Original Research

Assessment of Optimum Threonine Concentration in Diets of WL Layers at Peak Production

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Abstract

A trial was conducted to assess the requirement of digestible threonine concentration in the diets of WL layers. Pullets (390) were fed (25-44 weeks) with 13 test diets (6+6+1)containing protein at two levels i.e. 13.46 at 0.65% lysine and 15.56 at 0.60% lysine each with six graded concentrations of threonine (60, 63, 66, 69, 72,75) and a control with 17 % protein , 0.70 % lysine and 66% of threonine. Each diet was fed adlibitum to five replicates of six birds. Hen day egg production (HDEP) did not varied with threonine concentration in diet in low crude protein group (LCP), where as a significant increase in HDEP was noticed in medium crude protein (MCP) group. Feed intake (FI) was significantly decreased with increase in threonine in LCP group, but no variation was observed in FI in MCP group. Other production parameters like egg weight, egg mass, feed efficiency (g/g), mortality and body weight gain were not influenced (p>0.05) by the concentration of threonine in diet. Returns over the feed cost were highest at 63% threonine concentration at both the protein levels. It can be concluded that WL layers require approximately 63% of lysine as threonine in diet for optimum production.

Key words: Layer, Threonine, Production, Economics

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Introduction

Ideal protein concept is currently being used to determine the ideal amino acid profile in diets of poultry (Pastor *et al.*, 2013). Formulation of diets based on crude protein (CP) concept leads to a situation that lack or excess of certain amino acids. As a result the performance of the birds will get affected. Instead of providing CP as such, supplementation of essential amino acids to layer diet will fulfil the needs without affecting the performance. Low protein diets prepared with corn soy, threonine is the third limiting amino acid in low protein diets prepared with cornsoy for commercial poultry and the level of threonine in diet compromises the performance of the layers (Schmidt *et al.*, 2010). Threonine is important for formation of protein and maintenance of protein turnover. Threonine functions as a nutrient immunomodulator in maintaining the intestinal barrier function (Azzam *et al.*, 2011). Threonine participates in synthesis of protein and several important metabolic products *viz.* glycine, acetyl Co-A, pyruvate (Kidd and Kerr, 1996) and uric acid (Martinez *et al.*, 1999). Further, threonine aids in formation of collagen, elastin, and antibodies (Sa *et al.*, 2007). Threonine @470mg/d is required for layers as per NRC, 1994. Whereas, (Gheisar *et al.*, 2011) inferred that low protein diets with threonine supplementation had no negative effect on production in layers. But the exact requirement of digestible threonine for layers in corn soy ration at lower protein levels was not clear. Hence, a study was conducted to assess the threonine requirement for the layers at peak production as a ratio to lysine at various protein levels.

Materials and Methods

Experimental Birds

A total of 390 white leghorn layers (BV-300) at 24 weeks with body weight ranges form 1285-1315g were randomly distributed and housed in colony cages (3 birds/cage). Two adjacent cages with a common feeder were considered as one replicate. Birds were allotted into 13 treatment groups of 5 replicates each with 6 hens per replicate. The laying hens were offered experimental diets from 25-44 weeks of age. Hens were given free access to water and experimental diets. The protocol of the current study was approved by the Animal Ethics committee of the institute.

Experimental Diet

A total of 13 diets were prepared. A diet with 0.70 per cent digestible lysine (17.0% CP) and 66% of lysine as threonine was kept as control (Table 1). Two basal diets were prepared one with lysine at 0.65 per cent and 13.46 per cent CP second basal diet was with 0.60 per cent lysine at 15.56 per cent CP. To these basal diets crystalline threonine was supplemented at six graded concentrations (60, 63, 66, 69, 72 and 75% of lysine as threonine). Energy levels in all 13 diets were maintained at 2700 kcal / kg. The ratio between digestible methionine +cystine (M+C), tryptophan (Trp), arginine (Arg), isoleucine (Ile) and valine (Val) to digestible lysine (Lys) were 86, 19, 114, 72 and 80 per cent respectively and were maintained constant in all the diets. Diets 1-6 were prepared by adding crystalline L-threonine at 0,1.2, 2.4, 3.6, 4.8 and 6.0 per cent respectively, while diets 7-12 were prepared by adding crystalline L-threionine at 0, 0.9, 1.8, 3.6, 5.4 and 7.2 per cent respectively.

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Table 1: Ingredient composition (%) of dietary treatments fed to layers (25-44weeks**)**

Ingredient(in Kg)	Basal Diet-I	Basal Diet -II	Control
d. Thr (%)	60	60	66
d. Lysine (%)	0.65	0.60	0.70
	D1-D6	D7-D12	D13(Control)
Maize	412	362	364
Soy bean meal	70	27	108
Deoiled rice bran	126	192	150
Pearl Millet (Bajra)	190	120	98
Ground nut cake	41	103	95
Cotton seed meal	15	50	40
Stone	120	120	120
Salt	4	4	4
Lysine HCl	2.15	2.3	1.45
DL Methionine	1.45	1.29	1.8
L Threonine	0	0	0
Dicalcium Phosphate	12	12	12
Additives*	5.8	5.8	5.8
Total	**999.8**	**999.9**	**1000.5**
M.E (kcal/Kg)#	2704	2702	2706
Crude Protein (%) $	13.46	15.56	17.05

**Provided (/Kg diet): Vit. A 50MIU, Vit. D3 14miu, Vit. E 20g, Vit. K3 8g, Vit. B1 3.2g, B2 32g, B6 3.6g, B12 0.024g, Niacin 28g, Calcium pantothenate 16g, Folic acid 5.6g. Trace minerals (in each kg): Manganese 70g, Zinc 70g, Iron 50g, Cobalt 0.6g, Iodine 0.6g, Copper 10g, Selenium 0.06g. Saccharomyces and Lactobacillus species, Acidifier; # Calculated; $ Analyzed*

Parameters Studied

The body weight (BW) of three birds per replicate was recorded at the beginning and at the end of each period (28 days). Feed intake and feed efficiency (g/g) were measured weekly and pooled for the period. Egg production and mortality were recorded daily. Egg weight was calculated as mean weight of eggs collected in the last three consecutive days of each period. Egg mass (egg production × average egg weight) was calculated period wise. During the last 3 consecutive days in each period 2 eggs /replicate and a total of 130 eggs/day were collected and analyzed for egg quality traits.

Statistical Analysis

Data was statistically analysed by one-way ANOVA using SPSS for windows (SPSS Inc. 2002). The significant differences ($p< 0.05$) seen in between means was determined by Duncans multiple comparison test (Duncan, 1955). The effect of various concentration of d. threonine were determined by using orthogonal polynomials for linear, quadratic, and cubic effects. Quadratic regression and equation were also determined.

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Results and Discussion

Hen Day Egg Production (%)

Egg production (EP) was not influenced by the concentration of threonine in LCP group, whereas increase in production with increase in threonine concentration was observed in MCP group (Table 2). The mean egg production was higher at 74(R^2 =0.008) and 75% (R^2 =0.253) threonine in LCP and MCP groups (Table 6&7). Threonine concentration in LCP group had no influence on HDEP might be due to the increased intake of threonine in LCP group when compared to MCP group (Table 4). Availability of lysine and other amino acids (essential) are more in LCP group than MCP group may be the reason for lower production at medium protein group than LCP (Table 4).

Table 2: Effect of various concentrations of d. Threonine on production parameters in WLH layers (25-44 weeks)

Groups	d. Lysine / CP (%)	d. thr. (lysine)	HDEP	FI/B (g)	EW(g)	EM(g)	FE(g/g)	Mortality (%)	BWG (g)
LCP/	0.65/13.46	60	90.18[abc]	117.2[a]	54.7	1413	2.968	1.333	1377
High lysine	0.65/13.46	63	89.48[abcd]	111.1[abc]	54.95	1382	2.842	0	1439
	0.65/13.46	66	91.14[abc]	111.3[abc]	53.37	1362	2.878	0.667	1369
	0.65/13.46	69	93.86[a]	113.3[abc]	53.04	1434	2.854	3	1417
	0.65/13.46	72	90.13[abc]	110.8[bc]	53.59	1367	2.877	1.467	1381
	0.65/13.46	75	92.14[ab]	110.3[bc]	54.46	1414	2.755	0.667	1371
MCP/	0.60/15.56	60	86.40[cd]	109.1[c]	54.82	1327	2.968	0	1447
Low lysine	0.60/15.56	63	84.90[d]	109.1[c]	54.59	1297	3.025	0	1417
	0.60/15.56	66	88.93[abcd]	109.1[c]	54.97	1375	2.964	0.667	1473
	0.60/15.56	69	86.35[cd]	109.1[c]	54.2	1311	2.98	0	1384
	0.60/15.56	72	88.13[bcd]	109.1[c]	54.79	1342	2.846	0	1399
	0.60/15.56	75	89.99[abc]	109.1[c]	53.75	1359	2.985	0.667	1389
Control	0.70/17.05	66	92.19[ab]	116.3[ab]	53.92	1412	2.93	2.133	1360
SEM			0.498	0.596	0.182	9.756	0.021	0.234	9.443
N			5	5	5	5	5	5	5
P value			0.006	0.046	0.501	0.118	0.471	0.244	0.439

This results indicating the need of amino acids is equal or less than the lowest level tested in this work (60% of lysine as threonine) at LCP group. These are coincides with results of (Figueiredo *et al.*, 2012), who reported no effect on egg production by incorporation of various levels of lysine (0.675, 0.743,0.811 and 0.879%) in combination with various concentrations of threonine (0.542, 0.596 and 0.650%) in diets of Hy-Line W36 laying hens at 42-58 weeks of age. The increase in egg production in MCP group may be due to lesser the intake of threonine (Table 4) and low supplemental levels might not be sufficient to meet the production potential.

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Feed Intake (g)

Significantly lower feed intake/bird (FI/B) was observed at 72 % (R^2 =0.056) threonine concentration in LCP group, but no variations in FI in MCP group (R^2 =0.163). Higher the feed intake in LCP group might be for meeting the egg production potential at low threonine concentrations birds. Similar observations were noticed (Rocha *et al.*, 2013) by incorporation of various digestible threonine to lysine ratios (65, 70, 75, 80, 85 and 90%) at 14.2% CP in layers of 24 to 40 weeks age. Similarly, (Azzam *et al.*, 2011; Faria *et al.*, 2002 and Martinez- Amezcua *et al.*, 1999) also reported the same.

Table 3: Economics in WL layers fed with various concentrations of d.threonine at two levels of d. lysine

Groups	d.Lysine/ CP (%)	d.Thr./ d.lys.	Cost of feed (Rs./Kg)	Cumulative feed cost/ entire experiment/bird (□.)	Returns through sale of eggs (□.) @Rs 2.50/-	Returns over feed cost (□.)	Gain over control □.)
LCP/	0.65/13.46	60	16.98	278.61	388.11	109.5	46.05
High lysine	0.65/13.46	63	17.05	265.2	391.15	125.95	62.5
	0.65/13.46	66	17.15	267.23	384.02	116.79	53.34
	0.65/13.46	69	17.28	274.1	372.9	98.8	35.35
	0.65/13.46	72	17.38	269.6	388.33	118.73	55.28
	0.65/13.46	75	17.49	270.08	379.86	109.78	46.32
MCP/Low Lysine	0.60/15.56	60	17.68	277.47	405.09	127.62	64.17
	0.60/15.56	63	17.68	276.23	412.25	136.02	72.56
	0.60/15.56	66	17.78	288	393.57	105.57	42.11
	0.60/15.56	69	17.89	277.76	405.33	127.57	64.11
	0.60/15.56	72	18	275.69	397.14	121.45	58
	0.60/15.56	75	18.11	291.32	388.93	97.61	34.16
Control	0.70/ 17.00	66	19.42	316.2	379.65	63.45	0

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Table 4: Various levels of lysine and threonine in diet and their intake by WL layers (25-44weeks of age)

CP (%)	Lysine (%)	Lysine level in diet (mg)	Lysine intake (mg/b/d)	Threonine (%) in diet	Threonine level in diet (mg)	Threonine intake (mg/b/d)
13.46	0.65	482.91	565.97	60	390	457.08
13.46	0.65	482.91	536.51	63	409	454.95
13.46	0.65	482.91	537.48	66	429	477.48
13.46	0.65	482.91	547.14	69	448	508.15
13.46	0.65	482.91	535.06	72	468	518.54
13.46	0.65	482.91	532.65	75	487	537.71
15.56	0.6	385.6	420.69	60	360	392.76
15.56	0.6	385.6	420.69	63	378	412.4
15.56	0.6	385.6	420.69	66	396	432.04
15.56	0.6	385.6	420.69	69	414	451.67
15.56	0.6	385.6	420.69	72	432	471.31
15.56	0.6	385.6	420.69	75	450	490.95

Table 5: Egg quality parameters of eggs collected from WL layers (25-44weeks) fed with various concentrations of threonine in diet

Lysine/CP	Threonine (%)	Shape index	Haugh unit	Yolk Index	Shell Thickness(mm)	Yolk colour
0.65/13.46	60	79.27	71.44[a]	45.1	0.379	6.237
0.65/13.46	63	77.24	72.55[a]	44.9	0.353	5.515
0.65/13.46	66	77.47	71.42[a]	45	0.367	5.737
0.65/13.46	69	77.58	72.41[a]	45.2	0.368	5.205
0.65/13.46	72	77.18	70.17[ab]	45.2	0.367	5.732
0.65/13.46	75	77.12	70.96[ab]	44.9	0.363	5.593
0.60/15.56	60	77.27	70.17[ab]	45.4	0.397	5.762
0.60/15.56	63	76.58	71.59[a]	45	0.4	5.525
0.60/15.56	66	79.47	71.93[a]	45	0.372	5.499
0.60/15.56	69	75.62	69.84[b]	44.3	0.384	5.384
0.60/15.56	72	77	69.98[b]	45.2	0.38	5.485
0.60/15.56	75	76.78	67.66[c]	44.1	0.367	5.194
0.70/17.00	66	76.47	68.59[b]	44.9	0.356	5.412
SEM		0.274	0.264	0.928	0.542	
N		5	5	5	5	
P		0.345	0.007	0.141	0.151	

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Table 6: Quadratic equation summary of threonine (%) requirements of WL layers (25-44wks) at LCP (13.46%)

Parameters	Quadratic equation	R^2	Requirement of Threonine (%)
Egg production (%)	$Y= 88.772+1.073X-0.088X^2$	0.008	74
Feed intake/bird(g)	$Y= 120.384-4.716X+0.569X^2$	0.056	74
Egg weight (g)	$Y= 55.89-1.071X+0.120X^2$	0.041	60
Egg mass(g/period)	$Y= 1417.8-17.259X+2.446X^2$	0.044	60
Feed efficiency (g/g)	$Y= 3.028-0.087X+0.010X^2$	0.01	74
Mortality (%)	$Y= 0.524+0.263X-0.013X^2$	0.041	60
Body weight gain(g)	$Y= 1379.054+15.946X-2.773X^2$	0.023	74

Table 7: Quadratic equation summary of threonine (%) requirements of WL layers (25-44wks) at MCP (15.56%)

Parameters	Quadratic equation	R^2	Requirement of Threonine (%)
Egg production (%)	$Y= 86.641-0.603X+0.195X^2$	0.253	75
Feed intake/bird(g)	$Y= 112.189-2.640X+0.426X^2$	0.163	75
Egg weight (g)	$Y= 54.751+0.060X-0.028X^2$	0.002	75
Egg mass(g/period)	$Y= 1339.91-16.393X+3.585X^2$	0.084	60
Feed efficiency(g/g)	$Y= 3.021-0.024X+0.002X^2$	0.046	75
Mortality (%)	$Y= 0.724-0.573X+0.103X^2$	0.119	75
Body weight gain(g)	$Y=1448.03-2.366X-1.444X^2$	0.131	60

Feed Efficiency (FE)

Feed efficiency was not influenced by the concentration of threonine in diet irrespective to the level of protein and lysine in diet of WL layers from 25-44 weeks of age. Feed efficiency was calculated in this study on g/g basis. Even though increase in production with increase in threonine concentration in MCP group, but the HDEP values were lower than the HDEP in LCP group. Feed intake/b is higher in LCP group than MCP group. Higher feed intake and higher egg production in LCP group and low HDEP and low feed intake in MCP are the reasons for no effect of threonine on feed efficiency.

Other Parameters

Egg weight (EW), egg mass (EM), mortality and body weight gain (BWG) were not influenced by the concentration of threonine. Similarly (Biazzi *et al.*, 2014) reported that no effect on egg weight, egg mass, feed efficiency, mortality and body weight gain by incorporation of 4.6, 4.8, 5.2, 5.5 and 5.8 g/kg of digestible threonine in Sahver brown pullets at 75-90 weeks of age. These are in line with (Azzam *et al.*, 2014 and Figueiredo *et al.*, 2012). This indicating that the need for this amino acid is at or below the first level used in this work i.e. 60% of lysine as threonine or 457.08 and 392.76 mg/b/d in LCP and MCP groups respectively. Nunes *et al.*, 2015, inferred that no significant effect of digestible threonine levels (0.460, 0.490, 0.520, 0.550 and 0.580%) on feed intake, egg production, egg weight, feed conversion ratio and egg mass in shaver brown layers at 50-66 weeks of age.

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Economics

Higher the return over the feed cost through sale of eggs was observed at 63% threonine concentration in diet of layers at both the protein groups (Table 3).

Egg Quality Parameters

Except Haugh unit score other quality parameters were not influenced by the concentration of threonine in diet. Haugh unit score was not influenced by the concentration of threonine in LCP group whereas, decrease the HU with increasing the concentration of threonine, in diet was observed in MCP group. Haugh unit score indicates the quality of albumin. Higher the value indicates the best quality of albumin. Major portion of albumin is made up of proteins. There was no significant variation in HU scores in LCP group indicating that the diet /nutrients are optimum for maintaining egg quality. Whereas, decrease in HU score in MCP might be due to lower the availability of essential amino acids in MCP group. (Shim *et al.*, 2013) reported that higher the mean Haugh unit values in low protein diets (21.62, 19.05, 16.32, and16.05% are high protein diets, 2% less than is medium and 4% less than these are low protein diets) of Bovans (18-74weeks). Whereas, Gunawardana *et al.*, 2008, observed no effect on HU by various protein levels in layer diets.

Conclusion

Feed efficiency and economics will influence the profits in the poultry industry. Hence, basing on these two factors it can be concluded that the lowest concentrations supplemented i.e. 63% of lysine as threonine is sufficient enough to maximize the profits at both the protein levels (13.46% CP with 0.65% lysine and 15.56% CP with 0.60% lysine) in diet of WL layers at 25-44 weeks of age. The availability of threonine /bird/day is 454.92 and 412.90mg in 13.46 and 15.56% CP supplementation groups respectively.

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References

1. Azzam M M M, Yuan C, Liu GH and Zou XT. (2014). Effect of excess dietary threonine on laying performance, egg quality, serum free amino acids, and digestive enzymes of laying hens during post peak period. *Journal of Applied Poultry Research*. 23:605-613.
2. Azzam M M M, Dong X Y, Xie P, Wang C and Zou XT. (2011). The effect of supplemental threonine on laying performance, serum free amino acids, and immune function of laying hens under high-temperature and high-humidity environment climates. *Journal of Applied Poultry Research*. 20:361-370.
3. Biazzi H M, Ricardo V N, Vilela C G, Sabrina E T, Valter O V, Rachel S B and Clauber P. (2014). Nutritional levels of digestible threonine in brown-egg laying hens from 75 to 90 weeks of age. Semina: Ciências Agrárias, Londrina, 35(6):3449-3456.
4. Duncan D B. 1955. Multiple range and F-tests. Biometrics 11:1-42.

 DOI 10.5455/ijlr.20180716045157

5. Faria DE, Harms RH and Russell GB. (2002). Threonine requirement of commercial laying hens fed a corn soybean meal diet. *Poultry Science*, 81:809-814.
6. Figueiredo GO, Bertechim M, Fassam EJ, Rodrigues PB, Brito JAG and Castro SF. (2012). Performance and egg quality of laying hens fed with dietary levels of digestible lysine and threonine. Arq.Bras. *Med. Vet. Zootec* 64 (3):743-750.
7. Gheisar MM, Foroudi F and Ghazikhani A. (2011). Effect of using L-threonine and reducing dietary levels of crude protein on egg production in layers. *Iranian Journal of Animal Science* 1: 65-68.
8. Gunawardana P, Roland D A Sr and Bryant MM.(2008). Effect of energy and protein on performance, egg components, egg solids, egg quality, and profits in molted Hy-line W-36 hens. *Journal of Applied Poultry Research* 17:432-439.
9. Kidd MT and Kerr BJ. (1996). Threonine respones in commercial broilers at 30 to 42 days. *Journal of Applied Poultry Research*, 6:362-367.
10. Martinez-Amezcua C, Laparra-Vega JL, Avila-Gonalez E, Fuente B, Jinez T and Kidd MT. (1999). Dietary l-Threonine responses in laying hens. *Journal of Applied Poultry Research* 8: 236-241.
11. Nunes R V, Agustini MAB, Takahashi SE, Murakami AE, Souza CL, Schneiders SE and Polese C. (2015). Nutritional requirement of digestible threonine for brown-egg laying hens from 50 to 66 weeks of age. Semina: Ciências Agrárias Londrina, 36(6): 3877-3886.
12. Pastor A, Wecke C and Liebert F. (2013). Assessing the age dependent optimal dietary branched-chain amino acid ratio in growing chicken by application of a nonlinear modelling procedure. *Poultry Science* 92 (12):3184-3195.
13. Rocha T C, Gomes P C, Donele J L, Barreto S LT, Mello HHC and Brumano G. (2013). Niveisde lisinadigestivelemracoes para poedeiras no period de 24 a 40 semanas de idade. *Revista Brasileira de Zootecnia* 38(9):1726-1731.
14. Sa L M, Gomes P C G, Rostagno H S, Albino L F T and Dagostini P. (2007). Exigencia nutricional de lisina digestivel para galinhaspoedeiras no period de 34 a 50 semanas de idade. *Revista Brasileira de Zootecnia* 36 (6):1829-1836.
15. Schmidt M, Rostango HS, Nunes RV, Pupa JMR and Gomes PC. (2010). Niveis nutricionals de lisina, de metionnina+ cistina e de t reonina digestiveis para gallinhas poedeiras no2 ciclo producao. *Revista Brasileira de Zootecnia Vi cosa.* 39 (5): 1099-1104.
16. Shim MY, Song E, Billard L, Aggrey SE, Pesti GM and Sodsee P. (2013). Effect of balanced dietary protein levels on egg production and egg quality parameters of individual commercial layers. *Poultry Science* 92:2687–2696.
17. SPSS Inc. (2002). SPSS for Windows: Release 11.5, Standard Version, 1989-2002. SPSS Inc., Chicago, IL.

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