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PAPER

Effects of feed restriction and diet nutrient density during re-alimentation on growth performance, carcass traits, organ weight, blood parameters and the immune response of broilers

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Abstract

Two hundred and ten one-day-old male chickens of the Ross 308 strain were randomly allocated to one of seven treatments (five replicates of six birds per treatment). The control birds were fed *ad libitum* throughout the whole experimental period. In the remaining treatments (T15-5, T15-10 and T15-15, and T30-5, T30-10 and T30-15), the feed restriction was 15 and 30% *ad libitum* intake between 8 and 14 days of age (starting period), whereas during growing (15-28 days) and finishing periods (20-42 days), the feeds contained 5, 10 and 15% higher energy and protein contents than the respective control feeds. Compared with the controls, T15 and T30 chickens suffered 12% and 20% body weight reduction at the end of the starting period, but both groups were heavier ($P<0.05$) than the controls at the end of the experimental period, mainly due to a higher ($P<0.05$) body weight gain during the finishing period, irrespective of the diet nutrient density. When the whole experimental period was considered, the feed restricted broilers showed a lower ($P<0.05$) feed conversion ratio than the controls and no negative effects were observed on carcass traits. The abdominal fat relative to carcass weight was higher ($P<0.05$) in the T15 broilers than in the

controls and the T30 broilers. A positive correlation ($P<0.05$) was observed between the total, HDL and LDL cholesterol contents in the blood and abdominal fat percentage. The dietary treatments had no or a low impact on the relative weight of the organs and immune response of the broilers.

Introduction

The growth performance of broiler chickens has increased greatly over the last few decades due to improvements in genetics, nutrition and management. However, the fast growth rate of broilers has resulted in health problems, and the higher nutrient supply has led to an increased fat deposition (Tumova and Teimouri, 2010). To avoid those problems, the use of feed restriction programmes in poultry production has long been proposed (Yu and Robinson, 1992). During the period of feed restriction, the growth rate of restricted fed birds is slower than that of fully fed birds, but when access to food is again unrestricted, the former exhibit an accelerated rate of weight gain (Zubair and Leeson, 1996). This effect depends on the so called compensatory growth phenomenon. The term compensatory growth (*i.e.* a faster than normal rate of growth after a period of nutritional or environmental stress) was first used by Bohman (1955) to describe the effects of diet on the growth of beef cattle. Compensatory growth has been studied extensively in meat animals, but the physiological, nutritional, metabolic and endocrine mechanisms involved are not well known. Bauman *et al.* (1982) pointed out that in order to support a physiological state, such as growth and development, homeorhetic control mechanisms may change the flux of nutrients within the body and their metabolism in tissues.

Feed restriction programmes can be applied either through *ad libitum* access to less energy dense diets (qualitative feed restriction) or through control of the daily feed supply (quantitative feed restriction). Although the application of feed restriction in the production of broiler chickens reduces health problems (Gonzales *et al.*, 1998; Urdaneta-Rincon and Leeson, 2002), it has been considered controversial due to the varied responses observed with respect to the final body weight (BW), feed conversion ratio (FCR), and carcass fat deposition. However, such discrepancies may be related to the different feeding strategies that have been applied. Studies have shown that the animals' ability to compensate for prior undernutrition is affected by the severity

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Contributions: AS and MS designed the experiment, and SR, AS and MS carried it out. ALMM performed the statistical analysis. ALMM, FPB and AS prepared the manuscript with contributions from all the co-authors.

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and duration of the period of undernutrition, the stage of development of the animal, genotype, sex, as well as the level of feed intake and composition of the diet during the refeeding period (Mitchell, 2007).

A great deal of studies has focused on feed restriction (Jahanpour *et al.*, 2015; Novel *et al.*, 2009; Urdaneta-Rincon and Leeson, 2002), but few of them have taken into account the influence of the diet concentration during the refeeding period (Giachetto *et al.*, 2003; Leeson and Zubair, 1997; Santoso *et al.*, 1995). Moreover, changes in metabolism indicators are expected to occur, due to dietary manipulations and the relationship between nutrition and immunity in poultry (Klasing, 2007). Over the last decade, several authors have investigated the effects of feed restriction on some blood metabolite and enzyme contents (Boostani *et al.*, 2010; Jahanpour *et al.*, 2013; Mohebodini *et al.*, 2009) and on the immune response (Fassbinder-Orth and Karasov, 2006; Mahmood *et al.*, 2007; Jahanpour *et al.*, 2012). However, there is a lack of information on the combined effects of feed restriction and the nutrient concentration of the diets fed during

re-alimentation on those parameters. Therefore, the aim of the present work was to investigate the effects of an early period of mild or severe quantitative feed restriction followed by refeeding energy and protein dense diets on growth performance, carcass traits, organ weight, blood parameters and the immune response of broiler chickens.

Materials and methods

Animals, housing, diets and treatments

The use and care of the birds and procedures in this study were approved by the Islamic Azad University Ethics Committee. Before starting the trial, the research facility was thoroughly cleaned and disinfected. Two hundred and ten one-day-old male chickens of the Ross 308 strain (Aviagen, Newbridge, UK), purchased from a commercial hatchery, were used. The broiler chicks were placed in 1.5×1.0 m cages and the floor was covered with shredded paper. Each cage was equipped with a pan feeder and a manual drinker. The research facility was an open-sided poultry barn with thermostatically controlled curtains, and was equipped with thermostatically controlled gasoline rocket heaters, overhead sprinklers, wall-mounted fans at both ends of the barn, and fluorescent tubes in the ceiling fixtures.

Ambient temperature was set at 32°C at placement and then decreased gradually to achieve 24°C from week 3 onwards. Lighting was constant during day 1. From day 2 to the end of the study, the light regime was 23L:1D.

The experiment lasted 42 days. The feeding programme consisted of a starter diet, until the chicks were 14 days old, followed by a grower diet up to 28 days of age, and then a finisher diet until the end of the experiment. All the feeds were maize-soybean meal based and did not contain any antibiotic feed additives. The diets were formulated according to a standard commercial programme (Table 1). The chicks were randomly assigned to one of seven treatments, each of them with five replicates, thus a total of 35 groups of six birds each were obtained. No feed restriction was applied in the control treatment during the last part of the starter phase, nor diets with increased nutrient density were fed during growing and finishing periods. A 15% quantitative (ad lib -/15%) feed restriction was applied between d 8 and d 14 in treatments T15-5, T15-10 and T15-15, and the grower and finisher feeds contained 5, 10 and 15% higher energy and protein contents than the corresponding control feeds. A 30% quantitative (ad lib -/30%) feed restriction was applied between d 8 and d 14 in treatments T30-5, T30-10 and T30-15 and the grower and finisher feeds contained 5, 10 and 15% higher energy and protein contents than the corresponding control feeds. Feed restriction consisted of a daily feed supply adjust-

ment that was proportional to the feed intake of the control chicks during the previous day. The control broilers were fed ad libitum throughout the entire experimental period, while the broilers in the other treatments were fed ad libitum before and after the feed restriction period. All the birds had free access to water throughout the whole trial.

Growth performance and carcass measurements

The body weight (BW) of the chicks and feed consumption were recorded weekly per cage, and the body weight gain (BWG), feed intake (FI) and feed conversion ratio (FCR, feed to gain g/g) were determined. At the age of 42 days, after 4 hours of fasting for complete evacuation of the gut, ten chickens per treatment (two from each replicate) that had the closest weight to the mean weight of the cage were selected and euthanized to determine the carcass traits. The birds were fully plucked by means of the dry plucking method and the feet, head and wingtips were removed. The broilers were eviscerated before determining the carcass weight. The weight of the breast, drumsticks, wings, abdominal fat and various organs were recorded.

Blood sampling and analysis, and immune response study

At 42 days of age, ten chickens per treatment (two from each replicate) were selected

Table 1. Experimental diets fed to broiler chickens.

	Starter	Grower ^o				Finisher ^o			
		0%	+5%	+10%	+15%	0%	+5%	+10%	+15%
Ingredients, g/kg									
Maize	557	602	533	463	402	630	573	506	436
Soybean meal 48%	355	311	346	383	412	280	305	338	374
Concentrate 5% [†]	50	50	50	50	50	50	50	50	50
Soybean oil	16	15	49	82	114	18	50	84	118
Calcium carbonate	10	10	10	10	10	10	10	10	10
Dicalcium phosphate	7	7	7	7	7	7	7	7	7
Vitamin-mineral premix [§]	5	5	5	5	5	5	5	5	5
Calculated analysis [^]									
Metabolisable energy, MJ/kg	12.38	12.52	13.16	13.77	14.39	12.71	13.34	13.99	14.63
Crude protein, %	23.0	21.2	22.3	23.5	24.4	19.9	20.7	21.7	22.8
Lysine, %	1.36	1.24	1.32	1.41	1.48	1.15	1.21	1.29	1.38
Threonine, %	0.51	0.49	0.50	0.51	0.52	0.48	0.48	0.49	0.50
Methionine, %	0.90	0.85	0.88	0.90	0.92	0.82	0.84	0.86	0.88
Tryptophan, %	0.90	0.83	0.88	0.93	0.96	0.78	0.81	0.85	0.90
Calcium, %	0.33	0.30	0.32	0.35	0.36	0.28	0.30	0.32	0.34
Available phosphorus, %	1.19	1.18	1.19	1.20	1.20	1.17	1.18	1.19	1.19

^o5%, 10% and 15% increment in the diet energy and protein concentrations over the control (0%); [†]declared composition per kilogram: metabolisable energy, 1700 kcal; crude protein, 20%; lysine, 3.2%; threonine, 1.5%; methionine, 3.5%; tryptophan, 0.7%; calcium, 11%; available phosphorus, 5.5%. [§]Supplied per kilogram of feed: vitamin A, 12,500 U; vitamin D₃, 1250 U; vitamin E, 18 U; vitamin K₃, 3.7 mg; thiamine, 1.8 mg; riboflavin, 6.6 mg; calcium pantothenate, 10 mg; niacin, 37.5 mg; pyridoxine, 32.5 mg; vitamin B₁₂, 2.5 mg; Mn, 50 mg; Zn, 37.5 mg; Fe, 25 mg; Cu, 7.5 mg. [^]According to NRC (1994).

as explained above to collect blood from their wing veins in EDTA tubes. Samples were transferred to the laboratory for analysis within 2 hours of collection. After centrifuging the blood samples (3000 g, for 10 min at room temperature), plasma was collected and stored in Eppendorf tubes at -20°C until assayed. The biochemical analysis was conducted according to standard protocols using commercial laboratory kits (Pars Azmoon Co., Tehran, Iran). The measured parameters were glucose, total protein, albumin, globulin, uric acid, triglycerides, cholesterol (total, HDL, LDL and VLDL), aspartate amino transferase (AST) and alanine amino transferase (ALT).

Antibody production from different antigens was assessed during the experiment. First, the birds were vaccinated against infectious bronchitis disease (d 1), Newcastle disease (d 9 and d 16), influenza disease (d 9 and d 16) and Gumboro disease (d 13 and d 19). All the vaccines were provided by Razi Co. (Tehran, Iran). Additionally, two birds per replicate were injected under the breast skin with 0.5 ml of a 10 % suspension in phosphate buffered saline of sheep red blood cells (SRBC) at d 22 and d 29. In order to determine the systemic antibody response, blood samples were collected

from two chicks per replicate via the wing vein at d 19 and d 26 (Newcastle disease), at d 30 and d 39 (influenza disease), and at d 29 and d 36 (SRBC). The blood samples were processed and analysed as described by Pourhossein *et al.* (2014). A hemagglutination inhibition assay was used to determine the antibody response to influenza disease and Newcastle disease. The total immunoglobulin (Ig) and immunoglobulin G (IgG) titers to SRBC were determined by means of the hemagglutination assay; the immunoglobulin M (IgM) titers to SRBC were then calculated as the difference between the total Ig and IgG titers.

Statistical analysis

SAS 9.1 (SAS Institute Inc., Cary, NC) was used in the statistical analyses. ANOVA was performed with the GLM procedure. The statistical design was $Y_{ijk} = \mu + A_j + e_{ij}$, where Y_{ij} is the dependent variable; μ represents the overall mean; A_j is the fixed effect of the treatment; and e_{ij} is the residual error. The least square means were compared using Tukey's test. The responses to feed restriction were investigated through pre-planned orthogonal contrasts (control vs. the T15 and T30 treatment groups, and T15 vs. the T30 treatment group).

Correlations between the percentage of carcass fat and plasma lipids were investigated with the SAS CORR procedure. Statistical significance was declared at $P < 0.05$.

Results and discussion

Growth performance

The birds remained healthy for the whole experimental period, no signs of illness were observed, and the mortality rate was zero for all groups. Table 2 shows FI, BWG and FCR during the starting, growing and finishing periods (1-7 and 8-14 days, 15-28 days and 29-42 days of age, respectively) and over the entire experimental period. As expected, none of the variables showed differences ($P > 0.05$) during the first week of life, prior to the feed restriction period (8-14 days of age). Quantitative feed restriction caused a higher feed intake, lower body weight gain and lower feed conversion ratio ($P < 0.05$). No differences ($P > 0.05$) were found between the two feed restriction treatments, but at the end of the restriction period, the T30 broilers weighed 26 g less than the T15 broilers. Compared with the controls, the T15

Table 2. Feed intake, body weight gain, and feed conversion rate of broilers raised under a normal feeding programme (control), or two levels of quantitative feed restriction (15 and 30%) between 8 and 14 days of age followed by re-feeding energy and protein dense diets (5, 10 and 15% over the control diet) from 15 to 42 days of age.

Control	Treatments ^o						SEM	Probability			
	T15-5	T15-10	T15-15	T30-5	T30-10	T30-15		CxT15	CxT30	T15xT30	
Starter period (1-14 d)											
1-7 d											
FI, g d ⁻¹	16.87	16.91	17.21	17.13	17.63	17.22	17.09	0.095	ns	ns	ns
BWG, g d ⁻¹	11.79	11.67	11.86	11.86	12.29	12.05	11.91	0.092	ns	ns	ns
FCR, g g ⁻¹	1.43	1.45	1.45	1.45	1.44	1.43	1.44	0.003	ns	ns	ns
8-14 d (feed restriction)											
FI, g d ⁻¹	44.52 ^a	38.29 ^b	38.29 ^b	38.29 ^b	31.50 ^c	31.50 ^c	31.50 ^c	0.623	<0.001	<0.001	<0.001
BWG, g d ⁻¹	27.62 ^a	22.19 ^b	22.71 ^b	21.72 ^b	17.33 ^c	18.71 ^c	18.52 ^c	0.450	<0.001	<0.001	<0.001
FCR, g g ⁻¹	1.61 ^b	1.73 ^{ab}	1.69 ^{ab}	1.77 ^a	1.82 ^a	1.69 ^{ab}	1.71 ^{ab}	0.013	<0.01	<0.01	ns
Grower period (15-28 d)											
FI, g d ⁻¹	64.93 ^{ab}	67.95 ^a	69.43 ^a	70.45 ^a	61.04 ^{ab}	64.20 ^{ab}	59.80 ^b	0.726	<0.05	<0.10	<0.001
BWG, g d ⁻¹	36.97	37.33	39.67	39.10	35.60	37.79	38.12	0.408	ns	ns	<0.10
FCR, g g ⁻¹	1.77 ^{abc}	1.82 ^a	1.75 ^{abc}	1.80 ^{ab}	1.72 ^{bc}	1.70 ^c	1.58 ^d	0.013	ns	<0.001	<0.001
Finisher period (29-42 d)											
FI, g d ⁻¹	154.7	152.6	154.1	158.1	151.3	156.7	153.5	0.927	ns	ns	ns
BWG, g d ⁻¹	65.38 ^b	75.22 ^a	75.75 ^a	80.12 ^a	76.14 ^a	80.00 ^a	80.30 ^a	0.808	<0.001	<0.001	ns
FCR, g g ⁻¹	2.37 ^a	2.03 ^b	2.04 ^b	1.98 ^{bc}	1.99 ^{bc}	1.95 ^{bc}	1.91 ^c	0.019	<0.001	<0.001	<0.001
Total period (1-42 d)											
FI, g d ⁻¹	101.2	101.4	102.7	105.1	98.60	102.3	99.12	0.521	ns	ns	ns
BWG, g d ⁻¹	45.83 ^b	50.34 ^a	51.38 ^a	53.38 ^a	50.23 ^a	52.88 ^a	53.14 ^a	0.433	<0.001	<0.001	ns
FCR, g g ⁻¹	2.22 ^a	2.01 ^b	2.00 ^b	1.97 ^{bc}	1.96 ^{bc}	1.93 ^{bc}	1.86 ^c	0.016	<0.001	<0.001	<0.01

FI, feed intake; BWG, body weight gain; FCR, feed conversion rate; T15-5, 15% feed restriction and 5% nutrient density increase; T15-10, 15% feed restriction and 10% nutrient density increase; T15-15, 15% feed restriction and 15% nutrient density increase; T30-5, 30% feed restriction and 5% nutrient density increase; T30-10, 30% feed restriction and 10% nutrient density increase; T30-15, 30% feed restriction and 15% nutrient density increase. ^{a,b,c}In a row, least squares means without a common superscript are significantly different ($P < 0.05$) by Tukey's test; ns, not significant.

and T30 broilers suffered from a 12% and 20% BW reduction (310 g *vs* 273 and 247 g; $P < 0.05$). Greater body weight losses than 11-12% could jeopardise compensatory growth in broilers (Rosa *et al.*, 2000). However, in the present work, the T15 and T30 groups completely recovered the BW by the end of the growing period (1117 and 1100 g *vs* 998 g in the control treatment; $P < 0.05$), although BW recovery was not so obvious in the T15-5 and T30-5 treatments compared with the control treatment (1086 and 1078 g *vs.* 998 g; $P < 0.05$). Nevertheless, at the end of the experimental period, the T15 and T30 chickens were heavier ($P < 0.05$) than the controls (Table 3) due to a higher ($P < 0.05$) BWG during the finishing period in both groups, irrespective of the nutrient density levels (Table 2). Some authors have also reported full BW recovery at slaughter age after quantitative feed restriction (Butzen *et al.*, 2013; Demir *et al.*, 2004; Jahanpour *et al.*, 2015), while others have found no BW recovery (Gonzales *et al.*, 1998; Mohebodini *et al.*, 2009; Saleh *et al.*, 2005). The works of Lee and Leeson (2001), Novel *et al.* (2009) and Urdaneta-Rincon and Leeson (2002) indicate that the contradictory results found in the literature may be related to the intensity and duration of the quantitative feed restriction and the length of the refeeding period.

In the growing period, the best FCR ($P < 0.05$) was achieved in the T30 group, and was related to a lower FI ($P < 0.05$), because no differences ($P > 0.05$) were observed in BWG between treatments (Table 2). Again, in the finishing period, the best FCR ($P < 0.05$) was also achieved in the T30 group, but no differences ($P > 0.05$) were observed in FI between treatments, and BWG did not differ ($P > 0.05$) from that of the T15 group (Table 2). In both the growing and finishing periods, the lowest FCR ($P < 0.05$) was observed in the T30-15 treatment. In the T15 group, the effect of a higher nutrient density after the feed restriction was not so obvious, and was only observed in the finishing period (Table 2). When the whole experimental period was considered, the FCR was higher ($P < 0.05$) in the controls and lower ($P < 0.05$) in the T30 broilers (Table 2). Since no differences ($P > 0.05$) were observed in FI between treatments, the improvement in FCR in the T15 and T30 groups was only due to their higher ($P < 0.05$) BWG. Improvements in FCR after quantitative feed restriction have been reported in some works (Saleh *et al.*, 2005; Santoso, 2002; Urdaneta-Rincon and Leeson, 2002), whereas most other authors have not found any differences for the unrestricted treatments (Butzen

et al., 2013; Demir *et al.*, 2004; Novel *et al.*, 2009). Considering those works, it is striking that a better FCR does not always seem to be related to full BW recovery (Saleh *et al.*, 2005) nor full BW recovery is necessarily associated with a better FCR (Butzen *et al.*, 2013; Novel *et al.*, 2009). Again, the present results indicated that, within the restricted groups, FCR tended to be lower in the high nutrient density treatments (T15-15 and T30-15). In agreement with these results, Leeson and Zubair (1997) reported an improved BWG and FCR from d 12 to d 21 with increasing diet energy, regardless of the prior feeding method (ad libitum or 50% ad libitum from 6 to 12 days of age), but the protein level during refeeding had no effect on the growth characteristics. On the contrary, Giachetto *et al.* (2003) found no significant interaction between feed restriction (30% ad libitum) and energy level (2900 and 3000 kcal ME/kg) during the refeeding period. Santoso *et al.* (1995) also failed to show differences due to diet protein content (21 to 35%) during the first week after the feed restriction period. Overall, the present results and those from the literature suggest that early feed restriction improves nutrient utilization for growth, and that the response may be greater if the diet supplied after the restriction period is an energy dense one, provided that enough protein is supplied according to requirements. On the other hand, the improved FCR observed in T15-15 and T30-15 treatments might also be due to some extent to the higher oil content of the feeds, because of increased dietary energy metabolisability (De Groote, 1974).

It should be noted that in the present study the cost of feeds rose linearly as the nutrient density increased: feeds were about 7, 15 and 22% more expensive in the T5, T10 and T15 groups, respectively, than in the controls. Since feed intake was similar between treatments, the average daily feeding cost was about 4, 13 and 19% higher in the T15-5, T15-10 and T15-15 treatments, respectively, than in the control. However, the average daily feeding cost was about 0, 9, and 10% higher in the T30-5, T30-10 and T30-15 treatments, respectively, than in the control. The differences between the T15 and T30 groups were probably due to the lower intake of the latter group during the restriction period.

Carcass traits and organ weight

The carcass traits are shown in Table 3. No differences were observed ($P > 0.05$) in carcass weight, considered as the percentage of body weight and percentage of breast in the carcass, between the controls and T15 and T30 broilers.

In the T15 group, the drumstick and wing percentages in the carcass were lower ($P < 0.05$). The highest ($P < 0.05$) breast percentages in the carcass were observed in the T15-10 and T30-10 treatments. Mohebodini *et al.* (2009), Onbasilar *et al.* (2009) and Saleh *et al.* (2005) did not find any effects of quantitative feed restriction on carcass traits. In addition, Novel *et al.* (2009) and Jahanpour *et al.* (2015) observed no significant effects of feed restriction level or duration on the relative weights of the carcass parts. On the contrary, Azlina Azma and Engku Azahan (2011) found that feed restriction, at both 50 and 80% ad libitum intake for three weeks, adversely affected the dressing percentage and the relative weight of the edible carcass parts. Urdaneta-Rincon and Leeson (2002) observed that the negative effect of feed restriction on breast meat yield at 42 days of age was related to the duration of the feed restriction period. However, Lee and Leeson (2001) noted that when birds were able to compensate in growth, following a period of undernutrition, there was little effect on the carcass characteristics of economic importance.

The percentage of abdominal fat was higher ($P < 0.05$) in the T15 broilers than in the control and T30 broilers (Table 3). These results indicated that feeding a nutrient dense diet after a period of mild, but not severe, feed restriction might favour fat deposition, which could be related to differences in energy metabolism and lipogenic enzyme activities during the refeeding phase (Zubair and Leeson, 1996). On the other hand, Leeson and Zubair (1997) reported that the growth response of re-alimented birds to the diet energy level was associated with increased carcass fatness. However, Giachetto *et al.* (2003) failed to show a significant effect of the interaction between feed restriction and energy level on carcass fat during the refeeding period. Most studies have reported the absence of abdominal fat response to quantitative feed restriction (Mohebodini *et al.*, 2009; Onbasilar *et al.*, 2009; Saleh *et al.*, 2005; Urdaneta-Rincon and Leeson, 2002). Conversely, some authors have reported a reduction (Boostani *et al.*, 2009; Santoso *et al.*, 1993; Santoso *et al.*, 1995) or even an increase (Lippens *et al.*, 2000; Zhan *et al.*, 2007) in abdominal fat percentage. The studies by Boostani *et al.* (2009), Santoso *et al.* (1995) and Zhan *et al.* (2007) suggest that the feed restriction effect on abdominal fat may be related to the age at which feed restriction is applied, the intensity of restriction and the protein content of the diet fed in the refeeding period.

The treatment groups had little effect on the

relative weight of the organs, except for thymus weight, which was higher ($P < 0.05$) in the T15 and T30 groups than in the control group, and for liver weight, which was higher ($P < 0.05$) in the T30 group than in the T15 group (Table 3). Early studies (Ballay *et al.*, 1992; Palo *et al.*, 1995) as well as more recent ones (Butzen *et al.*, 2013; Mahmood *et al.*, 2007; Onbasilar *et al.*, 2009) have not found any negative effects of feed restriction on organ weight. Jahanpour *et al.* (2015)

observed no effects of quantitative feed restriction for 7 days on the relative weight of organs related to the immune system (spleen, thymus and bursa of Fabricius), but both 25 and 50% feed restriction over a period of 14 days decreased the relative weight of the bursa of Fabricius. On the other hand, Zubair and Leeson (1994) and Palo *et al.* (1995) found that feed restricted broilers had heavier pancreases after re-alimentation than fully fed broilers. In the present work, the lowest pan-

creas weight was observed in the T15-15 and T30-15 treatments (Table 3), which suggested an adaptive response of the body to the higher energy and protein content of the diet during the refeeding period, *i.e.* less effort to supply the organs was required to obtain nutrients from the diet in order to support elevated BWG.

Blood constituents

Table 4 shows the blood metabolite contents and enzyme activity values. No differences ($P > 0.05$) were observed in the blood contents

Table 3. Final body weight, carcass traits and organ weights of 6-week old broilers raised under a normal feeding programme (control), or two levels of quantitative feed restriction (15 and 30%) between 8 and 14 days of age followed by re-feeding energy and protein dense diets (5, 10 and 15% over the control diet) from 15 to 42 days of age.

	Control	Treatments ^o						SEM	Probability		
		T15-5	T15-10	T15-15	T30-5	T30-10	T30-15		CxT15	CxT30	T15xT30
Body weight, g	2000 ^b	2221 ^a	2267 ^a	2350 ^a	2221 ^a	2330 ^a	2340 ^a	18.82	<0.001	<0.001	ns
Carcass weight, % BW	73.84	73.81	70.30	73.90	69.00	71.37	71.17	0.715	ns	ns	ns
Breast, % CW	34.33 ^{abc}	32.90 ^c	36.33 ^{ab}	34.00 ^{bc}	31.91 ^c	37.08 ^a	34.22 ^{bc}	0.314	ns	ns	ns
Drumsticks, % CW	30.60 ^a	28.19 ^{ab}	29.31 ^{ab}	25.75 ^b	29.97 ^a	28.77 ^{ab}	29.46 ^{ab}	0.364	<0.01	ns	<0.05
Wings, % CW	11.17 ^{ab}	10.13 ^b	10.70 ^{ab}	10.57 ^b	11.83 ^a	10.74 ^{ab}	10.84 ^{ab}	0.116	<0.05	ns	<0.01
Abdominal fat, % CW	1.14 ^b	1.65 ^{ab}	1.30 ^{ab}	1.85 ^a	1.24 ^{ab}	1.01 ^b	1.25 ^{ab}	0.064	<0.05	ns	<0.01
Organ weight, % BW											
Gastrointestinal tract	7.25	8.72	8.62	7.66	8.20	8.60	8.60	0.242	ns	ns	ns
Liver and bile	2.38 ^{ab}	2.17 ^b	2.31 ^{ab}	2.14 ^b	2.16 ^b	2.76 ^a	2.40 ^{ab}	0.052	ns	ns	<0.05
Pancreas	0.227 ^{ab}	0.239 ^{ab}	0.253 ^{ab}	0.164 ^c	0.263 ^{ab}	0.307 ^a	0.189 ^{bc}	0.010	ns	ns	ns
Spleen	0.140	0.122	0.136	0.124	0.118	0.157	0.174	0.008	ns	ns	ns
Thymus	0.350	0.483	0.391	0.535	0.396	0.429	0.524	0.018	<0.05	<0.10	ns
Bursa of Fabricius	0.216	0.221	0.222	0.236	0.209	0.209	0.242	0.009	ns	ns	ns

BW, body weight; CW, carcass weight. T15-5, 15% feed restriction and 5% nutrient density increase; ^oT15-10, 15% feed restriction and 10% nutrient density increase; T15-15, 15% feed restriction and 15% nutrient density increase; T30-5, 30% feed restriction and 5% nutrient density increase; T30-10, 30% feed restriction and 10% nutrient density increase; T30-15, 30% feed restriction and 15% nutrient density increase. ^a*In a row, least squares means without a common superscript are significantly different ($P < 0.05$) by Tukey's test; ns, not significant.

Table 4. Blood plasma constituents of 6-week old broilers raised under a normal feeding programme (control), or two levels of quantitative feed restriction (15 and 30%) between 8 and 14 days of age followed by re-feeding energy and protein dense diets (5, 10 and 15% over the control diet) from 15 to 42 days of age.

	Control	Treatments ^o						SEM	Probability		
		T15-5	T15-10	T15-15	T30-5	T30-10	T30-15		CxT15	CxT30	T15xT30
Glucose, mg dL ⁻¹	237	225	243	252	244	275	258	5.024	ns	ns	ns
Total protein, g dL ⁻¹	4.39	4.78	4.62	4.82	5.09	5.62	5.54	0.197	ns	<0.05	ns
Albumin, g dL ⁻¹	1.58	1.54	1.70	1.67	1.70	1.75	1.82	0.041	ns	ns	ns
Globulin, g dL ⁻¹	2.81	3.24	2.92	3.16	3.39	3.87	3.72	0.159	ns	<0.10	ns
Uric acid, mg dL ⁻¹	4.10	3.50	2.80	3.23	3.50	4.83	5.33	0.291	ns	ns	<0.05
Triglycerides, mg dL ⁻¹	39.00	33.33	27.67	29.67	33.67	41.00	30.67	1.406	<0.05	ns	ns
Cholesterol, mg dL ⁻¹											
Total	110.3 ^{ab}	110.0 ^{ab}	90.33 ^b	108.3 ^{ab}	122.3 ^a	101.7 ^{ab}	94.33 ^b	2.185	ns	ns	ns
HDL	11.00 ^{ab}	12.67 ^{ab}	10.33 ^{ab}	11.67 ^{ab}	15.33 ^a	8.00 ^b	9.67 ^b	0.506	ns	ns	ns
LDL	60.65 ^{ab}	56.57 ^{ab}	42.67 ^c	54.66 ^{ab}	64.67 ^a	56.67 ^{ab}	50.33 ^{bc}	1.324	<0.05	ns	ns
VLDL	7.80	6.67	5.53	5.93	6.73	8.20	6.13	0.281	<0.05	ns	ns
AST, U L ⁻¹	220 ^{abc}	207 ^{bc}	236 ^a	196 ^c	227 ^{ab}	199 ^c	238 ^a	2.985	ns	ns	ns
ALT, U L ⁻¹	3.00	4.67	2.67	2.00	4.33	4.33	3.67	0.236	ns	ns	ns

HDL, high density lipoprotein; LDL, low density lipoprotein; VLDL, very low density lipoprotein; AST, aspartate aminotransferase; ALT, alanine aminotransferase. ^oT15-5, 15% feed restriction and 5% nutrient density increase; T15-10, 15% feed restriction and 10% nutrient density increase; T15-15, 15% feed restriction and 15% nutrient density increase; T30-5, 30% feed restriction and 5% nutrient density increase; T30-10, 30% feed restriction and 10% nutrient density increase; T30-15, 30% feed restriction and 15% nutrient density increase. ^a*In a row, least squares means without a common superscript are significantly different ($P < 0.05$) by Tukey's test; ns, not significant.

of glucose, total protein, albumin, globulin, uric acid and triglycerides between treatments. These results are in agreement with those of Azis *et al.* (2012) and Jahanpour *et al.* (2013), and partially in agreement with those of other authors who investigated quantitative feed restriction (Boostani *et al.*, 2010; Demir *et al.*, 2004; Mohebodini *et al.*, 2009). Blood metabolites reflect the immediate nutritional status of birds. The noted discrepancies among the authors may be due to the age of initiation of the feed restriction and to the type, intensity and duration of the feed restriction programme. As an example, Demir *et al.* (2004) found that the blood albumin and glucose contents increased for 25% feed restriction at 13-14 and 20-21 days of age, but decreased for 50% feed restriction at 13 and 21 days of age. Other authors have reported increased blood glucose and triglyceride concentrations in broilers that were feed deprived for 4 h per day from 7 to 21 days (Onbasilar *et al.*, 2009) or from 1 to 21 days (Zhan *et al.*, 2007).

No differences ($P>0.05$) were found in the total plasma or HDL cholesterol between treatment groups, but LDL and VLDL cholesterol were higher ($P<0.05$) in the control than in the T15 group (Table 4). Some authors have reported that feed restriction causes an increase in the total cholesterol content in the blood compared with *ad libitum* fed broilers (Demir *et al.*, 2004), but others have found no effects (Mohebodini *et al.*, 2009; Onbasilar *et al.*, 2009).

Jahanpour *et al.* (2013) observed that the feed restriction level (25 and 50% *ad libitum*) did not affect the plasma VLDL or HDL cholesterol contents at 42 days of age, but the LDL cholesterol was higher and the total cholesterol was lower in the low and high restriction treatments, respectively, without any effect of the length of the restriction period. Variations in lipoprotein lipase activity could account for the discrepancies in the results reported by different authors (Zhan *et al.*, 2007). The fact that, in the present work, the feeding of more nutrient dense diets, following the feed restriction period, did not elicit a clear response in the blood lipid contents, suggests an interaction between the two studied factors, since Choe *et al.* (2013) observed that triglycerides, and the total and HDL cholesterol contents tended to increase with the energy increments in the diet.

In the present work, the lowest ($P<0.05$) plasma contents of the total, HDL and LDL cholesterol within the T15 group were found in the T15-10 treatment, whereas the lowest ($P<0.05$) values of these parameters within the T30 group were observed in the T30-10 and T30-15 treatments. These were the same treatments that showed the numerically lowest contents of abdominal fat (Table 3). In fact, significant Pearson's correlations were found between the plasma contents of the total, HDL and LDL cholesterol, but not for the triglycerides or VLDL cholesterol, or for the percent-

age of abdominal fat in the carcass (0.37, 0.37 and 0.24, respectively, $P<0.05$). Some authors have reported comprehensive blood lipid profiles in feed restricted chickens. Chen *et al.* (2012) observed that a prolonged 30% energy restriction decreased both the HDL and LDL cholesterol contents in blood, with a concomitant reduction in the abdominal fat percentage. On the contrary, Zhan *et al.* (2007) reported that very early and mild feed restriction decreased VLDL cholesterol in the blood and increased abdominal fat percentage at 63 days of age.

The feed restricted treatments did not show elevated plasma contents of AST or ALT, compared with the control treatment. This might reflect no diet manipulation effects on the liver or muscle function. In female broiler breeder chickens, Rajman *et al.* (2006) observed no differences in the AST and ALT contents in the blood at 44 days of age due to feed restriction from 16 days of age onwards. However, Jang *et al.* (2009) found a higher AST content in the plasma of 35 days-old broilers that were fed 70 and 85% *ad libitum* intake between 8 and 14 days of age.

Immune response

Few effects and no clear trends were observed in the immune response to the vaccines due to feeding regimes (Table 5). The response to the Newcastle disease vaccine was lower ($P<0.05$) at 19 days of age in the T15 and T30 broilers than in the control, but the differ-

Table 5. Immune response after vaccination or injection of sheep red blood cells in broilers raised under a normal feeding programme (control), or two levels of quantitative feed restriction (15 and 30%) between 8 and 14 days of age followed by re-feeding energy and protein dense diets (5, 10 and 15% over the control diet) from 15 to 42 days of age.

	Control	Treatments ^o						SEM	Probability		
		T15-5	T15-10	T15-15	T30-5	T30-10	T30-15		CxT15	CxT30	T15xT30
Newcastle disease, log ₂											
19 d	7.33	4.33	4.67	6.00	3.67	4.67	4.33	0.422	<0.05	<0.05	NS
26 d	5.33	6.00	5.00	6.00	5.67	5.67	4.33	0.241	ns	ns	ns
Influenza disease, log ₂											
30 d	1.33	1.33	2.00	1.33	1.67	1.00	1.33	0.110	ns	ns	ns
39 d	4.33 ^{ab}	4.67 ^a	4.00 ^{ab}	4.00 ^{ab}	4.00 ^{ab}	4.00 ^{ab}	3.67 ^b	0.067	NS	<0.05	<0.05
SRBC, log ₂											
Total Ig 29 d	3.67	4.67	2.33	3.67	1.67	3.00	3.00	0.318	ns	ns	ns
Total Ig 36 d	6.67	7.33	5.67	5.33	6.33	7.00	6.00	0.333	ns	ns	ns
IgG 29 d	0.00 ^b	1.00 ^a	0.00 ^b	0.33 ^{ab}	0.67 ^{ab}	0.67 ^{ab}	1.00 ^a	0.075	<0.05	<0.001	<0.05
IgG 36 d	5.00	5.00	4.67	4.33	4.33	4.67	4.00	0.228	ns	ns	ns
IgM 29 d	3.67	3.67	2.33	3.33	1.00	2.33	2.00	0.170	<0.10	ns	ns
IgM 36 d	1.67	2.33	1.00	1.00	2.00	2.33	2.00	0.175	ns	ns	<0.10

SRBC, sheep red blood cells; ^oT15-5, 15% feed restriction and 5% nutrient density increase; T15-10, 15% feed restriction and 10% nutrient density increase; T15-15, 15% feed restriction and 15% nutrient density increase; T30-5, 30% feed restriction and 5% nutrient density increase; T30-10, 30% feed restriction and 10% nutrient density increase; T30-15, 30% feed restriction and 15% nutrient density increase. ^aIn a row, least squares means without a common superscript are significantly different ($P<0.05$) by Tukey's test; ns, not significant.

ences disappeared one week later. The T30 broilers showed lower ($P < 0.05$) antibody response to the influenza disease vaccine than the T15 broilers and the control. With regard to SRBC, the highest ($P < 0.05$) IgG responses at 29 days of age corresponded to the T30 group, and this was followed by the T15 group. Onbasilar *et al.* (2009) reported that antibody titers against SRBC were not significantly affected by quantitative feed restriction. On the contrary, Mahmood *et al.* (2007) observed that birds kept under feed restriction programmes had a lower immune response against Newcastle and Gumboro disease vaccines than those fed ad libitum, and the negative effect was higher in the most severe restriction treatments. It is well-known that nutritional status affects the immune function (Klasing *et al.*, 2007). Klasing (1988) showed that an acute period of feed deprivation, or overconsumption, increased and decreased, respectively, selected aspects of the immune response. However, Fassbinder-Orth and Karasov (2006) observed that during feed restriction and re-alimentation, the immune structure and function of the gut, including the bursa mass, spleen mass, and total IgA, remained unchanged.

Conclusions

The effects of a period of feed restriction followed by re-alimentation with diets of increased energy and protein content have been studied in broilers. Overall, the effects of feed restriction were more pronounced than the effects of the diet energy and protein contents during the growing and finishing periods. Feed restriction improved the final BW and FCR, compared with the controls, and no negative effects were observed on carcass traits. The obtained results suggest that feeding a nutrient dense diet after a period of mild, but not severe, feed restriction favours fat deposition. Moreover, the feeding programme affected the blood lipid profile, and a positive correlation between the total, HDL and LDL cholesterol contents in the blood and abdominal fat percentage was observed. The results presented herein indicate that the treatments had no or little impact on the relative weights of the organs and the immune response of broilers. More studies are needed to clarify some of the observed responses and establish the economic advantage of feed restriction under on-farm conditions.

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