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Effects of feed restriction and ascorbic acid supplementation on serum biochemical composition of Marshall broiler chickens

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Abstract

252 two-week old *Marshall* broiler chickens were used in a 4 x 3 factorial experiment to determine the effects of feed restriction and ascorbic acid supplementation on serum biochemical composition. The birds were distributed after balancing for live weights into 12 treatments with 3 replicates of 7 birds each, subjected to four feed restriction levels: full feeding (AD), skip a day feeding (S1D), skip two days feeding (S2D) and skip three days feeding every week (S3D) for 24 hours from 15th to 35th day of age and three levels of ascorbic acid supplementation (0, 150, 300 mg/kg feed). Feed was provided *ad libitum* to all the birds from 36 to 56 days of age. Birds on S2D fed diets containing 300 mg/kg ascorbic acid had the highest ($p<0.05$) serum glucose, total protein and albumin during feed restriction. Serum glucose and cholesterol was lowest ($p<0.05$) in birds on S2D fed diets containing 300 mg/kg ascorbic acid during realimentation. Dietary ascorbic acid supplementation at 300 mg/kg elicited positive effects on the serum biochemical composition of Marshall broiler chickens on skip two days feeding every week.

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Introduction

The early growth rate of broiler chickens can cause stress, increased metabolic diseases and high skeletal disorders which can result into economical losses due to reduced performance (Cuddington, 2004). Ascorbic acid plays a major role in gluconeogenesis to enhance energy supply during environmental, pathological and nutritional stress (Bains, 1996) such as feed restriction. Analysis of serum biochemical parameters of broiler chickens is very much essential in diagnosing the various pathological and metabolic disorders and can be used as a tool to assess the health status of an individual bird or a flock. Changes in serum biochemical parameters are often used to determine various status of the body and stresses due to environmental, nutritional and/or pathological factors. Ascorbic acid supplementation enhanced productivity, immune responses and survivability under nutritional stress (Zulkifli *et al*., 1996). However, little information is available on the combined effects of feed restriction and ascorbic acid supplementation on serum biochemical composition of broiler chickens. Therefore, this study was carried out to determine the effects of feed restriction and ascorbic acid supplementation on the serum biochemical composition of Marshall broiler chickens.

Materials and methods

Experimental animals and design

The experiment was carried out at the Poultry Unit of the Directorate of University Farms (DUFARMS), Federal University of Agriculture, Abeokuta, Nigeria. The area lies on latitude 7° 13' 49.46" N, longitude 3° 26' 11.98" E and altitude 76 metres above sea level (Google Earth, 2006). The climate is humid and located in the rainforest vegetation zone of western Nigeria. A total of 252 unsexed day-old Marshall broiler chickens were sourced and raised in deep litter pens of an open-sided poultry house. The birds were fed with a conventional corn soybean meal diet (2942.46 kcal ME/kg, 20.75% CP). Normal prophylactic medication and vaccination were administered as at when due. After two weeks, the chickens were randomly divided into 12 treatments with 3 replicates of 7 birds each. The dietary experiment was laid out in a 4 x 3 factorial arrangement consisting of 4 feed restriction levels: full feeding (AD), skip a day feeding (S1D), skip two days (S2D) feeding and skip three days (S3D) feeding every week and 3 ascorbic acid supplementation levels (0, 150, 300 mg/kg feed). The experiment was carried out in two phases namely restriction phase (days15-35) and realimentation phase (days 36-56) during which feed was provided *ad libitum* to all the birds. The experiment lasted for 56 days.

Blood collection and analysis

At the end of 35th and 56th days, blood samples were collected aseptically with sterile syringes and needles from the wing (brachial vein) of two birds per replicate into bottles without anticoagulant. Blood samples collected were subjected to serum biochemical analysis of serum total protein, albumin, globulin and glucose using bromocresol purple method of Varley *et al.* (1980). Serum uric acid (SUA) was determined according to the method of Wootton (1964). Serum cholesterol was estimated using the commercial diagnostic kits (Qualigens India. Pvt. Ltd., Catalogue number 72201-04). The blood samples were centrifuged at 4000rpm (revolutions per minute) for 3 minutes in a micro-centrifuge to obtain serum that is free from cell debris for the biochemical analysis using a spectrophotometer (Available Commercial Kits produced by Sentinel, Italy) at a wavelength of 500nm.

Statistical analysis

Data (values) obtained were subjected to Analysis of Variance (ANOVA) in a completely randomized design using SAS (2002) Software Package. Differences among treatments were separated using Duncan's Multiple Range Tests.

Results

The effects of interaction of feed restriction and ascorbic acid supplementation on serum biochemical composition of broiler chickens during feed restriction (days 15-35) are shown on Table 1. Birds on AD fed diets containing 150 mg/kg ascorbic acid had the lowest ($p<0.05$) glucose while the highest ($p<0.05$) was observed in birds on S2D fed diets containing 300 mg/kg ascorbic acid. Total serum protein and albumin were highest ($p<0.05$) in birds on S2D fed diets containing 300 mg/kg ascorbic acid. Serum cholesterol was highest ($p<0.05$) in birds on S2D fed diets containing 150 mg/kg ascorbic acid. Table 2 shows the effects of interaction of feed restriction and ascorbic acid supplementation on serum biochemical composition of broiler chickens during realimentation (days 36-56) Birds on S2D fed diets containing 300 mg/kg ascorbic acid had the lowest ($p<0.05$) glucose. Birds on S2D and S3D fed diets containing 0 mg/kg ascorbic acid had the lowest ($p<0.05$) total protein. Birds on AD fed diets containing 300 mg/kg ascorbic acid had the lowest ($p<0.05$) albumin. Globulin was lowest ($p<0.05$) in birds on S2D fed diets containing 0 mg/kg ascorbic acid. Birds on AD and S2D fed diets containing 0 mg/kg ascorbic acid had the highest ($p<0.05$) serum uric acid. Birds on S1D fed diets containing 300 mg/kg ascorbic acid and S2D fed diets containing 300 mg/kg ascorbic acid had the lowest ($p<0.05$) serum cholesterol.

Table 1. Effects of interaction of feed restriction and ascorbic acid supplementation on serum biochemical composition of Marshall broiler chickens during feed restriction (days 15-35)

Restriction levels	AD			S1D			S2D			S3D			SEM
Ascorbic acid levels / Parameters	0 mg/kg	150 mg/kg	300 mg/kg	0 mg/kg	150 mg/kg	300 mg/kg	0 mg/kg	150 mg/kg	300 mg/kg	0 mg/kg	150 mg/kg	300 mg/kg	
Glucose (mg/dl)	111.73^{ab}	106.70^{b}	111.70^{ab}	114.83^{ab}	120.57^{ab}	123.93^{ab}	118.43^{ab}	114.57^{ab}	127.33^{a}	121.27^{ab}	117.10^{ab}	114.50^{ab}	1.57
Total Protein (g/l)	39.47^{b}	40.53^{b}	42.27^{ab}	41.63^{ab}	42.43^{ab}	40.33^{b}	42.43^{ab}	42.97^{ab}	45.20^{a}	41.00^{b}	41.17^{b}	40.73^{b}	0.37
Albumin (g/l)	25.17^{b}	24.93^{b}	24.63^{b}	26.73^{ab}	24.90^{b}	25.27^{ab}	26.07^{ab}	26.53^{ab}	28.10^{a}	25.07^{b}	24.60^{b}	26.03^{ab}	0.27
Globulin (g/l)	14.30	15.60	17.63	14.90	17.53	15.07	16.37	16.43	17.43	15.93	16.57	14.70	0.32
Uric acid (mg/dl)	5.33^{ab}	5.00^{ab}	5.30^{ab}	6.03^{a}	4.40^{b}	4.60^{ab}	5.23^{ab}	5.57^{ab}	5.23^{ab}	5.17^{ab}	5.30^{ab}	5.47^{ab}	0.13
Cholesterol (mg/dl)	145.40^{b}	158.83^{ab}	144.43^{b}	164.07^{ab}	158.40^{ab}	144.10^{b}	158.30^{ab}	182.33^{a}	167.43^{ab}	149.90^{b}	145.77^{b}	163.97^{ab}	2.65

$^{a-e}$Means in the same row having different superscripts are significantly different ($P<0.05$)
SEM: Standard error of mean
AD- Full feeding S1D- Skip a day feeding S2D- Skip 2 days feeding S3D- Skip 3 days feeding

Table 2. Effects of interaction of feed restriction and ascorbic acid supplementation on serum biochemical composition of broiler chickens during realimentation (days 36-56)

Restriction levels	AD			S1D			S2D			S3D			SEM
Ascorbic acid levels / Parameters	0 mg/kg	150 mg/kg	300 mg/kg	0 mg/kg	150 mg/kg	300 mg/kg	0 mg/kg	150 mg/kg	300 mg/kg	0 mg/kg	150 mg/kg	300 mg/kg	
Glucose (mg/dl)	165.40^{a}	158.80^{a}	121.00^{b}	160.10^{a}	143.90^{a}	119.87^{b}	144.70^{a}	158.80^{a}	75.67^{c}	163.63^{a}	156.70^{a}	161.10^{a}	4.64
Total Protein (g/l)	61.30^{a}	60.17^{a}	51.90^{bcd}	62.50^{a}	57.37^{abc}	56.67^{abc}	46.00^{d}	49.90^{cd}	58.87^{ab}	48.47^{d}	51.97^{bcd}	56.67^{abc}	1.04
Albumin (g/l)	33.67^{ab}	32.90^{abc}	27.57^{e}	34.40^{a}	32.47^{abcd}	29.67^{abcde}	28.10^{de}	30.50^{abcde}	33.20^{ab}	28.23^{cde}	29.27^{bcde}	30.20^{abcde}	0.52
Globulin (g/l)	27.67^{a}	27.27^{a}	24.37^{ab}	28.10^{a}	24.90^{ab}	27.00^{a}	17.90^{d}	19.43^{cd}	25.67^{ab}	20.37^{cd}	22.70^{bc}	26.47^{ab}	0.63
Uric acid (mg/dl)	7.77^{a}	3.97^{c}	7.10^{ab}	6.50^{ab}	5.50^{bc}	5.57^{bc}	8.37^{a}	6.70^{ab}	6.97^{ab}	7.20^{ab}	7.00^{ab}	5.17^{bc}	0.25
Cholesterol (mg/dl)	180.40^{ab}	186.90^{a}	174.47^{bc}	185.37^{a}	169.47^{c}	159.87^{d}	170.00^{c}	173.90^{bc}	154.40^{d}	179.43^{ab}	168.50^{c}	179.37^{ab}	1.71

$^{a-e}$Means in the same row having different superscripts are significantly different ($P<0.05$)
SEM: Standard error of mean
AD- Full feeding S1D- Skip a day feeding S2D- Skip 2 days feeding S3D- Skip 3 days feeding

Discussion

Serum glucose (75.67-165.40 mg/dl) obtained was lower than the reference range of 197 to 299 mg/dl indicated by Clinical Diagnostic Division (1990). The observed difference may be due to breed and age difference. Birds on skip two days fed diets containing 300 mg/kg ascorbic acid had the highest serum glucose, total protein and serum albumin during feed restriction. However, recorded the least glucose level during realimentation. Borges *et al.* (2007) reported that an increase in glucose concentration was one of the direct response of birds to greater adrenaline, noradrenalin and glucocorticoid secretion in stressful conditions which was needed to prepare birds for a fight and flight response. Lower glucose observed in birds during realimentation may be attributed to improved carbohydrate metabolism. This concept is supported by the fact that there is a need for glucose to provide energy for different tissues in the body. This is because glucose is the easiest substrate for cells to obtain energy, since its level in blood is usually decreased (Rajgude *et al.*, 2005). Total serum protein values (39.47-62.50 g/l) are comparable with the reference values of 40 to 60 g/l (Simaraks *et al.*, 2004). The high total protein could be attributed to good protein reserve reflecting the ability of the chickens to store protein for tissue development. Sahin *et al.* (2003) reported that dietary ascorbic acid supplementation increased serum protein in broilers. In contrast to the present finding, Jang *et al.* (2009) found low total protein concentrations in broilers subjected to feed restrictions. Serum albumin (24.60-34.40 g/l) is in harmony with the values of 20.5 to 47.9 g/l (Gumaa, 2014). The increased serum albumin concentrations could be attributed to reduction in synthesis and secretion of corticoids in birds that received ascorbic acid. Serum globulin (14.30-28.10 g/l) is comparable with the values of 19.2 to 30.7 g/l (Gumaa, 2014). Globulin was lowest in birds on skip two days feeding fed diets containing 0 mg/kg ascorbic acid during realimentation. This may suggest poor immune response and insufficient antibody production as a result of being fed unsupplemented ascorbic acid diet. Serum cholesterol (144.10-186.90 mg/dl) obtained fell within the reference values of 129 to 297 mg/dl for adult chickens (Clinical Diagnostic Division, 1990). Serum cholesterol was highest in birds on skip two days feeding fed diets containing 150 mg/kg ascorbic acid during feed restriction. This could probably be due to increased lipid concentration in the birds. This result agrees with the findings of Mohebodini *et al.* (2009) who reported higher serum cholesterol for chickens that received restricted feeding compared with chickens on full feeding. McKee *et al.* 1997 reported that dietary ascorbic acid supplementation did not affect blood cholesterol in broilers. The fact that birds on skip a day feeding and skip two days feeding fed diets containing 300 mg/kg ascorbic acid had the lowest serum cholesterol during realimentation could be attributed to improved lipid mobilization in the birds. Reduction in serum cholesterol concentration by feeding ascorbic acid has been demonstrated in broilers (Gursu *et al.*, 2004). Serum uric acid values (3.97-8.37 mg/dl) fell within the reference values of 1.9 to 12.5 mg/dl for adult chickens (Clinical Diagnostic Division, 1990). The uric acid being highest in birds on full feeding and birds on skip 2 days feeding fed diets containing 0 mg/kg ascorbic acid during realimentation could be attributed to the quality of protein fed and high level of nutrient utilization.

Conclusion

Dietary ascorbic acid supplementation at 300 mg/kg elicited positive effects on the serum biochemical composition of Marshall broiler chickens on skip two days feeding every week.

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