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Performance, Carcass Characteristics and Immune Responses of Broiler Chickens Subjected to Sequential or Wet Feeding Programs Subsequent to Early Meal Feeding Regime

Research Article

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ABSTRACT

The current study was conducted to evaluate effects of sequential or wet feeding programs subsequent to and early meal feeding regime on performance, carcass characteristics and humoral immunity in broiler chicks. 192 Ross 308 chicks (seven-day old) were allocated to four treatments at four replicates (12 chicks per plot) based on a Completely Randomized Design. Treatments were included: control group (C) where birds had free access to feed throughout the experiment, meal fed groups (MF) where birds were meal fed from 7 to 14 d and subsequently meal fed by the control diet (MFC), meal fed a wet diet (MFW) or subjected to a sequential feeding program (MFS) until day 42. The results showed a statistical reduction of feed intake and increased feed conversion ratio during the meal feeding period ($P<0.05$). Consequently, meal fed chicks had a depressed body weight at 14 d, which was later, compensated ($P<0.05$). Following meal feeding, birds in the meal fed a wet diet (MFW) group exhibited the highest body weight, but the sequential fed group had significantly ($P<0.05$) lower feed intake, body weight and feed conversion ratio (FCR) compared to the other groups. The highest relative weight of small intestine and the lowest abdominal fat pad percentage were observed in the MFS group ($P<0.05$). Birds' antibody responses to Newcastle, Influenza viruses and sheep red blood cell were not influenced by feeding regimes. It is concluded that feeding a wet diet following meal feeding programs can be effectively used in broiler chicken production.

KEY WORDS broiler, meal feeding, performance, sequential feeding, wet feeding.

INTRODUCTION

Broiler chickens have a very fast growth rate and are generally fed *ad libitum* throughout their rearing period to reveal their full genetic potentials (Toghyani *et al.* 2011). In such conditions, body fat deposition and incidence of metabolic disorders such as ascites and Sudden Death Syndrome (SDS) may increase (Hocking *et al.* 2002). Feed restriction has been reported as a viable method to defer early-life fast growth rate in broilers and consequently reduces the incidence of such problems (Ozkan *et al.* 2006). Different methods of feed restriction are applied in practice such as reduced nutrients intake by means of diet dilution (Camacho Fernandez *et al.* 2002), appetite suppression (Oyawoye and Krueger, 1990), limiting the time of feed access through skip-a-day or meal feeding procedures (Saffar and Khajali, 2010), or limiting quantity of feed offered daily to the birds (Ocak and Sivri, 2008). Meal feeding is a feed restriction, which birds have daily free access to feed at specific times. Meal feeding has been used and

shown to be an effective feed restriction program in broiler production. Low stress is the advantage of meal feeding compared to skip-a-day feed restriction (Susbilla *et al.* 2003). Sequential feeding is a feeding program, which consists of giving several diets of different nutritional values, in one to several-day cycles. It might significantly reduce feed cost and also create the potential to incorporate higher amounts of cheap raw materials into broiler diets (Leterrier *et al.* 2006). Sequential feeding with distinct dietary concentrations of energy and amino acids on alternate days has resulted in a similar efficiency compared with a complete feeding regime (Bouvarel *et al.* 2004). In the wet feeding technique, a suitable amount of water is mixed with feed prior to distribution the feeders and is believed to improve feed intake and growth of broiler chicks (Scott, 2002). Different restriction regimes have successfully been used by many researchers to lower the early growth rate of broilers and consequently to benefit from its favorable effects on carcass fat contents, feed efficiency (Nielsen *et al.* 2003; Khetani *et al.* 2009) total mortality (Urdaneta Rincon and Leeson, 2002), and metabolic diseases (Lippens *et al.* 2000), however, the impact of feeding regimes on the re-alimentation period in broiler chicks is not fully investigated. Thus, the aim of the present experiment was to determine the effect of meal feeding regimes on subsequent sequential feeding or wet feeding programs on performance, carcass traits and immune responses of broiler chickens in a 42-day trial.

MATERIALS AND METHODS

Animals and diets

Five hundred sixty day-old male broiler chickens (42±0.9 g) of a commercial strain (Ross/Ross) were purchased. All the birds were fed and watered *ad libitum* to 7 d of age using the starter diet formulated to meet or exceed nutrient requirements provided by the Ross 308 Manual, (2007). At day 7 and after overnight fasting, 192 chicks were individually weighed and chicks of a similar body weight (96 g) were randomly assigned to one of four treatments with four replicates of 12 birds. Chicks in the control group were fed *ad libitum* throughout the experimental period (C), while chicks in other treatments were meal-fed three times per day (0800 to 1000 h, 1600 to 1800 h and 2400 to 0200 h) from 7 to 14 d. The feeding regimes subsequent to meal feeding included: *ad libitum* feeding (MFC), feeding a wet diet (MFW), and a sequential feeding program (MFS) to 42 d. Wet diets were prepared daily and a ratio of 1.3 g water g^{-1} feed was found to be optimum for the diets tested. A mold inhibitor was incorporated into the diet at the time of formulation. Sequential fed chicks received two diets including a high energy low protein diet and a low energy high protein diet over 24-h cycles (Table 1). Environmental temperature was reduced from 32 to 25 ˚C on day 21, and was kept constant. Chicks were raised on floor pens (2×1.5 m) covered with wood shaving to a depth of 2.5 cm and equipped with a bell drinker and trough feeder. The lighting program consisted of a period of 23 h light and 1 h of darkness.

Performance and carcass traits

Body weight and feed intake were monitored at 14, 28 and 42 d using pens as the experimental units, and feed conversion ratio was calculated by dividing the amount of feed intake (kg) of a particular period by the weight gain (kg) of that period for each replicate. Before weighing, the birds were fasted for 4 h. All the pens were checked for mortality twice a day and the birds that died during the experiment from every group were weighed and sent to the pathology laboratory for necropsy, and feed intake was adjusted accordingly. At 42 d, three birds per replicate were randomly chosen and slaughtered. Abdominal fat, liver, pancreas, gizzard, heart, caecum, small intestine, bursa and spleen were collected, weighed and calculated as a percentage of live body weight and also carefully examined to detect any pathological lesion or damages. The small-intestine length was also measured and recorded.

Immune parameters

At 24 d birds were water-vaccinated with Newcastle and Influenza viruses. Ten days later three chicks per replicate were randomly chosen, and blood samples were collected from the brachial vein and centrifuged at 2000 g for 15 minutes to obtain serum (SIGMA 4-15 Lab Centrifuge, Germany). Antibody titers against Newcastle and Influenza viruses were measured using a Hemagglutination Inhibition Test. At 22 d, 12 birds from each treatment groups were injected i.v. with 1 mL of a 7% suspension of sheep red blood cell (SRBC) prepared in phosphate-buffered saline. Blood samples were collected from challenged birds six days later to quantify anti-SRBC antibody titers. To obtain the Heterophil to Lymphocyte ratio (H/L) at 42 d, blood samples (3 samples per replicate) were collected by syringes containing heparin to avoid blood clot formation. Blood smears were prepared using May-Greenwald-Giemsa stain. Approximately 2 to 4 hours after fixation with Methyl Alcohol 100 Leukocytes per samples were counted by Heterophil to Lymphocyte separation under an optical microscope (100×oil immersion), then Heterophil to Lymphocyte ratio was calculated and recorded. To determine Albumin to Globulin ratio (A/G), blood sample (the same 3 birds per replicate) were collected at 42 d and after serum separation albumin and protein concentration was determined using a spectrophotometer and a kit package (Pars Azmoon Co;

Tehran, Iran). Globulin concentration in serum was computed by subtracting Albumin concentration from protein.

Statistical analysis

The data were subjected to analysis of variance procedures appropriate for a Completely Randomized Design (CRD) with four replicates, using the general linear model procedures of SAS, (2008). Means were compared using Tukey test, and statements of statistical significance are based on P<0.05.

RESULTS AND DISCUSSION

Data on chicks performance indices recorded for different periods have been summarized in Table 2. Meal feeding resulted in a significant reduction of feed intake during the restriction period (7-14 d) and consequently, a lower body weight was observed in MF chicks compared to *ad libitum* fed chicks (P<0.05). In the re-alimentation period, WF caused a marked increase of feed intake over 28-42 d period and the birds subjected to sequential feeding program exhibited the lowest feed consumption in different periods subsequent to meal feeding (P<0.05). Succeeding the restriction period, birds in meal fed by the control diet (MFC) and meal fed a wet diet (MFW) indicated a compensatory growth and, at 28 and, 42 d showed no statistical differences in terms of body weight compared to the control group, but sequentially fed birds gained significantly less weight (P<0.05). Feed conversion ratio (FCR) in the meal fed groups was depressed during restriction from 7 to 14 d (P<0.05) but improved marginally from 14-28 d. Over the other periods, birds in MFS exhibited the lowest FCR in comparison to the other treatments (P<0.05). In line with current results, Mohebodini *et al.* (2009) showed that meal feeding resulted in a statistical reduction of feed intake during the restriction period but during the grower period (22-42 days) no significant difference was observed between birds fed *ad libitum* and others subjected to feed restriction in terms of weight gain and feed conversion ratio. Similarly, Saffar and Khajali (2010) reported that body weight of birds subjected to meal feeding were significantly lower than full-fed controls, however, no significant differences were found among the treatments with respect to body weight on 42 and 49 d. The suppressed body weight affected by the meal feeding program was compensated by the end of experiment in MFC and MFW birds which indicate that a successful catch-up growth has occurred, and is consistent with previous reports (Susbilla *et al.* 2003). According to the study of Zubair and Leeson (1994), most weight loss during early feed restriction in birds can be normally compensated by 20 to 25 d of the re-feeding period.

Feeding a wet diet subsequent to meal feeding showed satisfactory results on performance parameters. Regarding the entire experimental period, MFW fed birds consumed more feed. This feed intake increase in the compensatory growth period (14-42 d) was not associated with a higher feed conversion ratio (FCR) compared to full fed groups as it resulted in a higher weight gain. Scott, (2002) also reported the same trend for wet fed broilers. He concluded a possible explanation for improvements in feed intake with wet feeding may relate to the faster rate that the diet would become soluble in the gut, thereby facilitating faster digestion, gut clearance and ultimately higher feed intake. The studies by Forbes and his co-workers (Yalda and Forbes, 1996; Yasar and Forbes, 1998; Yasar and Forbes, 2000), demonstrated consistent increases in feed intake and growth rate of broilers with wet feeding. However, chicks in MFS gained significantly less weight in different periods subsequent to meal feeding, when compared to the other treatments. The lower feed intake of chicks over the 14-42-d period in MFS may account for the body weight reduction observed following meal feeding and has been previously reported by other researchers (Bouvarel *et al.* 2004). Similar to our results, Bouvarel *et al.* (2004) reported that application of 24 h cycle sequential feeding regimens either reduced chickens' growth and feed efficiency compared to control complete diet or had no significant effect. Nevertheless, later Bouvarel *et al.* (2008) indicated that 48-h cycle sequential feeding varying in protein and energy contents, or both resulted in similar growth performance and carcass composition to the complete diet. The authors suggested that the lack of negative effects of sequential feeding on growth performances was related to sufficient feed intake (i.e., no differences in total feed intake between treatments). It emerges from these experiments that sufficient intake of distinct feeds is essential to reach an overall nutritional balance with sequential feeding. The re-alignment period is a crucial phase for restricted birds to compensate the retarded growth rate; subsequently birds offered sequential diets varying in energy and protein within this critical period are introduced to a more complicated situation and so are unable to show any signs of compensatory growth. Carcass yield of birds was not influenced by feeding regimes (Table 3).

However, birds in meal fed by the control diet (MFC) and meal fed a wet diet (MFW) had the highest (P<0.05) percentage of abdominal fat pad compared to the other treatments. The sequential feeding program subsequent to meal feeding resulted in a statistical increase of intestine relative weight (P<0.05). A higher abdominal fat percentage in meal fed by the control diet (MFC) and meal fed a wet diet (MFW) birds suggested that feed-restricted chickens tend to enhance fat deposits during the re-feeding period.

Table 1 Composition of the experimental diets

Ingredient (%)	Starter	Grower				
		C	E+	E−	P+	P−
Corn (8% CP)	54.98	54.49	50.38	58.21	39.95	69.03
Soybean meal (43% CP)	40.07	38.59	39.35	37.73	51.04	26.14
Soybean oil	0.68	3.3	6.68	0.1	5.51	1.1
Dicalcium phosphate	1.85	1.56	1.56	1.56	1.44	1.68
Calcium carbonate	1.10	0.99	0.98	1.32	0.97	1
DL-Methionine	0.29	0.22	0.22	0.21	0.29	0.14
L-lysine	0.18	0.05	0.03	0.07	0	0.11
Vitamin premix[a]	0.25	0.25	0.25	0.25	0.25	0.25
Mineral premix[b]	0.25	0.25	0.25	0.25	0.25	0.25
Salt	0.35	0.3	0.3	0.3	0.3	0.3
Calculated composition						
Metabolizable energy (kcal/kg)	2800	3000	3200	2800	3000	3000
Crude protein (%)	20.73	20.95	20.95	20.95	25.14	16.76
Lysine (%)	1.32	1.18	1.18	1.18	1.41	0.94
Met + Cys (%)	0.99	0.90	0.90	0.90	1.08	0.72
Calcium (%)	0.97	0.85	0.85	0.85	0.85	0.85
Available phosphorous (%)	0.48	0.42	0.42	0.42	0.42	0.42

C: control group; E+: high energy diet; E−: low energy diet; P+: high protein diet; P−: low protein diet.
[a] Vitamin premix provided per kg of diet: vitamin A: 2.7 mg; vitamin D_3: 0.05 mg; vitamin E: 18 mg; vitamin k_3: 2 mg; Thiamine: 1.8 mg; Riboflavin: 6.6 mg; Panthothenic acid: 10 mg; Pyridoxine: 3 mg; Cyanocobalamin: 0.015 mg; Niacin: 30 mg; Biotin: 0.1 mg; Folic acid: 1 mg; Choline chloride: 250 mg and Antioxidant: 100 mg.
[b] Mineral premix provided per kg of diet: Fe (FeSO4.7H2O, 20.09% Fe): 50 mg; Mn (MnSO4.H2O, 32.49% Mn): 100 mg; Zn (ZnO, 80.35% Zn): 100 mg; Cu (CuSO4.5H2O): 10 mg; I (KI, 58% I): 1 mg and Se (NaSeO3, 45.56% Se): 0.2 mg.

Table 2 Performance parameters of broiler chicks subjected to experimental feeding regimes at different periods

Performance indices	Dietary treatments[1]				
	Control	MFC	MFW	MFS	SEM
Body weight (g)					
14 d	220[a]	186[b]	180[b]	186[b]	2.22
28 d	797[a]	760[ab]	785[a]	694[b]	9.36
42 d	1909[a]	1871[a]	1928[a]	1605[b]	14.41
Body weight gain (g/d/bird)					
7-14 d	17.8[a]	12.9[b]	12.02[b]	12.8[b]	0.32
14-28 d	41.2[a]	41.03[ab]	43.23[a]	36.3[b]	0.57
28-42 d	79.4[a]	79.3[a]	81.6[a]	65.1[b]	0.61
7-42 d	51.8[a]	50.7[a]	52.3[a]	43.1[b]	0.41
14-42 d	60.3[a]	60.2[a]	62.4[a]	50.7[b]	0.47
Feed intake (g/d/bird)					
7-14 d	28.4[a]	23.4[b]	22.3[b]	23.6[b]	0.27
14-28 d	66.4[a]	62.4[ab]	64.1[ab]	58.7[b]	0.67
28-42 d	139[b]	136[b]	153[a]	93[c]	1.94
7-42 d	88[a]	89.4[a]	90.4[a]	65.4[b]	0.94
14-42 d	103[ab]	101[b]	107[a]	75[c]	1.04
Feed conversion ratio (g feed/g gain)					
7-14 d	1.6[a]	1.83[b]	1.86[b]	1.86[b]	0.028
14-28 d	1.62	1.52	1.47	1.62	0.021
28-42 d	1.76[a]	1.7[a]	1.88[a]	1.43[b]	0.027
7-42 d	1.7[ab]	1.78[a]	1.73[ab]	1.52[b]	0.026
14-42 d	1.71[a]	1.65[ab]	1.72[a]	1.49[b]	0.020

The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.
[1] Control group: *ad libitum* feeding; MFC: meal feeding with subsequent control diet feeding; MFW: meal feeding with subsequent wet diet feeding and MFS: meal feeding with subsequent sequential feeding.

Nielsen *et al.* (2003) reported early feed restriction led to a decreased fat deposition, whereas others researchers have reported opposite results (Zubair and Leeson, 1994; Lippens *et al.* 2000). The discrepancies might be due to the metabolic programming whereby early malnutrition leads to adult life obesity.

The higher weight of small intestine of birds from the MFS feeding regime, despite similar length (Table 3), implies a thicker intestinal lumen which may limit the absorption capacity and thus make birds unable to compensate for the alternation of high and low protein diets. The buffer storage of nutrients such as amino acids for over 24-h and

lower absorption rate may account for decreased feed intake which led to suppressed body weight gain relative to the other treatments. Nevertheless, according to Swennen *et al.* (2004) when comparing Isoenergetic diets varying in protein contents, the excessive energy relative to protein intake results in increased heat production and even energy retention as fat. In the present study and in Bouvarel *et al.* (2004), no changes in abdominal fat levels with sequential feeding were observed.

According to the data on Table 4, feeding regimes applied in the current study had no significant effects on birds' humoral immune responses. None of the parameters tested including antibody production against Newcastle, Influenza and sheep red blood cell, Heterophil to Lymphocyte and Albumin to Globulin ratios, bursa and spleen relative weight were statistically different among treatments (P>0.05). As generally known, one crucial factor contributing to immune function is nutritional status (Kidd, 2004).

Table 3 Carcass traits of broiler chicks subjected to experimental feeding regimes at 42 d old

Carcass components	Dietary treatments[1]				
	Control	MFC	MFW	MFS	SEM
Carcass yield (%)	70.94	70.65	71.50	71.51	0.359
Abdominal fat (%)	1.69^{b}	2.24^{a}	2.03^{ab}	1.66^{b}	0.069
Liver (%)	2.10	2.20	2.10	2.16	0.04
Gizzard (%)	1.88	1.9	1.82	2.04	0.051
Heart (%)	0.54	0.54	0.57	0.49	0.011
Pancreas (%)	0.24	0.24	0.23	0.19	0.006
Proventriculus (%)	0.358	0.268	0.356	0.394	0.009
Intestine (%)	3.55^{ab}	3.31^{b}	3.46^{ab}	4.15^{a}	0.093
Caecum (%)	0.42	0.605	0.407	0.58	0.019
Intestine (cm)	178	180	180	180	2.9
Caecum (cm)	39.7	39.1	37.8	40.6	0.496

The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.
[1] Control group: *ad libitum* feeding; MFC: meal feeding with subsequent control diet feeding; MFW: meal feeding with subsequent wet diet feeding and MFS: meal feeding with subsequent sequential feeding.

Table 4 Antibody titers against Newcastle and Influenza at 34 d and SRBC at 28 d, lymphoid organs weight, Heterophil to Lymphocyte and Albumin to Globulin ratios at 42 d

Immune parameters	Dietary treatments[1]				
	Control	MFC	MFW	MFS	SEM
Newcastle (log2)	3.88	3.75	3.63	3.63	0.139
Influenza (log2)	2.88	2.88	2.50	2.17	0.141
SRBC	7.00	7.13	6.67	7.38	0.218
H/L ratio	1.89	1.67	2.12	1.65	0.081
A/G ratio	0.15	0.14	0.18	0.17	0.044
Lymphoid organs weight (%)					
Bursa	0.11	0.058	0.061	0.090	0.006
Spleen	0.112	0.122	0.141	0.115	0.005

SEM: standard error of the means.
[1] Control group: *ad libitum* feeding; MFC: meal feeding with subsequent control diet feeding; MFW: meal feeding with subsequent wet diet feeding and MFS: meal feeding with subsequent sequential feeding.

Similar to the current findings Liew *et al.* (2003) also reported that antibody production and weights of immune organs did not differ between broiler chicks fed *ad libitum* and those fed 60% of *ad libitum*. It seems that, in general, the level of feed restriction is a determining factor to assess its effect on immunity in birds. Praharaj *et al.* (1999) indicated that broiler chicks fed moderately differing levels of energy (2800, 2650 and 2500 kcal/kg of ME) did not show any significant difference in antibody response and weights of immune organs that was due to dietary energy levels. However, there are studies reporting that severe or prolonged feed restriction in birds could impair systemic immune function and lower relative spleen and bursa weights (Payne *et al.* 1990; Cook, 1991; Hangalapura *et al.* 2005).

For instance, Mahmood *et al.* (2007) reported suppressed immune responses against Newcastle disease and Infectious bursal disease at 30 d in broiler chickens subjected to meal feeding from 8 to 28 d. These authors stated that as the duration of feed restriction increased, the immune response decreased.

The present findings are also in agreement with an earlier report indicating that processes leading to specific antibody secretion remain intact over a wide range of nutritional states (Moore *et al.* 2003).

However, our findings on anti-SRBC titers is in contrast with the results reported by Khajavi *et al.* (2003) who indicated an enhanced antibody responses to SRBC in feed-restricted broilers.

These apparent contradictions could be attributed to differences in animal models, severity of restriction, age of the experimental animals, and experimental design.

CONCLUSION

The results of the current study suggested that early age meal feeding procedure followed by wet feeding program could be used as a feeding regime in broiler chickens production, without any negative effects on carcass and immune responses. Furthermore, subjecting meal fed broiler chickens to sequential feeding programs may decrease productive traits of birds and results in suppressed body weight.

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