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Indian Journal of Animal Sciences **93** (5): 517–522, May 2023/Article
https://doi.org/10.56093/ijans.v93i5.129492

Assessment of digestible amino acid requirement in White leghorn Layers based on production performance at low protein levels in diet

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Received: 26 October 2022; Accepted: 12 April 2023

ABSTRACT

To understand the optimal requirement of d.lysine and threonine in White Leghorn (WL) layers (25-44 weeks), two independent experiments were executed. For the d. lysine study, birds (N=528) were categorized into eleven dietary groups (11×6×8) and were fed with two different protein levels (13.36 and 15.78%) each containing five concentrations of d. Lys. To optimize d. Thr, layers (N=390) were allotted to thirteen groups (13×5×6) in these diets were formulated by fixing the lysine concentrations at 0.65 and 0.60% with 13.36 and 15.78% protein levels respectively. Threonine was supplemented in both diets at graded levels of lysine. Basal diets with 0.70% lysine and 17% protein; threonine at 66% lysine served as corresponding controls. Egg production response to supplemental d. Lys was quadratic. Net feed efficiency and egg mass indices response were in both linear and quadratic. Supplementation of diets with Thr, the response of egg production was linear whereas, it was both linear and quadratic for economic parameters. In conclusion, supplementation of d. Lys at 0.65 and 0.60%; 69 and 72% d. Thr as % of d. Lys at low and high protein diets is optimal for better egg production with economic efficiency in WL layers in the tropics.

Keywords: Crude protein, Economic efficiency, Essential amino acids, Performance efficiency, White Leghorn layers

Poultry offers one of the largest sources of animal protein for human consumption in the world, with the more efficient conversion of feed into quality protein than other livestock. Of all the feed components, protein-based stuff often dictates higher feed prices that further impact the overall economics of poultry enterprises (Khater *et al.* 2020). Energy and amino acid balance in the diet is the most valuable factors which should be defined under variable climatic conditions for optimizing layer performance (Lima *et al.* 2018, Elsayed *et al.* 2019). Amino acids rather than protein per se fulfill the nutrient requirements for production and maintenance (Abdullah *et al.* 2019). Therefore, shifting priorities towards amino acids rather than protein not only addresses cost but also environmental issues (Kumari *et al.* 2016a,b; Torki *et al.* 2014).

Formulation of diets by incorporating amino acids of crystalline (Marco *et al.* 2021) and proteinogenic type enzymes of exogenous origin, growth promoters, probiotics, prebiotics, etc. would possibly achieve sustainable poultry production with minimum environmental impact (Bailey 2020).

The amino acid requirements in birds depend on sex, production status, body composition, and circumstances. The ideal ratio of indispensable amino acids to lysine remains largely unaffected by dietary, environmental, and genetic factors. However, accurate other amino acid requirement concentrations under a variety of circumstances could be expressed by the requirement for other amino acids in % of lysine. Lysine is the second limiting amino acid and threonine is the third limiting amino acid, particularly in diets reduced in protein (Rezaeipour *et al.* 2012) in chicken.

Currently, there is a need to redefine the nutrient requirements more importantly amino acids to support the production of rapidly evolving high-yielding genetic strains under different climatic conditions. Considering the aforementioned, the present study was undertaken to study the digestible lysine and threonine requirements for White Leghorn (WL) layers of BV-300 strain at different dietary protein levels in the tropics.

MATERIALS AND METHODS

All the experiments involving the handling of birds and their management were following the guidelines of the Institute Animal Ethics Committee.

Experimental birds: White Leghorn (WL) layer chicken of commercial BV-300 strain at the first phase of egg production (25 wks) were housed in open-sided high-rise houses comprising three-tier California cages having dimensions of 18" × 15" × 15". At 25 wks of age, four and three layers were placed in each cage for lysine and

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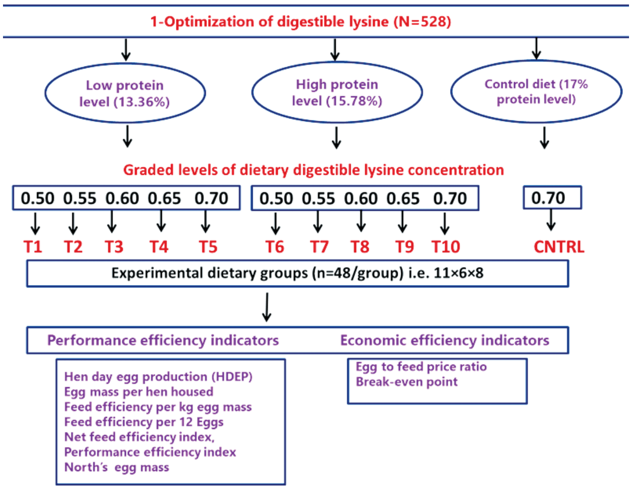

Fig. 1. Experimental design for optimization of d. Lys in WL layers during first phase of egg production.

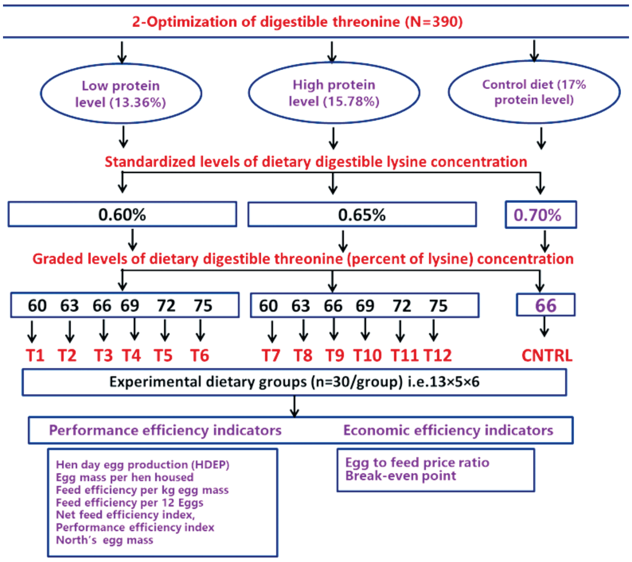

Fig. 2. Experimental design for optimization of d. Thr in WL layers during first phase of egg production.

threonine experiments respectively. Two adjacent cages were considered as one replicate and shared feeder. All the birds were maintained under uniform conditions with a 16 h photoperiod and were following prevailing industry standards. Microclimatic variables were recorded during the study period using digital meters fixed inside the house.

Experimental diets and design: The experimental design of lysine and threonine experiments is detailed in Figs 1-2. All the birds were fed with varied protein and amino acid concentrations for a period of 20 wks (25-44 wks age). For optimization of digestible (d.) lysine, WL layers (N=528) of 25 weeks were randomly distributed into 11 dietary experimental groups each comprising six replicates of eight birds. Different lysine (Ajinomoto, L-Lysine HCL, Brazil) concentrations at 0.5% increment were studied at low and high protein (13.36 and 15.78%) levels. For threonine optimization, initially, lysine concentrations were fixed (based on results in the previous experiment) for both low and high protein levels. Accordingly, graded levels of crystalline threonine (Ajinomoto, L-Threonine (98.5%), Brazil) was added relative to the percent lysine. WL layers (N=390) of 25 weeks were randomly distributed into 13 dietary experimental groups each comprising five replicates of six birds. All the experimental birds were fed with dietary energy of 2700 kcal/kg ME and essential amino acids at 86, 66, 19, 114, 72, and 80% of M+C, Thr, Trp, Arg, Ile, and Val relative to d. lysine respectively. The essential amino acid (EAA) profile in diets was fixed based on the ratio of other d. EAA to lysine as indicated by Lemme (2009).

Feed composition and analysis: Before feed formulation, dietary ingredients were analyzed for proximate principles (AOAC 2005) and amino acids content via Near-infrared spectroscopy using AMINONIR (Amino lab$^{®}$) at Amino Degussa Evonik Industries, Singapore. The ingredient and nutrient composition (Table 1, 2) of formulated experimental diets are given in Supplementary Table 1 and 2. The amino acid concentrations of various feed ingredients/feedstuff is presented in Supplementary Tables 3-4.

Table 1. Nutrient composition of diets formulated for d. lysine optimization in WL layers (25–44 weeks)

D-lysine (%)	0.50	0.55	0.60	0.65	0.70	0.50	0.55	0.60	0.65	0.70	0.70
CP (%)			13.36					15.78			17.00
Group	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	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
Ca (%)^	3.603	3.603	3.603	3.603	3.451	3.605	3.605	3.605	3.606	3.606	3.428
P (%)^	0.457	0.457	0.456	0.455	0.454	0.451	0.450	0.450	0.451	0.450	0.453
d. lysine	0.501	0.550	0.604	0.650	0.704	0.503	0.553	0.603	0.650	0.701	0.704
d. M + C	0.435	0.481	0.522	0.563	0.617	0.493	0.492	0.528	0.570	0.609	0.614
d. threonine	0.408	0.404	0.423	0.432	0.472	0.467	0.464	0.464	0.464	0.463	0.516
d. tryptophan	0.114	0.113	0.113	0.125	0.137	0.119	0.118	0.119	0.130	0.135	0.144
d. arginine	0.708	0.705	0.703	0.736	0.756	0.753	0.748	0.751	0.758	0.761	0.894
d. Iso leucine	0.463	0.458	0.457	0.450	0.444	0.543	0.540	0.540	0.538	0.537	0.604
d. valine	0.565	0.559	0.558	0.552	0.548	0.651	0.648	0.647	0.645	0.642	0.701

^ Analysed values.

Table. 2. Nutrient composition of diets fed to WL layers (25-44 weeks) for d. threonine optimization

		Basal Diet- II	Control
d.Thr % (as % Lysine) 60		60	66
d.Lysine (%) 0.65		0.60	0.70
	D1-D6	D7-12	D13(Control)
ME (kcal/Kg)	2704	2702	2706
CP^(%)	13.46	15.56	17.05
Calcium^ (%)	4.350	4.350	4.350
Av. Phosphorus (%)	0.430	0.430	0.440
d.Lys	0.650	0.604	0.701
d.M+C	0.563	0.530	0.630
d.Thr	0.391	0.360	0.470
d.Trp	0.137	0.140	0.170
d.Arg	0.840	1.070	1.200
d.Ile	0.470	0.430	0.540
d.Val	0.550	0.550	0.650

^ Analysed values.

Data collection: Body weight (BW) was recorded every four weeks in the morning before the feed was offered. Feed intake, residual feed, egg production, and mortality were recorded on daily basis. Various performance efficiency indicators, i.e. hen day egg production (HDEP), performance efficiency (PE), net feed efficiency index (NFEI), North's egg mass (NEM) and feed efficiency per kg egg mass (FE/EM) were calculated on a cumulative basis from 25-44 wks. The egg feed price ratio and break-even point were calculated to determine the economic efficiency as per the standard formulae (Supplementary Table 5).

Data analysis: All the generated experimental data were analyzed statistically by employing a one-way analysis of variance using Statistical Package for the Social Sciences. The significance of differences was calculated with Tukey's post hoc test. Values were considered significant and highly significant at 5 and 1% levels respectively.

RESULTS AND DISCUSSION

The recorded micro-environmental variables during the experimental period are presented in supplementary Table 6.

Varied concentrations of d. lysine and layer performance: The efficiency performance indices NFEI, EM/HH, and NEM indices in response to d. Lys was both linear and quadratic with significant variation (Table 3). Inclusion of d.Lys at different protein levels of layer diets observed a quadratic pattern and maximized ($p<0.05$) at 0.65 and 0.60% in low and high-protein diets. The lowest egg production was recorded in T1 with 0.5% Lys in a low-protein diet. Lys addition at varied levels did not influence performance efficiency, however, higher values were found in T3 and T4 groups than control counterparts. The response of egg mass-produced was significantly influenced by lysine concentration with T10 birds fed 0.70% having a higher ($p<0.01$) yield while the T6 had a lower yield (Table 3). North egg mass and egg mass on hen housed were significantly higher at 0.65 and 0.70% d. Lys in both low and high protein levels (T2, T3, T5, T7, T8, T9) were comparable. Feed efficiency did not reveal any significant change between the treatment and control groups. Feeding of layers with 0.65 and 0.60% Lys concentrations improved economic efficiency as witnessed by higher EFPR with better break-even points (Fig. 3) than control and lysine with high protein level feed.

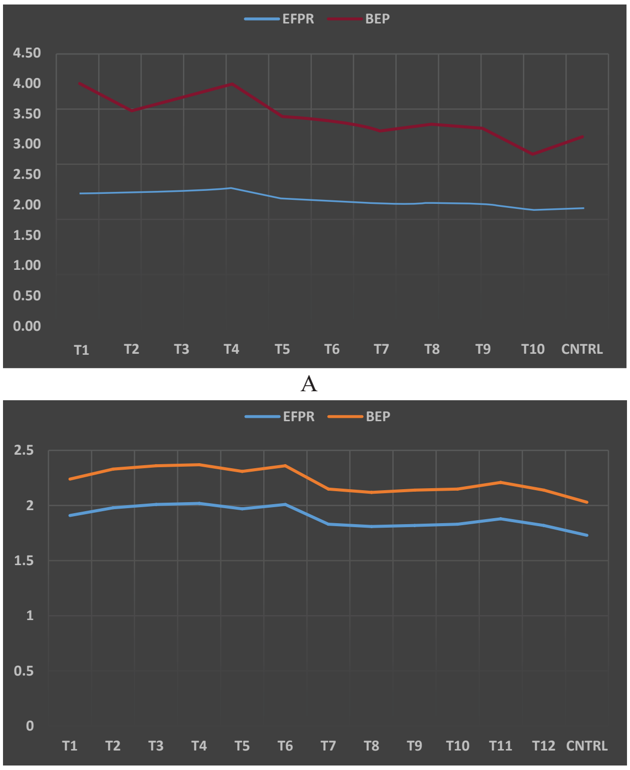

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Fig. 3. Economic efficiency during first production phase as indicated by EFPR and BEP of BV 300 strain WL layers fed on diets containing varied concentrations of d. lysine (A) and d. threonine (B)

Lys is especially important and denoted as the ideal AA. This gold standard amino acid is being used as the basis for setting the requirements for all other AA (Ayasan *et al.* 2010, Silva *et al.* 2015). Multiple factors like basal diet, genetic lines, ambient temperature, age, the carry-over effect of previous biological concentrations, etc. has an obvious influence on AA requirements (Ayasan *et al.* 2009). Lesser number of eggs produced at low levels of lysine in the low protein group in the present study can be attributed to AA imbalance, which might decrease the protein synthesis and consequently the egg production. Replacing the protein portion of layer diets with amino acids improves egg production more than conventional high-protein diets (Alagaany *et al.* 2020).

Similarly, concentrations of Lys up to 0.80% resulted in an enhancement of egg production (Panda *et al.* 2010, Spangler *et al.* 2019). Nevertheless, alteration of energy with varying concentrations of Lys had no effect ($p>0.05$) on egg production, egg mass, etc. Egg mass derivatives were significantly ($p<0.05$) increased with proportionate increments of Lys levels under both protein levels. This

Table 3. Performance efficiency indices of layer chicken fed with varied concentrations of d. Lysine at different crude protein levels (n=48)

CP (%)	d.Lys*	Treatment groups	FE/EM	HDEP (%)	NFEI	PEI	EM/HH	NEM
13.36	0.50	T1	2.19	83.57^{b}	94.3^{d}	53.02	14.42^{bc}	42.07^{b}
	0.55	T2	2.19	88.70^{a}	96.0^{cd}	51.39	14.65^{bc}	44.31^{ab}
	0.60	T3	2.03	89.78^{a}	104.6^{abcd}	57.58	15.30^{bc}	47.85^{a}
	0.65	T4	1.90	90.16^{a}	109.6^{abc}	59.93	15.55^{abc}	48.76^{a}
	0.70	T5	2.08	89.07^{a}	101.3^{abcd}	55.45	15.09^{bc}	46.41^{ab}
15.78	0.50	T6	2.10	87.11^{ab}	99.2^{bcd}	55.08	14.31^{c}	43.24^{b}
	0.55	T7	2.18	89.03^{a}	98.0^{cd}	51.21	14.56^{bc}	44.17^{ab}
	0.60	T8	2.09	89.99^{a}	102.9^{abcd}	55.31	15.00^{bc}	46.02^{ab}
	0.65	T9	2.45	90.26^{a}	102.3^{abcd}	54.12	14.85^{bc}	46.08^{ab}
	0.70	T10	2.04	91.18^{a}	114.2^{a}	55.86	17.22^{a}	48.21^{a}
17.00	0.70	CNTRL	1.94	89.59^{a}	113.1^{ab}	58.70	16.35^{ab}	48.18^{a}
SEM			0.044	0.487	1.417	0.874	0.198	0.471
p-value (Linear)			0.428	0.074	0.018	0.535	0.039	0.014
p-value (Quadratic)			0.932	0.013	0.002	0.468	0.010	0.029

^{abcd}Means bearing different superscripts within column differ significantly.

indicates the need for a higher Lys requirement of more than 0.65% for increased protein synthesis that contributes to higher egg mass (Table 5).

Varied d. threonine concentrations and layer performance: Supplementation of threonine in high lysine and low protein feed was witnessed to have a profound ($p<0.01$) effect on egg production (Table 4) during the first phase of the laying cycle. Layers fed with Thr at 69% (T4) and 75% (T12) of lysine resulted in higher ($p<0.01$) HDEP without compromising performance efficiency. Egg to feed price ratio and a breakeven point was also improved ($p<0.01$) in these groups at Thr concentrations from 63-75% (T1-T6) (Fig. 4). With regards to low lysine and high protein diets, the addition of Thr at 60-75% levels reduced ($p<0.01$) egg production on hen day basis (Table 5) than T12 and CNTRL group with T8 (63% Thr) being the lowest. Subsequently, the layer economics (EFPR and BEP) was also compromised significantly in T7-T10 groups with no significant variation from CNTRL (Fig. 3).

Although insignificant, the present study noticed comparatively better feed efficiency indices in high Lys and low protein level diets with threonine. As determined by kg egg mass produced and net feed efficiency index, T1-T6 groups were more efficient than T7-T12 and control groups. The performance efficiency index also revealed a similar trend. However, egg mass calculated on hen housed and North's index was not influenced by the amino acid concentrations in the diet.

Improvement in hen day egg production was noticed with a proportionate increase in threonine concentration irrespective of lysine/protein levels in diets. Numerically higher HDEP was recorded at 69% and 66% of Thr in

Table 4. Performance efficiency indices of layer chicken fed with varied concentrations of d.Threonine at two fixed levels of d.lysine and crude protein (n=30)

d. Lys/CP (%)	d.Thr*	Treatment groups	FE/EM	HDEP (%)	NFEI	PEI	EM/HH	NEM
0.65/13.36	60	T1	2.38	90.18^{abc}	83.98	50.27	15.66	49.33
	63	T2	2.27	89.48^{abcd}	87.69	50.81	15.44	49.19
	66	T3	2.29	91.14^{abc}	87.24	51.20	14.95	48.63
	69	T4	2.28	93.86^{a}	90.39	49.64	15.28	49.77
	72	T5	2.30	90.13^{abc}	74.69	51.21	15.17	48.29
	75	T6	2.20	92.14^{ab}	92.89	54.42	15.35	50.19
0.60/15.78	60	T7	2.37	86.40^{cd}	84.86	48.28	15.35	47.39
	63	T8	2.41	84.90^{d}	78.20	48.14	15.29	46.31
	66	T9	2.37	88.93^{abcd}	85.05	47.42	15.46	48.87
	69	T10	2.38	86.35^{cd}	82.09	49.61	15.17	46.83
	72	T11	2.26	88.13^{bcd}	85.99	51.90	15.22	48.30
	75	T12	2.38	90.00^{abc}	81.09	49.02	15.09	48.38
0.70/17.05	66	CNTRL	2.34	92.19^{ab}	86.80	51.50	15.31	49.74
SEM			0.017	0.498	1.11	0.533	0.058	0.303
p-value (Linear)			0.420	0.006	0.100	0.511	0.752	0.336
p-value (Quadratic)			0.293	0.167	0.434	0.629	0.389	0.326

^{abcd}Means bearing different superscripts within column differ significantly.

Table 5. Quadratic equation summary of layer chicken fed with varied concentrations of d. Lysine at different crude protein levels (n=48)

Parameter	Equation	R^2 value	Requirement of d. lysine
HDEP	$Y=84.944+1.187x-0.068x^2$	0.113	0.70
NFEI	$Y=98.524-0.137x-0.120x^2$	0.138	0.50
EM/HH	$Y=15.175-0.257x+0.034x^2$	0.129	0.50
NEM	$Y=44.089+0.320x-0.002x^2$	0.063	0.70
EFPR	$Y=2.273-0.015x-0.001x^2$	0.379	0.50
BEP	$Y=4.021-0.094x-0.00x^2$	0.372	0.50
d.Threonine at two fixed levels of d.lysine and crude protein (n=30)			
HDEP	$Y=90.243-0.556x+0.086x^2$	0.001	75
EFPR	$Y=1.960-0.042x+0.005x^2$	0.018	75
BEP	$Y=2.302-0.048x-0.006x^2$	0.018	75

high and low Lys groups respectively. The result of this study corroborates with the findings of Azzam *et al.* (2011) where Thr at 67% increased egg yield with 16.92% protein in brown layers. Egg mass was also found to be improved ($p<0.05$) at these concentrations indicating the synergistic action of Lys and Thr in low-protein diets (Table 4). Threonine is important for the proper gut functioning of birds (Nichols and Bertolo 2008), as well as threonine, plays a major role in performance indices (Al-Hayani 2017) which might be due to its effect on gut health. Threonine plays a major role in intestine mucin secretion (Kumari *et al*. 2020) and the production of antibodies (Cardoso *et al*. 2014) in poultry. Hence threonine requirement could be elevated under challenging conditions including early stages of production in layers. Based on FCR and EM average total and d. Threonine requirement in laying hens were calculated to be 457 and 486 mg/b/d, respectively (Macelline *et al*. 2021).

But the positive effect was not noticed up to the higher concentrations tested due to the interaction of other amino acids (antagonistic effect) as well as the inadequacy of amino acids at those concentrations.

Economic efficiency: Economic indicators (EFPR and BEP) were greatly ($p<0.01$) influenced by d. Lys and d. Thr without affecting feed intake. The higher feed efficiency indices obtained in both experiments might have resulted in better economic gains over the use of these amino acids at low protein concentrations. Simultaneous reduction of protein level without compromising the protein requirement and production could have been economically achieved with the addition of these amino acids.

In conclusion, supplementation of d. Lysine at 0.65 and 0.60%; 69 and 72% d. Threonine as % of d. Lysine at low and high protein diets (13.36 and 15.78%) are optimal for achieving better egg production with economic efficiency without compromising performance efficiency indices during the first phase of egg production in WL layers in the tropics.

Modulating concentrations of Threonine and Lysine showed to have synergistic effects in improving layer performance under tropics. This is an indication that requirements to maximize the performance of these laying hens may probably be met by digestible threonine levels at these levels.

ACKNOWLEDGEMENT

The authors gratefully acknowledge the Sri Venkateswara Veterinary University, Tirupathi, Andhra Pradesh, India, for providing the facilities.

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