled Belgian blue cattle is an example of this. Here a deletion in the gene producing myostatin has occurred. Due to lack of myostatin production, no inhibitory effects on proliferation take place causing increased proliferation of myoblasts and consequently more muscle fibres.

3.4. Regulation of myogenesis by proteolytic enzymes (the calpains)

Following proliferation, myoblasts align and start to fuse. In this process, myoblasts have to shear cytoplasm. This means that some disintegration of the cell membranes must occur. It is suggested that the proteolytic enzymes, the calpains, are involved in this process. Three isoforms of calpains exist in muscles: two are calcium dependent, i.e., μmol Ca^{2+} dependent Calpain (μ-Calpain) and mmol Ca^{2+} dependent Calpain (m-Calpain), and the third, termed p94, is independent of calcium. The calcium dependent calpains are further regulated by calpastatin, which inhibits the activity of calpains. μ-Calpain is believed to initiate the degradation of the myofibrillar protein. Two observations suggest that the m-Calpain is responsible for membrane degradation because cell culture studies have shown that m-Calpain is upregulated before onset of fusion, which is not the case for μ-Calpain and antisense towards m-calpain prevent fusion. Antisense is a molecular tool to downregulate a specific gene.

3.5. Intrauterine growth restriction and implications for livestock pig production

Intrauterine growth restriction (IUGR) can be defined as impaired growth and development of the foetus or its organs during gestation. In the pig (with a gestation length of 115 days), IUGR is naturally occurring like in other litter-bearing animals and causes a large variation in birth weight (1.37 ± 0.26 kg). Furthermore, we demonstrated that IUGR piglets develop fewer primary fibres (formed on day 25-50 of gestation) and secondary muscles fibres (formed on day 50-90 of gestation) during foetal development, and have a slower daily gain postnatal (Figures 15.5 and 15.6). Phenotypically, litter size is inversely related to the average birth weight, and a large litter size is associated with a higher number of IUGR piglets resulting in higher mortality rates. This phenomenon likely contributes to the increased mortality rates observed the last two decades in Denmark following selection for increased litter size, and it is therefore obvious that IUGR causes a large economic loss in pig production. It has been speculated that development of IUGR piglets is caused by a reduction in nutrients transfer across the placenta. Consequently, experiments aimed at increasing nutrients transfer across the placenta were carried out. These studies include increased maternal feed uptake, treatment with growth hormone, dietary inclusion with L-arginine and L-carnitine.

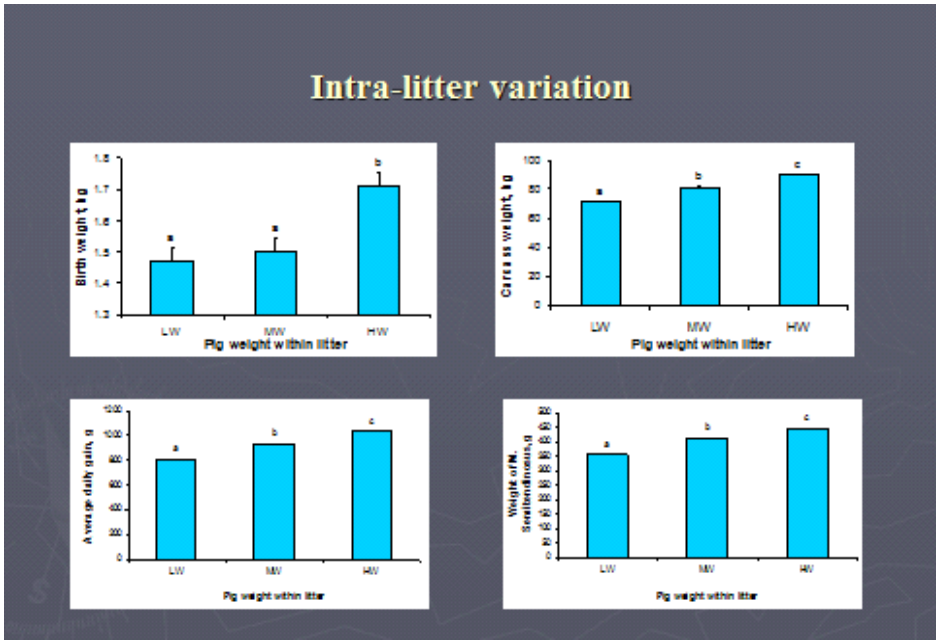


Figure 15.5. Birth weight, carcass weight at slaughter, average daily gain and weight of M. semitendinosus in lightest-weight pig (LW), middle-weight pig (MW) and heaviest-weight pig (HW) within a litter (data from Nissen et al. [5]).

3.6. Maternal feed intake

A gestating sow is only offered between 30 and 40% of her voluntary feed intake. Therefore, it could be suggested that this low level of feed intake restricts the transfer of nutrients across the placenta of the IUGR foetus. Thus, some experiments have been conducted where gestating sows were offered increased feed intake in specific windows during gestation associated with muscle fibre formation. However, increased feed intake from day 25 to day 50 (Primary muscle fibre formation), day 50 to day 80 (Secondary muscle fibre formation), or from day 25 to day 80 of gestation muscle fibre number had no consistent effect, and most studies revealed no effect on birth weight, muscle fibre number and postnatal performance. One study, however, showed an increased muscle fibre number in the smallest littermates.

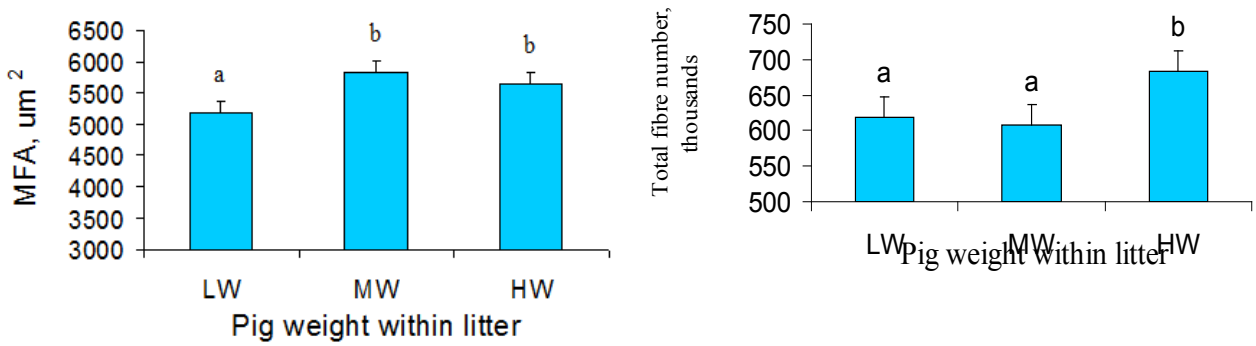


Figure 15.6. Variations in mean cross-sectional area (left figure) and total number of muscle fibres among low birth weight piglets, mean birth weight piglets and heavy birth weight groups within a litter. ([5]).

3.7. Maternal treatment with porcine growth hormone

Porcine growth hormone (pGH) is a protein produced in the anterior pituitary gland and is species-specific. Growth hormone has a significant effect on the growth of various tissues. In fat tissue it has a direct effect on lipid metabolism, while in muscles, the effect of GH is regulated via IGF-I level. Thus, in growing pigs, daily injections with pGH reduce fat accretion, but increase muscle growth. Besides the growth-promoting effect on muscles, pGH also has a diabetogenic effect, resulting in hyperglycaemia and hyperinsulineamia, because muscle tissue becomes less sensitive to insulin. Similar effects have been found in gestating sows in response to pGH treatment. Because glucose is the major energy substrate for the foetus, hyperglycaemia following maternal pGH may increase the maternal/foetal blood glucose gradient resulting in higher foetal uptake of glucose and thereby increase muscle fibre number and foetal growth.

Maternal treatment with pGH may influence foetal growth and birth weight depending on the treatment windows during gestation and maternal dietary protein level. The smallest piglets at birth within a litter may be expected to benefit most from treatment of the sows with pGH. It has been reported that the increase in piglet body weight (70 g) when their mothers had been treated with pGH in early gestation, although non-significant, was more pronounced in small littermates (+242 g) than in medium (+73 g) and larger littermates (+43 g).

Some studies concerning maternal treatment with pGH found increased number of muscle fibres of small littermates whereas others found no effect. The consequences in terms of postnatal growth have not been studied. There may be an interaction between GH treatment and dietary protein level. Thus, piglets from sows treated with pGH in the first quartile only increased their birth weight if the sows diet contained a high protein level (22.2%) compared to a normal level (13.5%). Thus, these studies warrant further research to establish the effect of maternal treatment with pGH on birth weight, muscle fibre number and the consequences for postnatal growth.

3.8. Dietary inclusion of L-Carnitine

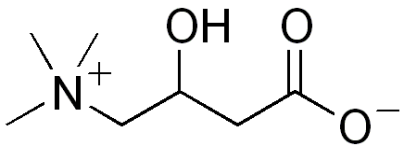


Figure 15.7. L-Cartinine.

The primary role of L-Carnitine (Figure 15.7) is to transport long and medium chain fatty acids across the mitochondrial membrane for β-oxidation, although it is also involved in protein synthesis and glucose homeostasis. Hundred to 125 mg/day of L-Carnitine in the diet for sows during gestation and lactation have a positive effect on litter weight as well as on birth weight and weaning weigth. Recently, it was demonstrated that the total number of muscle fibres was increased after weaning, but the extent to which this gives rise to an increased postnatal daily gain remains to be studied. However, these results are very promising and should be studied further.

3.9. Dietary inclusion of arginine

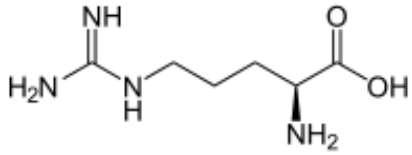


Figure 15.8. L-Arginine.

We have recently demonstrated that the concentration of glucose in umbilicus blood was higher in large littermates compared to small littermates on day 110 of gestation indicating that IUGR piglets have access to less glucose. The placental uptake of nutrient follows Fick's principle:

$$\text{Uptake of nutrients} = \text{blood flow } [A\text{-}V]$$

where [A-V] is the arterio-venous difference. However, studies in ewes and cows have demonstrated that the major change in nutrient uptake during normal gestation is mainly due to an increase in blood flow and only to a minor extent to an increased extraction of nutrients. Thus, adequate blood flow across the placenta is the major critical factor for normal foetal growth. Blood flow capacity is related to the placental size and vascular growth (angiogenesis).

Various animal models to study mechanisms of IUGR have been established encompassing over- and undernutrition of ewes, heat stress, and multiple pregnancies. Consistently these studies have shown that placental size and vascularisation (angiogenesis), blood flow, and expression of vascular endothelium growth factor (VEGF, a growth factor stimulating angiogenesis) were decreased, while the placental vasoactivity was increased in IUGR.

L-arginine (Figure 15.8) may prove to be a substance that can ameliorate IUGR without ethical and welfare problems as the use of L-arginine is approved for piglets. Arginine is a non-essential amino acid, but arginine is also the precursor for the production of nitric oxide (NO). This reactive compound stimulates angiogenesis and causes vasodilatation (increased diameter of blood vessels, which reduces the resistance and increases the blood flow). The conversion of arginine to NO is catalyzed by epithelial nitric oxide synthase (eNOS). In addition, arginine can also be converted to ornithine and further to polyamines (PA) (putrescine, spermidine, spermine) catalyzed by arginase and ornithine decarboxylase (ODC), respectively. The effect of PAs on cell proliferation and differentiation supports the utilization of increased nutrient transfer by increased blood flow. In the pig, some studies have examined the response to L-arginine metabolism. Recently, it was reported that L-arginine, eNOS (activity), ornithine, ODC (activity) and polyamines were reduced in muscles from IUGR foetuses compared to their larger littermates. These results indicate that low arginine status may be involved in IUGR via reduced eNOS activity and reduced production of polyamines. A few studies have investigated the effect of dietary inclusion of L-arginine in the maternal diet on reproduction. It was shown that dietary inclusion of L-arginine in feed for gestating sows increased the number of piglets born alive by 2, which was due to fewer piglets being stillborn and higher survival rates (1.2 and 0.7 piglets per litter, respectively). The average litter weight was increased by almost 3.2 kg in offspring from L-arginine treated sows compared to offspring from control sows. Moreover, the average birth weight increased non-significantly by 50 g/piglet. It is highly likely that the IUGR piglets gained more weight than piglets on average as it is expected that the IUGR piglets are the responders to maternal L-arginine. The reason for this statement is that we recently found that when gestating sows were fed a low dietary protein diets (to induce IUGR), the main effect was a reduction of foetal weight at d110 of gestation of the smaller foetuses. The effect of adding L-arginine to the maternal diet from day 14 to day 28 in gestation was an increase in the number of primary fibres and the number of viable foetuses increased by 3.7 foetuses at day 70 of gestation. In support of this hypothesis, it was found that parental administration of L-arginine prevents foetal growth restriction in undernourished ewes. Finally, L-arginine treatment increased foetal growth in women with IUGR.

However, research needs to clarify whether L-arginine is effective in preventing IUGR and how it affects the postnatal growth performance of IUGR piglets. Further, it is interesting to study L-carnitine and L-arginine in combination and separately.

In terms of meat quality, it has been demonstrated that muscle fibre number does not affect the drip loss of the meat, while the larger the fibres, the higher the drip loss. However, the higher the number of muscle fibres, the smaller the cross-sectional of the fibres. This will result in lower concentrations of myoglobin and consequently cause lighter and less red meat.

4. Postnatal growth - the growth of the individual fibre in length and girth

4.1. The satellite cell

During growth, DNA accumulates in muscles of mammals, and 60-70% of the DNA present at slaughter has been accumulated during postnatal growth. This increase can be attributed to the satellite cells. In 1961, a small mono-nucleated cell situated between the cell membrane and the basement membrane was identified.

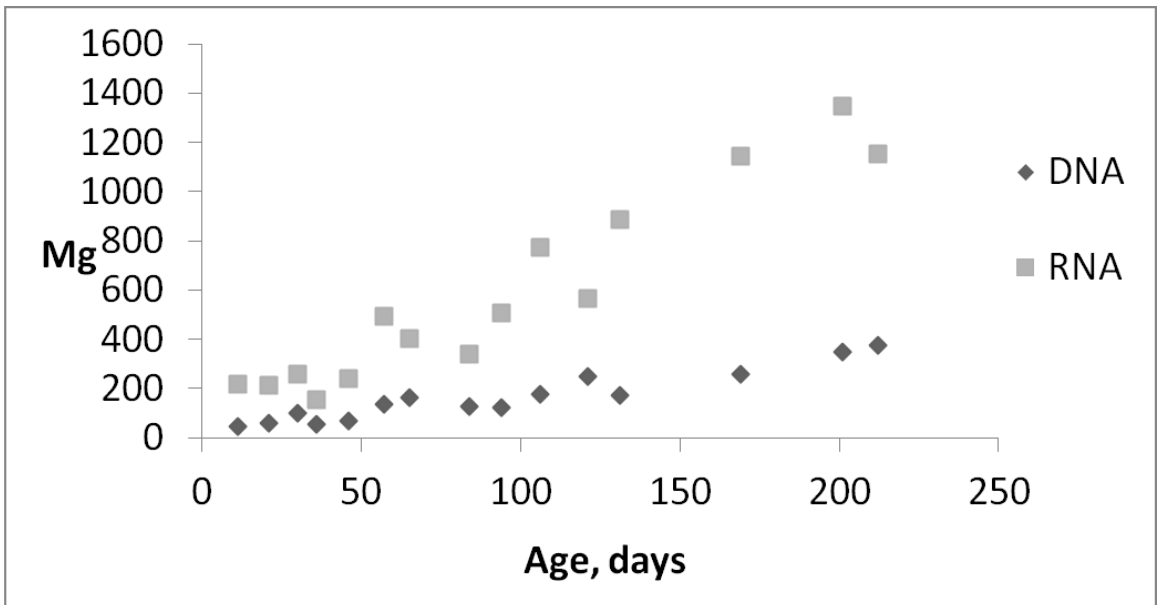


Figure 15.9. Changes in total DNA and RNA content by age in M. longissimus dorsi.

Because this cell is situated in the periphery, it was termed a satellite cell and it was shown that the satellite cell could divide and that one or both daughter cells fuse with the muscle fibres and become myo-nuclei. Thus, the satellite cell proliferation (or cell division) is responsible for the increase in muscle DNA during postnatal growth (Figure 15.9). The satellite cell is very important for postnatal muscle growth; firstly because the DNA provides the machinery for protein synthesis, and secondly, it has been shown that if gamma-irradiation is used, which kills satellite cell proliferation, postnatal muscle growth rate is reduced.

4.2. Dependency on age, fibre type, genotypes, feeding strategy, and growth factors

The number and proliferation rate of the satellite cells are dependent on the age of the animal. Thus the rate of proliferation of the satellite cell is high at birth and shortly after, while in mature animals, where the growth of muscle has ceased, the number and the rate of proliferation are markedly reduced and the satellite cell becomes quiescent. However, if the muscle fibres are injured, the adult satellite cells become activated and start to divide and repair injured muscle fibres or form new muscle fibres. In pigs, there is a linear increase in muscle DNA from birth to around 200 days of age (130-140 kg body weight) (Figure 15.9). By counting the number of nuclei in the M. longissimus dorsi muscle (mainly Type II fibres) on histochemical sections stained for nuclei, these studies confirmed a linear increase in nuclei per fibre (DNA) from birth to 200 days of age, whereas in M. vastus intermedius (mainly Type I fibres), the number of nuclei per fibre only increased linearly until 100 days of age (50-60 kg body weight). In addition, the number of nuclei per fibre is highest in Type I fibres in agreement with a higher protein turnover in muscles with mainly Type I fibres compared to muscles with mainly Type II fibres. Following a period of exercise training as well as single bout of exercise in untrained humans, the rate of satellite cell proliferation is enhanced.

It is possible to isolate the satellite cells from young pigs and grow them in vitro. Thus, we have isolated satellite cells from M. semimembranosus of the smaller birth weight, middle birth weight and heaviest birth weight piglets within litters. The results showed that the rate of proliferation was lowest in the smaller littermates and highest in the heavy birth weight littermate with the middle weight birth weight falling in between. Furthermore, satellite cells from the heaviest birth weight littermate differentiated faster into myofibres in vitro than the other piglets in the litter. These results indicate that the lower daily gain in IUGR pigs is not only explained by the number of fibres explain, but also by a lower cross-sectional area caused partly by a lower satellite cell proliferation (see section 3.1.).

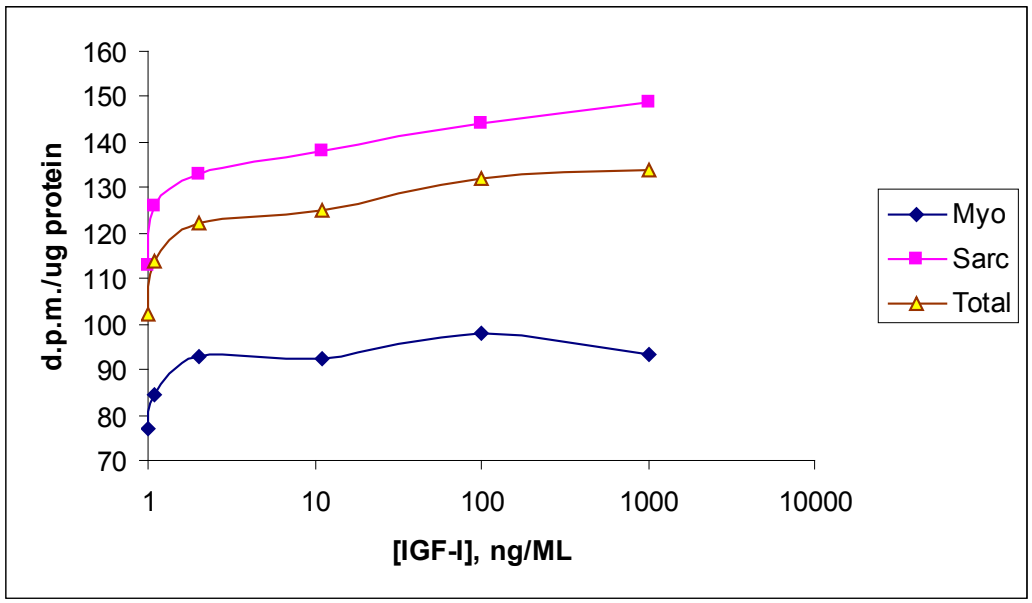
The feeding strategy may also affect the rate of satellite cell proliferation. Thus, in some production units, where the lean meat percentage of the carcass is low, restricted feeding is applied in order to increase lean meat percentage. This feeding strategy reduces muscle growth via a reduced satellite cell proliferation compared to ad libitum feeding. Finally, it has also been indicated that compensatory growth is supported by increased satellite cell proliferation (see section 5).

The link between alterations in the rate of proliferation of satellite cells by the factors described above may be mediated by growth factors, which may be either stimulatory or inhibitory. Insulin-like Growth Factor I and II (IGF-I, IGF-II), Fibroblast Growth Factor (FGF), and Transforming Growth Factors-β (TGF-β) stimulate proliferation, while myostatin is inhibitory as in myoblasts. There are several more known growth factors affecting proliferation of satellite cells. The IGFs are produced in the liver, but are also expressed in muscle tissue. Restricted feeding is associated with decreased satellite cell proliferation and lower serum level of IGF-I, and because the latter affect satellite cell proliferation IGF-I may, at least partly, mediate the effect of restricted feeding on satellite cell proliferation.

4.3. Protein turnover

The growth rate of livestock animals is related to muscle protein turnover. Thus, the more positive the muscle protein balance, the better the growth performance, the efficiency of growth, and the lean percentage; making this trait economically essential in meat production. Moreover, the efficiency is decisive for the environmental load of nitrogen and phosphorus during production. The proteolytic potential in the muscle at the time of slaughter has long been regarded as an important factor in the tenderisation process in meat, which claims high muscle protein turnover in healthy animals at the time of slaughter. Consequently, management of muscle protein turnover may enable control of the three meat quality attributes - price, tenderness and sustainability.

The muscle protein pool is not static, but dynamic with continuous turnover of proteins, which proceed throughout life even when growth has ceased (Chapter 14). The rate of muscle protein turnover is regulated through protein synthesis and protein degradation, and the rate of these processes in general exceed the actually protein accretion rate. Thus, e.g. in a 45 kg pig, the muscle protein synthesis was measured to be 169 g/day and the muscle protein degradation to be 119 g/day, resulting in a net muscle protein accretion of 29 g/day. This energy-consuming process allows the muscle fibres to adapt acutely and rapidly to environmental changes such as exercise and nutrition, and to prevent the accumulation of proteins with defects and proteins not in use. Because the muscle protein turnover is relatively large compared with the actual muscle protein accretion in growing animals, minor adaptations in either protein synthesis or degradation can have a profound influence on the net muscle protein accretion. Individual proteins have different turnover rates, ie. estimated values of protein synthesis and degradation are generally average turnover rates of the combination of individual proteins in the pool studied (e.g. skeletal muscles). In agreement we found in porcine satellite myotube cultures that the protein turnover was 2-fold higher in sarcoplasmic proteins compared to myofibrillar proteins (see Figure 15.10).



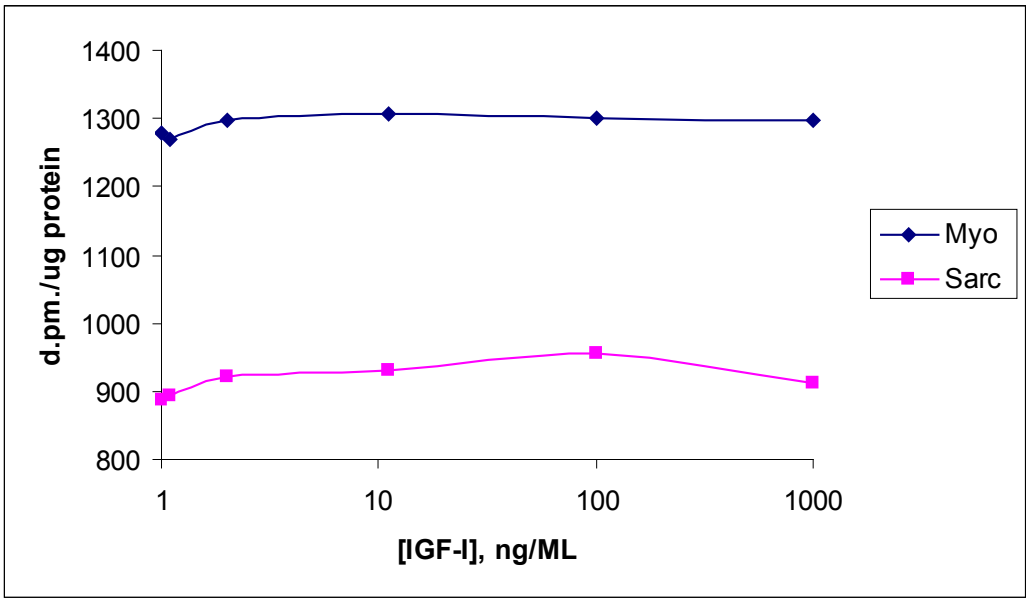


Figure 15.10. Effect of the addition of IGF-I on protein synthesis and degradation of various proteins in porcine satellite cell derived myotube cultures. Protein synthesis was measured by incorporation of tritiated tyrosine (disintegration per minute corrected for protein). The higher the d.p.m., the higher the protein syntheses. To measure protein degradation, myotubes were loaded with tritiated tyrosine and the remaining disintegration in the myotubes was measured. This means the higher the d.p.m, the lower the degradation.
Top: Protein synthesis. Bottom: Degradation.

4.4. Muscle protein synthesis

Muscle protein synthesis is the result of the transcription of DNA to mRNA and the translation of mRNA to protein (Figure 15.11). These processes involve a range of enzymes, and thus the activity of these enzymes, together with the amount of DNA and the availability of amino acids, determines the synthesis capacity (see also Chapter 14).

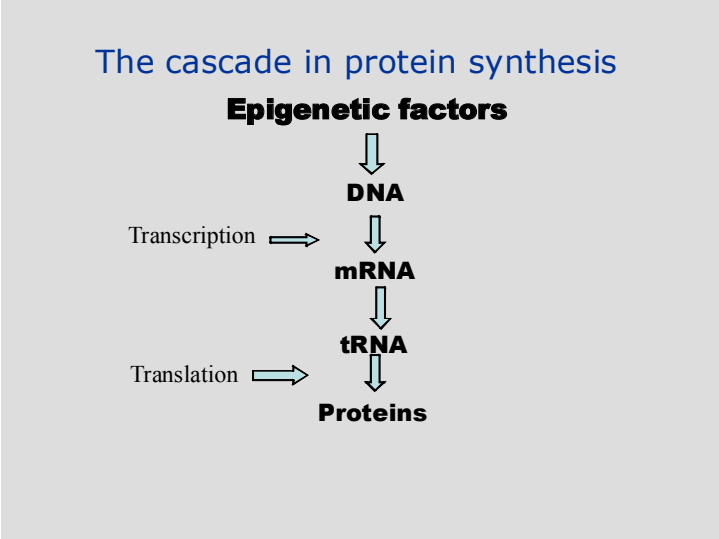


Figure 15.11. The cascade in protein synthesis.

Regulation of protein synthesis in the muscle fibre can be at the transcriptional stage (long-term) and (or) at the translational stage (short-term), i.e. in the synthesis and (or) breakdown of RNA and in the activity of existing. Protein synthesis and degradation at the muscle fibre level depend on the availability of substrates (amino acids) and on the availability of energy (ATP), as protein synthesis is an energy-consuming process. Furthermore, protein turnover is under endocrine control mainly by insulin, growth hormone/insulin-like growth factor I (IGF-I)/IGF-binding proteins (GH/IGF-I/IGF-BPs), the glucocorticoids, thyroid hormones and the sex hormones. The different hormones seem to act on different sites of the transcription–translation pathway. Hormones may act either directly on the synthesis and degradation or influence protein turnover indirectly by affecting satellite cell proliferation.

4.5. Muscle protein degradation

Proteolytic enzymes are responsible for protein degradation in the muscle fibres, and the rate of degradation varies largely in response to the physiological demands of the fibres. Three proteolytic enzyme systems have been suggested to play a major role in the proteolysis of muscle protein; the calpains, the proteasome, and the cathepsins (see also Chapter 14). Comprehensive evidence suggests that muscle protein degradation occurs in a sequential way, the calpain system acting on disassembling the myofibrillar matrix followed by the action of the proteasome and the cathepsins on the constituent myofibrillar proteins and the sarcoplasmic proteins (Figure 15.12).

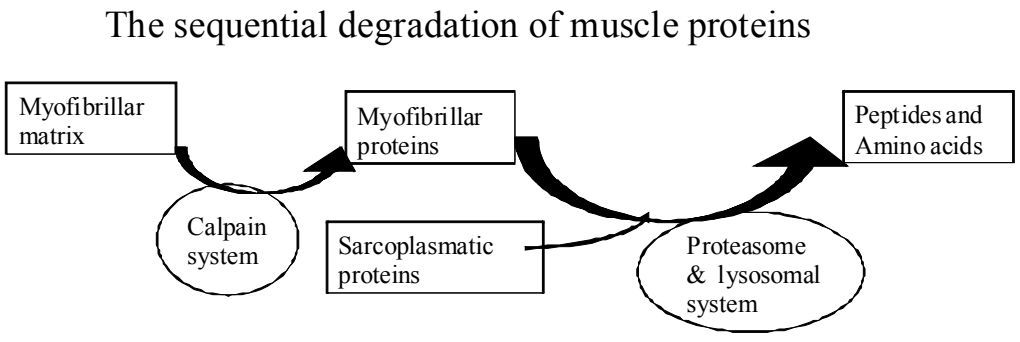
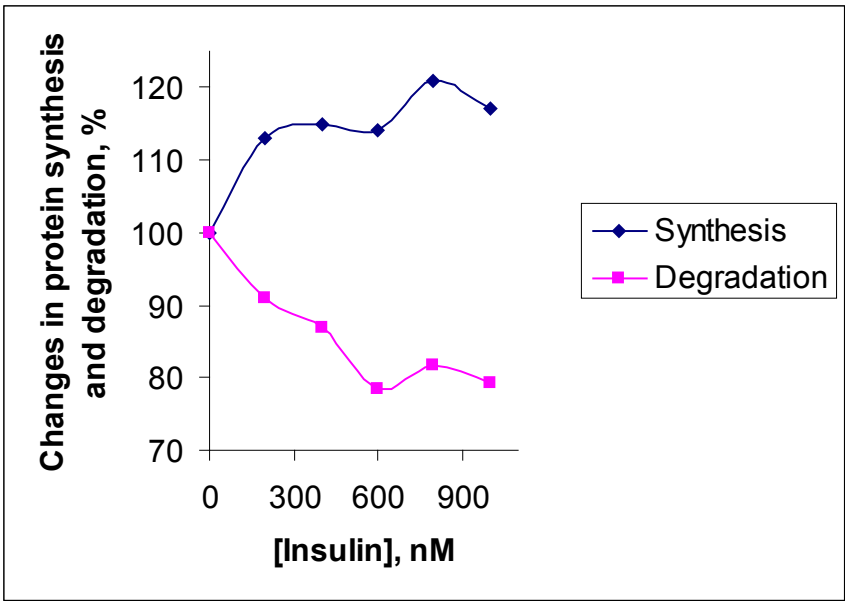


Figure 15.12. Disassembly and degradation of myofibrillar and sarcoplasmatic proteins to amino acids.

4.6. Regulation of muscle protein turnover

Insulin, an anabolic hormone tightly regulated by the influx of glucose to the body, stimulates protein synthesis. Thus, the increase in insulin caused by elevated glucose in response to feed intake may explain the increased protein synthesis observed in the same period. The response to insulin is rapid, which suggests that insulin stimulates the synthesis-activity of existing RNA (increased efficiency of translation) and that the role of insulin is to maintain a “normal” translational efficiency. However, infusion of insulin to postabsorptive rats did not restore the same protein synthesis or the same protein synthesis activity per RNA as in fed rats, although the insulin level exceeded physiological values. This has later been explained by results showing that insulin and amino acids seem to act together in stimulating muscle protein synthesis, and that amino acids change the insulin threshold concentration necessary for muscle protein synthesis. Insulin infusion has also been shown to have a depressing effect on muscle protein degradation in lambs, which is also supported by major reduction in protein degradation (both myofibrillar and total) in L6 myotubes, human skeletal muscle cells and porcine satellite cell culture upon insulin administration, see Figure 15.13. However, in cultured mammalian cells the major anabolic response to insulin is found on protein synthesis.



Figur 15.13. The influence of insulin on protein synthesis and degradation satellite cell derived myotube cultures.

Growth hormone and IGF-I are also anabolic hormones that stimulate muscle protein synthesis in several species as well as in porcine satellite cell culture (Figure 15.10). In addition, IGF-I administration to lambs has also decreased the rate of muscle protein degradation in some studies, whereas we found very little response of IGF-I administration on degradation in porcine satellite cell culture (see Figure 15.9). Treatment with GH in vivo increases the DNA content of muscle tissue, which suggests increased satellite cell proliferation after GH treatment.

The glucocorticoids are generally catabolic hormones and suppress protein synthesis and increase protein degradation. The site of action is suggested to be both at the transcriptional and the translational level.

The thyroid hormones stimulate whole body protein turnover. However, at thyroid levels above normal, the protein degradation is increased without an additional increase in synthesis. The proteolytic effect of triiodothyronine (T_3) might be linked to the proteolytic calpains as the stimulating effect of T_3 on muscle protein degradation was depressed if an inhibitor against calpain (leupeptin)

was added. Likewise, the thyroid hormones may have a regulatory effect on the activity of the cathepsins. The thyroid hormones are suggested to act at both the transcriptional and the translational level, but they are probably not involved in the acute regulation of translation as is insulin.

The sex hormones testosterone and estradiol stimulate muscle growth, and especially high levels of testosterone may explain the larger muscle mass in male animals. Testosterone stimulates protein synthesis and suppresses protein degradation. However, the effect of testosterone seems to depend on animal maturity, as no effect was found on protein synthesis and degradation in intact and castrated pigs from birth to 4 weeks of age. The anabolic effect of the sex hormones has been suggested to be indirect via the GH-IGF-axis, because the IGF-I concentration increased when steers were implanted with estradiol or a mixture of trenbolone acetate and estradiol. Lack of effect on the protein turnover when a skeletal muscle cell line was treated with either testosterone or estradiol supports this impression.

4.7. Dependency on age

Total daily protein synthesis and degradation (in gram) usually increase with increasing live weight, whereas the fractional synthesis rate (FSR=g protein synthesized/100 g protein/day) and fractional degradation rate (FDR=g protein degraded/100 g protein) decrease with age. A linear relationship between the fractional growth rate (growth rate in percentage of total weight) and the FDR has been found in well-fed rats. Accordingly, a high growth rate is accompanied by high rates of muscle protein breakdown. Consequently, in these situations the FSR may be elevated as well. The changes in the protein turnover with age can be related to changes in the activity of enzymes involved, e.g. a decreased activity of the calpain system with increased age.

4.8. Dependency on fibre types

The fibre type seems to influence the protein turnover rate. Protein synthesis and protein degradation rates increases in muscles rich in slow twitch fibres (type I) compared with muscles rich in fast twitch fibres (Type II). A general, positive relationship between fractional synthesis rate and per cent area of slow oxidative fibres has been found in studies with rats, with no relationship between the ratio of fast glycolytic:fast oxidative and glycolytic fibres (Type IIB:Type IIA) and protein synthesis. This suggests that the speed of contraction is the important factor determining protein turnover rates. Cattle possessing double muscling, i.e. Belgian white-blue breed, have a larger frequency of Type IIB fibres and fewer type IIA fibres compared with normal cattle and, in addition, a lower protein synthesis. This could, suggest a difference in the protein synthesis capacity between Type IIA and Type IIB fibres, which differ in their oxidative capacity. This is supported by results comparing protein synthesis in pork M. masseter characterized by solely Type I and Type IIA fibres with M. longissimus and M. biceps femoris characterized by a high frequency of Type IIB fibres; the M. masseter has a higher FSR compared with the other muscles (Figure 15.13). Likewise, the RNA concentration is higher in muscles rich in Type I fibres, i.e. red part of M. semitendinosusand M. masseter compared with M. longissimus dorsi (LD), rich in Type IIB fibres, which supports a larger synthesis capacity in Type I fibres. This coincides with a higher concentration of elongation factor 2 (eEF-2) in muscles rich in Type I fibres compared with muscles rich in type IIB fibres. eEF-2 works distally to both transcription and translation, and may be an even better indicator of muscle protein synthesis.

Muscles containing mainly Type I fibres show higher activities of μ-calpain than muscles containing mainly Type IIb, but at the same time there might also be increased activity of calpastatin. Thus, the ratio between calpain and the inhibitor, calpastatin, will explain the differences seen in protein degradation between fibre types.

4.9. Genotypes

Various genotypes are often characterized by their different growth potential, which is often explained by differences in muscle protein turnover, either protein synthesis, protein degradation or both.

Lambs selected over ten generations for high or low weaning weight differed with respect to protein synthesis and degradation: the lambs selected for high weaning weight had a higher protein synthesis and degradation in M. vastus lateralis. Likewise, pigs from a muscular line compared with an obese line had higher RNA concentration and RNA:DNA-ratio in the muscles and pigs from an obese compared with a lean strain had a higher activity of calpastatin suggesting a general depression of protein turnover in the obese pig strain.

Comparison of FSR in muscles from Landrace and Iberian pigs with a live weight of approx. 20 kg, revealed higher FSR in the Iberian pigs, but a smaller weight gain, which suggests that FDR is also increased in the Iberian pigs. This variation can probably be explained with variation in muscle fibre types, as unimproved and wild breeds have a higher capacity for oxidative metabolism than improved ones. Among ruminants, two genetic modifications i.e. callipyges gene in lambs and double muscling (DM) in cattle are known to induce hypertrophy in some muscles of the carcasses. In the callipyge lambs, decreased protein degradation caused increased muscle growth, whereas in the double muscling cattle both protein synthesis and degradation decrease.

The abovementioned examples of the genetic effect on muscle growth clearly demonstrate that muscle growth can be regulated in different ways by changes in protein synthesis and degradation, although the final result is an increased growth of the muscle.

4.10. Influence of exercise on muscle protein turnover

The effect of exercise on protein turnover in muscles is ambiguous, as it depends on the kind of exercise and the type of muscle studied. High-intensity resistance exercise training induces hypertrophy of the muscles involved in exercise by accumulation of myofibrillar proteins in the muscle fibres. Depending on the muscle, this accumulation has been associated with increased synthesis and either simultaneously increased or decreased degradation. With endurance training, which more resembles the exercise production animals get when they are loose-housed or grazed compared with tie-stall housed, the main change in the muscles is enhancement of the fatigue-resistance of the skeletal muscles, and only minor changes are seen in the muscle size. However, the muscle protein synthesis is decreased during the exercise, but is increased at the completion of the exercise. Lambs exercised up to 35 min. a day, five times per week on a treadmill for 30 days had higher concentration of fast-twitch isoform myosin light chain-1 ($MLC1_f$) mRNA in M. biceps femoris compared with control lambs. The $MLC1_f$ mRNA concentration was used as an index for myofibrillar protein synthesis. After 60 days of exercise, the difference had disappeared. Thus, the synthesis rate was increased with exercise, but adapted to the exercise with time. This adaptation is supported by results on rats trained for four weeks, where no difference was found in either protein synthesis or degradation. The pattern for skeletal muscle protein degradation during endurance training and in the recovery period is less well understood. However, a study with rats showed an increased degradation of total muscle protein, but no change in the degradation of myofibrillar protein immediately after treadmill exercise or during the recovery period. This agrees with recordings of the activity of μ-calpain and calpastatin as an index for myofibrillar protein degradation, where no effects from treadmill exercise of lambs on the activity of the enzyme system in M. biceps femoris were found. When slaughter pigs were injected with epinephrine and exercised on treadmill right before slaughter, activity of μ-calpain increased and no change in calpastatin was observed, which suggests increased muscle protein degradation. However the extreme energy depletion caused by the epinephrine injection would seldom be seen in a traditional production system.

4.11. Influence of feeding level on muscle protein turnover

The nutritional level has a marked influence on muscle protein turnover. Therefore, when the energy level is restricted below maintenance for a period of time (short-term), the protein synthesis decreases as expected, and the protein degradation increases in order to supply the animal with sufficient nutrients (i.e. glucose and amino acids). In contrast, when pigs are fed a restricted diet (long-term), both protein synthesis and degradation decrease, compared with pigs with free access to feed. In newborn lambs, a higher energy supply increased muscle protein synthesis, as expected, but had no effect on the protein degradation, so the developmental stage of the animal (maturity) seems to influence the effect of energy level on protein degradation.

The protein supply also has an effect on protein turnover rates. Several studies have shown a correlation between protein intake and protein synthesis and degradation. In a study with pigs fed only 61.4 % of the lysine requirement, the effect on protein turnover differed between muscles. In M longissimus only protein synthesis was depressed, whereas in M. masseter and M. biceps femoris both protein synthesis and degradation were depressed upon the lysine restriction (Figure 15.14). Thus, even though all muscles responded with a decreased fractional accretion rate, the rate of protein synthesis and degradation depends on the muscle. In the same study, the depression of the protein synthesis could be explained by a depression of the RNA activity, and not in the RNA capacity demonstrating that the regulation of the synthesis is in the translational step. In agreement with the effect of feeding sub-maintenance levels of energy, protein restriction below maintenance or protein-free rations increase the protein degradation in pigs.

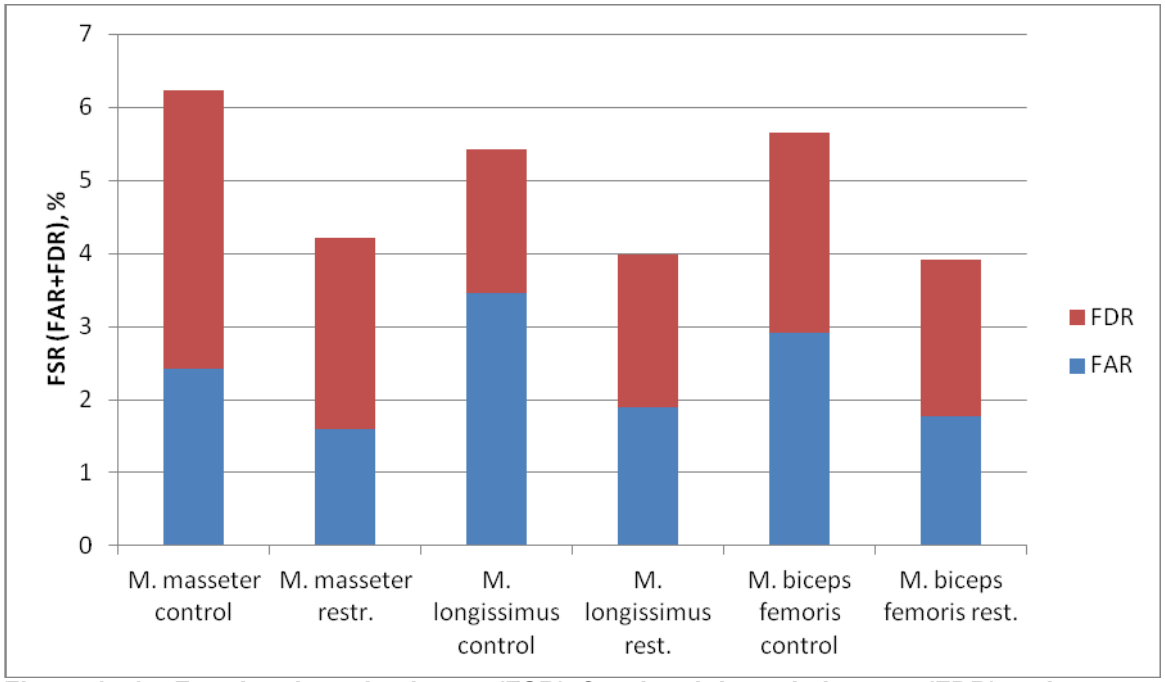


Figure 15.14. Fractional synthesis rate (FSR), fractional degradation rate (FDR) and fractional accretion rate (FAR) in M. masseter, M. longissimus and M. biceps femoris from pigs offered a diet containing 100% of recommended lysine requirement (control) or 61% of recommended lysine requirement (rest) for 17 days beginning at a live weight of 12 kg.

A model summarizing the expected relationship between muscle protein turnover, i.e. muscle protein synthesis and degradation, and the level of feed intake is presented in Figure 15.15.

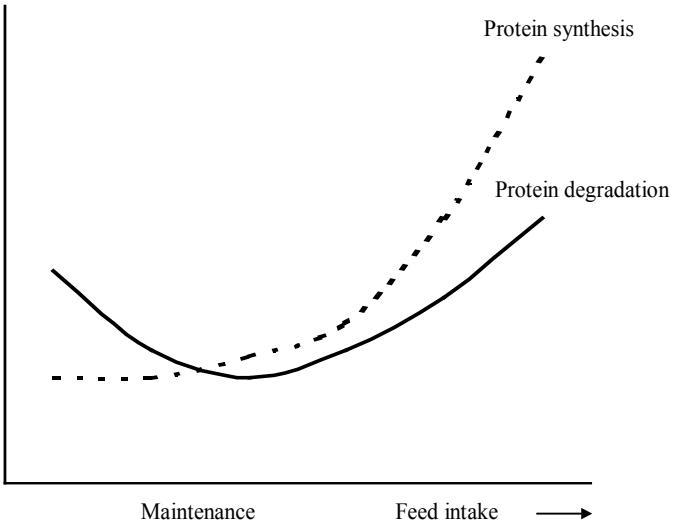


Figure 15.15. Model of the expected relationship between muscle protein synthesis and degradation, depending on level of feed intake in growing animals (modified [7]).

It was concluded that by increasing the amount of a well-balanced diet to animals in growth, both protein synthesis and degradation can be expected to increase, although at different rates, and give rise to an increased muscle protein accretion.

4.12. Muscle protein turnover and tenderness

Several reports suggest a relationship between the rate of muscle protein degradation and the rate and extent of tenderness development in meat (Table 15.1). Thus, a situation in which the rate of protein degradation is decreased may lead to increased muscle growth, but decreased tenderness, as has been found in e.g. i) treatment with b-adrenergic agonists (see Chapter 14), ii) restricted feeding iii) bulls versus steers, and iv) animals possessing the callipyge gene. In contrast, treatment with porcine growth hormone results in increased muscle growth by stimulating the rates of both synthesis and degradation without change in tenderness. Finally, short-term fasting for 5 days leads to increased rate of muscle protein degradation resulting in increased tenderness in lambs. The link between the rate of muscle protein degradation and tenderness development may be coupled to the calpain system, which is known to be the rate-limiting proteolytic system disassembling the myofibrillar proteins to their individual constitutive proteins. Thus, in cattle the inhibiting activity of calpastatin (inhibitor of calpain) is inversely related to the rate of muscle protein degradation and positively correlated with the shear force (toughness) of the meat. Having established a link between the rate of protein degradation and post mortem tenderisation, the challenge is to implement this into a feeding strategy, which will result in increased tenderness without compromising performance. This feeding strategy could be compensatory growth or catch-up growth.

Table 15.1. Factors affecting protein degradation parameters and shear force in muscle and meat, respectively, of meat-producing mammals.					
	Muscle growth	**FSR[a]**	**FDR[b]**	**Calpastatin activity**	**Shear force**
β-adrenergic agonist	↑	↔, ↑	↓	↑	↑
pGH[c]	↑	↑	↑		↔
Bull vs steer	↑	↔	↓	↑	↑
Fasting (short time)	↓	↓	↑	↓	↓
Fasting (long time)	↓	↓	↓	↑	↑
Callipyge gene	↑	↔	↓	↑	↑

a) Fractional Synthesis Rate. b) Fractional Degradation Rate. c) porcine Growth Hormone.

5. Compensatory growth

For decades, it has been recognised that pigs whose growth rate has been slowed by nutritional deprivation may exhibit an enhanced rate of growth when re-alimented. If this exceeds the maximal rate of gain when adequate nutrition is provided, the animal is said to have undergone compensatory growth or catch-up growth. An example of our experiments with compensatory growth is given in Figure 15.16. As can be seen, the restricted pigs were behind in body weight following the restrictive period (day 28-80). However, at the end of the experiment, the former restricted pigs had a body weight similar to the control pigs fed ad libitum throughout the experiment. For earlier reviews covering parts of our experiments on compensatory growth, see Andersen et al. ([1] & [2]).

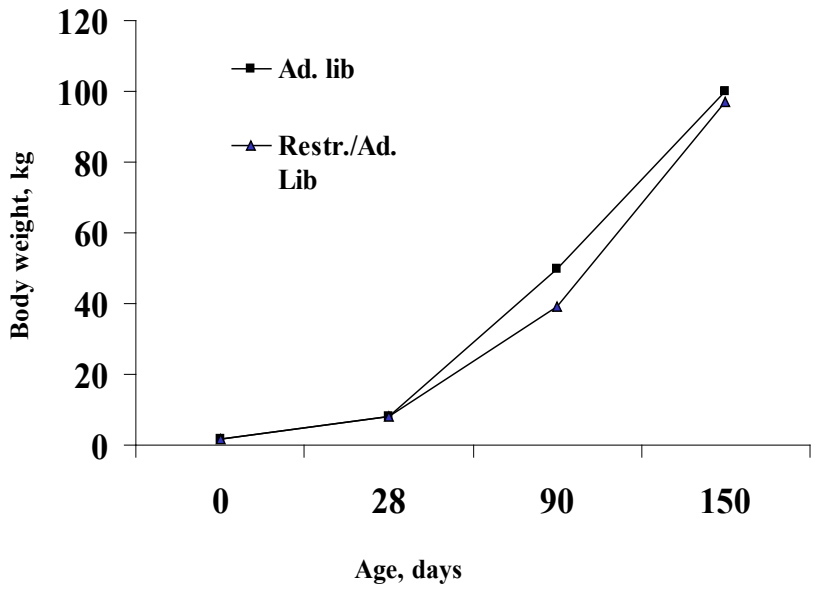


Figure 15.16. Compensatory response in daily gain following a period with restrictive feeding.

During compensatory growth, the rates of both protein synthesis and degradation are elevated according to findings in rats and cattle. Consequently, implementation of a compensatory growth strategy in meat-producing animals could be a way to improve tenderness of meat taking the production economy into consideration. However, the increase in protein turnover during compensatory growth is dynamic. Thus, initially during compensatory growth, increased protein synthesis is

evident, while protein degradation remains low as a consequence of the former restricted feeding regime. This causes a larger difference between the rates of synthesis and degradation. Later on, also the rate of protein degradation increases gradually and eventually exceeds the rate of protein degradation of control ad libitum fed animals. Thus, one of the goals to successfully implement a compensatory feeding approach in the production of meat of high quality is to establish the length of the compensatory period that results in the highest muscle protein degradation potential at the time of slaughter. This was examined and together with results using markers for protein synthesis (concentrations of RNA and elongation factor-2) and protein degradation (activity of μ-calpain and myofibrillar fragmentation index, MFI), we suggested that until 48 days of compensatory growth, the rate of protein turnover increased steadily, but the difference between protein synthesis and degradation diminished with every day on compensatory growth. Beyond 48 days of compensatory growth, the muscle protein turnover exceeded the turnover of control ad lib fed animals. Consequently, in our compensatory growth model, we fed pigs restrictedly (60-70% of ad lib intake) for 52-62 days from day 28 until they were 80 or 90 days of age and subsequently ad lib until 140 days of age (50-60 days). Feed intake in the restriction period was reduced by 60-70%, daily gain by 20-25% and kg feed:kg gain was reduced by 20%. At the end of the restriction period, the cross-sectional area (CSA) of the LD muscle and the fat layer were measured by ultra sonic equipment. In restrictedly fed pigs, the CSA of LD was reduced by 15% and the fat layer by 26%. During re-alimentation, feed intake was unchanged, while daily gain had significantly increased by 70-80 g/day due to an improved kg feed:kg gain of 6% compared to control pigs. During the entire growth period from day 28 to day 140 of age, compensatory growth resulted in similar daily gain, carcass weight and lean meat percentage, while efficiency expressed as the kg feed:kg gain ratio was reduced. The significance of the latter is that slaughter pigs on a compensatory growth strategy can be produced on 15 kg less feed per pig corresponding to an improvement in efficiency of 5 %. These experiments were carried out in a conventional production system, and the pigs reared in individual pens. However, when pigs were reared on pasture and fed restrictedly until transfer to indoor facilities with access to an outdoor concrete area at 40 kg live weight and fed ad lib until slaughter, compensatory growth was also observed.

5.1. Meat quality and compensatory growth

The technological meat quality (pH, drip loss, pigment and colour) was not affected by compensatory growth, although the pigment concentration was reduced after compensatory growth in partly outdoor reared pigs, probably due to higher intake of roughage. Consistently, the shear force of LD muscle was reduced, indicating better tenderness, or tenderness measured by a sensory panel increased by compensatory growth. This was true in female pigs, but not in castrated male pigs. The reason for this may be that compensatory growth reduced intramuscular fat (IMF) in castrated male pigs, but not in female pigs. IMF increased tenderness linearly in the range up to 2.5%. Consequently, decreased IMF may counteract the beneficial effect of compensatory growth on tenderness.

Our studies on compensatory growth show that this strategy reduces the kg feed:kg gain ratio. At the same time, the overall daily gain was unaltered as was also the lean meat percentage in both castrated male and female pigs. Moreover, the technological meat quality was unaltered, but the eating quality (tenderness) was increased in female pigs, but not in castrated male pigs.

6. Concluding remarks

Muscle fibres develop during foetal development from day 25 to day 90. From day 25 to day 50, the first population develops: this population is termed Primary fibres. From day 50 to day 90, a second population develops termed Secondary fibres. Until recently, it was accepted that the number of muscle fibres is constant at birth and remain constant during postnatal life, but research has now indicated that a third population may be formed perinatal. The number of muscle fibres is an important economic trait as it is positively related with lean meat percentage of the carcass and daily gain and inversely related with the kg feed/kg gain. The variation in muscle fibre number is

dependent on both environmental and genetic factors, and studies have estimated that selection for muscle fibre number increases performance without deteriorating technological meat quality traits unlike selection for increased cross-sectional area of the muscle fibres. Medium to high heritability has been demonstrated in pigs for muscle fibre number. However, at present no markers are available for muscle fibre number and consequently it is not possible to use muscle fibre number in selection strategies. Postnatal muscle protein turnover (accretion of muscle protein) consists of two dynamic processes: the rates of protein synthesis and protein degradation, where in growing animals the protein synthesis is larger than the protein degradation. The protein turnover is dependent on several factors such as satellite cell proliferation, fibre types, age of animal, genotype, exercise as well as feeding strategies. By using various feeding strategies, it is possible to manipulate the protein turnover. In this chapter, we have focussed on the compensatory growth response where we found protein degradation where both the rate of synthesis and degradation were increased. The enzymes that initiate muscle protein degradation in vivo are the same that tenderize meat, compensatory growth response also increases meat tenderness in female pigs, but not in castrated male pigs.

7. Literature

1. **Andersen, H.J., Oksbjerg, N., & Therkildsen, M.** (2005a) Potential control tools in the production of fresh pork, beef and lamb demanded by the European society. Livest. Prod. Sci., 94:104-124.

2. **Andersen, H.J., Oksbjerg, N., Young, J.F., & Therkildsen, M.** (2005b) Feeding and meat quality – a future approach. Meat Science. 70:543-554.

3. **Dwyer, C.M., Fletcher, J.M., & Stickland, N.C.** (1993) Muscle cellularity and postnatal growth in the pig. J. Anim. Sci. 71:3339-3343.

4. **Nissen, P. & Oksbjerg, N.** (2010) Quantification of prenatal effects on productivity in pigs. (In: Managing the prenatal environment to enhance livestock productivity; editors Greenwood, P.L., Bell, A.W., Vercoe, P.E., and Viljoen, G.J.) Springer Dordrecht Heidelberg London New York. ISBN 978-90-481-3134-1. (DOI 10.1007/978-90-481-3135-8), pp 37-69.

5. **Nissen, P.M., Jorgensen, P.F., & Oksbjerg, N.** (2004) Within-litter variation in muscle fiber characteristics, pig performance, and meat quality traits. J. Anim. Sci. 82:414-421

6. **Du, M. & McCormick, R.J.** (2009) Applied muscle biology and meat science. CRC Press, Taylor and Francis Group. ISBN 978-1-4200-9272-1.

7. **Oksbjerg, N. & Therkildsen, M.** (2007) Compensatory growth in pigs: effect on performance, protein turnover and meat quality (In Paradigms in pig science, editors: Wiseman, J., Varley, M.A., McOrist, S. and Kemp, B.) Nottingham University Press, Nottingham. ISBN 978-1-904761-56-3, pp 417-426.