Journal of Animal Feed Science and Technology 5 (2017) 09-14
DOI: http://dx.doi.org/10.21088/jafst.2321.1628.5117.2

Effect of level of protein and concentrations of amino acids on egg quality parameters in WL layers

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Keywords:

Egg Production
Haugh Unit Score
Lysine, Protein
Threonine.

Abstract

Quality of egg is important not only for processing market by also for long term storage of shell eggs. Protein/ amino acid levels in diet will influence the quality of egg. Hence two experiments were conducted to assess the quality of an egg at various levels of protein and different concentration of lysine and threonine. 528 and 390 birds at the age of 25 weeks were procured separately and randomly allotted into 11 and 13 treatment groups for experiment -1 and 2 respectively. In experiment 1 the birds were fed with low protein (13.36% CP) and medium protein (15.78% CP) diets each with 6 replicates of 8 birds each at 5 different concentrations of lysine. In experiment-2 two protein groups (13.46% CP/0.65% lysine and 15.56% CP/0.60% lysine) with two lysine levels, each protein was supplemented with 6 concentrations of threonine, fed to 5 replicates each with 6 birds. In both the experiments the control was with 17%CP, 0.70% lysine and 66% threonine. Two eggs/replicate/period a total of 132 eggs/period and 130 eggs/period was collected and brake open for quality assessment in experiment 1 and 2 respectively. Out of all egg quality parameters haugh unit score was increased significantly with increasing amino acid concentration in experiment one. But lower the Haugh unit score at high protein, low lysine and high threonine concentrations were observed in experiment 2. No change in other parameters in both the experiments. Basing on these results it can be concluded that concentration of amino acids especially lysine plays a major role in maintaining the egg quality.

Introduction

Sky rocketing feed ingredient cost in poultry production has been a matter of concern to the nutritionist and farmers. It has been reported that one percent reduction in dietary crude protein through improved amino acid formulations, there is a ten percent reduction in nitrogen losses in poultry waste. Low protein diets with required amino acid composition not only reduces the pollution but also minimize the cost of production [1] without effecting the performance of birds. The primary objective of poultry nutrition is to obtain highest level of performance, reduce nutrient burden on the environment and maximise the profits.

Now the concept has been changed from crude protein to ideal amino acid ratio. Lysine is the base for ideal amino acid profile. It emphasize the need to know the lysine requirement for optimal performance and profits. Lysine is the second limiting amino acid after methionine in corn soy diet [2].

Lysine requirement of hens varied from 650 to 900mg/hen per day [3,4] . Some studies [5,6,7] reported that the optimal Methionine+Cystine/Lysine (Met + Cys/Lys) ratio for laying hens was 0.75. If the protein level of diets changes, the natural lysine content also varies. Threonine is the third limiting amino acid for layers. Threonine participates in

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Received on 02.05.2017, **Accepted on** 15.05.2017

synthesis of protein and several important metabolic products *viz.* glycine, acetyl Co-A, pyruvate [8] and uric acid [9]. Further, threonine aids in formation of collagen, elastin, and antibodies [10].

Egg quality has become an important aspect of egg marketing. Egg quality comprises a number of factors related to the shell, albumin and yolk, quality may be divided into external and internal appearance [11].

Egg quality is determined by its consumer acceptance with respect to several characteristics including cleanliness, freshness, surface area, mass, volume and coefficient of packaging, egg weight, shell quality, yolk index, albumen index, haugh unit and chemical composition [12] .

All egg quality characteristics are affected by several factors including age and genotype of hen, nutrition, type of rearing system and the time of oviposition [13,14].

Retail outlets are now demanding high standards for conventional internal and external quality characteristics. Quality of protein in diet influences the protein constituents of albumen and yolk of egg. Works carried out so far on protein level, amino acid concentrations in diet focused mainly on egg production and economics. Hence there is a need to quantify the influence of protein and amino acid concentration in diets of layers on egg quality.

Therefore, two experiments (section I and II) were conducted to determine the effect of various dietary concentrations of lysine and threonine at various protein levels on egg quality in WL layers.

Materials and Methods

Birds and Dietary Treatments

In experiment I a total of 528 WL layers (BV-300) aged 25 weeks were randomly distributed into 11 treatment groups each with 6 replicates of 8 birds. They were fed 11 types of diets as illustrated in Table 1 such that treatment diets consisted of 5 different lysine concentrations (0.50, 0.55, 0.60, 0.65, and 0.70%) at 2 protein levels i.e. 13.36 (LCP) and 15.78%(MCP) and control diet with 17% CP and 0.70% lysine. All these diets were iso caloric (2700 Kcal/Kg). A constant ratio was maintained between digestible Mehtionine+ Cysitne, Threonine, Tryptophan, Arginine, Leucine, Iso Leucine and Valine (88, 66, 19, 114, 72 and 80) to lysine.

Where as in experiment –II a total of 390 WL layers aged 25 weeks were randomly allocated into 13 treatment groups of 5 replicates each with 6 hens per replicate. Basing on first experiment two basal diets were prepared with two levels of proteins with two levels of lysine (CP at 13.46 with 0.65% lysine and 15.56 with 0.60% lysine) were tested at various concentrations (60, 63, 66, 69,72 and 75% of lysine as threonine) of threonine in diet. To these basal diets L-threonine at 0, 1.2, 2.4, 3.6, 4.8 and 6.0; 0, 0.9, 1.8, 3.6, 5.4 and 7.2 percent was added to basal diets 1-6 and 7-12 respectively. In this experiment control diet was prepared with 17% CP, 0.70% lysine and 66% of lysine as threonine. All these diets were iso caloric (2700 Kcal/Kg). A constant ratio was maintained between digestible Mehtionine+ Cysitne, Tryptophan, Arginine, Leucine, Iso Leucine and Valine (88, 19, 114, 72 and 80) to lysine.

Fluorescent bulbs were used to provide 16 h of light daily including normal day light. The birds were housed in cages with 4 and 3 hens in each cage (18′′ × 15′′ × 15′′) in expt. I and II respectively. Two adjacent cages were used as treatment replicate. These trials were conducted for a period of 20 weeks. Feed and water were provided ad libitum. The protocol of the current study was approved by the Animal Ethics committee of the institute.

Data Collection

Production parameters were recorded. The number of eggs produced with defective or broken shells and the number of shell less eggs were recorded as egg shell defects (ESD), and expressed in relation to the total number of eggs produced.

Egg Quality

During the last 3 days in each period (28days), 2 eggs per replicate were randomly collected to assess egg quality parameters. A total of 132 eggs/period in experiment I (2x6x11) and 130 eggs/period in experiment –II (2x5x13) were utilised for the measurement of component (yolk, albumen, and shell) percentages, shape, and quality. The major axis of the egg was defined as the longest length of longitudinal diameter and minor axis was defined as the longest length of latitudinal diameter, and these 2 parameters for eggs were obtained using Vernier calipers (accurate to 0.01 mm) (SuCe Measuring Instruments Co., Ltd., China). Eggshell thickness was measured using a micrometer (accurate to 0.001 mm) on at least 5 sites, including 3 equatorial sites, one blunt, and one sharp ends of the shell. The yolk color was measure by using Roche yolk color fan. Haugh units were calculated using the following formula: HU = 100log10 (H -1.7 W0.37 + 7.56), where HU = Haugh unit, H = albumen height (mm), and W=egg weight (g).

Statistical Analysis

Data were statistically analysed by one- way ANOVA using SPSS for windows [15]. The significant differences (p< 0.05) seen in between means was determined by Duncans [16] multiple comparison test.

Results

Experiment I

There is no significant effect on, ESD, albumin index, yolk color, shell thickness, shell weight, egg shape index by offering the diets with various levels of protein and different concentration of lysine/ threonine in diet. Significant increase in haugh unit score was observed with increase in concentration of lysine at both the protein levels in experiment I, whereas, no significant variation of Haugh unit in low protein/HL group but significant decrease in high protein/LL group was observed in experiment II.

Significant increase in yolk index value with increase in lysine concentration in high protein group,

Table 1: Nutrient Composition (%) of different dietary treatments fed to WL layers (25-44 weeks) in experiment I

D Lysine (%)	0.50	0.55	0.60	0.65	0.70	0.50	0.55	0.60	0.65	0.70	0.70
Crude protein GROUP	CP 13.36%/LCP I					CP 15.78%/MCP II					Control
ME(kcal/Kg)	2700	2698	2699	2696	2702	2698	2698	2699	2699	2696	2697
CP(%)	13.39	13.34	13.40	13.36	13.38	15.76	15.75	15.80	15.79	15.80	17.06
Total Lysine(%)	0.539	0.537	0.537	0.538	0.539	0.571	0.567	0.571	0.578	0.583	0.705
Total M+C	0.488	0.484	0.483	0.473	0.467	0.570	0.569	0.567	0.562	0.559	0.581
Total Threonine	0.486	0.481	0.500	0.506	0.546	0.562	0.559	0.558	0.556	0.555	0.613
Total Tryptophan	0.132	0.131	0.131	0.203	0.248	0.142	0.141	0.141	0.152	0.158	0.167
Total Arginine	0.804	0.801	0.799	0.787	0.782	0.860	0.854	0.858	0.864	0.866	1.005
Total Isoleucine	0.510	0.504	0.502	0.498	0.494	0.604	0.601	0.600	0.598	0.596	0.668
Total Valine	0.633	0.627	0.625	0.614	0.607	0.737	0.733	0.733	0.728	0.725	0.787
Calcium	4.603	4.603	4.603	4.603	4.451	4.605	4.605	4.605	4.606	4.606	4.428
Available Phosphorus	0.457	0.457	0.456	0.455	0.454	0.451	0.450	0.450	0.451	0.450	0.453
Sodium	0.181	0.181	0.181	0.181	0.181	0.173	0.173	0.173	0.172	0.172	0.172
Chloride	0.186	0.186	0.186	0.185	0.185	0.187	0.187	0.186	0.186	0.186	0.185

Table 2: Nutrient Composition (%) of different dietary treatments fed to WL layers (25-44 weeks) in experiment II

Ingredients		Diet (in Kgs) Basal Diet-I	Basal Diet II	Control
	d.Thr	60LCP/HL	60 MCP/LL	66
	d.Lysine	0.65	0.60	0.70
		D1-D6	D7-D12	D13 (Control)
Metabolizable Energy (kcal/Kg)		2704	2702	2706
Crude Protien (%)		13.46	15.56	17.05
Total Lysine (%)		0.880	0.820	0.860
Total M+C (%)		0.690	0.630	0.740
Total Thr (%)		0.500	0.460	0.600
Total Tryptophan		0.160	0.170	0.180
Total Arginine		0.940	1.200	1.040
Total Isoleucine		0.560	0.510	0.630
Total Valine		0.650	0.660	0.760
Calcium (%)		4.350	4.350	4.350
Available Phosphorus (%)		0.430	0.430	0.440
Sodium (%)		0.190	0.190	0.180
Chloride (%)		0.170	0.160	0.180

Table 3: Effect of various levels of protein and concentration of lysine on egg quality parameters (Experiment I)

Groups	Lys. (%)	Protein (%)	Egg shell defects (%)	Albumin index	Haugh unit score	Yolk index	Yolk color index DSM 3/1108:5.0)	Shell thickness (mm)	Shell weight (%)	Egg shape index
LCP	0.50	13.36	0.684	0.089	84.13^{c}	0.441^{a}	4.179	0.352	9.883	75.23
	0.55	13.36	0.558	0.089	85.30^{bc}	0.437^{ab}	3.936	0.339	9.577	76.59
	0.60	13.36	0.763	0.089	86.58^{abc}	0.429^{ab}	3.776	0.350	9.788	76.99
	0.65	13.36	0.901	0.089	88.42^{ab}	0.432^{ab}	3.635	0.353	9.813	76.43

	0.70	13.36	0.705	0.090	90.23[a]	0.402[cde]	3.601	0.353	9.729	75.94
MCP	0.50	15.78	0.701	0.089	85.98[bc]	0.410[cd]	4.078	0.352	9.787	77.07
	0.55	15.78	1.110	0.089	86.74[ab]	0.387[e]	3.871	0.348	10.04	76.80
	0.60	15.78	0.834	0.089	86.58[abc]	0.397[de]	3.803	0.353	9.896	75.84
	0.65	15.78	0.866	0.089	88.73[ab]	0.399[de]	3.636	0.354	9.756	76.97
	0.70	15.78	0.981	0.089	88.14[ab]	0.418[bc]	3.501	0.349	9.671	76.80
Control	0.70	17.00	0.983	0.088	86.73[ab]	0.429[ab]	3.634	0.344	9.632	75.98
	SEM		0.052	0.145	0.467	0.022		0.933	0.335	0.072
	N		6	6	6	6		6	6	6
	P-value		0.558	0.111	0.020	0.002		0.106	0.177	0.149

Table 4: Effect of various levels of protein/lysine and concentration of threonine on egg quality parameters (Experiment II)

Groups	d. Lysine / CP (%)	d. Thr. (As % lys)	Egg shell defects	Albumin index	Haugh unit score	Yolk index	Yolk color index DSM 3/1108:5.0	Shell thickness(mm)	Shell weight (%)	Egg shape index
LCP/HL	0.65/13.46	60	0.506	0.087	91.44[a]	0.451	3.237	0.379	10.63	79.27
	0.65/13.46	63	0.556	0.088	92.55[a]	0.449	2.515	0.353	10.80	77.24
	0.65/13.46	66	0.728	0.091	91.42[a]	0.450	2.737	0.367	10.65	77.47
	0.65/13.46	69	0.690	0.093	92.41[a]	0.452	2.205	0.368	10.47	77.58
	0.65/13.46	72	0.932	0.089	90.17[ab]	0.452	2.732	0.367	10.70	77.18
	0.65/13.46	75	0.768	0.088	90.96[ab]	0.449	2.593	0.363	10.73	77.12
MCP/LL	0.60/15.56	60	0.664	0.085	90.17[ab]	0.454	2.762	0.397	10.28	77.27
	0.60/15.56	63	0.792	0.089	91.59[a]	0.450	2.525	0.400	11.26	76.58
	0.60/15.56	66	0.540	0.086	91.93[a]	0.450	2.499	0.372	10.02	79.47
	0.60/15.56	69	0.486	0.088	89.84[b]	0.443	2.384	0.384	10.67	75.62
	0.60/15.56	72	0.474	0.086	89.98[b]	0.452	2.485	0.380	10.73	77.00
	0.60/15.56	75	0.496	0.086	87.66[c]	0.441	2.194	0.367	10.70	76.78
Control	0.70/17.05	66	0.462	0.088	88.59[b]	0.449	2.412	0.356	10.26	76.47
	SEM		0.041	0.022	0.264	0.928		0.542	0.061	0.274
	N		5	5	5	5		5	5	5
	P value		0.439	0.118	0.007	0.141		0.151	0.133	0.345

whereas significant decrease in yolk index value with increase in lysine concentration in low protein group were observed in experiment I. No significant variation in yolk index values were observed due to variation in either protein/lysine or threonine concentration in diet in experiment-II.

Discussion

Egg Shell Defects

There is no significant influence on egg shell quality by alterations in diet of birds. This might be due protein /amino acid concentration had no effect on absorption of calcium and formation of shell.

Albumin Quality

Albumin index values were not influenced either by level of protein or concentrations of lysine/ threonine in both the experiment. Whereas significant increase in Haugh unit score with increase in lysine concentration was observed at both the protein levels in experiment I. Haugh unit is a measure of albumen quality and therefore freshness of the egg [17], while [18] proposed measuring albumen height to determine egg quality. These findings were in collaboration with [19], by altering the protein and lysine in diets of Isa-Brown laying hens. Increase in albumin quality during high ambient temperature was observed by supplementation of ascorbic acid [20] and increasing with vitamin E [21, 22].

In experiment II no significant variation in haughunit score at LCP/HL group, but significant decrease in Haugh unit was observed with increased threonine in diet in MCP/LL group. These are on par with the findings of [23], who observed decrease in albumen quality with increasing dietary protein and amino acid content, similarly [24], also reported decreased Haugh unit score with the dietary addition of neem kernel meal. Albumin quality also influenced by addition of different types or cultivars of grains such as pearl millet [25] or wheat [26] in diet.

Yolk Quality

Significant decrease in yolk index values were observed in low protein groups with increase in concentration of lysine in diet, whereas significant increase in yolk index with increase in concentration of lysine in MCP group. No variation in yolk index in experiment II with variation in protein, lysine and threonine in diet of birds. Dietary changes had no significant influence on yolk colour in both the experiments.

Yolk quality is determined by the colour, texture, firmness and smell of the yolk [27]. Although yolk colour is a key factor in any consumer survey relating to egg quality [27], consumer preferences for yolk colour are highly subjective and vary widely from country to country. The primary determinant of yolk colour is the xanthophyll (plant pigment) content of the diet consumed. Pale yolks can result from any factor which alters or prevents the absorption of pigments from the diet or the deposition of these pigments in the yolk. Yolk color has a considerable influence on egg marketing.

Shell Quality

Diet had no significant influence on shell thickness, shell weight and shell defects. Increase in egg weight over a production period while decrease in egg shell thickness and strength. Egg shell quality depends on egg size and egg weight. Shell strength and thickness were highly correlated to each other.

Shape Index

There is no significant variation in shape index. This may be due to all the birds are of same strain (same genetic potentiality). Same hatch, management also under uniform condition. This indicates that nutrition had no influence on shape index of the egg. This was coincides with the reports of [28].

Conclusions

Haugh unit score of an egg indicates the albumin quality. Albumin is the rich source of protein. This experiment has proven the dietary protein/amino acid concentration had influence on haughunit score. Basing on this trails it can be concluded that other than good management, best practice with respect to bird husbandry, careful egg collection, handling, processing the diet offered to the birds also will influence the quality of the final product.

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