Indian Journal of Animal Sciences **84** (5): 554–558, May 2014/Article

Effect of intensity and duration of quantitative feed restriction on broiler caecum microbiota

HOSSEIN JAHANPOUR[1], ALIREZA SEIDAVI[2], ALI AHMAD ALAW QOTBI[3], FERNANDO DELGADO[4] and SANDRA GAMBOA[5]

Islamic Azad University, Pol-e-Taleshan, Rasht, P.O. Box 41335–3516 Iran

Received: 23 November 2013; Accepted: 27 January 2014

ABSTRACT

This study evaluated the effects of quantitative feed restriction with different intensity and duration on broiler intestinal microbial flora. Using a completely randomized design, Ross 308 male broiler chicks (115; 3 replications/ treatment; 10 chicks/replication) were assigned to 5 experimental treatments. These included quantitative feed limitations in 50% (T2,T3) and 25% (T4,T5) lower than the recommended by the Ross 308 broiler catalogue in 2 periods of 7 (T2, T4) and 14 days (T3, T5), with one control treatment (T1; feed intake as *ad lib.*). All chicks were fed *ad lib.* before and after the limitation period, until slaughter (day 42). The results showed that quantitative feed restriction had significant effects on microbiota of ceca in broilers except for lactic acid bacteria. A feed restriction of 25% during 7 days (T4) resulted in the highest mean value for coliforms and *Escherichia coli*; in contrast, a feed restriction of 25% during 14 days (T5) resulted in the highest mean value for lactobacilli and enterobacteria. A feed restriction of 50% during 7 days did not affect microbial count when compared with the control group. The number of total bacteria was higher in T4 although the difference had not been statistically significant from T2 and T3. Major alterations occurred for *E. coli* and coliforms in T4, lactobacilli and enterobacteriaceae in T5 and enterecoccus in both T3 and T4. It is concluded that a feed restriction of 25% during 7 days (T4) gave rise to the growth of other species than those analyzed in this study whereas a feed restriction of 25% during 14 days increased lactobacilli and enterobacteriaceae.

Key words: Broiler, Caecum, Food intake, Microflora, Restriction

In broiler rearing industry, nutrition management techniques include restricted feeding strategies. The goal is to restrict growth in the early rearing period to reduce metabolic and skeletal disorders (Robinson *et al.*1992) and fat deposition (Yu and Robinson 1992). Zubair and Leeson (1994) indicated that feed restriction programmes applied early in the rearing period can alter the growth patterns of bird and decrease their maintenance requirements. The reduction in the overall maintenance requirements is responsible for an improvement in the feed efficiency ratio (FCR). Usually, following the restricted feed programme, and after feeding a diet of high quality, a faster growth occurs (Sahraei 2012), and this situation can provide many benefits to the farmer by decreasing feed costs without retarding overall growth till slaughter age. Although early feed restriction reduces growth performance, compensatory growth in the refeeding period will be attained in order to accelerate organism growth to reach the weight of animals (Hornick *et al.* 2000, Pinheiro *et al.* 2004).In broiler chicken, normal intestinal microbiota populations can be affected by different factors such as diseases of digestive tract and diet, use of antibiotics, nutritional deficiency, transport, high density herd, age, quantitative genotype and gender, vaccination and other stress inducer factors (Torok *et al.* 2008a, Yegani and Korver 2008, Yang and Choct, 2009, Zhao *et al.* 2013). These factors lead to an imbalance in gut flora and thereby weakens the body's defense mechanisms and thus the chance of the establishment of intestinal pathogens. Early feed restriction in broilers suggested that feed restriction has beneficial effects in protecting against the establishment of undesirable intestinal pathogens (Soleimani *et al.* 2012). Moreover, microbes capable of degrading uric acid are much abundant in gut but the cecum has received most of the attention since uric acid can be converted here into amino acids by microbial synthesis and then be reabsorbed by the host. This process is thought to be especially important for conserving nitrogen, especially in low protein diets (Kohl 2012). Therefore, the purpose of this research was to evaluate the effects of feed restriction

Present adress: [1]Young Researchers and Elite Club, (armin_jahanpoor@yahoo.com). [2,3]Department of Animal Science, College of Agriculture ([2]alirezaseidavi@iaurasht.ac.ir; [3]adian2000@yahoo.com). [4,5]Agricultural School of Coimbra; CERNAS, Centre for Natural Resources, Environment and Society. IPC, 3040–316, Portugal ([4]fdelgado@esac.pt, [5]scgamboa@esac.pt).

method with different intensity and duration on broiler ceca microbiota.

MATERIALS AND METHODS

Bird husbandry and performance traits

A 42-d experiment was conducted with day-old male broiler chicks of the Ross 308 strain according to institutional ethical norms. Animals were housed in a hall with a 4 m×40 m area with 5 ventilators and previously prepared as described by Jahanpour *et al.* (2012). The temperature inside the experimental room was maintained about 30–32°C from 1–7 days of age, at 26–30°C from 8–14 days of age, and at 24–26°C from15–42 days of age.

To provide moisture of at least 55 to 65%, in the early growing period, the hall was sprayed with water on the floor. A discontinuous lighting programme, 23 h of light and 1h of darkness per day, was adopted with 43 watt lamps that were installed in a row at a distance of 200 cm and a height of 2.2 m from the floor. The recommended vaccination programme against bronchitis, Newcastle and Gumboro diseases was observed in all cases. To reduce stress, 1:1000 multivitamin plus electrolytes dissolved in drinking water was used in the 24 h period after each vaccination. The chicks (150) were assigned at the first day of age, to a completely randomized design with 5 dietary treatments, 3 replicates/treatment and 10 birds/replicate; each replicate was considered an experimental unit. Broilers nutritional requirements were based on the National Research Council, Nutrient Requirements of Poultry (NRC 1994) recommendations and on Ross 308 broiler nutrition specifications (Aviagen 2007). Drinking water was freely available.

Diets and experimental design

The ingredient composition of the diets used as well as the nutrient composition are presented in Tables 1 and 2, respectively.

The broilers were fed *ad lib.* for the first 8 d. At eighth day of age, a group was maintained at *ad lib* intake (T1; control group) and 4 different feed restriction programmes (T2-T5) were implemented.

The T2 and T3 programmes included broilers fed with restricted amounts (50% of *ad lib.* intake) of the standard mash for Ross 308 broiler chicken from 8 to 14 days and from 8 to 21 days, respectively. The T4 and T5 programmes, in turn, fed with even more restricted amounts (25% lower than *ad lib.* intake) of the same standard mash for Ross 308 broiler chicken from 8 to 14 days and from 8 to 21 days, respectively. The chicks had access to *ad lib.* food intake after the completion of the restriction period. In addition, all diets in each of the 3 rearing periods were designed with the same raw energy and protein.

Cecum microbiota analysis

The microbial parameters examined in this study were: total bacteria, *Escherichia coli*, coliforms bacteria, lactobacilli bacteria, lactic acid bacteria, enterobacteriacea and enterococcus. For viable counts, samples were smeared over the appropriate selective agar and incubated. In the last day of the rearing period (42nd days of age), 1 bird from each replication was randomly selected and slaughtered by neck cutting. The colon segments were isolated, weighed and sent to the laboratory into sterile Petri plates. Samples were transferred to sterile tubes with phosphate buffer saline (PBS) and shaken for approximately half an hour to separate the gastrointestinal contents and the bacteria and to prepare the suspension. An amount of 1 ml was removed from the prepared suspension and was added to 9 ml PBS in another tube. Serial dilutions were obtained and a drop of each sample was spread over the appropriate agar surface. Inoculated plates were incubated

Table 1. Composition of the diets used during experimental periods

Ingredient (%)	Starter	Grower	Finisher
Corn	46.09	50.09	48.88
Soybean meal	40.00	35.00	39.97
Fish meal	3.00	3.00	-
Meat meal	3.00	3.00	-
Oil	4.56	5.45	7.39
Dl-methionine	0.29	0.23	0.17
L-threonine	0.30	-	-
Ca%22, P%18	0.99	0.75	1.64
$CaCo_3$	0.98	0.76	1.00
K-bicarbonate	0.05	0.03	-
NaCl	0.37	0.37	0.37
Vitamin and mineral mixture	0.60	0.50	0.50
Total	100	100	100

Table 2. Nutrients analysis of diets used during experimental periods

Nutrients	Starter	Grower	Finisher
Energy (ME) (kcal.kg)	3200	3200	3200
Crude protein	23.0	20.0	20.0
Lysine (SID) (%)	1.41	1.26	1.22
Methionine (SID) (%)	0.67	0.59	0.50
Met+Cys (SID) (%)	1.05	0.94	0.85
Threonine (SID) (%)	1.98	0.87	0.85
Tryptophan (SID) (%)	0.30	0.27	0.28
Arginine (SID) (%)	1.68	1.54	1.51
Iso-leucine (SID) (%)	1.04	0.95	0.94
Valine (SID) (%)	1.60	1.07	1.03
Leucine (SID) (%)	1.99	1.87	1.82
Calcium (%)	1.05	0.90	0.85
Available phosphorus (%)	0.50	0.45	0.42
Sodium (%)	0.23	0.23	0.20
Potassium (%)	1.00	0.90	0.93
Chloride (%)	0.30	0.30	0.30
DCAB (mEq.kg)	272.12	244.55	242.77
Choline (g.kg)	1.48	1.37	1.37
Linoleic acid (%)	1.21	1.27	1.24
Ether extract (%)	6.84	7.87	9.22
Crude fibre (%)	3.78	3.52	3.73

at 37°C in aerobic conditions for 48 h. A colony counter was used to enumerate bacterial colonies. The average number of live bacteria was calculated taking into account a gram of original sample. All quantitative data were converted into logarithmic colony forming units (cfu/g).

Statistical analysis

In order to study the effect of intensity and duration of diet restriction on ceca microbiota, the general linear model (GLM) procedure was applied according to the theoretical model $Y_{ij}= \mu + a_i + e_{ijk}$, in which the value of each observation (Y) relies on the treatment applied (a) plus the observed mean (μ) and the experimental error (e).The Duncan's post hoc test was used if the initial test result was significant at P<0.05.

RESULTS AND DISCUSSION

Mean values obtained for microbial counts of cecum in each treatment are presented in Table 3.

The feed restriction regimens applied resulted in significant differences for total bacteria (P<0.05), *Escherichia coli* (P<0.05), coliforms (P<0.05), lactobacilli (P<0.05), enterobacteriaceae (P<0.05) and enterococcus (P<0.01). No significant difference among treatments was found for lactic acid bacteria (P>0.05). The control group (T1) had the lowest mean value for *E. coli* and coliforms (despite no differences with T2, T3 and T5) as well as enterobacteriaceae (despite no differences with T2, T3 and T4); a feed of 25% restriction during 7 days (T4) resulted in the highest mean value for total bacteria (significantly different from T1 and T5; P<0.05), *E. coli* and coliforms counts (P<0.05) and, on contrary, in the lowest value for lactic acid bacteria (despite no significant differences with the other treatments) and for enterococcus (despite no significant differences with T3 and T5); in contrast, a feed of 25% restriction during 14 days (T5) resulted in the highest mean values for lactobacilli (P<0.05), lactic acid bacteria (despite no significant differences with the other treatments) and enterobacteriaceae (P<0.05). The counts of lactobacilli were likewise higher in T3 and T4 (P>0.05) while the counts of enterobacteriaceae were likewise lowest in T3 and T4 (P>0.05). The number of enterococci was lower in T3 and T4 and significantly different from T1 and T2 (P<0.05). Mean values for lactic acid bacteria remained stable across treatments.

The microbiota in the gastrointestinal tract of broiler chicks plays an important role during the rearing period. This microbial population is distributed throughout the digestive tract and is found in high concentrations in the ceca (Gabriel 2006). The ceca bacteria confer a health benefit by competition for specific nutrients with pathogenic organisms and undesirable microbes, by lowering pH through acid formation and by modulation of the intestinal epithelium. The gastrointestinal tract microbiota changes with age and ultimately reflects the diet adopted (Lu *et al.* 2003, Torok *et al.* 2008b). Studies from Lu *et al.* (2003) and Bjerrum *et al.* (2006) showed that the chicken ileum was dominated by lactobacilli, whereas the cecum harbored a more diverse microbial community dominated by clostridium-related species (Bjerrum *et al.* 2006, Lund *et al.* 2010). As expected, in our study we observed that lactobacilli accounted for 30% of the microbial community in the control group (Rehman *et al.* 2007). The chicken's

Table 3. Mean comparison (±SEM) of microbiota of cecum among 5 treatments*

Microbiota[cfu/gr]	Treatments				
	T1 (Control group)	T2 (Broilers fed with 50% of *ad lib.* from 8 to 14 days of age)	T3 (Broilers fed with 50% of *ad lib.* from 8 to 21 days of age)	T4 (Broilers fed with 25% lower than *ad lib.* from 8 to 14 days of age)	T5 (Broilers fed with 25% lower than *ad lib.* from 8 to 21 days of age)
Total bacteria	24.70×10^{7b} $\pm2.71\times10^{7}$	26.33×10^{7ab} $\pm3.28\times10^{7}$	27.43×10^{7ab} $\pm3.72\times10^{7}$	43.60×10^{7a} $\pm2.31\times10^{7}$	21.90×10^{7b} $\pm0.300\times10^{7}$
Escherichia coli	$3.53\text{x}10^{7b}$ $\pm0.64\times10^{7}$	4.00×10^{7b} $\pm0.58\times10^{7}$	$7.97\text{x}10^{7b}$ $\pm3.22\times10^{7}$	16.33×10^{7a} $\pm0.67\times10^{7}$	8.75×10^{7b} $\pm1.25\times10^{7}$
Coliforms bacteria	6.83×10^{7b} $\pm0.38\times10^{7}$	11.37×10^{7b} $\pm1.73\times10^{7}$	13.77×10^{7b} $\pm3.42\times10^{7}$	23.27×10^{7a} $\pm2.89\times10^{7}$	13.5010^{7b} $\pm1.20\times10^{7}$
Lactobacilli bacteria	6.00×10^{7c} $\pm0.58\times10^{7}$	5.00×10^{7c} $\pm1.15\times10^{7}$	7.00×10^{7bc} $\pm0.58\times10^{7}$	9.00×10^{7b} $\pm0.58\times10^{7}$	12.0010^{7a} $\pm0.00\times10^{7}$
Lactic acid bacteria	18.33×10^{7a} $\pm4.4095\times10^{7}$	18.00×10^{7a} $\pm4.6188\times10^{7}$	13.90×10^{7a} $\pm3.09\times10^{7}$	12.13×10^{7a} $\pm1.27\times10^{7}$	22.00×10^{7a} $\pm2.00\times10^{7}$
Enterobacteriaceae	10.00×10^{7a} $\pm0.23\times10^{7}$	14.80×10^{7a} $\pm1.3306\times10^{7}$	15.53×10^{7a} $\pm1.30\times10^{7}$	26.37×10^{7a} $\pm3.22\times10^{7}$	93.6510^{7b} $\pm75.35\times10^{7}$
Enterococcus bacteria	$0.70\times10^{7\,a}$ $\pm0.17\times10^{7}$	0.67×10^{7a} $\pm0.18\times10^{7}$	0.13×10^{7b} $\pm0.15\times10^{7}$	0.10×10^{7b} $\pm0.21\times10^{7}$	0.34×10^{7ab} $\pm0.4\times10^{7}$

*Means with same letter (superscript) in each line are not significantly different at a, 0.05 (Duncan post hoc test).

cecum is dominated by lactic acid bacteria and this bacterial population was not affected by the feed restriction regimen applied.

In this study, the feed restriction programmes had a statistically significant effect on total bacteria, coliforms and enterobacteriaceae, *Escherichia coli,* lactobacilli and enterococcus (P<0.05), without affecting lactic acid bacteria (P>0.05). Similar results were reported in broiler by Danicke *et al.* (1999) and Gong *et al.* (2002) as well as in other species, for total bacteria (Thompson and Spiller 1995, Maia *et al.* 2007), *Escherichia coli* (Ringo *et al.* 1998), lactobacilli (Ringo *et al.*1998, Maia *et al.* 2007) and enterococcus (Maia *et al.* 2007). Shapiro and Sarles (1949) observed that in chicken's cecum the numbers of enterococcus were lower than the numbers of coliforms, which is in agreement with our observations, independently of the treatment. Those authors also stated that enterobacteriaceae and enterococci decreased in the ceca when broilers grew older, but this decrease seems to be dependent on the diet fed to the broilers. Indeed, the feed restriction programmes used in our study revealed a significant increase in lactobacilli and enterobacteriaceae and this rise is stressed in T5, the less severe quantitative restriction regimen applied during 2 weeks. As stated by Yegani and Korver (2008) the composition of the microflora is not constant and there are many factors that can alter this composition including the age of the chicken, diet composition, breed, and geographic location.

The total bacteria imbalance in T4 followed by a normal value in T5 suggested a physiological adaptation along time. Results from experiments from Aliakbarpour *et al.* (2013) showed that intermittently fed birds were able to adapt to the feeding strategy for improving feed conversion and nutrient requirements. In this study, a feed with 25% restriction during 14 days (T5) leads to an increase in lactobacilli and enterobacteriaceae while a feed of same restriction level for a shorter period (7 days, T4) gave rise to the growth of other species than those identified in this study. Indeed, in the gastrointestinal tract of broiler chicken a large proportion of the bacterial population remain unidentified due to lack in knowledge on appropriate culturing conditions. From molecular studies on microflora of the chicken's cecum it is estimated that a bacterial population consisting of at least 640 species from 140 genera, of which 10% of the identified bacterial 16S rRNA gene sequences represent previously known bacterial species, and the remaining sequences belong to unclassified species or genera (Apajalahti *et al.* 2004). The *Faecali bacterium prausnitzii*, a clostridium-related bacteria within the gut, has been shown to produce butyrate and D-lactate by fermentation (Bjerrum *et al.* 2006). These volatile fatty acids produced in the ceca are proposed to have a bacteriostatic effect on enteropathogens. The decline in the number of Enterobacteriaceae (including *Salmonella)* in the ceca of broiler chickens during the growth of poultry seems to be due to volatile fatty acids, without affecting *Lactobacillus* strain (van der Wielen *et al.* 2000).

In conclusion, a feed restriction of 25% during 7 days (T4) gave rise to the growth of other species than those analyzed in this study whereas a feed restriction of 25% during 14 days lead to an increase in lactobacilli and enterobacteriaceae.

REFERENCES

Aliakbarpour H R, Chamani M, Rahimi G, Sadeghi A A and Durdi Q. 2013. Intermittent feeding program and addition of *Bacillus subtilis* based probiotics to the diet of growing broiler chickens: Influence on growth, hepatic enzymes and serum lipid metabolites profile. *Archiv Tierzucht* **56**:40

Apajalahti J, Kettunen A and Graham H. 2004.Characteristics of the gastrointestinal microbial communities, with special reference to the chicken. *World Poultry Science Journal* **60**: 223–32.

Aviagen. 2007. *Ross 308 BROILER: Nutrition Specification*. Scotland, UK.pp.8.

Bjerrum L, Engberg R M, Leser T D, Jensen B B, Finster K, Pedersen K. 2006. Microbial community composition of the ileum and cecum of broiler chickens as revealed by molecular and culture-based techniques. *Poultry Science* **85**(7): 1151–64.

Danicke S, Vahjen V, Simon O, Jeroch H. 1999. Effects of dietary fat type and xylanase supplementation to rye-based broiler diets on selected bacterial groups adhering to the intestinal epithelium, on transit time of feed, and on nutrient digestibility. *Poultry Science* **78**:1292–99.

Gabriel I, Lessire M, Mallet S, Guillot J F. 2006. Microbiota of the digestive tract: Critical factors and consequences for poultry. *World's Poultry Science Journal* **62**: 499–511.

Gong J, Froster R J, Yu H, Chambers J R, Sabour P M, Wheatcroft R, Chen S. 2002. Diversity and phylogenetic analysis of bacteria in mucosa of chicken ceca and comparison with bacteria in the cecal lumen. *FEMS Microbiology Letters* **208**:1–7.

Hornick J L, Van Eenaeme C, Gerard O, Dufrasne I and Istasse L. 2000. Mechanisms of reduced and compensatory growth. *Domestic Animal Endocrinology* **19**: 121–32.

Jahanpour H, Seidavi A and Qotbi A A. 2012. Effects of intensity and duration of feed quantitative restriction on broiler immune system. *Annals of Biological Research* **3**(6): 2656–61.

Kohl K D. 2012. Diversity and function of the avian gut microbiota. *Journal of Comparative Physiology B* **182**: 591–602.

Lu J, Idris U, Harmon B, Hofacre C, Maurer J J and Lee M D. 2003. Diversity and succession of the intestinal bacterial community of the maturing broiler chicken. *Applied and Environmental Microbiology* **69**(11): 6816–24

Lund M, Bjerrum L and Pedersen K. 2010. Quantification of *Faecali bacterium prausnitzii*- and *Subdoligranulum variabile*-like bacteria in the cecum of chickens by real-time PCR. *Poultry Science* **89**: 1217–24.

Maia M R, Chaudhary LC, Figueres L and Wallace R J. 2007. Metabolism of polyunsaturated fatty acids and their toxicity to the microflora of the rumen. *Antonie Van Leeuwenhoek* **91**: 303–14.

NRC.1994. National Research Council. *Nutrient Requirements of Poultry*. 9th Revised edn. National Academy Press. Washington, D.C

Pinheiro D F, Cruz V C, Sartori J R and Paulino M L V. 2004. Effect of early feed restriction and enzyme supplementation

on digestive enzyme activities in broilers. *Poultry Science* **83**: 1544–50.

Rehman H U, Vahjen W, Awad W A and Zentek J. 2007. Indigenous bacteria and bacterial metabolic products in the gastrointestinal tract of broiler chickens. *Archives of Animal Nutrition* **61**(5): 319–35.

Ringo E, Bendiksen H R, Gausen S J, Sundsfjord A, Olsen R E. 1998. The effect of dietary fatty acids on lactic acid bacteria associated with the epithelial mucosa and from faecalia of arctic charr, Salvelinusalpinus. *Journal of Applied Microbiology* **85**: 855–64.

Robinson F E, Classen H L, Hanson J A and Onderka D K. 1992.Growth performance, feed efficiency and the incidence of skeletal and metabolic disease in full-fed and feed-restricted broiler and roaster chickens. *Journal of Applied Poultry Research* **1**: 33–41.

Sahraei M. 2012. Feed restriction in broiler chickens production: a review. *Global Veterinaria* **8**(5): 449–58.

Shapiro S K, Sarles W B. 1949. Microorganisms in the intestinal tract of normal chickens. *Journal of Bacteriology* **58**: 531–44.

Soleimani A F, Zulkifli I, Hair-Bejo M, Omar A R, Raha A R. 2012.The role of heat shock protein 70 in resistance to *Salmonella* Enteritidis in broiler chickens subjected to neonatal feed restriction and thermal stress. *Poultry Science* **91**(2): 340–45.

Thompson L, Spiller R C. 1995. Impact of polyunsaturated fatty acids on human colonic bacterial metabolism: an *in vitro* and *in vivo* study. British *Journal of Nutrition* **74**: 733–41.

Torok V A, Ophel-Keller K, Hughes R J, Forder R, Alii M, Macalpine R. 2008a. Environment and age: impact on poultry gut microflora. *Proceedings of 19th Australian Poultry Science Symposium*. pp. 149–52. 12–14 February 2007. New South Wales, Australia.

Torok V A, Ophel-Keller K, Loo M and Hughes R J. 2008b. Application of methods for identifying broiler chicken gut bacterial species linked with increased energy metabolism. *Applied and Environmental Microbiology* **74**(3): 783–91.

van der Wielen P W J J, Biesterveld S, Notermans S, Hofstra H, Urlings B A P and van Knapen F. 2000. Role of volatile fatty acids in development of the cecal microflora in broiler chickens during growth. *Applied and Environmental Microbiology* **66**(6): 2536–40.

Yang Y, Iji P A and Choct M. 2009. Dietary modulation of gut microflora in broiler chickens: a review of the role of six kinds of alternatives to in feed antibiotics. *World's Poultry Science Journal* **65**: 97–114.

Yegani M and Korver D R. 2008.Factors affecting intestinal health in poultry. *Poultry Science* **87**(10): 2052–63.

Yu M W and Robinson F E. 1992. The application of short term feed restriction to broiler chicken production: a review. *Journal of Applied Poultry Research* **1**: 147–53.

Zhao L, Wang G, Siegel P, He C, Wang H, Zhao W, Zhai Z, Tian F, Zhao J, Zhang H, Sun Z, Chen W, Zhang Y and Meng H. 2013. Quantitative genetic background of the host influences gut microbiomes in chickens. *Scientific Reports* **3**:1163

Zubair A K and Leeson S. 1994. Effect of varying period of early nutrient restriction on growth compensation and carcass characteristics of male broilers. *Poultry Science* **73**(1):129–36.

Zubair A K and Lesson S. 1996. Changes in body composition and adipocyte cellularity of male broilers subjected to varying degrees of early-life feed restriction. *Poultry Science* **75**:719–28.