Journal of Animal Research: v.11 n.2, p. 291-297. April 2021

DOI: 10.30954/2277-940X.02.2021.9

Effect of Low Protein Diets on Blood Biochemical Parameters and Immunity in WL Layers

Naga Raja Kumari K*, S.V. Rama Rao, D. Srinivas Kumar and V. Chinni Preetham

Department of Poultry Science, NTR College of Veterinary Science, Gannavaram, Sri Venkateswara Veterinary University, Andhra Pradesh, INDIA

**Corresponding author: NRK Kumari; E-mail: nkkallam3@gmail.com*

Received: 06 Jan., 2021 **Revised:** 11 Feb., 2021 **Accepted:** 19 Feb., 2021

ABSTRACT

Two trials were conducted to assess the blood biochemical and immunity parameters in WL layers (25-44 weeks) at low protein and amino acid supplemented diets. First one is for assessment of digestible lysine and 2nd one is for digestible threonine at constant ratio of other essential amino acids at low protein levels. In experiment –I WL pullets (n=528) were randomly allotted into 11 treatment groups each with 6 replicates of 8 birds and fed with 2 levels of protein (13.36 and 15.78%) with five concentrations of d. lysine (0.50, 0.55, 0.60, 0.65 and 0.70%) and a control group with 17% CP and 0.70% lysine. In 2nd experiment pullets of 390 numbers were distributed into 13 treatment groups with 5 replicates of 6 birds. Basal diets with two d. lysine concentrations (0.65% and 0.60%) at two protein levels (from the experiment –I) and each lysine concentration was supplemented with 6 graded concentrations (60, 63, 66, 69, 72 and 75%) of crystalline threonine, and a control with 17 % CP, 0.70 % lysine and 66% threonine were fed to the birds. Results of these experiments revealed that there was no significant variation in total proteins, albumin globulin, A:G ratio, cholesterol, Alkaline phosphotase, Calcium, Phosphorus and HI titres in both the trials. It indicates that the levels of proteins 13.36 and 15.78% with 0.65%,0.60% lysine at 60% lysine as threonine are optimum for WL layers for production without altering health status.

HIGHLIGHTS

- Health status of the WL layers at Low protein diets supplemented with essential amino acids.
- Immunity levels in layers at low protein diet.
- Cholesterol levels in layers at various protein/aminoacid levels.

Keywords: Low Protein, essential amino acids, WL layers, serum biochemical parameters, immunity

Regular genetic selection trials to get highest productivity from the layers is one of the reasons for increased production in multifold in these 2 decades (Younas *et al*., 2019). For achieving maximum production from these superior germplasm birds need to be fed with good quality nutrients as per the requirement. So it makes the constant pressure on nutritionist to redefine the nutritional formulas every time according to local economic, environmental and management conditions (Lima *et al*., 2018; Abdullah *et al*., 2019; Khater *et al*., 2020). Among all the nutrients in diets of poultry cost of proteins is high (50-60%), even though as such protein in not needed, can be substituted with direct supplementation of essential amino acids in diets.

Breghendahl *et al*. (2008) estimated the "ideal amino acid profile" and determined that the ratio of methionine to lysine (47:100 respectively) to support maximum egg mass. And concluded that a constant ratio of all other amino acids relative to lysine for good egg size.

How to cite this article: Naga Raja Kumari, K., Rama Rao, S.V., Kumar, D.S. and Chinni Preetham, V. (2021). Effect of Low Protein Diets on Blood Biochemical Parameters and Immunity in WL Layers. *J. Anim. Res.,* **11**(2): 291-297.

Source of Support: None; **Conflict of Interest:** None

Now a day due to the availability of synthetic amino acids like methionine, lysine, threonine, and tryptophan, utilisation of these amino acids in poultry rations at low protein diets is the common practice to reduce the feed cost. Silva *et al*. (2012) inferred that layers provided low protein diet (14%) fortified with amino acids especially Methionine, lysine and threonine exhibited the performance on par with birds supplemented 18% protein.

While, Soares *et al*. (2019) suggested optimum amino acid requirements for HYline W-36 layers from 28-30 weeks of age by following deletion methods, as per Goettingen approach the amino acid requirements were Lys 100, Met+Cys 88, Trp 21, Thr 69, Arg 109, Val 90, Ile 75, Leu 127, Phe+Tir 110, Gly+Ser 73 and His 29%. Whereas, in Louvain approach was Lys 100, Met+Cys 88, Trp 21, Thr 69, Arg 104, Val 91, Ile 78, Leu 121, Phe+Tir 119, Gly+Ser 77 and His 29%.

The amino acid requirements of the laying hens are normally determined by measuring response in egg production, egg weight, feed consumption and feed conversion efficiency of hens fed on diet containing graded concentrations of the particular limiting amino acid.

But data on health status of the birds by serum biochemistry is scanty. The result of serum analysis is considered to assess the health status of an animal. Serum parameters have been observed as good indicators of the physiological status of animal and their changes are important in assessing the response of such animal to various physiological situations (Khan and Zafar, 2005). Hence, this study was carried out to determine the effects of graded levels of proteins with optimum concentrations of amino acids on serum biochemical profile and humoral immunity of WL Layers.

MATERIALS AND METHODS

Experiment I: A total of 528 WL layers with uniform body weight (1320-1328 g) were randomly distributed and housed in colony cages (4 birds/cage). Two adjacent cages with common feeder were considered as one replicate. Birds were allotted to 11 treatment groups of 6 replicates each with 8 hens. Two basal diets were prepared with 0.50% digestible lysine at 13.36% (LCP) and 15.78% (MCP) protein levels. Ten experimental diets were prepared by adding five different concentrations of crystalline lysine (0.50,0.55,0.60,0.65,0.70%) to the two basal diets. A diet with 17% CP, 0.70% lysine was prepared and fed to a group as control (Table 1). All the experimental diets were iso-caloric with 2700 kcal/kg ME. The ratio between digestible M+C, Thr, Trp, Arg, Ile and Val to digestible Lys were 86, 66, 19, 114, 72 and 80 %, respectively and were maintained constant in all the diets.

Experiment II: A total of 390 WL layers which were having similar body weight (1315-1330 g) were randomly distributed and housed in colony cages (3 birds/cage). Two adjacent cages with common feeder were considered as one replicate. Birds were allotted to 13 treatment groups of 5 replicates each with 6 hens. Basing on the performance of birds in first trial lysine @0.65, 0.60 at 13.36 and 15.78% CP respectively were taken for preparation of basal diets.

Basal diet I is with 0.65 per cent supplemental lysine at 13.36 per cent CP(HL/LCP) and basal diet II is with 0.60 per cent lysine at 15.78 per cent CP (LL/MCP) and control diet with 0.70 per cent digestible lysine at 17.0% CP (Table 2). 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 ME. The ratio between essential amino acids like digestible M+C, Trp, Arg, Ile and Val to digestible Lys were 86, 19, 114, 72 and 80 per cent, respectively and were maintained constant in all the diets.

The dried and ground samples of the feed ingredients (Maize, Maize glutein meal, Soy meal, De oiled rice bran, Rape seed meal, Ground nut cake, Guar meal, Sun flower cake and Bajra, Cotton seed meal) were analyzed for proximate principles (AOAC, 2005) before diet formulation, while the amino acid content of individual ingredients was analyzed via NIR using Amino NIR® by following the traditional wet chemistry (Amino lab®) at Amino Degussa Evonik industries, Singapore. The experimental diets were prepared based on the results obtained and the complete feed samples were analyzed for proximate principles. The amino acid composition of composite feed is calculated based on the data arrived and quantity used for preparation of the ration.

The ratio of other digestible essential amino acids (EAA) to digestible lysine reported by Lemme (2009) was taken as the base for fixing the EAA profiles in the diets. The synthetic amino acids (AA) including methionine (Met),

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

Ingredients	Basal Diet-I	Basal Diet II	Control
Lysine %	0.50	0.50	0.70
CP%	13.36	15.78	17.0
Diets	D1-D5(LCP)	D6-D10(MCP)	D11 (Control)
M E (kcal/Kg)	2700	2698	2697
CP*(%)	13.39	15.76	17.06
Calcium*(%)	4.603	4.605	4.428
Av. Phosphorus (%)	0.457	0.451	0.453
d.Lys	0.501	0.503	0.704
d.M+C	0.435	0.493	0.614
d.Thr	0.408	0.467	0.516
d.Trp	0.114	0.119	0.144
d.Arg	0.708	0.753	0.894
d.Ile	0.463	0.543	0.604
d.Val	0.565	0.651	0.701

*Analysed values.

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

Ingredients	Basal Diet-I	Basal Diet II	Control
d.Thr(% as lysine)	60	60	66
d.Lysine%	0.65	0.60	0.70
Protein %	13.36	15.78	17.00
Diets	D1-D6(HL/LCP)	D7-D12(LL/MCP)	D13 (Control)
M E (kcal/Kg)	2704	2702	2706
C P*(%)	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.

threonine (Thr), tryptophan (Trp) and lysine (Lys) were supplemented to adjust their dietary levels.

All the birds were managed in open sided houses in 3 tier cages under uniform environment and standard hygienic conditions throughout the experiment with a photoperiod of 16 L:8 D. The birds were offered feed and water *ad libitum*. The minimum and maximum ambient temperatures in the house were recorded every day at 06.00 and 14.30 h (IST), respectively.

The experiments were conducted as per the guidelines of Institutional Animal Ethics Committee.

Serum biochemistry

At the age of 44 weeks, blood samples were collected into eppendorf tubes (10 ml) from the axillary vein of 66 (1 bird per replicate) birds used during the 1st experiment and from 65 birds during the 2nd experiment. The tubes were kept in slanted position at room temperature to facilitate the separation of serum for estimation of serum cholesterol (Cat. No. CH 200, CH 201 & CH 202 for Cholesterol estimation), alkaline Phosphstase (Cat. No.AP 311& ap 313 for Alkaline phophotase estimation), Albumin (Cat.No. ALB 001 & ALB 002) and total protein (Cat. No. TP 245 for total protein estimation) by using spectrophotometer with commercially aviable kits (Erba diagnostic Mannheim GmbH, Germany).

Immunological parameters

At the age of 34th week, birds were vaccinated against RD. Blood samples were collected before vaccination and also 18 days (36th wk) after the vaccination into eppendorf tubes (10mL) from the axillary vein of 66 and 65 birds (1 bird per replicate) in 1st and 2nd experiments, respectivley. After the serum separated naturally , it was centrifuged for 10min (3000 rpm) at room temperature. The antibodies specific for Newcastle disease virus were measured in serum of birds by Haemagglutination Inhibition (HI) test (Allan *et al*., 1978).

Parameters studied

Serum Total proteins, albumin, globulins, A: G Ratio, choclestreol ALP, Ca and P, HI titers were analysed.

Statistical analysis

The data were analyzed by one way-ANOVA using Statistical Package for Social Sciences (SPSS), 15th version and comparison of means was done by Duncan's multiple range test (Duncan, 1955) at $p<0.05$.

RESULTS AND DISCUSSION

Experiment 1: The study indicated that either concentration of d. lysine or protein in layer diet had no effect ($p>0.05$) on serum biochemical parameters *viz.* total protein, albumin, globulin, A:G, cholesterol, ALP, calcium and phosphorus (Table 3). No effects on total protein observed in the present study suggest that the protein quality was optimum at all concentration of d. lysine inclusion. Dibner and Ivery (1990) stated that during stress periods especially diets with deficit in amino acids at low proteins showed reduction in albumin content and

Table 3: Concentrations of serum bio chemical variables and HI Titres in WL layers fed with various concentrations of d. lysine and two levels of CP in Experiment-I

Groups	Lysine %	Protein %	Total Protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	A:G	Cholesterol (mg/dl)	ALP* (IU/L)	Ca (mg/dl)	P (mg/dl)	HI antibody titres to RDV (log_2)
LCP	0.50	13.36	4.11	2.16	1.95	1.11	163	198	7.600	3.762	2.585
	0.55	13.36	4.27	2.15	2.12	1.01	164	214	6.225	3.118	2.807
	0.60	13.36	4.21	2.22	1.99	1.12	158	222	6.875	3.137	2.807
	0.65	13.36	4.28	2.33	1.95	1.19	157	234	7.575	3.634	3.000
	0.70	13.36	4.28	2.24	2.04	1.10	159	238	7.550	3.728	3.000
MCP	0.50	15.78	4.31	2.25	2.06	1.09	168	196	7.300	3.359	2.585
	0.55	15.78	4.28	2.24	2.04	1.10	152	216	6.725	3.337	2.807
	0.60	15.78	4.33	2.21	2.13	1.03	165	217	7.675	3.478	2.807
	0.65	15.78	4.39	2.46	1.93	1.27	170	239	6.675	3.338	3.000
	0.70	15.78	4.52	2.36	2.16	1.09	157	234	6.800	3.383	3.000
Control	0.70	17.00	4.78	2.27	2.51	0.90	168	241	7.450	3.770	3.000
SEM			0.708	0.459	0.710	0.481	0.828	131.8	0.618	0.673	0.365
N			6	6	6	6	6	6	6	6	6
P-value			0.167	0.995	0.126	0.577	0.993	0.177	0.367	0.561	0.127

*Alkaline Phosphotase.

suggested the lysine requirement @ 717 ± 22 (R^2 = 0.95) mg/b/d for maximum Egg mass.

Whereas Shahir *et al*. (2006) reported that, Serum albumin increased with increasing lysine intake up to 720 mg/hen/d and then remained relatively constant. Serum albumin is the major reserve protein of the laying hen that will be degraded in response to the needs of protein synthesis in the oviduct (Smith, 1978).

Significantly no variations in serum protein properties at various protein/lysine levels in this study indicate that the levels supplemented might be optimum enough.

Whereas, Garlich 2001 reported that increase in serum calcium concentration (20.84 to 24.63 mg/dl) with increase in lysine in diet (0.75 to 0.86%).

The HI titres were not influenced by increased concentration of lysine irrespective of protein (Table 3). In contrast to the current findings Panda *et al.* (2005 and 2007) and Rama Rao *et al.* (2003), reported increased HI titres with increased concentration of Methionine in the diet.

Experiment 2: Concentration of d. threonine in the diet had no effect (p>0.05) on serum bio chemical parameters *viz.* total protein (g/dl), albumin (g/dl), globulin (g/dl), ALP (IU/L), cholesterol (mg/dl), calcium (g/dl) and phosphorus (g/dl) content (Table 4), in both the groups irrespective of protein / lysine level. The serum biochemical values for different parameters obtained in the present study were comparable with those reported earlier (Azzam *et al.*, 2011; Waiebel *et al.*, 2000). Further, present study findings are in line with Abdel-Wareth and Esmail (2014) and Azzam *et al.* (2011) who reported that supplementation with L-threonine had no effect (p>0.05) on serum calcium, phosphorus and alkaline phosphatase in laying hens. Wang *et al*. (2017) reported that various protein sources (Soya, cotton seed meal, rape seed meal) in diet of layers did not affect the plasma Glucose, total proteins, albumin and globulin levels in 36 to 40 weeks of age. This study inferred that various sources of proteins mean variation in availability of amino acids to the birds. Even though availability is varied the birds are not showing any alterations in biochemical parameters indicated that birds can maintain the health status by changing the feed pattern.

Xiao *et al*. (2017) reported that the level of serum Ca was higher (p<0.05) in MHA-Ca supplementation group than that of the other forms of Methionine and control when

Table 4: Concentrations of serum bio chemical variables and HI Titres in WL layers fed with various concentrations of d. threonine at two levels of d. lysine (Experiment II)

Groups	d.Lys./ CP (%)	d.Thr/ d.lysine	Total Protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	A:G	Cholesterol (mg/dl)	ALP* (IU/L)	Ca (mg/dl)	P (mg/dl)	HI antibody titres to RDV (log$_2$)
HL/LCP	0.65/13.46	60	5.24	1.45	3.80	0.38	80.8	161.9	8.501	4.014	9.2
	0.65/13.46	63	5.37	1.72	3.53	0.49	110.6	216.6	11.98	4.992	9.0
	0.65/13.46	66	5.26	1.63	3.63	0.46	167.3	182.4	10.93	4.461	9.4
	0.65/13.46	69	5.52	1.89	3.63	0.53	132.0	153.0	10.50	4.251	9.0
	0.65/13.46	72	5.73	1.70	4.04	0.43	123.8	152.5	11.85	4.925	8.0
	0.65/13.46	75	5.25	1.39	3.98	0.35	126.5	154.8	10.35	5.175	9.0
LL/MCP	0.60/15.56	60	5.11	1.31	3.80	0.35	67.7	130.7	10.13	4.665	9.2
	0.60/15.56	63	5.56	1.64	3.91	0.45	88.4	222.6	9.962	4.881	9.0
	0.60/15.56	66	5.50	1.31	4.19	0.32	98.8	213.4	9.315	4.658	9.4
	0.60/15.56	69	5.60	1.33	4.27	0.33	155.5	217.5	10.73	4.365	9.0
	0.60/15.56	72	5.66	1.46	4.20	0.37	69.1	187.4	10.23	4.185	9.6
	0.60/15.56	75	6.69	1.50	5.19	0.33	153.4	151.0	10.09	4.084	9.6
Control	0.70/17.00	66	6.10	1.42	4.69	0.31	100.9	185.3	9.235	4.618	8.6
	SEM		0.116	0.045	0.112	0.015	7.85	7.13	0.266	0.423	0.103
	N		5	5	5	5	5	5	5	5	5
	P-value		0.446	0.244	0.204	0.076	0.059	0.117	0.148	0.121	0.187

*Analysed values.

fed various forms of Methioine in Broiler breeders ration. No difference (p>0.05) was observed among the groups on P level. Richards *et al*. (2005) reported that diets with added DLM increased the contents of Ca and P in broiler chickens.

The concentration of d. threonine in diet had no effect on immunoglobulin concentration in both the groups (Table 4). In contrast to this Azzam *et al.* (2011) reported increased immunoglobulins with increase in threonine concentration (0.03%). Furthermore, Kadam *et al.* (2008) observed that injection of 10, 20, or 30 mg of threonine into the yolk sac significantly increased the humoral response to SRBC in broilers at 21d post hatch. Tyler *et al.* (1996) reported increased IgG concentration with increase in total protein concentration. Azzam *et al.* (2014) observed a higher level of IgG at 0.3% l-threonine and concluded that immune system of the laying hen has a specific requirement for threonine that is much higher than those required for egg production (0.3% vs 0.2%).

CONCLUSION

Serum biochemical parameters like serum total protein (g/dl), albumin (g/dl), globulin, A:G ratio, ALP (IU/L), cholesterol (mg/dl), Ca (g/dl) and phosphorus (g/dl) and serum HI titres against NDV were not influenced by increase in concentration of lysine or d. threonine irrespective of protein in diet.

Based on the present study it can be concluded that WL layers at 25-44 weeks of age require supplementation of 0.65 and 0.60 percent digestible lysine at 13.36 and 15.78 percent protein respectively in diet i.e. 598.8 and 570 mg/h/d and d. threonine at 60 per cent of d.lysine i.e., 458.3 and 517.1 mg/h/d respectively for better production performance.

REFERENCES

Abdel-Wareth, A.A.A. and Esmail, Z.S.H. 2014. Some productive, egg quality and serum metabolic profile responses due to L-Threonine supplementation to laying hen diets. *Asian J. Poult. Sci*., **8**(3): 75-81.

Abdullah, H.M., Bielke, L.R. and Helmy. Y.A. 2019. Effect of arginine supplementation on growth performance and immunity of broilers: A Review. *J. Glob. Inno. Agri. Soci. Sci.,* **7**: 141-144.

Allan Reis. 2013. Digestible threonine to lysine ratio in diets for laying hens aged 24-40 weeks. *R. Bras. Zootec.,* **42**(12): 879-884.

AOAC. 2005. Official methods of analysis. 18th edn. Association of Official Analytical Chemists. Washington, DC.

Azzam, M.M.M., Dong X.Y., Xie, P., Wang, C. and Zou, X.T. 2011. The effect of supplemental l-threonine on laying performance, serum free amino acids, and immune function of laying hens under high-temperature and high-humidity environmental climates. *J. Appl. Poult. Res.,* **20**: 361–370.

Azzam, M.M.M., Zou, X.T., Dong, X.Y. and Xie, P. 2014. The effect of supplemental L Threonine on mucin 2 gene expression and intestine mucosal immune and digestive enzymes activities of laying hens in environments with high temperature and humidity. *Poutl. Sci.,* **90**: 2251-2256.

Bregendahl, K., Roberts, S.A., Kerr, B. and Hoehler, D. 2008. Ideal ratios of Isoleucine, methionine, methionine plus cystine, threonine, tryptophan and valine relative to lysine for white leghorn type laying hens of twenty –eight to thirty four weeks of age. *Poult. Sci.,* **87**: 744–758.

Dibner, J.J. and Ivey, F.J. 1990. Hepatic protein and amino acid metabolism in poultry. *Poult. Sci.*, **69**: 1188-1194.

Duncan, D.B. 1955. Multiple range and F-tests. *Biometrics,* **11**: 1-42.

Garlich, J., Brake, J., Parkhurst, C.R., Thaxton, J.P. and Morgan, G.W. 1984. Physiological profile of caged layers during one production year, molt, and postmolt: egg production, egg shell quality, liver, femur, and blood parameters. *Poult. Sci.,* **63**(2): 339-343.

Kadam, M.M., Bhanja, S.K., Mandal, A.B., Thakur, R., Vasan, P., Bhattacharyya, A. and Tyagi J.S. 2008. Effect of in ovo threonine supplementation on early growth, immunological responses and digestive enzyme activities in broiler chickens. *Br. Poult. Sc.,* **49**: 736-741.

Khan, T.A. and Zafar, F. 2005. Haematological study in response to varying doses of estrogen in broiler chicken. *Int. J. Poul. Sci.*, **10**: 748-751.

Khater, H.F., Ziam, H., Abbas, A., Abbas, R.Z., Raza, M.A., Hussain, K., Younis, E.Z., Radwan, T. and Selim, A. 2020. Avian coccidiosis: Recent advances in alternative control strategies and vaccine development. *Agrobiol. Records*., **1**: 11-25.

Lemme, A. 2009. Acid recommendation for laying hens. *Degusa AG Amino news,* **13**: 27-30.

Lima, M.B., Sakomura N.K., Silva E.P., Dorigam, J.C.P., Ferreira, N.T., Malheiros, E.B. and Fernandes, J.B.K. 2018. The optimal digestible valine, isoleucine and tryptophan intakes of broiler breeder hens for rate of lay. *Anim. Feed Sci. Tech.,* **238**: 29-38.

Panda A.K., Rama Rao, S.V., Raju, M.V.L.N. and Bhanja, S.K. 2007. Relative Performance and immune Response in White Leghorn Layers fed liquid DL-mehtionine Hydroxy Analogue and DL-methionine. *Asian-Aust. J. Anim. Sci.*, **20**(6): 948-953.

Panda, A.K., Rama Rao, S.V., Raju, M.V.L.N., Shyam Sunder, G. and Sharma, S.R. 2005. Performance of commercial layers fed diets with various levels of methionine. *Ind. J. Anim. Nutr.,* **22**(4): 229-232.

Rama Rao, S.V., Praharaj, N.K., Reddy, M.R. and Panda. A.K. 2003. G×E interaction between genotype and dietary levels of methionine for immune function in commercial broilers. *Br.Poult. Sci.,* **44**: 104-112.

Richards, J.D., Atwell, C.A., Vazquez-Anon, M. and Dibner, J.J. 2005. Comparative *in vitro* and *in vivo* absorption of 2-hydroxy-4 (methylthio) butanoic acid and methionine in the broiler chicken. *Poult Sci.,* **84**:1397-405.

Shahir, M.H., Shariatmadari , F., Mirhadi, S.A. and Chwalibog, A. 2006. Determination of lysine requirement of laying hen using serum biochemical indicators. *Arch.Geflügelk.,* **70**(2): 74-79.

Silva, Da., J.H.V. *et al.* 2012. A determination of order of limiting amino acids in a low crude protein diet for laying hens. *Poult. Sci.,* **91**(Suppl. 1): 329, 113.

Smith, W.K. 1978. The Amino acid requirement of laying hen: Models for calculation .1. Physiological Background. *Worlds Poult. Sci. J.,* **34**: 81-96.

Soares, L., Sakomura, N.K., Dorigam, J.C.P., Liebert, F., Sunder, A., Nascimento, M.Q. and Leme, B.B. 2019. Optimal in-feed amino acid ratio for laying hens based on deletion method. *J.of Ani. Phy. and Ani. Nutri*., **103**: 170-181.

Tyler J.W., Hancock, D.D., Parish, S.M., Rea, D.E., Besser, T.E., Sander, S.G. and Wilson, L.K. 1996. Evaluation of 3 assays for failure of passive transfer in calves. *J. Vet. Intern. Med.,* **10**: 304-307.

Waibel, P.E., Carlson, C.W., Brannon, J.A. and Noll, S.L. 2000. Limiting amino acids after methionine and lysine with growing turkeys fed low protein diets. *Poult. Sci.,***79**(9): 1290-1298.

Wang, Xiaocui., Zhang, Haijun., Wang, Hao., Wang, Jing., Shugeng, Wu. and Guanghai, Qi. 2017. Effect of dietary protein sources on production performance, egg quality, and plasma parameters of laying hens. *Asian-Austr. J. Anim. Sci*., **30**(3): 400-409.

Xiao, Xue., Yongxia, Wang., Weilong, Liu., Tingting, Ju. and Xiuan, Zhan. 2017. Effects of different methionine sources on production and reproduction performance, egg quality and serum biochemical indices of broiler breeders. *Asian-Australas J. Anim. Sci.,* **30**(6): 828-833.

Younas, M., Rahman, S., Shams, S., Salman, M.M. and Khan. I. 2019. Multidrug resistant carbapenemase-producing Escherichia coli from chicken meat reveal diversity and co-existence of carbapenemase encoding genes. *Pak. Vet. J.,* **39**: 241-245.