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Research Article

Effect of Different Levels of Dietary Crude Protein, Lysine and Methionine on Performance of White Leghorn Laying Hens

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Abstract

Objective: An experiment was conducted to study the effect of feeding of different levels of dietary crude protein (17.5, 16.5 and 15.5%) with varying levels of lysine (0.77, 0.73 and 0.68%) and methionine (0.36, 0.34 and 0.32%) on performance of white leghorn layers from 34-53 weeks of age. **Methodology:** Each diet was fed *ad libitum* to 12 replicates consisting of 88 birds in each replicate. Egg Production (EP), Feed Intake (FI), gram of feed to produce gram of egg mass (FE), Egg Weight (EW) and Egg Mass (EM) were recorded at 28 days intervals. **Results:** Dietary reduction in protein levels had reduced ($p < 0.001$) EP, FE, EW and EM during most of the period and overall experimental period whereas, FI was not affected by dietary CP. The EP was lower ($p < 0.001$) in the group fed with 15.5% CP diet than other groups. The FE was lower ($p < 0.001$) in the group fed 17.5% CP. The EW was decreased ($p < 0.001$) with reduction in dietary CP levels during different periods (except period 3 and 4) and overall experiment (34-53 weeks). **Conclusion:** From the results it was evident that a diet with 16.5% CP with 0.73% lysine and 0.34% methionine were essential to elicit optimum performance in the WL-layers.

Key words: Layer, crude protein, lysine, methionine, performance

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Poultry production is one of the world's most technically advanced agricultural industries, contributing significantly to the global food basket. During the past 10 years, most of the laying hen strains have shown considerable increases in productivity, primarily stimulated by advances in genetics and nutrition. Despite this development, there are still issues related to nutrition that require more study¹. The potential for reducing dietary protein has become a reality because of the availability of Lys, Met, Thr and Trp in the market^{2,3}. The best and most economical method of poultry feeding is to use the sub-optimal levels of dietary CP and provision of balanced amino acids by synthetic amino acid supplements^{4,5,6}.

Attention has been focused on dietary protein as it constitutes the most expensive component in poultry diets and it is necessary to understand the relationship between the minimum protein needs of laying hens and their daily feed consumption⁷. Unless the nutrient profile of the diet is carefully matched to the bird's feed intake, shortage or wastage of many nutrients occurs resulting in significant economic loss⁸ and environmental pollution. Manju *et al.*⁹ revealed that optimum level of crude protein and lysine for WL-laying hens was 16 and 0.59%, respectively. The DL-methionine will be the first limiting amino acid in corn-soy based diets and also a synthetic source of methionine is costlier. So, feeding optimum levels of methionine will reduce the cost of production and increases the production performance. Lysine is the second limiting amino acid in the diets of poultry playing a vital role in egg production¹⁰ hence, it is normally supplemented in the diets. Egg weight will be improved by increasing the levels of methionine¹¹ and lysine¹². In this context, the proposed study was carried out to test the effect of different levels of dietary crude protein, lysine and methionine in the WL-layers on production performance.

MATERIALS AND METHODS

Bird's husbandry: Thirty two weeks old WL-laying hens of Babcock-BV300¹³ strain were selected at M/S Sri Lakshmi Narasimha Poultry Research Farm and were kept on the test diets for 2 weeks continuously, by gradually replacing the previous diet. An experiment was conducted from 07 November, 2014 to 20 February, 2015. Selected birds were reared in four birds colony cages, fitted on the elevated platform in an open sided house. All birds were reared under uniform management conditions except diets. The

experiment was conducted by following the guidelines of the Institute Animal Ethics Committee (Directorate of Poultry Research, Hyderabad, India).

Diets: Three diets (Table 1) were formulated with CP levels of 17.5, 16.5 and 15.5% having uniform levels of energy (2550 kcal kg⁻¹). Synthetic sources of lysine and methionine were supplemented to all diets. The concentrations of lysine (0.77, 0.73 and 0.68%) and methionine (0.36, 0.34 and 0.32%) in three diets were maintained at different levels as mentioned in parenthesis. Three test diets were fed *ad libitum* to respective three groups of birds. In this experiment a total of 3168 birds were utilized with 12 replicates in each group of diet consisting of 88 birds per replicate.

Parameters studied: Egg Production (EP) was recorded twice daily and expressed on a hen-day-basis. A measured quantity of feed was offered daily two times and the daily Feed Intake (FI) per bird and the quantity of feed consumed to produce an egg (FE) was calculated. At the end of every

Table 1: Ingredients and nutrient composition (%) of experimental diets

Ingredients	Diet 1 (17.5% CP)	Diet 2 (16.5% CP)	Diet 3 (15.5% CP)
Maize	57.5	59.2	59.7
Soybean	6.3	1.5	0
Sunflower	16.9	19.9	17.1
DORB	0	0	3.6
MBM	3.0	3.0	3.0
Fish meal	6.0	6.0	6.0
Salt	0.3	0.3	0.3
Stone grit	7.4	7.5	7.8
DCP	2.0	2.0	2.0
DL met	0.05	0.041	0.036
Lysine	0.04	0.105	0.106
AB2D3K (g)	0.015	0.015	0.015
B-plex (g)	0.01	0.01	0.01
CC (50%)	0.05	0.05	0.05
Tox bind	0.2	0.2	0.2
*TM premix	0.11	0.11	0.11
Liv tonic	0.050	0.050	0.050
VitEse	0.010	0.010	0.010
Probiotic	0.010	0.010	0.010
Nutrient composition (percentage as fed basis)			
ME (kcal kg ⁻¹)	2550	2550	2550
CP (%)	17.5	16.5	15.5
** Methionine (%)	0.36	0.34	0.32
** Lysine (%)	0.77	0.73	0.68
Calcium (%)	3.6	3.6	3.6
NPP (%)	0.36	0.36	0.36

ME: Metabolizable energy, DORB: De oiled rice bran, MBM: Meat and bone meal, DCP: Dicalcium phosphate, CC: Choline chloride, B-plex: B-complex vitamins, *Trace mineral premix supplied mg kg⁻¹ diet, Mg: 300, Mn: 55, I: 0.4, Fe: 56, Zn: 30, Cu: 4, **Calculation based on amino analyzed feed ingredients

28th day period, the left residue was deducted from the total feed offered. The average EW was recorded by weighing 50 randomly selected eggs per replicate during the last 3 days of each period. Egg Mass (EM) was calculated by multiplying the average egg weight with the total number of eggs produced in each replicate during each 28th day period. The FE was also calculated by dividing the g FI/g EM per day.

Statistical analysis: The data were subjected to one-way analysis of variance¹⁴ and the significant difference was compared with Duncan's multiple range test¹⁵ at $p < 0.05$.

RESULTS AND DISCUSSION

Egg production: The EP was found to be decreased as that dietary CP level reduced (17.5-15.5%) along with proportionate reduced levels of lysine (0.77-0.68%) and methionine (0.36-0.32%). The reduction in EP (Table 2) was significant ($p < 0.0001$) during a period 1 and 2 (34-42 weeks), whereas, after 42 weeks only a numerical reduction was observed. The overall egg production during the total period of study was also decreased ($p < 0.01$) with reduction in dietary CP content, which ranged from 94.1% (17.5% CP) to 89.9% (15.5% CP). The decrease in CP content (17.5-15.5%) has resulted in a decrease in lysine content (0.77-0.68%) and methionine (0.36-0.32%). Lowest EP was observed in 15.5% CP diet, might be due to insufficient amounts of essential amino acids and further partial conversion of essential amino acid nitrogen into non-essential amino acid nitrogen. Initially, we had started the trial with four diets having a fourth diet with 14.5% CP, 0.64% lysine and 0.30% methionine. After observing the poor performance ($p < 0.0001$) during the period 1 (34-37 weeks) in all economic parameters like egg production (82.2%), feed efficiency (2.74), egg weight (49.7 g) and egg mass (1144 g), 14.5% CP diet was not carried out for further studies. Only the diets with 17.5, 16.5 and 15.5% CP were studied.

The results of the present study were in agreement with the findings of Manju *et al.*⁹, Adeyemo *et al.*¹⁶, Roberts *et al.*¹⁷

and Novak *et al.*¹⁸ who also reported a decrease in EP with reduction in dietary CP level, which might be due to decreased target value of one/more amino acids required during particular laying period. Bouyeh and Gevorgian⁶ stated that reduced EP was obtained in laying hens fed low dietary CP (13%) than dietary group with 14% CP, however reducing dietary protein and addition of synthetic amino acids like lysine (0.76%) and TSAA (85% of lysine) to the diets resumed the EP to normal levels. Contrary to our observations⁵, found that there was no change in EP with increased dietary CP (15-18%) and recommended a dietary level of 15% CP with 0.75% lysine and 0.33% methionine to maintain optimum EP in layers during the production cycle of 33-72 weeks, whereas in our experiment, 15.5% diet has lower levels of lysine (0.68%) and methionine (0.32%) compared to Rama Rao *et al.*⁵. Ji *et al.*¹⁹ also found that EP was not affected in the low-protein diets with lysine levels ranging between 0.46 and 0.66%. Similarly, Mousavi *et al.*³ noted that EP was not affected by the reduction in dietary protein level from (18.5-15.5%), without any alteration in digestible total sulfur amino acids and threonine:lysine ratio.

Feed consumption per bird: Generally, feeding low-protein diets has been found to increase feed consumption in chicken. In the present study feed consumption (Table 3) was almost similar in all diets during different periods of production and also overall (34-53 weeks). Similar findings were reported by Manju *et al.*⁹, Rama Rao *et al.*⁵, Adeyemo *et al.*¹⁶, Roberts *et al.*¹⁷, Meluzzi *et al.*²⁰ and Leeson and Caston²¹, who found no change in feed consumption in birds with various levels of dietary CP (14-18%). However, increased feed consumption as protein content in the diet decreased was reported by Latshaw and Zhao²², Gheisar *et al.*²³, Keshavarz and Jackson²⁴, Wu *et al.*²⁵ and Bartov²⁶ which might be due to an amino acid appetite. In spite of above findings³, observed increased ($p < 0.01$) average daily feed intake in birds fed with low CP diets consisting constant amounts of digestible amino acids. Decrease in feed consumption as dietary CP level decreased was reported by Bouyeh and Gevorgian⁶, Novak *et al.*¹⁸,

Table 2: Effect of feeding different levels of crude protein, lysine and methionine on egg production

Treatments			Egg production (%)					
CP (%)	Lys (%)	Met (%)	P-1 (34-37 weeks)	P-2 (38-41 weeks)	P-3 (42-45 weeks)	P-4 (46-49 weeks)	P-5 (50-53 weeks)	Mean (34-53 weeks)
17.5	0.77	0.36	93.30 ^a	94.10 ^a	92.80	91.50	86.80	92.10 ^a
16.5	0.73	0.34	93.20 ^a	93.50 ^a	92.30	91.70	87.00	91.90 ^a
15.5	0.68	0.32	89.90 ^b	90.60 ^b	91.60	91.40	87.80	90.40 ^b
N			12.00	12.00	12.00	12.00	12.00	12.00
SEM			0.699	0.386	0.364	0.242	0.294	0.242
p-value			0.0001	0.0001	0.271	0.848	0.297	0.006

^{a,b,c} Means with different superscripts in a column differ significantly ($p < 0.05$) and SEM: Standard error mean

Table 3: Effect of feeding different levels of crude protein, lysine and methionine on feed intake

Treatments			Feed intake per bird gram per day					
CP (%)	Lys (%)	Met (%)	P-1 (34-37 weeks)	P-2 (38-41 weeks)	P-3 (42-45 weeks)	P-4 (46-49 weeks)	P-5 (50-53 weeks)	Mean (34-53 weeks)
17.5	0.77	0.36	120.60 ^a	113.70	113.10	110.10	105.60	112.70
16.5	0.73	0.34	120.90 ^a	113.30	113.50	111.00	105.00	112.90
15.5	0.68	0.32	120.10 ^a	112.80	113.70	110.50	105.30	112.70
N			12.00	12.00	12.00	12.00	12.00	12.00
SEM			0.293	0.321	0.257	0.329	0.327	0.199
p-value			0.0001	0.480	0.592	0.577	0.761	0.918

^{a,b,c}Means with different superscripts in a column differ significantly (p<0.05) and SEM: Standard error mean

Table 4: Effect of feeding different levels of crude protein, lysine and methionine on feed efficiency

Treatments			FE (g FI/g EM) per day					
CP (%)	Lys (%)	Met (%)	P-1 (34-37 weeks)	P-2 (38-41 weeks)	P-3 (42-45 weeks)	P-4 (46-49 weeks)	P-5 (50-53 weeks)	Mean (34-53 weeks)
17.5	0.77	0.36	2.310 ^b	2.160 ^c	2.120 ^b	2.13	2.14	2.17 ^c
16.5	0.73	0.34	2.340 ^b	2.210 ^b	2.150 ^{ab}	2.16	2.14	2.20 ^b
15.5	0.68	0.32	2.520 ^a	2.320 ^a	2.170 ^a	2.17	2.15	2.27 ^a
N			12.00	12.00	12.00	12.00	12.00	12.00
SEM			0.017	0.0156	0.008	0.009	0.067	0.008
p-value			0.0001	0.0001	0.028	0.262	0.847	0.0001

^{a,b,c}Means with different superscripts in a column differ significantly (p<0.05), SEM: Standard error mean and EM gram per day = (EW×EP%)/100

Table 5: Effect of feeding different levels of crude protein, lysine and methionine on egg weight

Treatments			Egg weight (g)					
CP (%)	Lys (%)	Met (%)	P-1 (34-37 weeks)	P-2 (38-41 weeks)	P-3 (42-45 weeks)	P-4 (46-49 weeks)	P-5 (50-53 weeks)	Mean (34-53 weeks)
17.5	0.77	0.36	56.00 ^a	55.90 ^a	57.40	56.40	55.90	56.30 ^a
16.5	0.73	0.34	55.30 ^b	54.80 ^b	57.20	56.60	55.70	55.80 ^b
15.5	0.68	0.32	53.10 ^c	53.70 ^c	57.20	56.30	55.30	55.10 ^c
N			12.00	12.00	12.00	12.00	12.00	12.00
SEM			0.210	0.205	0.089	0.084	0.075	0.105
p-value			0.0001	0.0001	0.627	0.252	0.001	0.0001

^{a,b,c}Means with different superscripts in a column differ significantly (p<0.05) and SEM: Standard error mean

Keshavarz and Austic²⁷ and Harms and Russel²⁸, which might be due to adequate levels of lysine, methionine, threonine and tryptophan in the supplemented diets.

Feed efficiency (gram of feed/gram of egg mass): Feed efficiency (Table 4) was increased (p<0.0001) as the dietary protein content was reduced, ranging from 2.17 (17.5% CP) to 2.27 (15.5% CP) but significant during initial three periods (34-45 weeks of age) and overall experimental period. This might be subjected to similar feed intake associated with low EP and EW in low CP diets. In consonance with our findings, Manju *et al.*⁹, Adeyemo *et al.*¹⁶, Meluzzi *et al.*²⁰ and Liu *et al.*²⁹ also reported increased FE in low CP diets in their respective studies. Poor feed efficiency (1.836-1.908 gram of feed/gram of egg) with decreased dietary CP from (18.5-15.5%) was noticed by Mousavi *et al.*³, who assumed from increased feed consumption and a decrease in egg weight. Whereas, Rama Rao *et al.*⁵ and Bouyeh and Gevorgian⁶ reported that dietary

CP (15-18% CP) had no significant effect on FE. On contrary, Novak *et al.*¹⁸ reported that FE was significantly improved as dietary protein decreased during initial phases of laying and non-significant improvement was observed during the latter periods which might be due to significant reduction in feed consumption associated with a slight decrease in EW. Roberts *et al.*¹⁷ also observed significant reduction in feed utilization in the latter stages (32-58 weeks) of production with diet of 17.5% CP than with diet of 18.72% CP, which might be due to significant reduction in EM and slight reduction in feed consumption.

Egg weight: The EWs were higher in diets with high dietary CP and AAs and there was significant reduction in EW as dietary CP and AA levels were decreased during initial 2 periods and overall experiment, which might be due to inadequate intake of total nitrogen. Range of EW (Table 5) during entire trial was 56.3 g (17.5, 0.77 and 0.36%) to 55.1 g (15.5, 0.68 and 0.32%).

Table 6: Effect of feeding different levels of crude protein, lysine and methionine on egg mass

Treatments			Egg mass, gram egg per period					
CP (%)	Lys (%)	Met (%)	P-1 (34-37 weeks)	P-2 (38-41 weeks)	P-3 (42-45 weeks)	P-4 (46-49 weeks)	P-5 (50-53 weeks)	Mean (34-53 weeks)
17.5	0.77	0.36	1462 ^a	1475 ^a	1491	1447	1359	1453 ^a
16.5	0.73	0.34	1446 ^a	1434 ^b	1478	1454	1357	1436 ^a
15.5	0.68	0.32	1336 ^b	1361 ^c	1466	1440	1359	1395 ^b
	N		12.00	12.00	12.00	12.00	12.00	12.00
	SEM		14.451	9.498	4.921	3.860	4.691	5.358
	p-value		0.0001	0.0001	0.123	0.347	0.982	0.0001

^{a,b,c}Means with different superscripts in a column differ significantly ($p < 0.05$) and gram EM per period = (gram EM per day) $\times$ 28

Similar findings were reported by Mousavi *et al.*³, Rama Rao *et al.*⁵, Bouyeh and Gevorgian⁶, Adeyemo *et al.*¹⁶, Novak *et al.*¹⁸ and Wu *et al.*²⁵, who had observed that EW reduced linearly ($p < 0.01$) as CP level reduced in the diets. Generally high dietary protein is needed for high EW than required for EP. Rama Rao *et al.*⁵ also reported that a dietary CP content of 16.5% was required for optimal EW in layers at 21-32 weeks of age and later 15% CP is enough to maintain optimum EW. Contrary to these findings Leeson and Caston²¹, Roberts *et al.*¹⁷ and Manju *et al.*⁹ reported that dietary protein levels had no effect on EW, whereas²⁰ reported that EW was unaffected during first 8 weeks of start of lay, later heavier eggs were obtained with high dietary CP groups, which was probably related to uniform conditions of the pullets at the beginning of the period.

Egg mass per period: As dietary CP and corresponding AAs levels were decreased in the diet, EM per period (Table 6) was also decreased ($p < 0.0001$) during phase 1, 2 and overall period. Range of EM during entire trial was 1453 g (17.5, 0.77 and 0.36%) to 1395 g (15.5, 0.68 and 0.32%) per one laying period (28 days). The EM was found to be medium (1436 g) with an average intake of dietary protein and AAs (16.5, 0.73 and 0.34%).

These results were in agreement with the previous findings of Mousavi *et al.*³, Roberts *et al.*¹⁷, Novak *et al.*¹⁸, Wu *et al.*²⁵, Harms and Russel²⁸, Yakout³⁰ and Summers *et al.*³¹, who also observed lower EM from the hens consuming low-CP diets compared with hens on normal CP diets. They also attributed that with a combination of low EP and decreased EW were contributed for reduced EM. In contrast, Rama Rao *et al.*⁵ reported that dietary CP level of 15-18% had no effect on EM during different periods and overall egg laying period.

CONCLUSION

Based on the above results, it was concluded that a diet containing 16.5 CP with proportionate concentrations of

limiting amino acids like 0.73 lysine and 0.34% methionine is adequate for WL-layers during 34-53 weeks of age to elicit optimum production performance.

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