Response of Japanese quail (*Coturnix coturnix japonica*) Laying Hens to Dietary Threonine Levels in the Tropical Northern Savannah of Nigeria

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Abstract— In order to describe the response of Japanese quails to dietary amino acids after sexual maturity, accurate estimates of the amounts of each amino acid required for maintenance and egg production are needed to exploit its production potentiality. This experiment was therefore carried out to investigate the response of Japanese quail Laying Hens to Optimum Dietary Threonine Levels in the Tropical Northern Savannah of Nigeria. Two hundred and twenty five, six weeks old female quails were weighed and randomly allotted to five treatments of three replicates with fifteen birds per pen in a completely randomized design. The results from the study showed that daily feed intake, hen house egg production, hen day egg production and egg number were significantly ($p<0.05$) influenced with increasing dietary threonine levels. Similar ($p>0.05$) values were observed for daily feed intake of quail hens fed control (0.71), 0.80, 0.85 and 0.90% threonine diets. Those birds on control (0.71%) and 0.80% threonine differed ($p<0.05$) significantly from the values obtained for those on 0.76% threonine diet. Quail hens fed 0.80, 0.85 and 0.90% total threonine diets had similar ($p>0.05$) values for hen house egg production, hen day egg production and egg number. Those fed control (0.71), 0.76 and 0.85% threonine diets were also similar ($p>0.05$). The cost of threonine increased the cost per kilogram of diet. Feed cost per dozen egg and feed cost per crate of eggs differed ($p<0.05$) significantly for dietary treatments. Birds fed 0.80, 0.85 and 0.90% threonine diets had similar and significantly ($p<0.05$) better feed cost per dozen egg than those fed other diets. Those on control (0.71) and 0.76% threonine diets were also similar ($p>0.05$). Feed cost per crate of eggs favoured birds on 0.80% threonine which was significantly ($p<0.05$) better than those on 0.90 and 0.85% threonine diets that were similar. However, birds fed control (0.71), 0.76 and 0.85% were also similar ($p>0.05$). Therefore, this study concludes that the group fed with total threonine diet at 0.80% was best for growth, egg production performance, feed cost per dozen egg and feed cost per crate of egg. Egg weight, egg length, albumen weight, yolk height, yolk diameter, shell weight and egg shape index were statistically ($p>0.05$) similar for all dietary treatments. Dietary threonine levels significantly ($p<0.05$) influenced egg diameter, albumen length, albumen height, yolk weight, shell thickness, egg specific gravity, Haugh unit and egg shell index. The result of this present study also suggests that recommendations above 0.74% threonine of NRC (1994) for laying quails is required to support optimal laying performance of quails in the tropical northern savannah of Nigeria. It is therefore suggested that quail farmers should be encourage in the use of threonine in the diet of laying quails in addition to lysine and methionine for improved egg productivity and quality traits.

Index Terms— egg, Japanese quail, quality, threonine, tropical savannah.

1 Introduction

The protein intake of most Nigerians is inadequate and often lacks protein of high biological value derived from animal products [1]. The protein requirement of the increasing population will continue to increase. The need to increase animal production aggressively is an understatement if the already shortfall in protein requirements of the average Nigerian and continuous increase in the nation's population are considered [2].

In recent years, due to lower cholesterol content, consumer preference for poultry products has increased [3]. Other advantages associated with the poultry industry are that, they require less investment than other livestock, more efficient and quick yield, which has led to major investment and growth in the poultry industry [3]. According to Bawa *et al*. [4], the productive and reproductive performances of animals depend largely on the quality and quantity of their dietary nutrients intake. It is therefore imperative to ensure that the proportions of each of the nutrients required for optimum performance at the least cost are adequately provided in their diets. Currently, Japanese quail had gained attention in poultry industry as they are resistant to pathogens and a good producer of egg and meat for healthy nutrition in human and is being used as beneficial animal model in researches [5].

Dietary protein requirement of quail is influenced by the amount of energy metabolism, and other components used in making the diet, while amino acids requirements are influenced by many factors including dietary, environmental, genetics and productive purposes [6], [3]. According to Ogundipe *et al*. [7], lysine, methionne, threonine and tryptophan and sometimes arginine are the critical amino acids as they are the most difficult to supply in proper amount in feed proteins. Knowledge of the dietary essential amino acids requirement of Japanese quail is of great importance in formulating cost-effective feeds. In order to describe the response of Japanese quails to dietary amino acids and to develop an effective model for the precise feeding of these birds after sexual maturity, accurate estimates of the amounts of each amino acid required for maintenance and egg production are needed for tropical environments to exploit its production potentiality.

In poultry diets based on corn and soybean meal, threonine is the third limiting amino acid [8]. It is required for the protein synthesis and maintenance of body protein turnover, and acts on collagen and elastin synthesis and production of antibodies [9]. The pure L-threonine is totally digestible and is commercially available, offering flexibility in diet formulation. Its supplementation allows for inclusion of lower-protein feeds, preventing nitrogen excretion and thereby environmental pollution [10].

2 Materials and Methods

The experiment was carried out at the Poultry Unit of Iflosam Integrated Farm, Kilometre 28, Kano-Katsina Road, Bichi. Bichi is located on A9 highway on coordinate proximity "69.6105610 – 149.5870380" in North – Western Nigeria. It has an area of 612 Km^2 and a population of 277, 099 at the 2006 Census Kano State [11]. Bichi is a Local Government Area in Kano State. The State is located within the Northern Guinea Savannah Zone on the latitude 11^0 14′ 44′′ N and longitude 7^o 38′ 65′′E, at an altitude of 610 m above sea level. The climate is relatively dry with a mean annual rainfall of 700-1400mm. [12]. Two hundred and twenty five, six weeks old quail hens were used for the study. The birds were randomly allotted to five dietary treatments of three replicates with fifteen birds per pen in a completely randomized design. All routine management procedures were strictly adhered. Performance characteristics measured included body weights of birds in each replicates, which were taken at the beginning and end of the trial while percentage change in body weight was determined. Average daily feed intake was determined by obtaining the differences between the quantity of feed offered and the left over. Feed conversion ratio was calculated weekly and feed cost per dozen eggs laid was calculated based on prevailing market prices of feed ingredients. Egg production was recorded daily and pooled to calculate hen-day egg production and hen-housed egg production. The age at peak of egg production and percentage egg production at peak were also determined. For egg internal and external quality assessment, nine freshly laid eggs from each treatment were weighed bi-weekly from the third week into lay (ten weeks of age) to twentieth week. The following Egg quality traits: Weight of each egg sampled and the albumen, yolk and shell weight were measured respectively using a sensitive digital scale (PD-Series Scale 200g) to the nearest 0.01g. The egg length, egg breadth, egg yolk width, egg yolk height, albumen height, albumen width were measured respectively using sensitive electronic digital Vernier calliper (SR44 with 1.5m/sec speed & + - 0.01mm accuracy). Egg shell thickness was measured using sensitive electronic digital micrometer screw gauge (AEROSPACE SR44 with 0.001mm accuracy). Egg shape index (ESI) was calculated according to Sauver [13] using the formula:

$$ESI = \frac{EB}{EL}$$

Where:

ESI = Egg Shape Index
EB = Egg Breadth (mm)
EL = Egg Length (mm)

The egg shells were dried for three days and weighed to determine the shell weight. The Egg Shell Index was calculated according to Iposu *et al* [14] using the formula:

$$I = \frac{100SW}{S}$$

Where:

I = Egg Shell Index
SW = Shell Weight (g)
S = Surface Area (cm^2)

S will be calculated from egg weight (EW) using the equation

$$S = K.EW^{2/3}$$

Where:

K has a constant value of 4.67 for a range of egg weights less than 60g.

Formula for estimating the egg specific gravity (ESG) was based on weight of egg and shell as used by Poultry Adviser [15]:

$$ESG = \frac{EW}{\{0.9680(EW - SW) + (0.4921\ SW)\}}$$

Where:

ESG = Egg Specific Gravity
EW = Egg Weight
SW = Shell Weight

Shell thickness was measured using a micrometer screw gauge. Egg yolk height and width were measured using vernier caliper and the values obtained were used to calculate the yolk index.

The freshly collected eggs were broken out one after the other into a Petri dish, care being taken to keep the yolk intact and with this, the albumen heights was measured for the calculation of Haugh unit values. These interior egg quality traits were measured in terms of Haugh unit according to the method of Haugh [16] as outlined by Oluyemi and Roberts [17].

$$HU = 100\log 10\ (H-1.7W^{0.37} + 7.6)$$

Where:

HU = Haugh Unit
H = Observed height of the albumen in millimetres
W = Weight of the eggs in grams

Data obtained were subjected to analysis of variance using [18] general linear model procedure. Significant differences among treatment means were compared using the Tukey's Studentized Test. Optimum value of threonine was computed by fitting the dietary inclusion of threonine against the egg production parameters using bivariate analysis to generate the quadratic function that gave the optimal value.

3 RESULTS

The dry matter, crude protein, crude fibre, ether extract and ash used in the study are presented in Table 2. The dry matter, crude protein content was highest in 0.76% threonine diet while those in other diets ranged between 22.86 to 23.68%. Crude fibre in 0.76% was higher than that of 0.80%, which was higher than that of 0.85%, which was higher than that of the control (0.71%) and 0.90% threonine diets. Ether extract in 0.90% was higher than that of 0.85% threonine diet, while those in other diets ranged between 3.59 to 3.86%. The percentage ash in the control (0.71%) diet was higher than that of 0.90%, which was higher than that of 0.85%, which was higher than those of 0.76 and 0.80%.

Table 3 showed the laying performance of quail hens fed diets with threonine. There were significant ($p<0.05$) differences between treatment means for percent change in weight, daily feed intake, hen-house egg production, hen-day egg production and egg number. Final weight, feed conversion ratio, egg weight, age at peak egg production and peak egg production percent were similar ($p>0.05$) for all dietary treatments. Similar ($p>0.05$) values were observed for daily feed intake of quail hens fed control (0.71), 0.80, 0.85 and 0.90% threonine diets. Those birds on control (0.71%) and 0.80% threonine differed ($p<0.05$) significantly from the values obtained for those on 0.76% threonine diet. Quail hens fed 0.80, 0.85 and 0.90% total threonine diets had similar ($p>0.05$) values for hen house egg production, hen day egg production and egg number. Trend is also shown in figure 4 and 5. Those fed control (0.71), 0.76 and 0.85% threonine diets were also similar ($p>0.05$). The cost of threonine increased the cost per kilogram of diet. Feed cost per dozen egg and feed cost per crate of eggs differed ($p<0.05$) significantly for dietary treatments. Birds fed 0.80, 0.85 and 0.90% threonine diets had similar and significantly ($p<0.05$) better feed cost per dozen egg than those fed other diets. Those on control (0.71) and 0.76% threonine diets were also similar ($p>0.05$). Feed cost per crate of eggs favoured birds on 0.80% threonine which was significantly ($p<0.05$) better than those on 0.90 and 0.85% threonine diets that were similar. However, birds fed control (0.71), 0.76 and 0.85% were also similar ($p>0.05$).

Egg quality parameters of laying hens fed dietary levels of threonine are presented in Table 4. Egg weight, egg length, albumen weight, yolk height, yolk diameter, shell weight and egg shape index were similar ($p>0.05$) for all dietary treatments. Birds fed 0.80, 0.85 and 0.90% had similar ($p>0.05$) egg diameter which was significantly ($p<0.05$) higher than those fed control and 0.76% threonine diets. However, birds fed control (0.71) and 0.76% threonine diets were also similar ($p>0.05$). The albumen length of birds fed control (0.71), 0.76, 0.80 and 0.85% threonine diet were similar ($p>0.05$) and significantly ($p<0.05$) higher than those fed 0.90% threonine diet. There was observed increase in albumen height with increasing dietary threonine levels. Similar ($p>0.05$) values were observed for yolk weight of quail hens fed control (0.71), 0.80, 0.85 and 0.90% total threonine diets which were significantly ($p<0.05$) higher than those fed 0.76% threonine diet. Birds fed the control (0.71), 0.85 and 0.90% threonine diet had similar ($p>0.05$) shell thickness values which were significantly ($p<0.05$) higher than that recorded for quails hens fed 0.76 and 0.80% threonine diets. Similar ($p>0.05$) values were observed for egg specific gravity of quail hens fed control (0.71), 0.76, 0.80 and 0.90% threonine diets which were indicated by significantly ($p<0.05$) higher values than those fed 0.85% threonine diet. The Haugh unit values of quails fed 0.80, 0.85 and 0.90% threonine diets did not vary ($p>0.05$) statistically but were significantly ($p<0.05$) higher than those fed control (0.71) and 0.76% threonine diet which were also similar ($p>0.05$). Significant ($p<0.05$) increase in egg shell index reported for quail hens fed 0.80 and 0.90% threonine diets were similar and significantly ($p<0.05$) higher than those fed other dietary treatments. The birds fed control (0.71) had significantly ($p<0.05$) higher egg yolk index than those on other threonine diets that were similar ($p>0.05$).

4 DISCUSSION

The proximate composition of experimental diet for Japanese quail hens showed that the experimental diets were significant source of proteins, essential fatty acids, carbohydrates and other vitamins and minerals.

The significantly higher feed intake observed could be as a result of proper absorption of threonine amino acid in the gastrointestinal tract of the birds, while birds fed 0.76% of threonine in the diet had a contrast action. The report of this study disagreed with the study of [19] in which 0.66, 0.70, 0.74, 0.78, 0.82 and 0.86% threonine levels were used and observed that feed intake was not influenced by dietary level of threonine. The important observation in this study was that quails were able to utilize available threonine by increasing feed intake. This might be that threonine was able to promote gut health by several possible mechanisms

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including altering gut pH, maintaining protective gut mucus, selecting beneficial intestinal organisms, enhancing fermentation acids and enhancing nutrient uptake [20].This concur with the report of [21] who asserted that quails are better converters of feed to egg than the chicken.

The higher egg number, hen - housed and hen - day egg production observed for quails fed 0.80, 0.85 and 0.90% threonine based diet might be linked to balanced amino acid profile of the diets. This was because in laying hen, the essential amino acid profile could have some influence over their voluntary feed consumption and utilization to produce eggs. The findings of this study agreed with [4] who observed increased egg production with increasing protein in the diet. Trend of increased egg number, hen-housed and hen-day egg production with increasing levels of threonine in the diets observed in this study agreed with the reports of [22], [23] and [24] who reported that egg production and egg weight improved as threonine increased above the [25] requirement for laying hens.

Azzam *et al*. [24] reported that the addition of L-threonine at 0.2% resulted in optimal egg production. Crystalline l-threonine was added to the basal diet at 0 (control), 0.1, 0.2, 0.3, and 0.4%. Zollitsch *et al*. [22] reported an increase in egg production when laying hens were fed diets containing 14% CP and supplemented with threonine and arginine. Faria *et al*. [23] reported that egg weight increased significantly from 55.18 g at 26.7°C when the diet contained 0.35% threonine to 58.36 g when the diet containing 0.53% Threonine in experiment 1 (31 to 38 wk of age), whereas egg weight was similar in hens fed different dietary threonine levels in experiment 2 (45 to 52 of age) when the temperature of the house ranged from 28.3 to 32.2°C, while the temperature range during the period of this study was between 34^{o} C to 41^{o}C.

However, the results obtained in this study disagreed with those of [26] who reported no effect of supplemental L-threonine on egg production in laying hens. The peak egg production range of 97.78 - 100% observed in this present study suggest that Japanese quails could reached their peak of production six weeks after point of lay for 0.76% – 0.90% threonine supplemented diets. These values were higher than the peak egg production values (78.77% - 81.97%) reported by [4] when laying birds were fed varying protein levels. The cheaper cost of feed per dozen egg and cost of feed per crate of egg observed in this study corroborated the report of [10] who asserted that there is observed effect of threonine on feed conversion per dozen egg at optimal threonine in quail diet. The result of this present study suggests that the current [25] recommendation of 0.74% threonine for laying quails is not adequate to support optimal laying performance of quails in the tropics.

The observed increase in yolk weight in birds fed control diet, 0.80, 0.85 and 0.90% threonine diets could be attributed to better nutrient absorption resulting in heavier eggs laid in these treatments as observed by [27]. Higher levels of threonine at 0.80, 0.85 and 0.90% enhanced the internal quality of the eggs (Haugh units). The Haugh unit is a measure of the internal quality of eggs and it indicates the freshness or ages of eggs [16]. Egg shell index of birds fed 0.80 and 0.90% threonine diets were similar ($p>0.05$) and significantly higher than those fed other dietary treatments. This might be due to relatively very high feed intake recorded in birds fed these diets which indicated that the hens consumed almost all the calcium sources in the diets which was adequately absorbed and this corroborates the findings of [28] who reported that calcium absorption in laying hens was affected by stage of egg formation and the birds consumption of the quantity required at time it was needed most.

The observed difference in most of the egg quality traits disagreed with the reports of [29] who observed no significant ($p>0.05$) effect on the shell thickness, shell index across dietary treatments. Eggs of inferior quality according to [30] have Haugh unit values of less than 40 for poultry. The Haugh unit values (84.06 – 86.67) recorded corroborates the results of [31]; 2010 [32], [19] and are greater than the values (78.73 – 80.31 and 0.24mm – 0.26mm) reported by [33], [30], [4] for Haugh unit and shell thickness respectively. This might be that threonine was able to increase calcium binding in shell leading to increase in shell thickness. The values (0.48 – 0.49mm) obtained from this study were greater than the values (0.30 – 0.36mm) reported by [34]. This could be as a result of the welfare measures adopted in this present study for nutrition, housing and environment on farm as well as season in which the study was carried out.

This study was carried out in a typical tropical hot season. It was reported that high temperature reduces feed intake by the birds [35] and which affected egg production performance and quality traits. The house was properly ventilated and the space per quail hen was adequate (3.5cm per bird).The studies by [34] were conducted in an enclosed structure in the tropical rainforest environment of the South West, Nigeria with conventional cages. This confirms the finding of [36] who observed that stressors may influence egg shell quality. The variation recorded in this study could be due to the differences in environmental factors and fluctuations of the temperature during the period of conducting the experiment. The range of values obtained for external and internal egg quality traits in this work were comparable and similar to values obtained by [37].

5 Conclusion

Generally, it was observed that threonine did improve productive performance of Japanese quail laying hens. It shows therefore that threonine at levels above [25] recommendation could be used safely in quail nutrition without adverse effect on egg production performance. The optimal requirement of threonine based on this study was determined to be 0.80% for laying phase for Japanese quails in the tropical northern savannah of Nigeria.

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Table 1: Composition of Experimental Diets Containing Varying Levels of Threonine. (6-20 weeks).

	Dietary Levels of Threonine (%)				
Ingredients (%)	**0.71**	**0.76**	**0.80**	**0.85**	**0.90**
Maize	46.94	46.94	46.94	46.94	46.94
Groundnut cake	22.50	22.50	22.50	22.50	22.50
Soya cake	10.00	10.00	10.00	10.00	10.00
Wheat Offal	7.50	7.50	7.50	7.50	7.50
Fish meal	6.50	6.50	6.50	6.50	6.50
Limestone	5.62	5.62	5.62	5.62	5.62
Bone meal	0.30	0.30	0.30	0.30	0.30
Common Salt (Nacl)	0.30	0.30	0.30	0.30	0.30
Broiler Finisher Premix**	0.25	0.25	0.25	0.25	0.25
Lysine	0.01	0.01	0.01	0.01	0.01
Methionine	0.07	0.07	0.07	0.07	0.07
Tryptophan	0.01	0.01	0.01	0.01	0.01
Threonine	0.00	0.05	0.10	0.15	0.20
Total	100.00	100.00	100.00	100.00	100.00
Calculated Analysis					
ME Kcal/kg	2786	2786	2786	2786	2786
Crude protein (%)	22.6	22.6	22.6	22.6	22.6
Ether extract (%)	4.09	4.09	4.09	4.09	4.09
Crude fibre (%)	4.18	4.18	4.18	4.18	4.18
Calcium (%)	2.63	2.63	2.63	2.63	2.63
Lysine (%)	1.08	1.08	1.08	1.08	1.08
Methionine (%)	0.42	0.42	0.42	0.42	0.42
Cysteine (%)	0.28	0.28	0.28	0.28	0.28
Meth + Cyst	0.70	0.70	0.70	0.70	0.70
Sodium	0.15	0.15	0.15	0.15	0.15
Potassium	0.70	0.70	0.70	0.70	0.70
Available P (%)	0.35	0.35	0.35	0.35	0.35
TSAA	0.96	0.96	0.96	0.96	0.96
Tryptophan (%)	0.21	0.21	0.21	0.21	0.21
Threonine (%)	0.71	0.76	0.80	0.85	0.90
Feed cost (₦)	87.79	88.54	89.29	90.04	90.79

Met = Methionine; P=Phosphorus; ME=Metabolizable Energy; Cys= Cysteine **Biomix premix supplied per kg of diet: Vit. A, 10,000iu; Vit.D_3, 2000 iu; Vit E, 23 mg;Vit. K, 2mg: Vit.B_1,1.8;Vit. B_2, 5.5mg; Niacin, 27.5mg; Pantothenic acid, 7.5mg; Vit. B_{12}, 0.015mg: Folic acid, 0.75mg; Biotin, 0.06mg; Choline chloride, 300mg; Cobalt, 0.2mg; Copper, 3mg; Iodine, 1 mg; Iron, 20 mg; Manganese, 40 mg; Selenium, 0.2 mg; Zinc, 30mg; Antioxidant, 1.25mg.TSAA = Total sulphur amino acid

Table 2: Proximate Composition of Experimental Diets for Japanese quail hens (6 – 20 weeks)

	Dietary Levels of Threonine (%)				
Parameters	**0.71**	**0.76**	**0.80**	**0.85**	**0.90**
Dry matter (%)	94.12	94.40	94.36	94.58	94.66
Crude protein (%)	23.68	23.94	23.52	22.86	23.19
Crude fibre (%)	6.13	6.52	6.44	6.39	6.00
Ether extract (%)	3.86	3.59	3.84	3.98	3.99
Ash (%)	6.98	5.92	5.48	6.58	6.70
Nitrogen free extract (%)	59.35	60.03	60.72	60.19	60.12

Table 3: Effect of dietary threonine levels on egg laying performance of Japanese quail hens (6-20weeks).

	Dietary Levels of Threonine (%)						
Parameters	**0.71**	**0.76**	**0.80**	**0.85**	**0.90**	**SEM**	**Pr>F**
Initial weight (g/b)	133.30	133.30	133.30	133.30	133.30	0.00	0.00
Final weight (g/b)	174.39	165.00	157.35	169.69	171.41	12.50	0.88
Percent change in weight (%)	41.09^{a}	31.70^{d}	24.05^{e}	36.39^{c}	38.11^{b}	0.02	0.001
Daily feed intake (g/b/d)	22.96^{a}	21.18^{b}	21.93^{a}	21.83^{ab}	21.83^{ab}	0.66	0.04
Feed conversion ratio	2.27	2.12	2.14	2.12	2.14	0.06	0.49
Hen house production (%)	59.39^{b}	59.52^{b}	77.51^{a}	64.31^{ab}	67.65^{a}	2.77	0.01
Hen day production (%)	59.39^{b}	59.52^{b}	77.51^{a}	64.31^{ab}	67.65^{a}	2.71	0.01
Egg weight (g/d)	10.13	9.99	10.26	10.32	10.19	13.09	0.64
Age at peak egg production (days)	60.33	47.33	42.67	53.67	44.00	47.00	0.41
Egg number (total period/bird)	62.36^{b}	62.50^{b}	81.39^{a}	67.53^{ab}	71.03^{a}	2.90	0.01
Peak egg production (%)	95.55	100.00	100.00	100.00	97.78	1.41	0.17
Feed cost/dozen egg (₦)	34.53^{b}	33.94^{b}	25.00^{a}	30.91^{ab}	29.05^{a}	2.02	0.04
Feed cost/crate of egg (₦)	86.33^{a}	84.85^{b}	62.50^{e}	77.28^{c}	72.63^{d}	0.08	0.01

[abc] Means with different superscripts within the same row are significantly($P<0.05$) different. SEM= Standard error of the means

Table 4: Effects of dietary threonine levels on egg quality characteristics of Japanese quail hens (6-20weeks).

	Dietary Levels of Threonine (%)						
Parameters	**0.71**	**0.76**	**0.80**	**0.85**	**0.90**	**SEM**	**Pr>F**
Egg weight (g)	9.82	9.55	9.99	9.79	9.72	0.24	0.75
Egg length (mm)	31.40	31.54	31.44	31.08	31.02	0.42	0.86
Egg diameter (mm)	23.99^{b}	23.90^{b}	24.54^{a}	24.23^{a}	24.21^{a}	0.21	0.02
Albumen length (mm)	48.86^{a}	50.88^{a}	48.93^{a}	50.07^{a}	48.18^{b}	0.95	0.04
Albumen height (mm)	3.30^{b}	3.34^{b}	3.51^{a}	3.71^{a}	3.71^{a}	0.13	0.04
Albumen weight (g)	4.94	5.02	5.16	4.89	5.03	0.17	0.13
Yolk height (mm)	24.81	23.96	24.90	24.10	24.73	0.48	0.84
Yolk weight (g)	3.27^{a}	3.15^{b}	3.41^{a}	3.47^{a}	3.27^{a}	0.10	0.02
Yolk diameter (mm)	9.42	9.59	10.20	9.77	9.75	0.27	0.38
Shell weight (g)	0.79	0.80	0.84	0.76	0.82	0.05	0.71
Shell thickness (mm)	0.49^{a}	0.48^{b}	0.48^{b}	0.49^{a}	0.48^{a}	0.00	0.03
Egg shape index	0.77	0.76	0.78	0.78	0.78	0.01	0.37
Egg specific gravity	1.08^{a}	1.08^{a}	1.08^{a}	1.07^{b}	1.08^{a}	0.01	0.02
Haugh unit	84.06^{b}	84.59^{b}	85.20^{a}	86.58^{a}	86.67^{a}	0.86	0.04
Egg shell index	3.65^{c}	3.76^{b}	3.85^{a}	3.52^{d}	3.84^{a}	0.03	0.02
Egg yolk index	2.64^{a}	2.50^{c}	2.44^{d}	2.48^{cd}	2.54^{b}	0.01	0.01

[abc] Means in the same row with different superscript are significantly ($p<0.05$) different. SEM= Standard error of the means.