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EFFECT OF FEED RESTRICTION ON PERFORMANCE, CARCASS COMPOSITION AND PHYSICOCHEMICAL PROPERTIES OF THE *M. PECTORALIS SUPERFICIALIS* OF BROILER CHICKENS*

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Abstract

The aim of this study was to evaluate the effect of feed restriction on carcass composition, breast meat quality and microstructure of the *m. pectoralis superficialis* of broiler chickens. A total of 315 day-old female broilers (Ross 308) were randomly assigned to 3 groups of 105 birds each in 3 pen replicates. They were fed *ad libitum* as the control (I), and restricted for 6 h/day (from 08:00 to 14:00 h) as group II (3 and 4 weeks of age) and group III from 4 to 5 weeks of age. On day 42 of growth, 6 broilers with average body weight were chosen from each group for slaughter to analyse dressing percentage, carcass quality, physicochemical properties of breast muscles, including pH, colour (CIE L*a*b*), expressible juice, drip loss, thawing loss, cooking loss, Warner-Bratzler shear force, texture (TPA) and chemical composition, as well as histochemical profile of the *m. pectoralis superficialis*. Temporary feed restriction did not significantly alter the final body weight, dressing percentage and the proportion of breast and leg muscles, but increased the proportion of abdominal fat. No differences were found in the microstructure of the *m. pectoralis superficialis* of restricted and *ad libitum* fed chickens. Restricted feeding had an effect on water holding capacity and tenderness of breast muscles, and a non-significant effect on texture parameters and chemical composition. Restricted feeding of chickens changed their meat quality to a greater extent when applied from 3 to 4 weeks compared to analogous procedures introduced from 4 to 5 weeks of age.

Key words: broiler chickens, female, feed restriction, meat quality, carcass composition

The remarkable progress that has been made over the last few decades in the body weight of broiler chickens was possible through a genetic increase in feed

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intake and conversion ratio paralleled by improvements in nutrient availability and absorption. Unfortunately, the rapid growth rate of modern broilers contributes to the incidence of metabolic disorders (ascites, sudden death syndrome), bone abnormalities and increased heat stress (Garner et al., 2002; Scott, 2002; Sahraei, 2014; Olukomaiya et al., 2015), resulting among others in meat defects and deterioration of meat quality. Furthermore, excessive body fat accumulation of full-fed broilers is one of the important factors of carcass and meat quality which attracts more and more attention from processors and consumers (Hocquette et al., 2005; Richards and Proszkowiec-Weglarz, 2007).

Research results produced to date show that some negative consequences of selection in meat poultry can be offset through quantitative or qualitative feed restriction. Several feed restriction programmes have been tried to reduce total mortality, metabolic diseases, intestinal and leg bone abnormalities (Gonzales et al., 1998; Zulkifli et al., 2001; Sahraei, 2012; Tsiouris et al., 2014). Quantitative feed restriction has been also used to limit early growth, reduce feed intake, improve nutrient utilization with compensatory growth, and reduce abdominal fat (Benyi et al., 2009). Nevertheless, according to Tumova et al. (2002) and Camacho et al. (2004) the effect of restricted feeding on growth, feed conversion ratio, fatness and carcass characteristics depends on a number of factors such as market age, intensity of restriction, and age at which quantitative feed restriction was applied. Some studies indicate that restricted feeding of broilers reduces carcass fat content and increases protein deposition, thus improving carcass composition (Nielsen et al., 2003; Omosebi et al., 2014). Some authors, however, do not confirm this phenomenon (Lippens et al., 2000; Zhan et al., 2007) and provide evidence that early feed restriction in chickens may lead to increased fat deposition later in the rearing period (Velleman et al., 2014). Similarly, the effect of feed restriction on the yield of different carcass parts is not conclusive, although several researchers have determined the proportion of body components at the end of the rearing period of previously restricted broilers (Yu et al., 1990; Fontana et al., 1993; Jahanpour et al., 2015).

Feed restriction has been adopted to avoid rapid growth rate, which, in turn, is considered responsible for poor meat quality. Research suggests that late quantitative feed restriction of broiler chickens most often results in a non-significant decrease in final body weight and may decrease carcass fatness (Cristofori et al., 1997; Camacho et al., 2004; Omosebi et al., 2014). It is not known, however, how this procedure affects meat quality. Although some studies have determined the effect of temporary late feed restrictions on productive traits and carcass fatness in broilers, there is almost no information available concerning the effect of temporary restricted feeding on physicochemical properties of poultry meat. Therefore, the objective of this study was to determine the effect of temporary lack of access to feed in broiler chickens from 3 to 4 and from 4 to 5 weeks of age on production results, and carcass and meat quality.

Material and methods

A total of 315 day-old female broiler chickens (Ross 308) of similar initial body weight were randomly assigned to 3 treatments each in 3 replicates of 35 birds per pen, in a deep-litter facility, with a stocking density of 15 birds/m^2. They were reared under the same, optimal, electronically controlled environmental conditions (temperature, lighting regime, air humidity). Broilers were fed ad libitum throughout the experiment as the control (I) and the other two groups were feed-restricted: as group II (6 h/day from 3 to 4 weeks of age) and group III (6 h/day from 4 to 5 weeks of age). Birds were feed restricted from 08:00 to 14:00 h and had free access to feed during the remaining time. Water was available ad libitum throughout the experiment. Chickens were provided with commercial broiler diets, starter (1–21 days), grower (22–35 days) and finisher (36–42 days), the nutritive value of which is given in Table 1. During the experiment, individual body weight, group feed consumption and mortality were determined at weekly intervals. At the end of rearing the European Efficiency Index (EEI) was calculated for each pen replicate (RMRiGŻ, 1999).

$$EE1\ (pts) = \frac{livability\ (\%) \times live\ weight\ (kg)}{length\ of\ rearing\ period\ (days) \times FCR\ (kg)} \times 100$$

Table 1. Chemical composition of feed mixture

Item	Feed mixtures (day of age)		
	starter (1–21)	grower (22–35)	finisher (36–42)
Dry matter (%)	89.77	88.48	87.46
Crude protein (%)	18.90	18.37	17.12
Crude fat (%)	3.73	4.00	4.76
Crude fibre (%)	3.47	3.57	3.52
Crude ash (%)	5.20	3.91	3.76

On day 42 of rearing, 6 birds with average body weight were selected from each group. Immediately after slaughter, samples of left breast muscle (*pectoralis superficialis*) were collected to determine its microstructure. Samples were frozen in liquid nitrogen and stored at –86°C until analysis. The microstructure of the collected and frozen samples was evaluated to distinguish the types and measure muscle fibres. Muscle samples were cut in a Slee HR cryostat into 10 µm thick pieces at –25°C and were subjected to a reaction to detect NADH-TR using the Pears method and a myosin ATP-ase reaction in accordance with the Brooke and Kaiser (1970) and Guth and Samaha (1972) methodology. Histometric measurements and the percentage of individual fibre types were determined using the transverse cross-sections of the muscle in 10 first-order bundles performed by means of image analysis software Multi Scan v.14.02. Initial (pH_{15min}) and final pH (pH_{24h}) of breast muscle (*pectoralis superficialis*) was determined using a portable pH-meter (CyberScan10) equipped with a glass electrode. Muscle colour readings were taken in the L*a*b* colour space (CIE, 1976) using a Minolta CR 310 Chroma Meter (Japan). CIE L*a*b* values were measured on the inner side of *m. pectoralis superficialis*, immediately after it was detached from the bone, with four measurements per muscle and calculation of the mean for individual colour parameters of L* (lightness), a* (redness) and b* (yellowness). Areas were selected that were free of any obvious blood-related defects, such as bruises, haemorrhages, or full blood vessels (Fletcher et al., 2000). Expressible juice was determined based on the volume of free water squeezed from a ground meat sample using the filter paper method (Grau and Hamm, 1953). Drip loss was determined from meat weight loss after 24-h cold storage at 4°C. Thawing loss was determined from meat weight loss after 28-day storage at –18°C. Cooking loss was determined from percentage loss of meat weight as a result of cooking in a water bath at 100°C until a core temperature of 78°C was obtained in the thickest part of the sample. After cooling and weighing for cooking loss determination, samples were prepared for the shear force and texture measurements. Two 1.27 cm diameter cores and about 3 cm (for shear force determination) and 1.5 cm lengths (for texture analysis) were removed from each sample parallel to the fibre orientation through the thickest portion of the cooked muscle. Warner-Bratzler shear force was determined using an Instron 5542 (England) as maximum force (N) perpendicular to the fibres. Texture analysis, which included hardness (N), springiness (mm), cohesiveness, gumminess (N) and chewiness (mJ), was performed with the same instrument fitted with a 50 mm compression attachment using Texture Profile Analysis (TPA) software. A double compression test with 40% deformation was performed with a crosshead speed of 200 mm/min, an interval of 2 s between compressions, and a detection threshold of 0.01 N. Samples were compressed perpendicular to the muscle fibre orientation. Dressing percentage with and without giblets was determined as the proportion of the weight of chilled eviscerated carcass (with and without giblets, respectively) to the preslaughter body weight of the chickens. The difference between the weight of warm and chilled carcass was used to calculate the loss due to 24-hour cold storage of the carcasses at 4°C. The proportion of breast muscles, leg muscles, leg bones, giblets and abdominal fat were calculated to the weight of chilled carcass with giblets. Samples were collected from the dissected breast muscles to determine the content of dry matter, crude protein, crude fat, and crude ash. Chemical composition of the meat and feed mixtures was analysed at the Central Laboratory of the National Research Institute of Animal Production.

The results were analysed statistically with Statistica 10 PL using one-way analysis of variance. Significant differences between the means were determined with Duncan's multiple range test (Duncan, 1950). In addition, Pearson's linear correlation coefficients were calculated to determine the relationship between physicochemical characteristics of the meat.

Results

Production results of 42-day-old Ross 308 pullets are shown in Table 2. No significant differences were found between their final body weight and feed conversion ratio. Forty-two-day-old pullets, which were feed-restricted from 3 to 4 weeks of age, achieved the same body weight as those fed *ad libitum*, while the introduction of feed restriction from 4 to 5 weeks of age caused a non-significant increase in body weight (by 70 g) and reduced feed conversion ratio (by 90 g) compared with the control group. As a result, birds from group III had the highest European Efficiency Index (EEI) of 232 pts. Restricted feeding had no significant effect on dressing percentage and on the proportion of breast and leg muscles (Table 3). It was found, however, that pullets from group II, which had their feed restricted from 3 to 4 weeks of age, showed a trend for higher dressing percentage compared to birds from the other groups, with differences between the former and birds from groups I and III averaging 1.88 percentage points for dressing percentage with giblets, and 2.33 percentage points for dressing percentage without giblets. In addition, chickens from group II were characterized by the highest percentage of breast muscles (24.20%, $P>0.05$) and the lowest carcass weight loss during chilling (1.22%, $P>0.05$). At the same time, however, pullets from this group had the highest proportion of abdominal fat in the carcass (2.78%, $P\leq0.05$). A higher proportion of abdominal fat than in the control group was also noted in chickens from group III, but the difference was not significant ($P=0.17$).

Table 2. Production results of 42-day-old female broiler chickens

Item	Group			SEM
	I	II	III	
Body weight (kg)	1.77	1.77	1.84	0.29
Feed conversion ratio (kg/kg gain)	1.95	1.97	1.87	0.03
Total mortality (%)	0	4.76	0.95	0.82
0–3 weeks (%)	0	4.76	0.95	0.82
4–6 weeks (%)	0	0	0	
European Efficiency Index (pts)	216	204	232	

Table 3. Effect of feed restriction on carcass quality of 42-day-old female broiler chickens

Item (%)	Group			SEM
	I	II	III	
Carcass weight loss during chilling	1.47	1.22	1.52	0.06
Dressing percentage with giblets	74.80	76.52	74.48	0.45
Dressing percentage without giblets	70.52	72.60	70.03	0.54
Breast muscles	23.51	24.20	23.70	0.45
Leg muscles	20.19	19.31	20.69	0.32
Leg bones	5.41 a	4.52 b	4.53 b	0.17
Giblets	5.72	5.15	5.96	0.24
Liver	3.90	3.61	4.24	0.20
Gizzard	1.08	0.88	1.06	0.04
Heart	0.74	0.65	0.66	0.03
Abdominal fat	1.86 a	2.78 b	2.40	0.17

Means in rows with different letters a, b differ at $P\leq0.05$.

The results of histochemical examination of *m. pectoralis superficialis* (Table 4) show that restricted feeding had no significant effect on the cross-section area of IIB muscle fibres, which were the only type of fibre in the analysed muscle. Also the differences between the groups in most physicochemical properties of meat were not statistically significant (Table 4). Regardless of the procedure, both the pH and colour of the muscles were similar. The results show, however, changes of some WHC parameters of the meat. The breast muscles of feed-restricted chickens were characterized by significantly lower thawing loss and lower cooking loss, with the most favourable values for all these traits obtained in group II ($P \leq 0.05$). Furthermore, the introduction of temporary feed restriction reduced expressible juice from 16.53% in the control group to an average of 14.89% in groups II and III, with drip loss in both restricted experimental groups being almost half that in the control group on average (P=0.07). The results presented in Table 4 also suggest that temporary feed restriction contributed to a marked reduction in meat shear force. Moreover, compared to the *ad libitum* group, the breast muscles of restricted broilers were characterized by slightly lower values of hardness (group III) and cohesiveness, gumminess and chewiness (group II). At the same time, the breast muscles of feed-restricted chickens tended to accumulate more protein, the level of which averaged 23.22% in both restricted groups compared to 22.52% in the control group (Table 5). The fat content of breast muscles ranged from 1.90 to 2.42% and was the lowest, although non-significantly, in birds restricted from 3 to 4 weeks of age. In addition, analysis of our data showed the presence of significant negative correlations between pH_{24h}, drip loss, thawing loss and total losses ($P \leq 0.01$, data not shown). We also found non-significant negative coefficients of correlation between the level of crude protein and expressible juice (r=–0.53, P=0.07) and between the level of protein and cooking loss (r=–0.55, P=0.06).

Table 4. Microstructure and physicochemical properties of *m. pectoralis superficialis* of 42-day-old female broiler chickens

Item	Group			SEM
	I	II	III	
Diameter of IIB muscle fibres (µm)	51.90	50.86	47.63	1.48
pH_{15min}	6.19	6.24	6.15	0.06
pH_{24h}	5.66	5.74	5.70	0.02
L*	57.67	56.92	58.57	0.73
a*	11.49	10.81	10.99	0.28
b*	7.25	7.11	6.98	0.35
Expressible juice (%)	16.53	14.84	14.94	0.41
Drip $loss_{24h}$ (%)	1.26	0.67	0.76	0.13
Thawing loss (%)	6.24 a	3.37 b	5.50	0.53
Cooking loss (%)	24.81 a	21.34 b	21.47 b	0.61
Total losses (%)	32.31 Aa	25.38 B	27.73 b	0.92
Shear force (N)	16.75 a	13.00 b	15.12	0.74
Hardness (N)	5.32	5.30	5.06	0.17
Springiness (mm)	2.04	2.00	2.07	0.03
Cohesiveness	0.63	0.55	0.63	0.02
Gumminess (N)	3.37	2.90	3.27	0.19
Chewiness (mJ)	6.89	5.80	6.80	0.43

Means in rows with different letters a, b differ at $P \leq 0.05$; A, B at $P \leq 0.01$.

Table 5. Chemical composition of *m. pectoralis superficialis* of 42-day-old female broiler chickens

Item (%)	Group			SEM
	I	II	III	
Dry matter	25.43	25.76	25.91	0.18
Crude protein	22.52	23.39	23.05	0.18
Crude fat	2.17	1.90	2.42	0.15
Crude ash	1.13 A	1.14 a	1.19 Bb	0.01

Means in rows with different letters a, b differ at $P \leq 0.05$; A, B at $P \leq 0.01$.

Discussion

Many authors report that feed-restricted broilers are more efficient in feed conversion compared to birds fed *ad libitum*, although significant differences are confirmed very rarely. Our results partially agree with this observation. The introduction of restricted feeding caused a non-significant worsening of feed conversion ratio only in birds subjected to this procedure from 4 to 5 weeks of age. Similar findings were obtained by Urdaneta-Rincon and Leeson (2002) for male broiler chickens restricted to 90% of *ad libitum* intake from 14 to 29 days. Slightly better feed conversion by starved chickens, regardless of the date on which quantitative feed restriction was introduced, was reported by Camacho et al. (2004). Meanwhile, Benyi et al. (2010) showed that withholding feed from Ross 308 chickens from 8 to 28 days of age for 6 h daily had no effect on feed conversion ratio. At the same time, all the authors cited above demonstrated that feed-restricted chickens achieved slightly lower body weight ($P>0.05$) compared to birds fed *ad libitum*. Likewise, Cristofori et al. (1997) and Cornejo et al. (2007) showed that broilers restricted from 7 to 28 days and 21 to 35 days did not compensate in final body weight. Saber et al. (2011), who withdrew feed for 8 h/day from 21 to 28 days of age obtained a 143 g higher final body weight ($P>0.05$) and non-significantly more favourable feed conversion ratio in feed-restricted broilers. Also in the study of Lippens et al. (2000), restricted female broilers reached a somewhat higher body weight than the *ad libitum* group, which is consistent with our study.

One of the most controversial effects of restricted feeding of broiler chickens is its influence on carcass quality, especially its fat content. Camacho et al. (2004) reported that carcass, breast, leg, and abdominal fat weights of the restricted groups were not different from those of the control group. On the other hand, Urdaneta-Rincon and Leeson (2002) reported that the yield and proportion of breast meat was reduced for restricted birds. Scheideler and Baughman (1993) found a reduction in dressing percentage of 42-day-old restricted broilers in one experiment but not in the second. Different results were obtained by Al-Taleb (2003), who reported a higher proportion of breast and leg muscles in restricted broilers. Also data of Saleh et al. (1996) showed a trend of increasing dressing percentage for the restricted birds as well as improvement in breast meat yield. Our study revealed no significant differences in muscle percentage and dressing percentage of the chickens, but showed a trend towards improved meat:bone ratio in restricted broilers. We also found a significantly higher percentage of abdominal fat in restricted broilers, which is consistent with the tendencies observed by Urdaneta-Rincon and Leeson (2002). Also the results obtained by Zhan et al. (2007) show higher abdominal fat ($P \leq 0.05$) and no statistically significant differences in carcass yield and breast muscle of restricted broilers. However, in their study feed restriction was applied earlier than in ours, from 1 to 21 days of age for 4 h a day. According to these authors, early feed-restricted chickens tend to enhance fat deposits during the refeeding period, and the long period and low intensity, as well as initial day of feed restriction might induce metabolism programming, thus contributing to excessive fat deposition in adult life of broilers. Also in our study, pullets from both restricted groups were characterized by greater body fatness compared to the control birds, and the significantly higher level of abdominal fat that we found in group II may result from longer excessive fat deposition after an earlier return to *ad libitum* feeding than in group III. In a study with rabbits reared to 84 days of age, Tumova et al. (2003) found that feed restriction reduced renal fat only when started on day 56. The rabbits restricted between days 42 and 49 had more fat that those fed *ad libitum*. The higher level of abdominal fat in restricted chickens was also demonstrated by Lippens et al. (2000). The same authors also found that the increase in the abdominal fat percentage was more pronounced with the female broilers than with the males. In contrast, total body fat content in their study was not affected by the feed restriction. Meanwhile, Santoso (2001) showed that compared to broilers fed *ad libitum*, feed-restricted birds were characterized by lower proportion of abdominal fat and lower concentration of fat in meat. Our study showed different trends regarding fat deposition in these two body parts. Unlike the situation with abdominal fat, the non-significantly lowest level of fat in breast muscle was found in chickens from group II. At the same time, the muscles of these birds contained most crude protein ($P>0.05$). These results are consistent with Zhan et al. (2007). In their study fat content was decreased and crude protein content was increased in breast meat of feed-restricted broilers, which they believe shows a tendency for increased yield of muscle during the refeeding period after early feed restriction. Our study seems to support this observation, because this group of birds showed a trend for the greater proportion of breast muscle in the carcass. On the other hand, however, no significant differences were found between the analysed groups in muscle fibre size, and the revealed tendency rather shows that fibre diameter decreased in response to feed restriction. The trend for the higher concentration of crude protein in muscles compared to the control group was also evident in group III, but the muscles of these birds were at the same time characterized by higher levels of fat than in the other groups. The higher concentration of crude protein and crude fat in breast muscles of feed-restricted broilers is also suggested by the findings of Al-Taleb (2003). The higher level of ash that we observed in the meat of restricted broilers was previously reported by Santoso (2001) and by Tumova et al. (2003) and Bovera et al. (2008) in rabbits.

In contrast to the situation with carcass, little information is available about the effects of feed restriction on meat quality, in particular its physicochemical properties. Lippens et al. (2000) showed no effect of restricted feeding of chickens on the pH and colour of breast muscles, which was also confirmed by our study. However, despite the lack of statistical significance, our restricted broilers showed a tendency for higher final pH, the highest value of which was noted in group II. At the same time, the breast muscles of these birds were characterized by significantly lower cooking loss and lower thawing loss as well as a tendency for lower drip loss and expressible juice, and, as a result, the lowest loss of free water. Also in group III, the breast muscles of restricted chickens had better parameters of water holding capacity compared to the muscles of chickens fed *ad libitum*. Unlike our observations, Lippens et al. (2000) showed no differences in cooking loss from breast muscles between restricted and control chicks, but, as in our study, the breast muscles of the former showed a trend for lower drip loss. The lack of significant differences in the water holding capacity of meat, with almost identical pH_{24h} values in restricted and *ad libitum* fed rabbits were reported by Bovera et al. (2008). Similarly, Therkildsen et al. (2002) found that restricted feeding of pigs had no effect on pH_{24h}, drip loss and CIE L*a*b* colour. These authors showed, however, that compared to the *ad libitum* fed control group, the meat of restricted pigs showed a tendency towards greater tenderness in sensory evaluation. What is more, they showed that the muscles of the latter had a higher concentration of elongation factor 2 (eEF-2), which is essential for protein synthesis and indicates an increased muscle protein turnover. Moreover, restricted pigs also showed higher activity of μ-calpain and myofibrillar fragmentation index (MFI) 24 h postmortem, being indicative of higher protein degradation during aging. According to the authors cited above, compensatory growth may be used to improve meat tenderness in pigs, but the final *ad libitum* feeding period must exceed 42 days. Our study seems to confirm these observations, because the breast muscles of chickens in which restricted feeding was terminated 14 days before slaughter were characterized by the lowest shear force and the most favourable texture parameters, with a parallel tendency towards the highest concentration of protein. Restricted chickens, whose slaughter was preceded by 7-day *ad libitum* feeding only slightly surpassed the control group in meat tenderness, with most texture parameters being at a similar level. In the study by Lippens et al. (2000) cited above, no effect of feed restriction on broiler chicken meat tenderness was found.

The results of our study show that the introduction of restricted feeding produced no significant changes in final body weight, feed conversion ratio, dressing percentage and muscle percentage while increasing carcass fatness. This procedure had no effect on muscle fibre diameter but altered some physicochemical properties of breast muscles (water holding capacity and shear force) and non-significantly modified texture parameters and chemical composition, with the exception of crude ash. Restricted feeding of the chickens changed their meat quality to a greater extent when applied from 3 to 4 weeks compared to analogous procedures introduced 7 days later. However, more research is needed to understand this and confirm these results.

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