FEED RESTRICTION AND MUSCLE FIBRE CHARACTERISTICS OF *PECTORALIS MAJOR* IN BROILER CHICKENS*

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The aim of the study was to evaluate the effect of one-week quantitative feed restriction (in days 8–14 of age) on histological characteristics of *musculus pectoralis major* in broiler chickens during the fattening period. Cockerels of Ross 308 (1215 chicken) were divided into 3 groups: *ad libitum* fed (AL; 3 × 135), restricted 80% of AL (R80; 3 × 135), and with limited feed intake 65% of AL (R65; 3 × 135). Eight cockerels per group were slaughtered in weekly intervals from day 14 of age till the end of experiment at 35 days to determine changes in the muscle fibre characteristics. The number of muscle fibres in *pectoralis major* decreased ($P \leq 0.001$) with increasing age. Fibre cross sectional area was significantly ($P \leq 0.027$) affected by the interaction of group and age. At the end of feed restriction at 14 days all groups had similar fibre area (519–539 μm^2), the differences between groups were observed at 35 days of age with the largest fibre area in R65 chickens (2296 μm^2), while R80 did not differ from AL (1728 μm^2 and 1667 μm^2). There was no effect of feeding regime on giant muscle fibre incidence.

breast; giant fibre; limited feed intake; muscular tissue; poultry

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INTRODUCTION

Growth performance has been increased in broiler chickens due to genetic progress, improvement of nutrition, and controlled environment. This rapid growth is associated with higher body fat deposition, mortality, and increasing metabolic and skeletal disorders (Saleh et al., 2005; Barbut et al., 2008). The feed restriction is applied as a prevention of these problems (Mench, 2002; Sahraei, 2012). Early feed restriction in broiler chickens stimulates compensatory growth in the realimentation period (Plavnik, Hurwitz, 1990) and induces efficiency of feed utilization (Zubair, Leeson, 1996).

Growth performance has been associated with changes in meat quality. Meat quality depends on muscle fibre characteristics, of which number and fibre cross-sectional area (CSA) are the most important. In poultry, muscle fibre formation and number of muscle fibres are complete before hatching (Smith, 1963) and subsequent muscle growth is dependent on the hypertrophy of existing fibres because of the fusion of satellite cells with the fibres (Picard et al., 2002).

Pectoralis major, the main muscle of the breast, is in chickens composed of entirely glycolytic muscle fibres of type IIB (Verdiglione, Cassandro, 2013; Velleman et al., 2014). Fast-growing chickens have larger CSA of muscle fibres in *pectoralis major* muscle than the slow-growing ones (Verdiglione, Cassandro, 2013). This increase can be associated with a higher number of giant fibres, which have fibre CSA three to five times larger than normal muscle fi-

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bres (Dransfield, Sosnicki, 1999), are usually rounded in shape, and stain darker when coloured with haematoxylin-eosin (Miraglia et al., 2006). Giant fibres consist of fibres exhibiting structural and metabolic anomalies as a hypercontraction (Verdiglione, Cassandro, 2013). The presence of giant muscle fibres is an indicator of interrupted metabolism and abnormal contraction of muscle fibres during *rigor mortis*. Dalle Zotte et al. (2001) reported that every normal muscle fibre can be converted into giant one. Increased occurrence of giant fibres is an indicator for the development of insufficient meat quality (Dransfield, Sosnicki, 1999; Rehfeldt et al., 2004).

The aim of this study was to evaluate the effect of one-week feed restriction of two intensities applied from 8 days of age on basic muscle fibre characteristics and presence of giant muscle fibres in broiler chickens during the fattening period.

MATERIAL AND METHODS

In the experiment, male broiler chickens of genotype Ross 308 (n = 1215) were divided into three groups with three replicates (3 × 135 per each treatment): the control group was fed *ad libitum* (AL), the second group (R80) received 80% of AL (50.0 g/day per chicken), and the group R65 had feeding reduced at 65% of AL (40.6 g/day per chicken). The feed restriction was applied in days 8–14 of age. Feeding of the restricted groups was derived from feeding of the *ad libitum* fed group, in which daily feed intake was registered. The amount of feed was calculated from the feed intake of the previous day. Composition of the experimental feed mixture is given in Table 1. Chemical composition of the feed was analyzed according to AOAC (1995) procedures. Before and after the feed restriction the chickens were fed *ad libitum*. Water was available free throughout the whole experiment. Birds were housed in 9 littered pens with environmental conditions corresponding with requirements for broiler chickens. Live weight was determined at weekly intervals, mortality was detected daily. Eight chickens from each group were slaughtered at 14, 21, 28, and 35 days of age. Immediately after slaughter samples of *musculus pectoralis major* were taken for detection of histological characteristics of muscle fibres. The samples were frozen in 2-methylbutan cooled by liquid nitrogen and then stored at –80°C until analysis. Cross-sections (12 μm) of samples were cut using a cryostat Leica (Leica Microsystems, Nussloch GmbH, Nussloch, Germany) at –20°C. Subsequently, staining by haematoxylin and eosin for basic histological characteristics of muscle fibres was performed. Image analysis NIS Elements AR 3.1 (Laboratory Imaging, Nikon, Tokyo, Japan) was used to detect the number of muscle fibres per 1 mm^2, their CSA and diameter. The number of giant fibres per 1 mm^2 was counted and related to the total fibre number per 1 mm^2 according to Verdiglione, Cassandro (2013).

Data were processed by two-way ANOVA, group and age using ANOVA procedure of the SAS software (Statistical Analysis System, Version 9.0, 2003). The t-test was used to evaluate the differences between the values of group and age interactions. All data were expressed as mean ± standard deviation values. $P \leq 0.05$ was considered significant for all traits.

RESULTS

The effect of feed restriction on the weight of chicken, daily weight gain, feed conversion ratio, and mortality were summarized in Table 2. The characteristics of productive performance were not significantly influenced by feeding regime. Feed restriction had positive effect on mortality rate. The lowest mortality was observed in the group R65 with the most intensive feed restriction.

The results of one-week feed restriction affecting muscle fibre characteristics of *pectoralis major* are shown in Table 3. The interaction of group and age was not manifested in the number of muscle fibres per 1 mm^2. However, the number of muscle fibres significantly ($P \leq 0.001$) decreased with advancing age.

The CSA was significantly influenced by the interaction of group and age ($P \leq 0.027$). After the end of feed restriction at 14 days of age similar CSA was measured in all groups (519, 539, and 521 μm^2 for AL,

Table 1. Chemical composition of the diet

	Starter (days 1–14)	Grower (days 15–28)	Finisher (days 29–35)
Dry matter (g/kg)	906.4	899.4	907.2
Crude protein (g/kg)	233.9	208.9	199.8
Ether extract (g/kg)	63.5	80.0	82.9
Crude fibre (g/kg)	28.2	31.7	27.7
Ash (g/kg)	57.7	56.8	53.2
Metabolizable energy (MJ/kg)	12.6	12.6	13.2

Table 2. Effect of feed restriction on live weight, daily weight gain, feed conversion ratio, and mortality of broilers

Group	Live weight at 1 day (g)	Live weight at 35 days (g)	Daily weight gain (g)	Feed conversion ratio (%)	Mortality (%)
AL	43.9	2098	58.3	1.96	8.55[a]
R80	43.8	2026	56.7	1.87	6.30[b]
R65	43.9	1965	54.8	1.88	4.95[c]
RMSE	0.2	244	2.5	0.14	2.46
Significance	0.736	0.501	0.683	0.888	0.025

AL = *ad libitum* fed group, R80 = 80% feed of AL group, R65 = 65% feed of AL group, RMSE = root mean square error

[a–c]$P \leq 0.05$

R80 and R65, respectively), but during the realimentation period the CSA was insignificantly higher in both restricted groups compared to AL. At the end of the experiment R65 group with the most intensive feed restriction had the largest CSA (2296 μm^2), while R80 group with moderate feed restriction had not significantly larger CSA compared to AL group (1728 μm^2 and 1667 μm^2 for R80 and AL, respectively). In our experiment, the CSA significantly increased with advancing age ($P \leq 0.001$).

The diameter was significantly affected by the feeding regime ($P \leq 0.041$) with greater diameter in both restricted groups at 35 days of age, when the differences were the most noticeable. The diameter, similarly to CSA, increased with advancing age ($P \leq 0.001$).

Some fibres from *pectoralis major* exhibited histological features of giant fibres and also CSA of these fibres were typical for giant fibres. However, no excessive incidence of giant muscle fibres in restricted groups was detected and also age did not affect the giant fibre percentage.

DISCUSSION

Results of performance revealed that the final live weight was not affected by the feeding regime,

Table 3. Effect of feed restriction on broiler *pectoralis major* muscle fibre characteristics

Group	Age (days)	Number of muscle fibres per 1 mm^2	Fibre cross-sectional area (μm^2)	Diameter (μm)	Giant fibres percentage (%)
AL	14	1629 ± 304	519^{g} ± 100	24.7 ± 2.5	0.09 ± 0.07
	21	1019 ± 192	777^{fg} ± 154	29.9 ± 3.0	0.10 ± 0.05
	28	594 ± 121	1361^{cd} ± 317	39.6 ± 4.6	0.05 ± 0.09
	35	465 ± 86	1667^{bc} ± 256	44.0 ± 3.5	0.02 ± 0.06
R80	14	1574 ± 67	539^{g} ± 44	25.3 ± 1.1	0.04 ± 0.06
	21	944 ± 188	853^{ef} ± 217	31.4 ± 4.2	0.06 ± 0.11
	28	651 ± 96	1144de ± 248	37.0 ± 3.7	–
	35	441 ± 59	1728^{b} ± 239	45.1 ± 2.9	0.03 ± 0.08
R65	14	1626 ± 507	521^{g} ± 133	24.6 ± 3.4	0.05 ± 0.04
	21	982 ± 112	815^{fg} ± 105	31.2 ± 2.2	–
	28	577 ± 98	1419^{bcd} ± 282	41.0 ± 4.3	0.08 ± 0.19
	35	392 ± 130	2296^{a} ± 821	51.7 ± 10.0	0.04 ± 0.09
RMSE		210	303	4.3	0.09
Significance					
Group		0.830	0.026	0.041	0.334
Age		≤ 0.001	≤ 0.001	≤ 0.001	0.711
Group * age		0.966	0.027	0.088	0.460

AL = *ad libitum* fed group, R80 = 80% feed of AL group, R65 = 65% feed of AL group, RMSE = root mean square error

[a–g]$P \leq 0.05$

which corresponds with the data of Velleman et al. (2014) and Poltowicz et al. (2015). These authors detected also similar values of daily weight gain and feed conversion ratio. Lower mortality rate in restricted chickens from our experiment is in agreement with Saleh et al. (2005) and Sahraei (2012).

Results of the current study showed that feeding regime had no effect on the number of muscle fibres per 1 mm^2 in *pectoralis major*. In chickens, the number of muscle fibres is stabilized at the hatching time. The CSA depends on the type of muscle fibres; nevertheless, *pectoralis major* muscle is composed only of type IIB fibres. These glycolytic fibres are larger than oxidative type. Subsequently, the growth of the muscle occurs by an increase in the muscle fibre size (Dransfield, Sosnicky, 1999; Rehfeldt et al., 2004; Werner et al., 2008), not by hyperplasia after hatching.

The CSA corresponds with the number of muscle fibres (Ryu, Kim, 2005). In the current study, the CSA immediately after the end of feed restriction was similar for both restricted and *ad libitum* fed groups, which is in contrast with Li et al. (2007), who detected reduced glycolytic muscle fibres CSA in *lateral gastrocnemius* muscle of restricted group after feed restriction at 14 days of age. These inconsistent results may be affected by various methods of feed restriction and different muscles the fibres of which were measured. The *gastrocnemius* muscle has highly fast-twitch glycolytic fibres, while *pectoralis major* is composed of slow-twitch oxidative muscle fibres and is less sensitive to changes in nutritional status (Tesseraud et al., 1996). However, at the end of feed restriction at 14 days, Velleman et al. (2014) observed similar muscle fibre size of *pectoralis major* in the control group and in chickens with feed restriction realized in the second week posthatch. Nevertheless, when these authors applied limited feed intake at the first week posthatch, restricted chickens had smaller muscle fibres at 7 days of age than the *ad libitum* fed group. In the present experiment, during the realimentation period, restricted chickens showed fibres with the numerically larger CSA. At the end of the experiment at 35 days of age the CSA of chickens with the most intensive feed restriction increased, but groups R80 and AL did not differ. In contrast with our results, Rehfeldt et al. (2004) stated that qualitative and quantitative feed restriction reduced final CSA of muscle fibres at slaughter age. Also Li et al. (2007) determined that chickens with restricted feeding time had smaller CSA at the end of experiment at 63 days of age compared to control group. These inconsistent results of CSA can be affected by different methods of feed restriction or its experimental timing.

The increase of CSA and diameter can lead to pathological muscle alterations as for example giant fibres and thus to deterioration of meat quality. The giant fibres are determined only *post mortem* during transition from muscle to meat (Dalle Zotte et al., 2001; Werner et al., 2008). These structurally abnormal fibres are considered to arise from hypercontraction of the muscle fibres or parts of them that are not able to undergo normal relaxation after initial *rigor mortis* (Rehfeldt et al., 2004). Velleman et al. (2010) in study with 20% feed restriction for the first 2 weeks posthatch showed that *pectoralis major* morphological structure was altered with increasing pathological muscle fibres. However, in the current experiment the percentage of giant fibres was very low without the effect of feeding regime. Results are not comparable with the data from literature, because the authors who have studied the effect of feed restriction on the characteristics of muscle fibres have not included the percentage of giant fibres. According to Rehfeldt et al. (2004) giant fibre percentage lower than 0.5% is not associated with impairment in meat quality.

CONCLUSION

The results of the current study indicated that early feed restriction in broiler chickens may not affect the number of muscle fibres per 1 mm^2, but the fibre cross sectional area can be enlarged in restricted chickens and affected by the feed restriction intensity. The incidence of giant fibres was very low and was not affected by feeding regime and age.

REFERENCES

AOAC (1995): Official Methods of Analysis of AOAC International. 16th Ed. Association of Official Analytical Chemists, Arlington.

Barbut S, Sosnicki AA, Lonergan SM, Knapp T, Ciobanu DC, Gatcliffe LJ, Huff-Lonergan E, Wilson EW (2008): Progress in reducing the pale, soft and exudative (PSE) problem in pork and poultry meat. Meat Science, 79, 46–63. doi: 10.1016/j.meatsci.2007.07.031.

Dalle Zotte A, Rémignon H, Ouhayoun J (2001): Effect of some biological and zootechnical factors on appearance of giant fibres in the rabbit. Consequences on muscle fibre type, morphology and meat quality. World Rabbit Science, 9, 1–7. doi: 10.4995/wrs.2001.439.

Dransfield E, Sosnicki AA (1999): Relationship between muscle growth and poultry quality. Poultry Science, 78, 743–746. doi: 10.1093/ps/78.5.743.

Li Y, Yuan L, Yang X, Ni Y, Xia D, Barth S, Grossmann R, Zhao RQ (2007): Effect of early feed restriction on myofibre types and expression of growth-related genes in the gastrocnemius muscle of crossbred broiler chickens. British Journal of Nutrition, 98, 310–319. doi: 10.1017/S0007114507699383.

Mench JA (2002): Broiler breeders: feed restriction and welfare. World's Poultry Science Journal, 58, 20–29. doi: 10.1079/WPS20020004.

Miraglia D, Mammoli R, Branciari R, Ranucci D, Cenci Goga BT (2006): Characterization of muscle fibre type and evaluation of the presence of giant fibres in two meat chicken hybrids. Veterinary Research Communications, 30 (Suppl.1), 357–360. doi: 10.1007/s11259-006-0080-2.

Picard B, Lefaucheur L, Berri C, Duclos MJ (2002): Muscle fibre ontogenesis in farm animal species. Reproduction Nutrition Development, 42, 415–431. doi: 10.1051/rnd:2002035.

Plavnik I, Hurwitz S (1990): Performance of broiler chickens and turkeys poults subjected to feed restriction or to feeding of low-protein or low-sodium diets at an early age. Poultry Science, 69, 945–952. doi: 10.3382/ps.0690945.

Poltowicz K, Nowak J, Wojtysiak D (2015): Effect of feed restriction on performance, carcass composition and physicochemical properties of the *m. pectoralis superficialis* of broiler chickens. Annals of Animal Science, 15, 1019–1029. doi: 10.1515/aoas-2015-0036.

Rehfeldt C, Fiedler I, Stickland NC (2004): Number and size of muscle fibers in relation to meat production. In: Te Pas MFW, Haagsman ME, Everts HP (eds): Muscle development of livestock animals: physiology, genetics, and meat quality. CAB International, Wallingford, UK, 1–30.

Ryu YC, Kim BC (2005): The relationship between muscle fiber characteristics, postmortem metabolic rate, and meat quality of pig longissimus dorsi muscle. Meat Science, 71, 351–357. doi: 10.1016/j.meatsci.2005.04.015.

Sahraei M (2012): Feed restriction in broiler chickens production. Biotechnology in Animal Husbandry, 28, 333–352. doi: 10.2298/BAH1202333S.

Saleh EA, Watkins SE, Waldroup AL, Waldroupo PW (2005): Effects of early quantitative feed restriction on live performance and carcass composition of male broilers grown for further processing. Poultry Science Association, 14, 87–93. doi: 10.1093/japr/14.1.87.

Smith JH (1963): Relation of body size to muscle cell size and number in the chicken. Poultry Science, 42, 283–290. doi: 10.3382/ps.0420283.

Tesseraud S, Maaa N, Peresson R, Chagneau AM (1996): Relative response of protein turnover in three different skeletal muscles to dietary lysine deficiency in chicks. British Poultry Science, 37, 641–650. doi: 10.1080/00071669608417893.

Velleman SG, Nestor KE, Coy CS, Harford I, Anthony NB (2010): Effect of posthatch feed restriction on broiler breast muscle development and muscle transcriptional regulatory factor gene and heparan sulfate proteoglycan expression. International Journal of Poultry Science, 9, 417–425. doi: 10.3923/ijps.2010.417.425.

Velleman SG, Coy CS, Emmerson DA (2014): Effect of the timing of posthatch feed restrictions on broiler breast muscle development and muscle transcriptional regulatory factor gene expression. Poultry Science, 93, 1484–1494. doi: 10.3382/ps.2013-03813.

Verdiglione R, Cassandro M (2013): Characterization of muscle fiber type in the pectoralis major muscle of slow-growing local and commercial chicken strains. Poultry Science, 92, 2433–2437. doi: 10.3382/ps.2013-03013.

Werner C, Riegel J, Wicke M (2008): Slaughter performance of four different turkey strains, with special focus on the muscle fiber structure and the meat quality of the breast muscle. Poultry Science, 87, 1849–1859. doi: 10.3382/ps.2007-00188.

Zubair AK, Leeson S (1996): Compensatory growth in the broiler chicken: a review. World's Poultry Science, 52, 189–201. doi: 10.1079/WPS19960015.

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