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EFFECTS OF ASCORBIC ACID SUPPLEMENTATION IN THE DIET OF HEAT STRESSED LAYING HENS

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ABSTRACT

An experiment was conducted to determine the effects of synthetic ascorbic acid (AA) in laying hens during hot dry season of the year. One hundred and eighty ShiksBrown commercial layers (age 30 weeks) obtained from National Animal Production Research Institute (NAPRI) were used in this study. The birds were divided into 4 groups with each having 45birds. The groups were allotted to four dietary treatment groups in completely randomized design replicated three times with each replicate consisting 15 birds. The dietary groups consisted of various levels of Ascorbic Acid as follows: 0, 100, 200 and 300mg designated as the treatment groups 1, 2, 3 and 4 respectively. The study lasted for 12 weeks duration. Increase in final weight, body weight change, percent Hen-day-egg production, feed intake and lower feed cost per dozen eggs were noticed as dietary level of AA increased from 100 to 300 mg per Kg diet. The 200 mg and 300 mg AA 1/kg diet levels resulted in better shell weight, haugh unit, packed cell volume, haemoglobin content and total blood protein. The same levels also resulted in reduction of rectal temperature. It was concluded that supplemental feeding of AA at the rate of 200 mg/kg diet was adequate for the performance, egg quality characteristics and hematological status of laying hens during hot-dry season.

Key words: Rectal temperature, Heat stress, laying hens, ascorbic acid, ShiksBrown, egg quality

INTRODUCTION

Inclusion of feed additive has been reported by many researchers (Kolb, 1985 and Abdulrashid, *et al.,* 2010, Adeosun *et al.,* 2013 and Adeosun *et al.,* 2015). Various studies have shown that supplemental ascorbic acid (AA) in the diets of poultry has beneficial effects on growth, production and defensive performance of immune system. Alisheikov (1980) reported that inclusion of 75 AA/kg diet of broilers had beneficial effect on growth and it reduced death losses. Shulga (1980) stated that 50mgAA/kg diet improved the development of immunity after vaccination. It has been reported that Ascorbic acid has anti-stress effects and its production is reduced during stress, it is important that the diets of poultry be supplemented with it (Syke, 1978, Kassim nad Norziha 1995). A positive response to supplemental ascorbic acid in growth rate and feed intake of heat stress broiler chicken was observed at 200 mg/kg diet (Njoku, 1990; Balogun *et al.,* 1996). Similarly, Kutlu and Forbes (1993) stated that broiler chicks reared under heat stress at 36^{o}C experienced reduced growth rate, feed intake and feed efficiency but ascorbic acid supplementation at 250 mg/kg diet improved these parameters. Ladmakhi *et al.*

(1997) indicated that addition of vitamin C at 500 mg/Kg diet reduced the incidence of pulmonary hypertension syndrome in commercial broilers reared under high ambient temperature. Most of the reported studies on supplemental feeding of ascorbic acids had focused on traits of commercial importance. However, there is little information on actual effects of ascorbic acid on the serum biochemistry and/or physiological status of the birds. Therefore this study was carried out to determine the effects of ascorbic acid on the performance, egg quality, haematological status and rectal temperature of laying hens.

MATERIALS AND METHODS

The experiment was carried out during the hot-dry season of the year. One hundred and eighty, 30 weeks old ShikaBrown commercial layers obtained from National Animal Production Research Institute, Shika Zaria were used for the study. The birds were divided into four treatment groups with each group further divided into three replicates that contain 15 birds per replicate. The groups were randomly allocated to four dietary treatments consisting of difference levels of ascorbic acid inclusion in the diets as follows; 0, 100, 200, 300 mgAA/kg diet (Table 1).

Table 1: Composition of experimental layer diets with ascorbic acid supplementations during hot-dry season study

Ingredients (%)	**Ascorbic Acid Level (mg/kg diet)**			
	Control (0)	100	200	300
Maize	56.21	56.20	56.19	56.18
Groundnut Cake	18.09	18.09	18.09	18.09
Soybean Cake	5.00	5.00	5.00	5.00
Wheat Offal	10.00	10.00	10.00	10.00
Limestone	6.83	6.83	6.83	6.83
Bone Meal	3.00	3.00	3.00	3.00
Salt	0.35	0.35	0.35	0.35
Vit. Trace Mineral Premix	0.25	0.25	0.25	0.25
Methionine	0.15	0.15	0.15	0.15
Lysine	0.12	0.12	0.12	0.12
Ascorbic Acid	-	0.01	0.02	0.03
Total	**100.00**	**100.00**	**100.00**	**100.00**
Calculated Analysis:				
Metabolizable energy (Kcal/kg)	2632.00	2632.00	2632.00	2632.00
Crude protein (%)	17.00	17.00	17.00	17.00
Ether extract (%)	4.59	4.59	4.59	4.59
Crude fibre (%)	3.05	3.05	3.05	3.05
Calcium (%)	3.75	3.75	3.75	3.75
Available phosphorus (%)	0.61	0.61	0.61	0.61
Lysine (%)	0.79	0.79	0.79	0.79
Methionine (%)	0.74	0.74	0.74	0.74

Biomix layers premix supplied the following per kg diet Vit. A, 8500I.U, Vit. D_3, 1500 I.U; Vit. E, 10mg; Vit. K, 1mg; Thiamine, 1.5mg; Riboflavin, 4.5mg; Pyridoxine, 3mg; Niacin, 15mg; Vit B_{12}, 0.015;Pantothenic acid, 4.5mg; Folic Acid, 0.6mg; Biotin, 0.5mg; Choline Chlorides 175mg; Antioxidant, 1.25mg;Manganese, 40mg; Zinc, 30mg; Iron, 20mg; Copper, 3mg; iodine; 1mg; Selenium, 0.2mg; cobalt 0.2mg.

Complete randomized design was the layout of the experiment which lasted for 12 weeks duration. Feed and water were supplied to the birds without restriction. The parameters measured included initial weight, final weight, body weight change, Feed intake, Hen-Day-egg production, feed cost/kg, kgfeed/dozen eggs, income above feed expense (IAFE), Feed cost/dozen eggs and mortality. Eggs were collected 3-days to the end of every 28 days and these were used to estimate egg weight. Five eggs from each replicate were broken out and used to study egg quality such as shell weight, shell thickness yoke index and Haugh unit value.

Six (6) weeks after commencement of the experiment, three birds from each replicate were selected and blood samples taken which was used for the haematological studies in which packed cell volume (PCV), haemoglobin content (Hb) and total protein (TP) were determined using Cheesbrough 2006 procedure. Rectal temperatures were recorded from 3 birds per replicate three times a day (every other day) at the hours of 09:00, 12:00 and 15:00. The clinical digital thermometer was inserted into the claoca about 2-3 cm into the rectum. The readings were taken as soon as the value of the thermometer remained constant. All data collected were summarized and subjected to the analysis of variance using general linear model SAS (2007). And treatment means were separated using Duncan's Multiple Range Test (Steel and Torrie 1980)

RESULTS

The effect of dietary levels of ascorbic acid supplementation on the performance, egg quality characteristics, rectal temperatures and blood chemistry of laying hens during the hot – dry season were as shown in Tables 2, 3, 4 and 5.

Dietary ascorbic acid had significant ($P<0.05$) effect on final weight body weight change, feed intake % Hen-day-egg production, kg feed/dozen egg, feed cost/dozen eggs (₦), and percent mortality. The three levels of ascorbic acid (AA) have similar performance and are better than the control in their final body weight and low percent mortality (Table 2). Birds on 200 and 300 mg/AA/kg diet had similar performance with respect to body weight change, feed intake, Hen-day-egg production, kg feed/dozen egg, income above feed expensive and feed cost per dozen eggs. Their performances were better than control and 100 mg AA. As the level of AA increases from 0 to 300 mg/kg in the feed of the laying birds corresponding increase in final weight, body weight change, feed intake, Hen-day-egg production, and better kgfeed/dozen eggs were noticed. The diet containing 300 mg/AA/kg showed the best income above the feed expenses and feed cost per dozen eggs. There was no mortality recorded for all treatments with ascorbic acid supplementation except for the control diets.

Table 2: Effects of dietary levels of ascorbic acid on the performance of laying hens during hot-dry season

Parameters	Ascorbic Acid level (mg/kg)				SEM
	0	100	200	300	
Initial Weight (g/bird)	1676.67	1676.33	1676.33	1676.33	0.41
Final weight (g/bird)	1840.00^{b}	1869.67^{a}	1900.00^{a}	1922.67^{a}	51.2
Body weight change (g/bird)	163.67^{b}	193.33^{b}	223.67^{a}	246.69^{a}	30.87
Feed intake (g/bird/day)	117.93^{b}	118.84^{b}	120.28^{ab}	121.74^{a}	0.81
Hen-Day-Egg Production (%)	61.84^{c}	67.09^{b}	74.58^{a}	75.69^{a}	0.93
Feed cost/kg (₦)	41.80	43.30	44.80	46.30	2.11
Kg Feed/dozen egg	2.22^{b}	2.12^{b}	1.94^{a}	1.91^{a}	0.04
IAFE (₦)	87.20^{b}	88.20^{b}	93.09^{a}	93.09^{a}	1.51
Feed cost/dozen eggs (₦)	92.80^{b}	91.80^{b}	86.91^{a}	86.19^{a}	1.8
Mortality	4.45^{a}	0.00^{b}	0.00^{b}	0.00^{b}	1.11

^{a,b,c}Means with different superscripts in the same row are significantly (P<0.05) different.
SEM: Standard Error of Means. IAFE: Income above feed expenses at ₦15 per egg

Similarly, dietary level of ascorbic acid had significant (P<0.05) effect on egg weight, shell weight, and Haugh unit but had no significant (P>0.05) effect on shell thickness and yolk index. For egg weight, it was noticed that 200 mg and 300 mg/AA/kg diets gave the highest and similar values (Table 3). Birds on 200 and 300 mg/AA/kg diet had higher egg weight than birds on 0 and 100 mg/AA/kg diet. All the ascorbic acid level performed better than the control in their shell weight and Haugh unit. There was no significance difference in the shell thickness and yoke index among treatment means.

Table 3: Effects of dietary levels of ascorbic acid on the egg quality characteristics of laying hens during hot-dry season

Parameters	Ascorbic Acid level (mg/kg)				SEM
	0	100	200	300	
Egg weight (g)	53.42^{b}	56.27^{b}	59.65^{a}	59.76^{a}	0.95
Shell weight (g)	7.93^{c}	8.20^{b}	8.33^{b}	8.65^{a}	0.15
Shell thickness (mm)	0.47	0.47	0.48	0.48	0.01
Yolk index	0.47	0.47	0.47	0.47	0.01
Haugh Unit Value	80.13^{b}	81.64^{a}	81.61^{a}	82.27^{a}	0.68

^{abc}Means with different superscripts in the same row are significantly (P<0.05) different
SEM: Standard Error of Means

Effects of ascorbic acid supplementation in the diet of heat stressed laying hens

Table 4 shows the effect of ascorbic acid on the haematological status of laying hens during the hot dry season. Significant (P<0.05) effect of ascorbic acid supplementation were observed on packed cell volume (PCV), hemoglobin content (Hb) and on total protein (TP). The highest percent pack cell volume was attained at 300 mgAA/kg diet. The control, 100 mg AA/kg and 200 mg AA/kg dietary treatments had similar results for haemoglobin and total blood protein. It was also observed that as the dietary level of ascorbic acid increased, the PCV, Hb and TP also increased.

Effect of ascorbic acid supplementation on the rectal temperature of laying hens during the hot – dry season is as presented in Table 5. There were significant effects (P<0.05) of ascorbic acid on rectal temperature of the birds at 12:00 hours and 15:00 hours. At 12:00 hours and 15:00 hours, the rectal temperature decreased as the level of ascorbic acid increased from 0 to 300 mg AA/kg diet. Table 6 shows the relative humidity and minimum and maximum temperature of the study area.

Table 4: Effects of dietary levels of ascorbic acid on the haematological status of laying hens during hot-dry season

Parameter	Ascorbic Acid level (mg/kg)				SEM
	0	100	200	300	
Packed Cell Volume (%)	28.33^{b}	28.67^{b}	30.00^{a}	31.00^{a}	1.31
Haemaglobin Content (%)	9.63^{b}	9.90^{b}	10.23^{b}	12.30^{a}	0.19
Total blood protein (g/dl)	6.60^{b}	6.67^{b}	7.27^{ab}	8.83^{a}	0.52

[ab]Means with different superscript in the same row are significantly (P<0.05) different
SEM: Standard Error of Means

Table 5: Effects of dietary levels of ascorbic acid on the rectal temperature (^{o}C) of laying hens during hot-dry season

Parameter	Ascorbic Acid level (mg/kg)				SEM
	0	100	200	300	
9:00Hr	40.1	40.05	40.06	40.1	0.09
12:00Hr	41.83^{b}	41.80^{ab}	41.62^{ab}	41.57^{b}	0.07
15:00Hr	42.08^{c}	42.03^{c}	41.87^{b}	41.73^{a}	0.1

[abc]Means with different superscripts in the same row are significantly (P<0.05) different
SEM: Standard Error Means

Table 6: Relative Humidity and Ambient Temperature During the Experiment at Samaru

Month	Relative Humidity (%)	Temperature (^{o}C)	
		Maximum	Minimum
January	20	29	16
February	12	32	20
March	20	39	22
April	37	37	22
May	64	35	21
June	73	33	21
July	79	31	20
August	82	30	19
September	77	31	25
October	58	33	18
November	21	34	13
December	21	32	15

Source: Institute of Agricultural Research Metrological Station Ahmadu Bello University, Zaria

DISCUSSION

Ascorbic acid supplementation in layer diets resulted in better performance in laying hens during the hot-dry season. There was a progressive increase in final weight, body weight change, % Hen-day egg production, feed intake and lower feed cost per dozen eggs as dietary level of AA increased up to the 300 mg AA/kg diets. This is a reflection of the vital role that ascorbic acid could play in enhancing the performance of laying hens. This finding agreed with Adekola and Ayo (2006) who reported that AA supplementation alleviates the effect of heat stress in poultry birds.

Improvement in egg weights, better shell egg weight and haugh unit in birds fed diets with 200 mg and 300 mgAA/kg observed in this study agrees with the reports of Adeosun *et al*. (2013), Thomson and Moreng (1959) who reported that AA enhanced egg quality under high ambient temperature.

The values of the haematological parameters obtained in this study fell within the established normal values of 22-35% (PCV), 7-13% (Hb) and 6-9 g/dl TP respectively. It has been established that packed cell volume (PCV), Haemoglobin content and total protein are

important parameters used to access the health and nutritional status of animals (Oladele and Ayo 1999).

The difference in values of haematological parameters might not be unconnected with effects of high ambient temperature (AT) experienced by the layers during the hot-dry season of the year. It has been reported that laying hens exposed to heat stress had low concentrations of Ascorbic acid in the blood plasma (Pardue *et al.,* 1985; Ayo *et al.,* 2007). These authors further reported that during stress, generations of reactive oxygen species (ROS) was high to the level that overwhelmed tissue anti-oxidant defense system. The high values for PCV, Hb and TP observed in birds fed diets with 200 and 300mgAA/kg in this study is an indication that AA at these levels is adequate for protecting laying hens against stress induced damages to the biological system (Minka and Ayo, 2007).

The results of the present study indicated that rectal temperature (RT) of ShiksBrown® layers fell within the established normal range of 39.5-42^{o}C for the avian species (Freeman, 1971), however, the small variation observed in the RT values in this study agreed with the findings of Ayo *et al.* (1998) that RT varies with ambient temperature (AT) and the hours of the day during the hot-dry season. AA supplementation at 200 and 300mg/kg diets was able to cause significant reductions in RT values in the laying hens in this study which corroborate the reports of Ayo *et al.* (1996), Bottje and Harrison (1985), Njoku (1990) and Balogun *et al.* (1996) that high AT induce stress in poultry could be combated by AA supplementations in diets.

There was zero mortality recorded in the ascorbic acid supplemented groups. With regard to egg quality, non-significant response was observed in yolk index and shell thickness. The significant positive responses observed in birds fed 200 and 300 mg AA/kg in all egg quality parameters is an indication that ascorbic acid supplementation at those levels were sufficient for reduction of stress in laying hens. Is has been reported that when stress factor is high and prolonged, the overall body defensive mechanism begins to breakdown hence, diseases and death may follow (Ayo *et al.,* 1996; Babsky *et al.,* 1990); however, the gradual increases in packed cell volume (PCV), haemoglobin content (Hb) and total protein (TP) along with reduced body temperature observed in this study is an indication that ascorbic acid supplementation helped in combating stress.

CONCLUSION

Since both 200 mg and 300 mg/kg diet produced similar effects, it is therefore concluded that the optimum dietary supplementation of ascorbic acid in laying hen diets during the hot-dry season is 200 mgAA/kg diet.

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