Myogenesis – Possibilities of its Stimulation in Chickens

Adrianna SOBOLEWSKA, Gabriela ELMINOWSKA-WENDA, Joanna BOGUCKA, Michał SZPINDA, Konrad WALASIK, Marek BEDNARCZYK, and Monika PARUSZEWSKA-ACHTEL

Accepted May 19, 2011


Due to selection for increased body weight, modern broilers are 3-4 times heavier as compared with chickens of the laying type. The muscle mass is mainly determined by the total number of muscle fibres (hyperplasia), their thickness (hypertrophy) and different fibre types. Hyperplasia occurs during either embryogenesis or the early posthatch period. Skeletal muscles originate from the dermomyotome, which differentiates into four myogenic cell populations: myotomal cells, embryonic myoblasts, fetal myoblasts and satellite cells; the latter are the adult myoblasts, present within adult skeletal muscles to serve as a cell source for both muscle regeneration and self-renewal. Pax3 keeps migratory precursor cells non-differentiated, thereby controlling transcriptions of the MyoD gene, whereas Pax7 is a significant regulator of the satellite cell population. Manipulation of temperature and light quality and quantity have been proposed as methods of both pre- and postnatal myogenesis stimulation. Being thermogenic stimulants, both thyroid and adrenal hormones substantially stimulate metabolism. Short-term exposure of embryos to increased temperature between days 16 and 18 of incubation directly influences the proliferation and differentiation of muscle fibres, which manifest themselves in increased hyperplasia. Ultraviolet radiation is an effective means for disinfection of hatching eggs, resulting in a change of embryonic mortality rate during breeding. Especially, green light influences both body weight and the satellite cell number in the first days posthatch, thereby enhancing the growth of embryos, and causing a significant increase in both muscle and body weight. In vivo green stimulation probably enhances the proliferation and differentiation of myoblasts, subsequently causing an increase in muscle weight. The present paper highlights the possibilities of enhancing growth and development of skeletal muscles in birds by manipulation of many aspects of their regulation, thereby contributing to a further increase in production efficiency.

Key words: Myogenesis, skeletal muscle, myoblasts, satellite cells, broilers.

As a result of selection for increased body weight and due to achievements in the field of feeding and rearing, modern broilers are 3-4 times heavier (including breast muscle and thigh muscles) as compared with chickens of the laying type (DAMME & RISTIC 2003; GERKEN et al. 2003). Simultaneously, the time of rearing has been shortened to 35-42 days. Because of this, further breeding research will be primarily related to the selection of qualitative features such as flavour and tenderness, carcass fatness, thickness and types of muscle fibres, organoleptic features, etc. The evaluation of features under discussion is either impossible or difficult in vivo. Moreover, conventional selection programmes concerning these traits are expensive and extend the distance between generations. Greater understanding of both the molecular and cellular basis of growth and development of muscle tissue, which substantially determine the muscle mass, will enable us to compile methods and selection programmes involving improvement in both quantity and quality of meat (KLONT et al. 1998).
Muscle mass is mainly determined by the total number of muscle fibres, their thickness and different types. The skeletal muscle is a heterogeneous tissue composed of individual muscle fibres, diversified in size, shape and contractile protein content, which differ in terms of morphology, metabolism and physiology. Finally, these differences substantially determine both quantity and quality of meat. The selection of animals for growth rate and slaughter performance involves a choice of individuals, muscles of which contain more and more muscle fibres (hyperplasia – an increase in the number of cells in a given muscle determined mainly genetic) or their thickness (hypertrophy – an increase in thickness of cells). Another possibility of enhancing muscle mass involves its structure conversion, e.g. the distribution of differentiated muscle fibre types which differ in thickness, resulting in the higher share of white fibres with a larger diameter in a given muscle (REMIGNON et al. 1995; ELMINOWSKA-WENDA 2007).

General mechanisms of myogenesis

The total number of skeletal myofibres is defined by hyperplasia which mostly occurs during embryogenesis (pig, cattle, sheep), but may continue postnatally (rabbit). (OKSBJERG et al. 2004). An increase in the total myofibre number was confirmed by HALEVY et al. (2004, 2006a, b) during either embryogenesis or early posthatch in relation to breast muscle in chickens.

Stem myogenic cells are derived from the paraxial mesoderm which is subsequently segmented into somites. The somites initially divide into the sclerotome and the dermatomyotome. Skeletal muscles arise from a pool of premyoblastic cells which originate in the dermatomyotome of the forming somites and then differentiate into the four following myogenic cell populations: myotomal cells, embryonic myoblasts, fetal myoblasts and satellite cells. Both the myotomal cells and embryonic myoblasts migrate from the dermatomyotomal compartment, proliferate and differentiate into myoblasts which fuse to form the myotubes that are precursors of primary muscle fibres. The fetal myoblasts give rise to secondary muscle fibres. The normally quiescent satellite cells (adult myoblasts) are present within adult skeletal muscles and serve as a cell source for both muscle regeneration and self-renewal (GRZEKLKOWSKA-KOWALCZYK & SADKOWSKI 2009; KIELBOWA & KACPerczyK 2008; BUCKINGHAM et al. 2003).

Morphologically identified in 1961 by Katz and Mauro (cited after ZAMMIT et al. 2006), satellite cells constitute a population of non-differentiated mononucleated cells (MAROU 1961; KAHN et al. 1974; GAMBLE et al. 1978; POPIELA 1976; HARTLEY et al. 1992; GRABOWSKA 2007). The satellite cells, so-called because of their position on the edge of the fibre, are finally located outside the sarcolemma and beneath the basal lamina, surrounding each individual myofibre. The satellite cells are the sole source of additional muscle fibre nuclei postnatally (HALEVY et al. 2006a, b). After satellite cells divide, one of the daughter cells fuses with the growing fibre, the other remains as a satellite cell capable of further rounds of division (BUCKINGHAM et al. 2003).

The satellite cells provide a reservoir of myoblasts capable of initiating regenerations of an adult muscle after damage (MORGAN 2003). Some activated satellite cells proliferate to become incorporated into damaged muscle fibres, whereas others do not differentiate to replenish the satellite cell population. In non-damaged muscles, these cells retain the ability to divide mitotically and replicate DNA throughout life of each individual (GRABOWSKA 2007). However, some studies have indicated that the satellite cells can account for a reserve cell population within the growing muscle mass during the early postnatal period (HALEVY et al. 2004).

Two transcription factors, Pax3 and Pax7, play a critical role in the formation of skeletal muscles. These proteins are characteristic of precursor cells developing during myogenesis (GRÖS et al. 2005; MCKINNEL & RUDNICKI 2005). Pax3 plays an essential role in both the formation and specification of myogenic cells during embryogenesis (CHI & EPSSTEIN 2002; see PRZEWÓźniAK & BRZÓSKA 2008). This protein keeps migrated precursor cells non-differentiated, thus controlling transcription of the MyoD gene which regulates cell differentiation into myoblasts and myocytes (RELAX et al. 2006; see PRZEWÓźniAK & BRZÓSKA 2008). The transcription factor Pax7 is a significant regulator of the satellite cell population (OUSTANINA et al. 2004; RELAX et al. 2006). It is produced at a high level by non-differentiated satellite cells, thereby controlling expression of MyoD, a myogenic regulatory factor (OLGUIN & OLWIN 2004).

The differentiation of myoblast precursors into muscle fibres in vertebrates – including birds – is mainly controlled by four myogenic determination factors: MyoD, Myf5, myogenin and mrf-4 (herulin or Myf6) part of the MyoD family (DAVIS et al. 1987). MyoD genes have in common a 70-amino-acid, basic helix-loop-helix (bHLH) domain that is crucial for protein-protein interactions and DNA binding (BUCKINGHAM 1992; OLSON 1990). The myogenic bHLH factors divert non-differentiated cells to the myogenic lineage. They activate transcription of a wide variety of muscle-specific
genes by binding to conserved DNA sequence motifs (-CANNTG-known as E-box), that are found in the promoter and regulatory regions of these genes. In such a way, the myogenic regulatory factors (MRF) activate genes of skeletal muscle proteins such as $\gamma$-actin, isoforms of the myosin heavy chains, an acetylcholine receptor and the phosphokinase creatine gene, etc. All myogenic factors do not appear at the same stage of myogenesis. The specific sequence of myogenic factors is typical of myogenesis as follows: Myf5 is expressed first (before myotome formation), followed by myogenin and MyoD, and eventually Myf 6, the latter becomes the major transcript postnatally (BUCKINGHAM et al. 2003).

Experiments with mutant mice deficient in bHLH proteins (gene “knock-out”) have identified their function and division into primary (Myf 5, MyoD) and secondary (myogenin, Myf 6) genes. Myf 5 and MyoD are crucial for distinguishing myogenic precursors. Myogenin and Myf 6 participate in the final formation into myotubes (multinucleated cylindrical syncytia). Moreover, the expression of MyoD genes occurs exclusively in muscle fibres or myogenic precursors (GRZELKOWSKA-KOWALCZYK & SADKOWSKI 2009). HALEVY et al. (2004) analysed the expression of Pax7 within satellite cells from the pectoralis muscle of chickens at different ages in relation to the expression of MyoD and myogenin. Their results indicated that the number of Pax7 expressing cells was the greatest on the 1st day after hatch, declining successfully with age. Pax7 and MyoD expressing cells were dominant until the 4th day. A significant decrease in the number of these cells during early stages of posthatch growth is correlated with robust development of avian skeletal muscles, thereby incorporating them into existing muscle fibres. Myogenin expression occurred when cells started differentiating and peaked on day 3 posthatch of chickens. Further studies (2006a, b) confirmed the greatest number of satellite cells on days 2 and 3 posthatch, and a subsequent decrease on the 8th day. Because the total fibre number within the pectoralis muscle of chicken is mainly determined prenatally or during early posthatch, factors that influence avian embryogenesis, and whether it is possible to modify them, still need to be determined.

The factors influencing both embryogenesis and hatching results can be divided into the three following groups:

- factors related to the origin, age, feeding, and maintenance of parental flocks,

- factors dependent on the weight, structure and chemical composition of eggs,

- factors resulting from hatching technology (DEEMING & FERGUSON 2004).

Manipulation of temperature and light quality and quantity have been proposed as methods of both pre- and postnatal myogenesis stimulation.

The effect of thermal and light stimuli on myogenesis

The influence of many stimuli on early growth stages is relatively well-known. However, there is little information on the long – term effect of these stimuli and alternative influences on the posthatch development of muscles and production features. Particularly interesting are recent observations that suggest the influence of some mitogen factors, especially thermal radiation (temperature) and electromagnetic radiation, not only at the cellular, tissue or organ levels at early developmental stages in birds, but also their influence on production features.

Temperature is a well-established physical factor that significantly influences incubation. The optimum temperature of incubation (for wild birds: 33-30°C, for domestic birds 37-38°C) determines both high hatchability and the good quality of chicken (VISSCHEDIJK 1991). Exposure of embryos to different temperatures during the hatching period was found to enhance their posthatch adaptability to either high or low environmental temperature (YAHAV et al. 2004). PIESTUN and colleagues (2009) observed the influence of increased temperature (39.5°C) on both myoblast proliferation and skeletal muscle ‘hypertrophy’ in late – term (from embryonic days 16 to 18) chick embryos. These findings suggest that a temperature of 39.5°C enhances the diameter of muscle fibres posthatch. As compared to the control group, in the experimental group both a greater body weight and more pronounced effect on breast muscle growth was found. The results show that short – term exposure of embryos to increased temperature between days 16 and 18 of incubation directly influences the proliferation and differentiation of muscle fibres which manifest themselves in increased hyperplasia, resulting in greater skeletal muscle in older chickens. COLLIN et al. (2007) proved that the application of increased temperature at an early and/or late phase of embryogenesis in chickens did not increase their adaptability to different environmental temperatures at the 6th week. Instead, the effect of the same temperature towards the end of embryogenesis significantly increased the size of the pectoralis muscles, without deteriorating their quality.

Thermoregulation is mainly attributed to the development of hormonal interactions such as the

Myogenesis – Possibilities of its Stimulation in Chickens
hypothalamic – pituitary – adrenal axis (HPA-axis) and the hypothalamic pituitary – thyroid axis (HPT – axis). Both thyroid and adrenal hormones substantially stimulate metabolism, thereby being thermogenic stimulants (heat production). The influence of the environmental factors under discussion on the postembryonic development of birds is of great importance (YAHAV et al. 2004). The optimum thermal conditions during rearing of broiler chickens significantly influence the economical aspect, i.e. the cost of feed consumption and further the prize of meat (YAHAV & MC MURTRY 2001). YAHAV et al. (1995) found that broiler chickens have the ability to aclimatize to variable temperatures. The consequences of this are both an increased demand for energy needed for thermoregulation processes and a decrease in the body weight of birds. According to some authors (URDANETA -RINCON & LEESON 2004; REZAEL et al. 2006), lysine, an exogenous amino-acid, and the level of total protein in blood exert a significant influence on the muscle distribution in the carcass, and especially on the thickness of muscle fibres in broilers. The participation of the breast muscles was found to increase with an increased level of lysine and/or proteins in the diet. Instead, low levels of the feeding factors under discussion cause a decrease in participation of the breast muscles in the carcass of slaughtered chickens, resulting from a decrease in diameters of muscle fibres. A good touchstone of demand for exogenous amino-acids (lysine) is the measure of the total activity of protease in the alimentary contents of the small bowel, intestinal epithelium, liver and muscles. Generally, an increase in supply of lysine is accompanied by a decrease in the activity of protease, and vice-versa.

The influence of electromagnetic radiation on embryogenesis is diverse, depending on different wavelengths. The ultraviolet radiation is an effective means for disinfection of hatching eggs, resulting in a change of embryonic mortality rate during breeding (BEDNARCZYK 1983). There is usually a decrease in the percentage of hatching embryos, thereby improving results of the hatching.

The other kind of electromagnetic radiation is fluorescent light, which stimulates embryogenesis in chickens, being expressed by the number of somites, the body weight of embryos, the level of metabolites, and the incubation period. It influences haemopoiesis, especially both an increase in the total number of erythrocytes and an early occurrence of leucocytes in the peripheral blood of embryos, thereby stimulating the development of the immune system (BEDNARCZYK & COU DERT 1984; BEDNARCZYK et al. 1984).

Light source and spectrum are determinant factors in manipulating the growth of meat-type chickens, the effect of which is dependent on the duration, intensity and colour. The reaction of adult birds exposed to different light is diverse, and despite many trials, there was no evidence of an unambiguous influence of a defined light colour on utilitarian features of adult birds, production indexes of young slaughtered chickens, and the quality of poultry meat (ANDREWS & ZIMMERMANN 1990; GWARA et al. 1999; ROZENBOIM et al. 1999; GWARA & HODER 2001; ROZENBOIM et al. 2004a, b). Some research suggests a relationship between the light colour used during the rearing of broilers and both their muscle and body weight (ROZENBOIM et al. 1999). These parameters were highly correlated with the number of satellite cells in skeletal muscles on day 5 of chicks (HALEVY et al. 1998), which are of crucial importance for muscle growth during the early posthatch period. A statistically significant influence of blue, and in particular green light, on both body weight and the satellite cell number in the first days posthatch persuaded these authors to hypothesise that in ovo photostimulation enhanced not only the growth of embryos, but also caused a significant elevation in both muscle and body weight, and would even improve during posthatch tissue features such as flavor, tenderness, colour, and so on.

The studies concerning the effect of photostimulation during incubation of turkey eggs confirm that females reared under green light were heavier between 28 and 59 days as compared with both a white monochromatic light stimulated group and a control (no light) group (ROZENBOIM et al. 2003). Also HALEVY et al. (2006a, b) observed a greater increase in body weight and breast muscle weight in chickens after their in ovo photostimulation with a green and blue monochromatic light combination. This synergistic effect was observed on days 1 and 3 posthatch that was highly correlated with the larger number of Pax7+ cells in skeletal muscles of chickens. Therefore, it is probable that in ovo green stimulation enhances the proliferation and differentiation of myoblasts, subsequently causing an increase in muscle weight. These authors also suggested that mainly green light was responsible for growth acceleration during the early posthatching period. Similar results in birds selected for growth and meatiness were observed in chickens by WABECK and SKOGLUND (1974) and in quails by PHOGAT et al. (1985). However, adult Japanese quails raised under a green and blue monochromatic light combination were found to gain significantly less body weight than those reared under red or white monochromatic light (ROZENBOIM et al. 2003). The results of these authors suggest that the diversified effect of light on postnatal muscle growth is probably dependent on both its source and the species, breed, gender and age of birds examined.
The present paper highlights that in birds, enhanced growth and development of skeletal muscular muscles can be achieved by many potential regulatory possibilities that may contribute to a further increase in production efficiency.

References


REMIGNON H., GARRAHUHT H., MARCHE G., RICARD F. H. 1995. Selection for rapid growth increase the number and

Myogenesis – Possibilities of its Stimulation in Chickens 89


