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Response of Broiler Chickens to Quantitative Feed Restriction with or without Ascorbic Acid Supplementation

Research Article

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ABSTRACT

Ascorbic acid (vitamin C) supplementation has been documented as a useful tool to improve the performance of laying birds reared under harsh environmental conditions. However, there is limited information on the use of ascorbic acid as a means of ameliorating the stressful conditions imposed by feed restriction. To this effect a 3 × 2 factorial experiment was carried out to evaluate the effect of ascorbic acid inclusion in the diet of broiler chickens under quantitative feed restriction. A total of 96 unsexed 22 days-old Marshall broiler chickens of a commercial strain were distributed to six treatments, birds on treatments 1 and 2 were fed *ad libitum*, and those in treatments 3 and 4 were placed on 85% *ad libitum* while those on treatments 5 and 6 were offered 70% *ad libitum*. Diets fed to birds on treatments 1, 3 and 5 were formulated without ascorbic acid while those on treatments 2, 4 and 6 had their diets supplemented with 200 $mgkg^{-1}$ ascorbic acid. Blood samples were collected from one bird per replicate for haematological and serum biochemistry on the 35th and 49th days of the study. Performance indices (feed intake, weight gain and intake:gain ratio) were significantly (P<0.05) influenced by quantitative feed restriction. Feed intake (3256.93, 2799.74 and 2360.05 g/bird) and weight gain (1134.70, 1023.88 and 968.86 g/bird) had highest values recorded for the birds fed *ad libitum* while those on 70% *ad libitum* feeding recorded the best intake: gain ratio (2.87, 2.74 and 2.44). Ascorbic acid supplementation significantly (P<0.05) affected the growth performance of broilers. Birds fed diets containing ascorbic acid had relatively high values in the growth performance indices considered. Quantitative feed restriction and ascorbic acid supplementation did not affect the haematological and serum biochemical parameters studied. It can be concluded that quantitative feed restriction and ascorbic acid supplementation influenced the growth performance of broilers.

KEY WORDS ascorbic acid, broiler, haematological, restriction, supplementation.

INTRODUCTION

The improvement in body weight of broiler chickens, as a result of improved growth rate, led to more frequent metabolic and skeletal disorders (Robinson *et al.* 1999), sudden death syndrome (Gonzales *et al.* 1998), increased fat deposition (Yu and Robinson, 1992) and ascites (Julian, 1997). Feed restriction has been used as a management tool to control these problems to some extent by tempering with growth rate of fast-growing birds. It has been used to modify the patterns of bird growth by decreasing their maintenance requirements, which is likely to improve feed efficiency. It has been reported that feed-restricted animals have lower carcass fat content at market age than those fed *ad libitum* (Njoku *et al.* 2012). Excess fat does not only reduce carcass yield but also causes rejection of the meat by consumers (Kessler *et al.* 2000) and this causes difficulties in carcass processing (McMurty *et al.* 1988). Thus, feed restriction is a vital management tool used in the manipulation of the growth curve of fast-growing birds. Quantitative feed restriction has led to a reduction in maintenance costs of birds through improved feed efficiency and minimization of feed wastage (Tumova *et al.* 2002; Tolkamp *et al.* 2005).

Despite the above listed merits of feed restriction, it has been reported that physiological indices of stress, such as heterophil / lymphocyte ratio, basophil and monocyte frequencies, and plasma corticosterone concentration are higher in feed-restricted birds than in *ad libitum* fed birds (Maxwell *et al.* 1990; Maxwell *et al.* 1992; Hocking *et al.* 1993). These physiological indices of stress have been found to be positively correlated with the level of feed restriction imposed on livestock (Hocking *et al.* 1996). According to Mench (2002), broiler breeders show evidence of physiological stress as well as an increased incidence of abnormal behaviours during feed restriction.

Ascorbic acid (vitamin C) leads to the production of anti-stress hormones which help to combat the effects of stress in domestic animals. It is known to combine with toxins, helping to eliminate the toxins from the body. Ascorbic acid is essential in collagen formation. Under stress conditions, ascorbic acid demands become greater than that provided by synthesis in tissues (McDonald *et al.* 1988). Njoku (1986) concluded that during periods of heat stress in the tropics, supplementation of broiler diets with 200 mg/kg of ascorbic acid was necessary and economically advantageous as body weight and feed gain responses were improved. Several reports have documented benefits of supplementing poultry diets with ascorbic acid on growth rate, egg production, egg shell strength and thickness, fertility and sperm production, on counteracting unfavourable climatic and housing conditions and in cases of intoxication or disease (Bashir *et al.* 1998). However, there is a dearth of information on the use of ascorbic acid supplementation as to alleviate the effect of stress posed by quantitative feed restriction. Therefore, this study was carried out to evaluate the response of broilers to quantitative feed restriction with or without ascorbic acid supplementation.

MATERIALS AND METHODS

Experimental site

The experiment was carried out at the poultry unit of the directorate of university farms (DUFARM) of federal university of agriculture, Abeokuta, Ogun State, Nigeria. The farm lies at latitude 7 ˚10´ N, longitude 3 ˚2´ E and altitude 76 mm. It is located in the derived savannah zone of south-western Nigeria. It has a humid climate with mean annual rainfall around 1037 mm and temperature around 34.7 ˚C. The relative humidity is 63-96% in the rainy season (late March to October) and 55-82% in the dry season (November to early March) with an annual average of 82%. rainfall is around 44.96 mm in the late dry season (January-March), 212.4 mm in the early wet season (April-June), 259.3 mm in the late wet season (July-September) and 48.1 mm in the early dry season (October-December) (Google Earth, 2013).

Experimental animals and their management

A total of 96 unsexed day-old Marshall broiler chicks of a commercial strain were purchased from a reliable hatchery in Abeokuta, Ogun State. On arrival, they were given water containing glucose as an anti-stress measure before feeding. The chicks were brooded for the first 21 days in a brooding house littered evenly with wood shavings. Feed and water were supplied *ad libitum* throughout the brooding period. Daily routine management was carried out appropriately. On the 22nd day of rearing, the birds were weighed and distributed based on weight equalization into six experimental treatments in wire batteries on a three-tier system. Each treatment was replicated four times with four chicks per replicate. Each replicate of birds was housed in a 100 × 40 × 43 metre cage. Birds on treatments 1 and 2 were fed *ad libitum* with and without ascorbic acid supplementation in their feed respectively. Treatments 3 and 4 consisted of birds whose daily rations were restricted at 85% *ad libitum* (calculated based on the breeder's recommendation) with and without ascorbic acid supplementation respectively. Birds on treatments 5 and 6 were fed 70% *ad libitum* feed (calculated based on the breeder's recommendation) with and without 200 mgkg^{-1} ascorbic acid supplementation respectively. This feeding was carried out at 22-42 days of age, after which all birds were fed *ad libitum* for fourteen days (43-56 days of age). All experimental birds were maintained under similar environmental conditions: lighting was continuous and water was provided *ad libitum* throughout the experiment. Chicks were fed with compounded broiler starter diet (1-28 days of age) with the following characteristics: crude protein 22.55%, crude fibre 3.19%, ether extract 4.47% and metabolizable energy 11.48 MJ/kg; and then broiler finisher diet (29-56 days of age) with the following description: crude protein 20.32%, crude fibre 3.12%, ether extract 4.22% and metabolizable energy 11.69 MJ/kg as shown in Table 1.

Data collection

Feed intake was determined daily by subtracting the feed left over from the feed supplied. Initial body weight of chicks was taken on the 22nd day of the study and documented while weekly records of change in body weight were taken. The feed conversion ratio was calculated as feed / gain. On the 35th day (during restriction) and the 49th day (after realimentation), a bird per replicate were randomly selected. Blood samples were collected for the measurement of haematological and serum parameters.

About 2.5 mL blood was collected into sample bottles containing the anticoagulant ethylene diamine tetra-acetic acid (EDTA). This blood sample was used to determine packed cell volume (PCV), haemoglobin concentration (Hb), heterophil and lymphocyte counts following standard procedures (AOAC, 2005).

Another 2.5 mL of blood was collected into sample bottles without anticoagulant for the measurement of serum protein serum albumin and serum globulin (Baker and Silverton, 1985).

Statistical analysis

Data were subjected to a two-way analysis of variance in 3x2 factorial layouts ware (SAS, 2000). Significantly different (P<0.05) means were separated using new duncan's multiple range test as contained in the SAS (2000) package.

RESULTS AND DISCUSSION

All growth performance parameters considered were significantly (P<0.05) influenced by levels of feed restriction (Table 2). The highest feed intake value of 3256.93 g was recorded for birds fed *ad libitum* compared with 2799.74 g and 2360.05 g observed for those on 85 and 70% *ad libitum* feed respectively. The average daily feed intake decreased (P<0.05) when feed was restricted. Feed restriction resulted in 14.04% and 27.54% reduction in average daily feed intake of broilers subjected to 85% and 70% *ad libitum* feeding respectively. Total feed intake during the restriction and realimentation periods were different (P<0.05).

Table 1 Percentage composition of experimental starter and finisher diets for broilers

Ingredients	Broiler starter composition (%)	Broiler finisher composition (%)
Maize	47.00	53.50
Wheat offal	10.00	10.80
Groundnut cake (GNC)	17.50	13.80
Soybean meal	18.50	16.50
Fish meal (72%)	2.00	0.40
Bone meal	3.00	3.00
Oyster shell	1.00	1.00
Vitamin-mineral premix*	0.25	0.25
Lysine	0.25	0.25
Methionine	0.25	0.25
Salt	0.25	0.25
Calculated analysis		
Crude protein (%)	22.55	20.32
Crude fibre (%)	3.19	3.12
Ether extract (%)	4.47	4.22
Ash (%)	3.23	2.73
Metabolisable energy (MJ/kg)	11.48	11.69

* Vitamin and mineral premix: vitamin A (retinol-acetate): 12000000 IU; vitamin D_3 (cholecalciferol): 2000000 IU; vitamin E: 30000 mg; vitamin K_3: 2000 mg; vitamin B_1 (thiamine nitrate): 2000 mg; vitamin B_2 (riboflavin): 5000 mg; vitamin B_6 (pyridoxine hydrochloride): 4000 mg; vitamin B_{12} (cyanocobalamin): 20 mg; Pantothenic acid: 10000 mg; Nicotinic acid: 40000 mg; Folic acid: 1000 mg; Biotin: 150 mg; Choline chloride: 150000 mg; Iron (Fe): 40000 mg; Manganese (Mn): 80000 mg; Copper (Cu): 10000 mg; Zinc (Zn): 60000 mg; Iodine (I): 1000 mg; Cobalt (Co): 200 mg and Selenium (Se): 150 mg.

Table 2 Effect of quantitative feed restriction and ascorbic acid supplementation on performance of broiler chicken

Parameters	Feed restriction levels			SEM	Ascorbic acid		SEM
	Ad libitum	85% *ad libitum*	70% *ad libitum*		+	-	
Total feed intake (g/bird)	3256.93[a]	2799.74[b]	2360.05[c]	29.83	2848.59[a]	2762.55[b]	24.36
Feed intake during restriction (g/bird)	1572.38[a]	1381.10[b]	1153.60[c]	18.21	1391.54[a]	1346.51[b]	14.87
Feed intake during re alimentation (g/bird)	1684.55[a]	1418.64[b]	1206.45[c]	18.16	1457.05[a]	1416.04[b]	14.83
Average daily feed intake (g/bird) entire period	93.06[a]	79.99[b]	67.43[c]	0.85	81.39[a]	78.93[b]	0.70
Total weight gain (g/bird)	1134.70[a]	1023.88[b]	968.86[c]	2.56	1088.61[a]	996.35[b]	2.09
Weight gain during restriction (g/bird)	546.12[a]	440.83[b]	392.45[c]	1.09	484.90[a]	434.70[b]	0.89
Weight gain during realimentation (g/bird)	588.58[a]	583.05[a]	576.41[b]	2.04	603.71[a]	561.65[b]	1.67
Average weight gain during restriction (g/bird)	26.01[a]	20.99[b]	18.69[c]	0.05	23.09[a]	20.70[b]	0.04
Average weight gain during realimentation (g/bird)	42.04[a]	41.65[a]	41.17[b]	0.15	43.12[a]	40.12[b]	0.12
Average weight gain during entire period (g/bird)	32.42[a]	29.25[b]	27.68[c]	0.07	31.10[a]	28.47[b]	0.06
Feed:gain (entire period)	2.87[a]	2.74[b]	2.44[c]	0.03	2.61[b]	2.76[a]	0.02
Feed:gain (restriction)	3.14[a]	2.88[b]	2.95[b]	0.04	2.87[b]	3.11[a]	0.03
Feed:gain (realimentation)	2.87[a]	2.44[b]	2.09[c]	0.03	2.41[b]	2.52[a]	0.03

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 mean.
+: with ascorbic acid supplementation.
-: without ascorbic acid supplementation.

The *ad libitum* fed birds had the highest feed intake throughout the entire period due to the fact that they had excess feed at all times. Some earlier reports have demonstrated a higher feed intake by control birds compared to restricted groups (Khetani *et al.* 2009; Benyi *et al.* 2010; Lanhui *et al.* 2011). Increase in the duration or severity of restriction tends to decrease feed intake (Plavnik and Hurwitz, 1988). From this study, restricted broilers consumed less feed (457.49 g and 896.88 g for broilers on 85% and 70% *ad libitum*, respectively) than their counterparts on unrestricted feeding. This is in line with the observation of Yu *et al.* (1990) who noted a difference of 0.8 kg/bird in consumption by birds fed *ad libitum* in comparison to those on restricted feeding. Mehmood *et al.* (2005) and Hassanien (2011) reported that birds subjected to longer periods and higher levels of feed restriction consumed relatively little feed. However this observation was contrary to the reports of Beer and Coon (2007) and Onbasilar *et al.* (2009) that restricted feeding had little effect on feed consumption.

Weight gain by broilers on different levels of feed restriction followed the same patterns as feed intake. Total weight gain (1134.70 g, 1023.88 g, 968.86 g) and average weight gain (32.42 g, 29.25 g, 27.68 g) during the entire period decreased with percentage increase in feed restriction (P<0.05), as did weight gain and average weight gain during the restriction period. The highest values for these parameters were documented for broilers on *ad libitum* feeding. Broilers fed full and 85% *ad libitum* had similar mean values (588.58 g and 583.05 g) for weight gain during the realimentation period, which differs (P<0.05) from 576.41g for those fed 70% *ad libitum*. Makinde (2012) reported that feed restriction negatively influenced the growth performance of broilers as the severity of restriction increased. This difference in weight gain could be attributed to the difference in feed intake between treatment groups. The birds on *ad libitum* feeding must have obtained sufficient nutrient for their tissue growth and development. It has been reported that feed-restricted birds gained less weight than fully-fed birds (Benyi *et al.* 2010; Benyi *et al.* 2011; Jalal and Zakaria, 2012; Njoku *et al.* 2012). The result of the present study contrasts with those of Fontana *et al.* (1992) and Zubair and Leeson (1996) who observed similar weight gain in feed-restricted and *ad libitum* fed birds. On the other hand, Lee and Leeson (2001) reported higher weight gain in birds subjected to feed restriction than in those fed *ad libitum* during the realimentation period. The contrasting results may be due to the intensity or level of feed restriction. Broilers on 70% *ad libitum* feeding recorded the best (P<0.05) feed to gain ratio of 2.44, compared to 2.74 and 2.87 obtained for those on 85% *ad libitum* and full feeding respectively during the entire experimental period. Feed to gain ratio during the realimentation period decreased (P<0.05) with increase in the feeding level. The poorest feed to gain ratio (2.87) was observed for broilers fed *ad libitum* while the best (2.09) was documented for those on 70% *ad libitum* feeding. This suggests that birds subjected to feed restriction utilized their feed more efficiently than those fed *ad libitum*. The improvement in feed efficiency of birds on restricted feeding was in agreement with the overwhelming majority of published data (Boostani *et al.* 2010; Njoku *et al.* 2012; Mehmood *et al.* 2012; Mehmood *et al.* 2007). They all explained that birds subjected to the stress of feed restriction generally have the potential to utilize their feed more efficiently than those on a full-feeding programme. However, Fanooci and Torki (2010), Saber *et al.* (2011) and Sahraei (2013) reported that feed efficiency was not affected in broilers maintained under restricted feeding compared to birds fed *ad libitum* at 42 days of age.

Ascorbic acid supplementation affected total feed intake, average daily intake, and intake during the restriction and realimentation periods (P<0.05). Birds fed ascorbic acid supplemented diets consumed more feed during the entire period: 2848.59 g/bird compared with 2762.55 g/bird by those without ascorbic acid supplementation. Average daily feed intake ranged from 78.93 g/bird (birds fed without ascorbic acid) to 81.39 g/bird (birds fed with ascorbic acid). The mean values obtained for feed intake during the estriction and realimentation periods followed the same trend. Birds whose diets were supplemented with ascorbic acid had higher mean values of 1391.54 g/bird and 1457.05 g/bird compared with 1346.51 g/bird and 1416.04 g/bird respectively in birds without ascorbic acid supplementation. The improvement in the feed intake of broilers fed diets containing ascorbic acid could be linked to its effect on the birds' basal metabolic rate. A similar observation was reported by Khan and Sardar (2005) who stated that ascorbic acid in feed improved the feed consumption of laying birds raised under heat stress-prone environment. These results contrasted with the observation of Tuleun and Njoku (2000), who reported decreased in feed consumption of birds fed diets supplemented with ascorbic acid. The differences between these results may be due to the differences in ascorbic acid inclusion level, environmental conditions and breeds of birds used.

Differences (P<0.05) were observed in weight gain during the entire experimental, realimentation and restriction periods. The supplemented group had a higher weight gain of 1088.61 g/bird for the entire period compared with 996.35 g/bird noted for those fed diets without ascorbic acid supplementation. These findings are in agreement with those reported by Pardue *et al.* (1985) and Edrise *et al.* (1986) who observed relatively high weight gain in birds fed ascorbic acid supplemented diets. Average weight gain per bird (31.10 g, 28.47 g) was higher in the ascorbic acid supplemented group during the entire experimental period. Also, weight gains during restriction (484.90 g, 434.70 g), realimentation (603.71 g, 561.65 g) and their averages (23.09 g, 20.70 g during restriction and 43.12 g, 40.12 g during realimentation) were relatively high in the ascorbic acid supplemented groups. These findings are in accordance with previous findings of Vathana *et al.* (2002) and Nagra *et al.* (2005).

A better feed:gain ratio was observed in the ascorbic acid supplemented group than in the non-supplemented group. The feed:gain ratios during restriction (2.87, 3.11), realimentation (2.41, 2.52) and the entire experimental period (2.61, 2.76) were better in the ascorbic acid supplemented group than in the non supplemented group. This result is in accordance with the findings of Nagra *et al.* (2005), Njoku and Nwazota (1989) and Tuleun and Njoku (2000) who reported an improved feed conversion ratio or feed:gain ratio with ascorbic acid supplementation.

Results suggested a positive interaction between quantitative feed restriction level and ascorbic acid supplementation on feed intake, weight gain and feed:gain ratio during the entire, restriction and realimentation periods (Table 3). Increasing the restriction level from 0% (*ad libitum*) to 30% (70% *ad libitum*) led to decreased feed intake and weight gain (P<0.05) at different periods of the experiment. However, the addition of ascorbic acid improved feed intake weight gain. The better feed intake and weight gain observed in the supplemented group could be attributed to the fact that ascorbic acid is necessary for the maintenance of normal collagen metabolism. Collagen formation is needed for normal bones and muscles formation (McDonald *et al.* 1992).

Considering the *ad libitum* fed birds, those with ascorbic acid in their diet had a lower mean feed:gain ratio (2.79) than those without ascorbic acid (2.96). Thus ascorbic acid supplementation made the bird's better feed converters. The same trend was observed in 15% feed restricted birds with lower and better feed conversion ratio (2.63) in birds fed with ascorbic acid, and higher values in birds without ascorbic acid (2.85). Their values were lower during the period of feed restriction (2.99) and realimentation (2.35) compared with those without ascorbic acid supplementation that had 3.29 and 2.52 recorded during restriction and realimentation periods. Similar results have been reported by Tuleun and Njoku (2000). Birds fed at 30% restriction also showed improved feed conversion ratio during the period of feed restriction. Ascorbic acid supplemented birds had a feed:gain ratio of 2.83 which is lower than that of the non-supplemented group (3.07). During realimentation, there was no significant difference in the values of feed conversion ratio of the ascorbic acid supplemented birds and the non-supplemented birds.

The results of haematological analysis and serum biochemistry are presented in Table 4. These parameters were not affected by the level of feed restriction and ascorbic acid supplementation. The packed cell volume (PCV) decreased with increases in feed restriction. The recorded values were 29.33 for the *ad libitum* fed birds, 28.00 for birds fed 85% *ad libitum* and 26.33% for those fed 70% *ad libitum*. Boostani *et al.* (2010) explained that broilers fed *ad libitum* had a higher hematocrit values due to their higher growth rate and oxygen requirement. Mean values of heterophil ranged from 24.67% (birds fed *ad libitum*) to 27.50% (birds fed 70% *ad libitum*).

Table 3 Interaction between levels of feed restriction and ascorbic acid supplementation on broiler performance

Feed restriction levels (%)	0		15		30		
Ascorbic acid supplementation	+	-	+	-	+	-	SEM
Parameters							
Total feed intake (g/bird)	3286.15^{a}	3227.70^{a}	2818.55^{b}	2780.93^{b}	2441.08^{c}	2279.03^{d}	42.19
Feed intake during restriction (g/bird)	1606.68^{a}	1538.08^{a}	1387.93^{b}	1374.28^{b}	1180.03^{c}	1127.18^{c}	25.75
Feed intake during realimentation (g/bird)	1679.48^{a}	1689.63^{a}	1430.63^{b}	1406.65^{b}	1261.05^{c}	1151.85^{d}	25.68
Average daily feed intake (g/bird)	93.89^{a}	92.22^{a}	80.53^{b}	79.46^{b}	69.75^{c}	65.12^{d}	1.21
Total weight gain (g/bird)	1177.17^{a}	1092.23^{b}	1072.24^{c}	975.52^{e}	1016.42^{d}	921.29^{f}	3.61
Weight gain during restriction (g/bird)	573.40^{a}	518.83^{b}	463.99^{c}	417.67^{d}	417.30^{d}	367.59^{e}	1.54
Weight gain during realimentation (g/bird)	603.77^{a}	573.40^{c}	608.25^{a}	557.85^{d}	599.12^{b}	553.70^{d}	2.89
Average weight gain during restriction (g/bird)	27.31^{a}	24.71^{b}	22.10^{c}	19.89^{d}	19.87^{d}	17.50^{e}	0.07
Average weight gain re alimentation (g/bird)	43.13^{a}	40.96^{c}	43.45^{a}	39.85^{d}	42.79^{b}	39.55^{d}	0.21
Average weight gain (g/bird)	33.63^{a}	31.21^{b}	30.64^{bc}	27.87^{e}	29.04^{d}	26.32^{f}	0.10
Feed:gain (entire experiment)	2.79^{b}	2.96^{a}	2.63^{c}	2.85^{a}	2.40^{d}	2.47^{d}	0.04
Feed:gain (restriction period)	2.80^{d}	2.96^{b}	2.99^{b}	3.29^{a}	2.83^{c}	3.07^{b}	0.06
Feed:gain (realimentation period)	2.78^{b}	2.95^{a}	2.35^{d}	2.52^{c}	2.11^{e}	2.08^{e}	0.05

The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of mean.
+: ascorbic acid supplemented diet.
-: non supplemented diet.

Table 4 Effects of quantitative feed restriction and ascorbic acid supplementation on blood haematology and serum biochemistry of broiler Chickens

Levels of feed restriction (%)	0	15	30	SEM	Ascorbic acid supplementation		SEM
Parameters					+	-	
Packed cell volume (%)	29.33	28.00	26.33	2.92	28.11	27.67	3.11
Haemoglobin (g/dL)	9.72	9.37	8.83	0.95	9.50	9.11	0.96
Heterophil (%)	24.67	27.33	27.50	4.59	26.33	26.67	4.63
Lymphocyte (%)	73.50	72.00	71.17	4.53	72.44	72.00	4.46
Heterophil / lymphocyte	0.35	0.38	0.40	0.11	0.37	0.39	0.11
Serum total protein (g/dL)	5.78	5.47	5.20	0.56	5.61	5.36	0.58
Serum albumin (g/dL	3.12	2.88	2.93	0.47	3.10	2.86	0.45
Serum globulin (g/dL)	2.67	2.58	2.27	0.34	2.51	2.50	0.39

The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error mean.
+: with ascorbic acid supplementation.
-: without ascorbic acid supplementation.

The same non-significant difference was observed in lymphocyte values obtained for birds at different restriction levels. These results are in concordance with Bratte (2011) who noted no difference in haematological parameters between full-fed and feed-restricted birds at 56 days of age. This implies that experimental chickens fed *ad libitum* and those subjected to quantitative feed restriction were in normal physiological and nutritional states.

Heterophil:lymphocyte ratio has been reported to be highly heritable (Al-Murrani *et al.* 1997) and a reliable index for assessing stress in poultry (Gross and Siegel, 1983). Psychological and physical stressors such as fasting, frustration, water restriction and high stocking density increase the heterophil:lymphocyte ratio in birds (Jones, 1989; Cravener *et al.* 1992; Hocking *et al.* 1993). In this study, there was no difference in that ratio of broilers on different feed-restriction levels. *Ad libitum* fed broilers had the least mean value of 0.35 while those on 70% *ad libitum* had the highest mean value of 0.40. The slight increase in heterophil:lymphocyte ratio with increasing intensity of feed restriction agreed with the observation of Zuldhof *et al.* (1995) who reported small increases in that ratio with increasing feed restriction. The non-significant effect observed in this parameter indicates that broilers are able to adapt to the stress of a short feed-restriction period.

In serum, total protein, albumin and globulin did not differ between the three levels of feed restriction. Values for these biochemical parameters fell within the normal range for broilers (Mitruka and Rawnsley, 1977). It has been reported that total protein and creatinine levels in the blood of birds depend on the quality and quantity of protein intake (Iyayi and Tewe, 1998). The total protein level in this study decreased numerically with increase in intensity of feed restriction, due to lower feed intake in the birds on limited feed. Blood parameters are good indicators of the physiological, pathological and nutritional status of livestock. Variation in haematological indices can be used to evaluate the effect of nutritional factors and feed additives. This study found no difference in the PCV and haemoglobin concentration (Hb) of broilers with or without ascorbic acid supplementation. This result is similar to the observation of Mehmet *et al.* (2004) that blood parameters in Japanese quails were not affected by ascorbic acid supplementation under hot environmental condition. The mean values of PCV ranged from 27.67% (without ascorbic acid) to 28.11% (with ascorbic acid). Hb followed the same trend. The slight increase in the PCV and Hb of the supplemented group could be attributed to the ability of ascorbic acid to protect erythrocyte membrane integrity as reported by Canadan and Gultekin (2002) and Adekola *et al.* (2010). The Hb and PCV values obtained in the present study were consistent with the reported range for broilers (Mitruka and Rawnsley, 1977). Iheukemene and Herbert (2003) reported values of 6.0-13.0% and 29.0-38.0% for Hb and PCV respectively. Islam *et al.* (2004) noted that commercial and local chickens reared in the Sylhet region of Bangladesh had Hb values of 7.06-9.37% and PCV values of 26.56-34.60%.

There was no difference in the values of heterophil, lymphocyte and heterophil:lymphocyte ratio of birds in this study. The heterophil values for supplemented and non-supplemented groups were 26.33 and 26.67% respectively. Lymphocyte values were 72.44% in birds whose diet was supplemented with ascorbic acid and 72.00% in birds without ascorbic acid. Heterophil:lymphocyte ratios ranged from 0.37 (birds whose diet was supplemented with ascorbic acid) to 0.39 (birds without ascorbic acid). The values obtained in this present study were within the recommended values for avian species as documented by Jain (1993).

No difference was observed in the values of the total protein, serum albumin and serum globulin. The obtained values were 5.61 and 5.36 g/dL for total protein, 3.10 and 2.86 g/dL for serum albumin, and 2.51 and 2.50 g/dL for serum globulin. The mean values of PCV, Hb, heterophil, lymphocyte and heterophil:lymphocyte ratios were not influenced by the interaction between feed restriction and ascorbic acid inclusion, as shown in Table 5.

Table 5 Interaction effect of quantitative feed restriction and ascorbic acid supplementation on blood haematology and serum biochemistry of broiler chickens

Levels of feed restriction (%)	0		15		30		SEM
Ascorbic acid supplementation	-	+	-	+	-	+	
Parameters							
Packed cell volume (%)	28.00	30.67	28.67	27.33	27.67	25.00	2.79
Haemoglobin (g/dL)	9.43	10.00	9.63	9.10	9.43	8.23	0.89
Heterophil (%)	22.67	26.67	29.00	25.67	27.33	27.67	4.53
Lymphocyte (%)	75.33	71.67	70.00	74.00	72.00	70.33	4.41
Heterophil / lymphocyte	0.30	0.40	0.43	0.33	0.37	0.43	0.10
Serum total protein (g/dL)	5.67	5.90	5.73	5.20	5.43	4.97	0.60
Serum albumin (g/dL)	3.20	3.03	3.07	2.70	3.03	2.83	0.46
Serum globulin (g/dL)	2.47^{ab}	2.87^{a}	2.67^{ab}	2.50^{ab}	2.40^{ab}	2.13^{b}	0.31

The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error mean.
+: with ascorbic acid supplementation.
-: without ascorbic acid supplementation.

That interaction also had no effect on serum total protein and serum albumin. However, serum globulin was influenced by an interactive effect of ascorbic acid supplementation and quantitative feed restriction. The optimum value was obtained for birds fed *ad libitum* with diet containing ascorbic acid.

CONCLUSION

In this study, broilers' performance indices were influenced by feed restriction. Ascorbic acid supplementation during feed restriction improved birds' feed intake as well as their overall weight gain. Quantitative feed restriction and ascorbic acid supplementation did not affect blood haematology and serum biochemistry, but the interaction between them affected birds' serum globulin. A higher inclusion level of ascorbic acid is recommended since no effect was observed in the blood differential count at the present inclusion level.

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پاسخ جوجه‌های گوشتی برای تغذیه کمی با / یا بدون محدودیت مکمل اسید اسکوربیک

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چکیده

مکمل‌سازی اسید اسکوربیک (ویتامین C) به عنوان یک ابزار مفید برای بهبود عملکرد پرندگان تخمگذار پرورش یافته در شرایط سخت زیست محیطی اثبات شده است. با این حال، در استفاده از اسید اسکوربیک به عنوان وسیله‌ای برای اصلاح و رفع شرایط تنش‌زای اعمال شده در شرایط محدودیت غذایی، اطلاعات کمی وجود دارد. به این منظور یک آزمایش فاکتوریل ۳ × ۲ به برای بررسی اثر اسید اسکوربیک گنجاندن در جیره غذایی جوجه‌های گوشتی تحت محدودیت کمی غذایی انجام شد. در مجموع ۹۶ جوجه گوشتی ۲۲ روزه سویه تجاری مارشال مخلوط دو جنس در شش تیمار توزیع شد، پرندگان در تیمار ۱ و ۲ تا حد اشتها تغذیه شدند، و تیمار ۳ و ۴ در ۸۵ درصد اشتها تغذیه شدند، در حالیکه تیمارهای ۵ و ۶ در ۷۰ درصد اشتها تغذیه شدند. جیره‌های تغذیه شده به پرندگان در تیمارهای ۱، ۳ و ۵ بدون اسید اسکوربیک فرموله شدند در حالیکه آنهایی که در تیمارهای ۲، ۴ و ۶ بودند جیره غذایی با ۲۰۰ میلی‌گرم در کیلوگرم اسید اسکوربیک دریافت نمودند. در روز ۳۵ و ۴۹ نمونه خون از یک پرنده در هر تکرار برای مطالعه خون شناسی و بیوشیمی سرم جمع‌آوری شد. شاخص‌های عملکرد (مصرف خوراک، افزایش وزن و ضریب تبدیل) به طور معنی داری (P<۰/۰۵) تحت تأثیر محدودیت غذایی کمی بود. مصرف خوراک (۳۲۵۶/۹۳، ۲۷۹۹/۷۴ و ۲۳۶۰/۰۵ گرم/پرنده) و افزایش وزن (۱۱۳۴/۷۰، ۱۰۲۳/۸۸ و ۹۶۸/۸۶ گرم/پرنده) بالاترین عدد را در پرندگان تغذیه شده در حد اشتها داشت در حالیکه آنهایی که در ۷۰ درصد حد اشتها تغذیه شدند بهترین ضریب تبدیل (۲/۸۷، ۲/۷۴ و ۲/۴۴) را داشتند. مکمل اسید اسکوربیک به طور معنی داری (P<۰/۰۵) عملکرد رشد جوجه‌های گوشتی را تحت تأثیر قرار داد. پرندگان تغذیه شده با جیره‌های حاوی اسید اسکوربیک مقادیر نسبتا بالایی در شاخص‌های عملکرد رشد داشتند. محدودیت کمی غذایی و مکمل اسید اسکوربیک بر پارامترهای خونی و بیوشیمیایی سرم مورد مطالعه تأثیر نداشت. می‌توان نتیجه گرفت که محدودیت کمی غذایی و مکمل اسید اسکوربیک عملکرد رشد جوجه‌های گوشتی را تحت تأثیر قرار می‌دهد.

کلمات کلیدی اسید اسکوربیک، جوجه گوشتی، پارامترهای خونی، محدودیت، مکمل سازی.