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Effect of Dietary Ascorbic Acid Supplementation on Egg Production, Egg Quality and Hatchability of Indigenous Venda Chicken Hens

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ABSTRACT
An experiment was conducted to evaluate the effect of ascorbic acid supplementation on egg production, egg quality and hatchability of Venda hens. A total of 75 indigenous Venda chickens hens were randomly assigned to five ascorbic acid supplementation levels with three replications, each having five birds. The study was a completely randomized design. The experimental diets were isocaloric and isonitrogenous but with different ascorbic acid supplementation levels ranging between 0, 200, 500, 1000 and 1500 mg of ascorbic acid per kg DM feed. Level of dietary ascorbic acid supplementation had effect ($p<0.05$) on Venda hens egg weight, egg albumen weight, egg yolk weight, egg shell weight and hatchability values. Venda hens supplemented with 200 and 500 mg of ascorbic acid per kg DM feed produced heavier ($p<0.05$) eggs than those from hens supplemented with 1000 and 1500 mg of ascorbic acid per kg DM feed as well as those from the unsupplemented birds. Venda hens supplemented with 200 mg of ascorbic acid per kg DM feed produced eggs with higher ($p<0.05$) hatchability values than eggs from hens supplemented with 1500 mg of ascorbic acid per kg DM feed and the unsupplemented birds. Additionally, optimum ascorbic acid supplementation level of 700 mg kg^{-1} DM feed supported optimum egg production while a higher ratio of 750 mg kg^{-1} DM feed supported optimum hatchability. It is concluded that ascorbic acid supplementation to the diets of indigenous Venda chicken hens is desirable, mainly because of its positive effect on egg weight and hatchability.

Key words: Laying venda chickens, feed intake, egg albumen weight, egg yolk weight, egg shell weight

INTRODUCTION
Indigenous poultry production in South Africa and elsewhere has assumed greater importance on the background of rural unemployment, population explosion and vagaries of nature (Mbajiorgu *et al.*, 2011a). Importantly, to the poor majority in rural areas, indigenous chickens contributes significantly to food security and serves as an immediate source of meat and income when money is need for urgent family needs (Mbajiorgu *et al.,* 2011b). However, little is known about the effect of dietary ascorbic acid supplementation on egg weight, egg qualities and hatchability values of the indigenous Venda chicken hens. In broiler chickens, studies have nonetheless demonstrated improved productivity with ascorbic acid supplementation to the diets under various circumstances (Sahin *et al.*, 2003, 2002; Gous and Morris, 2005; Lin *et al.*, 2006; Attia *et al.*, 2009). In contrast, reports from Bell and Marion (1990) showed that laying hens failed to show a significant effect of various doses of dietary ascorbic acid on the number of eggs laid by broiler hens. Similarly, Kecnik and Sykes (1974) did not find any relationship between ascorbic acid

supplementation to the diets and productive parameters of the produced eggs of broiler chicken hens. Thus, data on the effect of ascorbic acid supplementation to the diets of broiler chicken hens are variable. However, no such studies on indigenous chickens were found. It is, therefore, important to ascertain such responses in indigenous Venda chicken hens. Hence, the objective of this study was to determine the effect of dietary ascorbic acid supplementation on egg production, egg quality and hatchability of indigenous Venda chicken hens.

MATERIALS AND METHODS

This study was conducted at the Experimental farm of the University of Limpopo in South Africa in 2009. A total of 75 Venda laying hens, aged 33 weeks were randomly assigned to five dietary ascorbic acid supplementation treatment levels with three replications, each having five hens. Thus, 15 laying pens were used in total for the experiment. A completely randomized design (SAS, 2008) was used for the experiment. The nutrient composition of the experimental diet were determined as explained below: Thus, the feed contained 900 g DM kg^{-1}, 13.5 MJ ME kg^{-1} DM; 150 g CP kg^{-1} DM; 10.65 g calcium kg^{-1} DM; 5.5 g phosphorus kg^{-1} DM but with similar inclusion level of 17.1 mg per kg DM feed of ascorbic acid/Vitamin C (Table 1). The five dietary ascorbic acid supplementation levels used ranged between $A_{0,}$ $A_{200,}$ $A_{500,}$ A_{1000} and $A_{1500,}$ respectively. Thus, ascorbic acid supplementation levels of $A_{0,}$ $A_{200,}$ $A_{500,}$ A_{1000} and A_{1500}represents the five dietary treatments used in the experiment as follows; diet A_0 had no ascorbic acid supplementation, while diets $A_{200,}$ $A_{500,}$ A_{1000} and A_{1500} were supplemented with 200, 500, 1000 and 1500 mg of ascorbic acid kg^{-1} DM feed (Table 1). The hens were on the above diets for 34 days. Feed and water were offered on *ad libitum* basis. All flocks used for egg collection were maintained under similar environmental and management conditions. At commencement of the study on day 35, the eggs produced per replica were numbered per replica according to treatment group and egg collection lasted for 10 days. Thereafter, eggs collected during the last 10 days from each replicate were pooled together per treatment group and set in a commercial multi-stage incubator. This was done in December, 2009. The eggs were fumigated with formalin on potassium permanganate in the ratio of 1:2 for 15 min and then were randomly set into the multi-stage incubator at specific dry bulb temperature of 37.5°C and wet bulb temperature of 28.3°C with the broad ends pointing upwards. On the 18 day of incubation, all eggs were candled and those with evidence of living embryos were transferred from the turning trays to hatcher baskets. Number of eggs that hatched per replicate within each treatment group was recorded at 21.5 day of incubation.

Table 1: Nutrient composition of the experimental diet

	Diet				
Nutrients	V_0	V_{200}	V_{500}	V_{1000}	V_{1500}
Dry matter (g kg^{-1}eed)	900	900	900	900	900
Energy (MJ kg^{-1}DM)	13.5	13.5	13.5	13.5	13.5
Protein (g kg^{-1} feed)	150	150	150	150	150
Calcium (g kg^{-1} DM)	10.65	10.65	10.65	10.65	10.65
Phosphorus(g kg^{-1} DM)	5.5	5.5	5.5	5.5	5.5
Vitamin C in feed (mg kg^{-1} DM)	17.1	17.1	17.1	17.1	17.1
Vitamin C_1 supplementation level (g kg^{-1} DM)	0	200	500	1000	1500

Diet A_0 had no ascorbic acid supplementation while diets $A_{200,}$ $A_{500,}$ A_{1000} and A_{1500} were supplemented with 200, 500, 1000 and 1500 mg of ascorbic acid kg^{-1} DM feed. Vit C : Vitamin C (ascorbic acid) in the diet. Vit C_1 : Vitamin C (ascorbic acid) supplementation levels

Data collection: The average voluntary feed intake per bird was measured daily by subtracting the weight of leftovers from that of the feed offered per day and the difference was divided by the total number of birds in the pen. Feed conversion ratio per pen was calculated as the total amount of feed consumed divided by the weight gain of live birds plus the weight gain of dead or culled birds in the pen (Lehmann *et al.*, 1996). Total egg production was determined by collecting eggs laid in each replicate everyday and pooling together for the period of 10 days. The daily mean number of eggs produced in each replicate was calculated in relation to the number of laying hens as follows: Eggs/hen/day = Total number of eggs/total number of laying hens/10 days. Mean egg weight per replicate was determined by an egg weighing digital balance. Thereafter, representative of the egg from each replica were then carefully broken on a glass plate (30 × 21 cm) to measure the egg quality characteristics. Thus, representative egg albumen, yolk and shell contents were carefully separated and weighed on an electronic weighing scale. The hatchability percentage was determined in each treatment as follows:

$$\text{Hatchability} = \frac{\text{Number of eggs hatched per treatment}}{\text{Total number of eggs set in each treatment}} \times 100$$

Chemical analysis: Dry matter (DM) contents of eggs, feeds, feed refusals and faeces were determined by drying the sample at 105°C for 24 h. Gross energy values for feeds and faeces were determined using an adiabatic bomb calorimeter and semi-micro Kjeldahl method was used to analyze nitrogen contents of feeds and faeces according to the method described by AOAC (2002). (University of Limpopo Laboratory, South Africa). Thereafter nitrogen contents of the egg contents, diets, feed refusals and faeces were analyzed to calculate crude protein using the formula: CP = % N x 6.25 (Schroeder, 1994). High performance liquid chromatography (HPLC) was used to determine the quantity of ascorbic acid contained in the experimental Venda hens feed (LATS, University of Limpopo, Polokwane) according to the method described by Swa-Choo and Siong (1996).

Statistical analysis: Data on feed intake, egg production, egg weight, egg content and egg hatchability of Venda hens were analyzed using the General Linear Model (GLM) procedure of the statistical analysis of variance. The Duncan's Multiple Range Test was used to test the significance of differences between treatment means ($p<0.05$) (SAS, 2008). Optimal responses in feed intake, egg production, egg weight and egg hatchability of Venda hens to the dietary ascorbic acid supplementation level were modelled using the following quadratic equation:

$$Y=a+b_1x+b_2x^2$$

Where Y = feed intake, egg production, egg weight or egg hatchability a = intercept; b_1 and b_2 = coefficients of the quadratic equation x = dietary ascorbic acid supplementation level and $-b_1/2b_2$ = x value for optimum response. The quadratic model was fitted to the experimental data by means of NLIN procedure of SAS.

RESULTS

Dietary ascorbic acid supplementation level had no effect ($p>0.05$) on feed intake and egg production however, level of dietary ascorbic acid supplementation had effect ($p<0.05$) on Venda hens egg weight and hatchability values (Table 2). Venda hens supplemented with

200 and 500 mg of ascorbic acid per kg DM feed produced heavier ($p<0.05$) eggs than those from hens supplemented with 1000 and 1500 mg of ascorbic acid kg^{-1} DM feed as well as those from the unsupplemented birds. Venda hens supplemented with 200, 500 and 1000 mg of ascorbic acid kg^{-1} DM feed produced eggs with higher ($p<0.05$) hatchability values than eggs from hens supplemented with 1500 mg of ascorbic acid kg^{-1} DM feed and the unsupplemented birds. There were no differences ($p>0.05$) in hatchability values between Venda hens supplemented with 200, 500 and 1000 mg of ascorbic acid kg^{-1} DM feed. However, Venda hens supplemented with 200 mg of ascorbic acid kg^{-1} DM feed produced eggs with higher ($p<0.05$) hatchability values than eggs from hens supplemented with 1500 mg of ascorbic acid kg^{-1} DM feed and the unsupplemented birds. Venda hens supplemented with 500, 1000 and 1500 mg of ascorbic acid kg^{-1} DM feed had similar ($p>0.05$) hatchability values. Similarly, Venda hens supplemented with 1500 mg ascorbic acid per kg feed had almost the same ($p>0.05$) hatchability values with the unsupplemented ones. Venda hens offered a diet supplemented with 200 mg of ascorbic acid per kg DM feed had higher ($p<0.05$) albumen weight than those from hens supplemented with 500, 1000 and 1500 mg of ascorbic acid per kg DM feed as well those from the unsupplemented birds (Table 3). However, Venda hens offered a diet supplemented with 500 mg of ascorbic acid per kg DM feed produced eggs with higher ($p<0.05$) egg yolk and egg shell weights than those on unsupplemented diets and those supplemented with 200, 1000 and 1500 mg of ascorbic acid per kg DM feed (Table 3).

Table 4 presents a series of quadratic regressions that predict optimum ascorbic acid supplementation level for feed intake, egg production, egg weight and hatchability of indigenous

Table 2: Effect of ascorbic acid supplementation level to the diets of Venda chicken hens on feed intake, egg production, egg weight and egg hatchability

	Diet					
Variable	V_0	V_{200}	V_{500}	V_{1000}	V_{1500}	SE
Feed intake (g/bird/day)	115	117	118	116	115	2.08
Egg production (eggs/hen/10 days)	5.9	6.3	6.2	6.1	6.0	0.06
Egg weight (g egg^{-1})	49.1^{c}	51^{a}	51^{a}	50^{b}	49^{c}	0.00
Hatchability (%)	31^{c}	64^{a}	57^{ab}	61^{ab}	40^{bc}	6.70

Means in the same column not sharing a common superscript are significantly different ($p<0.05$). SE: Standard error

Table 3: Effect of ascorbic acid supplementation level to the diets of Venda chicken hens on albumen weight, yolk weight and shell weight

	Diet					
Variable	V_0	V_{200}	V_{500}	V_{1000}	V_{1500}	SE
Albumen weight (g)	25.78^{e}	28.26^{a}	26.36^{d}	27.26^{c}	27.55^{b}	0.002
Yolk weight (g)	15.00^{d}	15.58^{c}	16.99^{a}	15.90^{b}	14.47^{c}	0.001
Shell weight (g)	6.05^{e}	6.44^{b}	6.93^{a}	6.30^{d}	6.41^{c}	0.001

^{a,b,c,d,e}: Means in the same column not sharing a common superscript are significantly different ($p<0.05$). SE: Standard error

Table 4: Effect of ascorbic acid supplementation level to the diets of Venda hens on optimal feed intake (g/bird/day), egg production (egg/hen/10 days), egg weight (g/egg) and hatchability (%)

Trait	Formula	AA	r^2	P
Intake	$Y = 115.583 + 0.005x + 0.00000402x^2$	621	0.681	0.319
Egg production	$Y = 6.022 + 0.00006x + -0.0000004x^2$	700	0.439	0.561
Egg weight	$Y = 49.600 + 0.004x + 0.000002928x^2$	683	0.694	0.306
Hatchability	$Y = 38.727 + 0.07x +-0.00004663x^2$	750	0.670	0.330

r^2: Regression coefficient P: Probability. AA: Ascorbic acid supplementation (mg kg^{-1} DM feed) for optimum variable

Venda chicken hens. Results indicate that feed intake was optimized at ascorbic acid supplementation level of 621 mg kg^{-1} DM feed (r^2= 0.681). egg production at 700 mg kg^{-1} DM feed (r^2 = 0.439), egg weight at 683 mg kg^{-1} DM feed (r^2= 0.694) and hatchability at 750 mg kg^{-1} DM feed (r^2 = 0.670), respectively. These optimum levels were predicted using the quadratic equation given under materials and methods. Thus, the optimum levels were calculated as $-b_1/2b_2$ where b_1 and b_2 are the co-efficients of the quadratic equation.

DISCUSSION

Results of the present study indicate that ascorbic acid supplementation level had no effect on feed intake and egg production of indigenous Venda chicken hens. In support of the present findings, Al-Taweil and Kassab (1990) and Mbajiorgu *et al.* (2007) also observed that supplementation with ascorbic acid had no effect on feed intake in broiler chickens. Also, in agreement with results of the present study, Bell and Marion (1990) reported that broiler chicken laying hens failed to show a significant effect of various doses of dietary ascorbic acid on egg production. However, the present findings is contrary to the findings of Gous and Morris (2005) and Lin *et al.* (2006) who observed that dietary ascorbic acid supplementation had effect on feed intake of broiler chickens. Similarly, Njoku and Nwazota (1989) and Cheng *et al.* (1990) also showed that ascorbic acid supplementation increased the number of eggs in laying broiler chicken hens, thus, contradicting results of the present findings. The physiological explanations for these contradictions are not clear and merits further investigation. However, it is known that ascorbic acid is associated with the conversion of body proteins and fat into energy for production through increased corticosterone secretion (Bains, 1996). Such transient changes in body protein and fat level do not appear to alter feed intake and egg production in laying Venda chicken hens. As such feed intake and egg production remained unchanged irrespective of the ascorbic acid supplementation level.

Ascorbic acid supplementation in the diets of Venda chicken hens improved egg weights. This is similar to the findings of Lazar *et al.* (1983) and Slinger (1985) who reported that ascorbic acid supplementation in broiler chicken breeders increased egg weight. Perek and Kendler (1963) also observed similar results. These authors observed that ascorbic acid supplementation to the diets of White Leghorn hens improved egg weight. However, contrary to the present findings, Kecnik and Sykes (1974) did not find any relationship between ascorbic acid supplementation to the diets of breeder hens and productive parameters of the produced eggs. The reason for this contradiction is not known. However, more research is required to explore the biochemical reasons for these differences.

Ascorbic acid supplementation in the diets of Venda hens improved egg hatchability. This is similar to the findings of Lin *et al.* (2000) who reported that ascorbic acid supplementation increased egg hatchability in broiler chicken breeders. Similar results were also observed by Peebles and Brake (1985) and Kontecka *et al.* (2001) in broiler chicken breeders and ducks, respectively. Monsi and Onitchi (1991) also observed similar results in broiler chicken breeders. Contrary to this, Creel *et al.* (2001) observed that ascorbic acid supplementation in the diets of broiler chicken breeders neither improved egg production nor enhanced egg hatchability.

The present study indicates that ascorbic acid supplementation in the diets of Venda hens increased egg albumen, egg yolk and egg shell weights. This is in agreement with Whitehead and Keller (2003) who reported a positive role of ascorbic acid on egg quality in broiler chickens. Improvements due to ascorbic acid supplementation in egg albumen and egg yolk qualities have also been reported elsewhere (Cheng *et al.*, 1990) in broiler chickens. Similar results were also reported by Rashid and Ahmed (1991). These authors stated that ascorbic acid supplementation in broiler chicken hens improved egg shell thickness. This is expected since ascorbic acid is necessary for the maintenance of normal collagen metabolism which is required for normal structure of egg shells (McDonald *et al.*, 2003). However, Kecnik and Sykes (1974) did not find any relationship between ascorbic acid suplementation and egg contents in broiler chickens.

The present study indicates that feed intake, egg production, egg weight and egg hatchability were optimized at different ascorbic acid supplementation levels of 621, 700, 683 and 750 mg kg^{-1} DM feed, respectively. The value of 700 mg kg^{-1} DM feed for optimum egg production in indigenous Venda chicken hen is lower than the value of 800 mg kg^{-1} feed of Whitehead and Keller (2003) estimated for optimum number of eggs laid in laying broiler chicken hens. Additionally, optimum ascorbic acid supplementation level of 750 mg kg^{-1} DM feed optimized hatchability in Venda chicken hens. However, Torgowski and Kontecka (1998) reported lower value of 500 mg of ascorbic acid per kg DM feed for optimum egg fertility in pheasants. Thus, the possible explanation for these differences in optimum requirements might be attributed to the genetic variations among the different strains of birds under study (Acar *et al.*, 1991).

CONCLUSION

The range of dietary ascorbic acid supplementation level used in this study did not have significant effect on feed intake and egg production of Indigenous Venda chicken hens. However, Level of dietary ascorbic acid supplementation had effect ($p<0.05$) on Venda hens egg weight, egg albumen weight, egg yolk weight, egg shell weight and hatchability values. Venda hens supplemented with 200 and 500 mg of ascorbic acid per kg DM feed produced heavier ($p<0.05$) eggs than those from hens supplemented with 1000 and 1500 mg of ascorbic acid per kg DM feed as well as those from the unsupplemented birds. Venda hens supplemented with 200 mg of ascorbic acid per kg DM feed produced eggs with higher ($p<0.05$) hatchability values than eggs from hens supplemented with 1500 mg of ascorbic acid per kg DM feed and the unsupplemented birds. Furthermore, optimum ascorbic acid supplementation level of 700 mg kg^{-1} DM feed supported optimum egg production in indigenous Venda chicken hens while a higher ratio of 750 mg kg^{-1} DM feed supported optimum hatchability. It is concluded that ascorbic acid supplementation to the diets of indigenous Venda chicken hens is desirable, mainly because of its positive effect on egg weight and hatchability.

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