Reasearch Article

Effect of Protein Levels in Diet on Production and Emissions in WLH Layer

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Abstract | The present study was conducted to evaluate the effect of various levels of protein, in the diets of WLH layers, on production performance and on nitrogen (N) and phosphorus (P) levels in the excreta. The trial was conducted in a total of 108 WLH layers (BV-300) at 25 weeks of age. The birds were allocated into three dietary treatment groups; six replicates of six hens per group. Diets with 2700 Kcal/kg of metabolizable energy (ME)and a constant ratio between digestible Methionine+Cystine (M+C), Threonine (Thr), Tryptophan (Try), Arginine (Arg), Isoleucine (Ile) and Valine (Val) to digestible Lysine (Lys) (86, 66,19, 114, 72, 80 respectively) were prepared based on corn-soy and were fed to birds from 25 to 44 weeks of age. During the final stage of feeding trial, metabolic trial was held in 3 consequent days to record the N and P levels in the excreta of WLH layers. Results demonstrated that egg production, egg weight and feed conversion ratio (FCR) (g/g) were not influenced by the level of protein in diet. Body weight was increased with the increase of protein levels in diet. Gain over feed cost compared to control (17% CP) was recorded as \$1 and \$ 0.67 in low (13.38%) and medium (15.58%) protein groups, respectively. The percentage of N was increased and percentage of phosphorus was decreased significantly ($P<0.05$) in the excreta with increasing the level of protein in diet. Taken together, decreasing the level of protein in diet of WLH layers with supplementation of essential amino acids improves the production and also reduces the nitrogen excretion into the faeces in turn emissions from poultry to environment can be minimized.

Keywords | Dietary Protein, Layers, Performance, Nitrogen, Phosphorus retention

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INTRODUCTION

In recent decades Indian poultry industry has made tremendous adjustments to meet the increasing demand of inexpensive and safe supply of meat and eggs. India has occupied 3rd and 5th rank in the world with the annual production of 70 billion eggs and 3.0 million metric tonns of poultry meat respectively (BAHS, 2010). Commercial egg production is being practised under intensive rearing system at high concentrated poultry areas resulting in accummulation of manure in exposed condition to environment. Poultry manure majorily contians two principal elements i.e. nitrogen (N) and phosphorus (P), which are potentially dangerous to environement in the form of ammonia (NH_3) dinitrogen(N_2),nitrous oxide (N_2O), nitrate ($NO3^-$) and phosphate (P_2O_5).

Emissions from intensive poultry production (indirectly and directly) contributes to 2% and 3% of the total greenhouse gas (GHG) emissions from livestock and atmosphere (Gerber et al., 2015). The ideal protein concept play (Kaur et al., 2008) an integral role in precision protein nutrition to minimize the loss of N and dietary P indirectly by improving growth and production. Nutritional management is one of the way to reduce pollution load by limiting excess nutrient intake and / or improving the nutrient utilization efficiency of the birds. Nutritional stratagies have achieved success in providing a partial solution for several of the prominent environmental issues (Beauchemin and Ginn, 2005). Adoption of nutritional strategies often results in a cost-savings or break even situation. Protein and amino acids are some of the most expensive nutrients in feed formulations, hence identification of optimum level of amino acid in feed formulation is essential not only in economics of production but also play a key role in reduction of nitrogen loss in poultry waste which indirectly helps in mitigation of environmental pollution. Reduction of CP level by 1% in layers diet from 21 to 31 weeks of age inturn reduces 30% of nitrogen excretion (Roberts et al., 2007). Growth, production or performance in birds were not influenced by the alteration in concentration of nitro compounds as nitro ethanol or nitro propanol under controlled conditions (Moraes et al., 2014). Relatively high concentration of protein and excessive levels of amino acids were found in diets of layers when formulated using some feedstuffs like maize, soybean meal, corn gluten, sunflower meal and meat meal to meet the nutrient requirements other than the first (methionine) and second (lysine) limiting amino acids (Gill et al., 2012; Burley et al., 2013).

Protein intake was reduced from 18.9 to 17.0g/hen/day (20 to 43 wks of age) and from 16.3 to 14.6g/hen/day (43 to 63wks of age) in layers reduces the N excretion without resulting in any change in egg production (Novak et al., 2008). N_2O emission was initially high with flux ranging from 2.05-29.15mg/m^3/d. Whereas, CH_4 emission form poultry manure reached high at 30 days. CO_2 emission which indicated microbial respiration decreased in its value over time indicating the maturity of compost. Green gas losses during composting of poultry manure were 0.37CH4-C mg/kg and 1.86 N_2O-N mg/kg. All these can be minimized by feeding low protein diets to the birds. The combined N retention in the egg and the hen amounted to about 42% of the N intake. In the entire nitrogen offered to the layers only 24% of N remained in manure, 34% in the eggs, and 1% in the carcass; hence, 40% was presumably lost, probably as NH_3 (Patterson and Lorenz, 1996).

From the provided Nitrogen/Protein in diet of the birds the excess amino acids over the requirement will be de aminated and the amino acid–derived N is removed in the urine in the form of uric acid (80%), ammonia (10%) and urea (5%) (Corzo et al., 2009).

But Ammonia emissions are reduced (15%) by reducing the crude protein in diet (2% units) of the birds (Powers and Angel, 2008). However, on regular basis the Nitrogen and Phosphorus retention are 45.6% and 29.1% respectively (Applegate et al., 2007).

The potential for nitrate or nitrite pollution is directly related to the nitrogen content of the excreted faecal material. Ample data is available with broilers on the effect of the

Table 1: Ingredient and Proximate Composition (%) of experimental diets (Kg) used in this study

Attributes	Diet-I (LCP)	Diet II (MCP)	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
Grit	120	120	120
Salt	4	4	4
Lysine Hcl	2.15	2.30	1.45
DL-Methionine	1.45	1.29	1.80
Vitamin E	0.20	0.20	0.20
Phytase	0.10	0.10	0.10
Penta Forte*	0.40	0.40	0.40
Soda bicarb	1.00	1.00	1.00
Toxin binder	0.50	0.50	0.50
Choline chloride	1.00	1.00	1.00
Dicalcium Phosphate	12.0	12.0	12.0
Trace Minerals**	0.50	0.50	0.50
Lactomax gold***	0.10	0.10	0.10
H plus #	2.00	2.00	2.00
Veg.Oil	0.28	0.42	0.41
Total	999.8	999.9	1000.5
Proximate composition (%)			
Metabolizable Energy (Kcal/Kg)	2704	2702	2706
Crude Protein	13.46	15.56	17.05
Calcium	4.350	4.350	4.350
Available Phosphorus	0.430	0.430	0.440
Total Phosphorus	0.470	0.540	0.550
Potassium	0.560	0.620	0.500
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

LCP: Low crude protein; **MCP:** Medium crude protein; **d:** digestible; ***Provided (/Kg diet):** Vit A 50 IU,Vit D3 14 IU, 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 (per kg diet):** Manganese 70g, Zinc 70g, Iron 50g, Cobalt 0.6g, Iodine 0.6g, Copper 10g, Selenium 0.06g; ***Saccharomyces and Lactobacillus species # Acidifier

Table 2: Production performance of WLH layers fed varying protein levels in diet from 25- 44 wks

Dietary protein level (CP %)	HDEP (%)	FI (g/b/d)	Egg weight (g)	Body weight (g)	FCR(FI/EM) (g/g)	Economics ($)
13.38 (LCP)	89.48	111.1	53.37	15.37[c]	2.27	1
15.58 (MCP)	88.90	115.7	54.97	38.80[a]	2.38	0.67
17.00 (Control)	92.19	116.3	53.92	21.13[b]	2.34	0.00
SEM	0.498	0.664	0.182	9.87	0.471	
P value	0.067	0.354	0.501	0.055	0.21	

HDEP: Hen Day Egg Production; **FI:** Feed Intake; **FCR:** Feed conversion ratio; **EM:** Egg mass; **SEM:** Standard error of mean

low protein on nitrogen excretion but the information about effect low protein diets on nitrogen, phosphorus excretion of layers is limited.

Thus, interest is growing in studying minimum dietary protein levels to optimize product output rather than using input levels to maximize outputs. Hence, the study was planned with low protein diets to test the performance as well as nitrogen and phosphorus excretion in WLH layers.

MATERIALS AND METHODS

Ethical Approval

Experiments were carried out in accordance with the guidelines laid down by the institute Animal Ethics Committee for the use of Poultry birds.

Experimental Birds and Protocol Design

The present investigation was conducted on BV-300 strain of White Leghorn layers (n=108) from 25 to 44 weeks of age at ILFC, NTR College of Veterinary Science, Gannavaram, Andhra Pradesh. Birds were randomly assigned to three treatments as control (C), T_1 and T_2 each comprising six replicates of six birds. All the birds were reared in wire cages under uniform management conditions with feed and water *ad libitum.*

Experimental Diets

Iso-caloric diets with varied protein level of 13.38 % (LCP), 15.58% (MCP) and 17% were fed to T_1, T_2 and control groups respectively (Table 1). Irrespective of protein level, a constant ratio between lysine and other essential amino acids such as digestible methionine (M) and cystine (C), Threonine (Thr), Tryptophan (Try), Arginine (Arg), Isoleucine(Ile) and Valine (Val) as 86, 66, 19, 114, 72 and 80 of lysine were maintained in all the diets. Hens were kept on the test diets for 5 periods (28d/period) and during the last week (44^{th} week) of the test a metabolism trial was conducted.

Metabolism Trial

At 44^{th} week, 3 birds from each treatment (1 bird/replicate) were selected randomly and placed in individual cages. Faecal matter was collected on aluminium foil placed under the cages. Feed intake and faecal collection were continued for 72 hrs. In all cases 3 replicates were used to determine faecal nitrogen. For determination of faecal elements, collected excreta samples were freeze dried to a constant weight and finely grounded. Faecal nitrogen, calcium and phosphorus were determined accordingly by Kjeldahl, methods as per AOAC (2005). Similarly, left over test diet was also analysed to arrive at percent nutrient retention.

Statistical Analysis

Data obtained were subjected to analysis completely randomized design with the one way analysis of variance (ANOVA) as per Snedecor and Cochran (1980) using Statistical package for the Social Sciences. Resultant means were compared using Duncan's (1955) multiple range test.

RESULTS

Production attributes along with economic efficiency of layers fed with experimental diets were presented in Table 2. Hen day egg production, FI/B/day, egg weight and FCR (g/g) were not influenced by either the level of protein or concentration of lysine in diet. Whereas, increased the body weight with increase in protein level in diet were observed. Gain/Bird over 20 weeks was $1 and $ 0.67 over traditional control in LCP and MCP protein groups, respectively.

Nitrogen excretion was nearer to 64.18, 84.60 and 92.42% of the percent absorbed nitrogen into the body. However nitrogen excretion was higher (28%) in control followed by medium protein (20%) diet when compared to low protein diet. Calcium absorption and excretion was not varied significantly by the level of protein in diet. Phosphorus excretion was more (41.19%) from low protein fed group when compared to medium protein (33.44%) and control (29.16%) groups.

DISCUSSION

Hen day egg production, FI/B/day, egg weight and FCR (g/g) were not influenced by either the level of protein or concentration of lysine in diet. Similar to these, Rama Rao et al. (2011) observed no significant effect on egg production, FI and FCR in WLH layers by incorporating various levels of protein (15-18%) in diet. The calculated average daily intake of protein by layers fed 13.38% CP was 14.84g/b/d. Which may be sufficient to meet the production requirements at that protein levels, hence feed intake may not be enhanced. In low protein diets all the essential amino acids are met as per the requirement of production threshold. Hence performance in terms of EP, FI, EW were not influenced by variation in levels of protein in diet of WLH layers from 25-44 weeks of age. Similarly, Khajali et al. (2008) reported that layers can perform well on diets containing approximately 14 to 15 % balanced protein compared with those fed a diet with 17% CP. Latshaw and Zhao (2011) reported that diets with different levels of CP (13, 15, 17) had no effect on EP, EM/D, Ash or nitrogen retention from 29 to 57 weeks of age in egg type hens. Whereas, Zeweil et al. (2011) inferred that no significant variation in egg production and egg mass by the level of dietary CP (12, 14 and 16%) in Baheij laying hens.

Lower the feed intake (99.27g/day vs.111.95g/day) values were observed by Bouyeh and Gevorgian (2011) in Hy-line layers when fed with various (13% and 14% CP) levels of protein in diet and also observed poor feed efficiency in high protein diet fed groups. In contrast to these, Mousavi et al. (2013) observed linear increase in egg production (92.32 to 93.63%) with increase in crude protein (15.5 to 17.5%) in diet.

Body weight gain was significantly (P< 0.05) higher in medium protein diets when compared to low protein diet. Similar to current findings Rama Rao et al. (2011) reported increased egg weights with increase in level of protein in diet from 15-16.5% during early laying phase. Similarly increase in egg weight, egg production and egg output were observed by Saki et al. (2015) in layers fed with high protein (15 vs. 14% of CP).

When egg size was considered as the criteria the calculated CP intake in the 15.58% CP was 18.26g/b/d. Similarly, Novak et al. (2008) and Khajali et al. (2008), have also suggested 16 to 16.3% CP for layers during the initial laying phase (up to 32 weeks) to sustain performance similar to that of layers fed 17% CP. The higher levels of CP required during the initial laying periods may be due to a constant increase in the rate of EP to reach the peak lay with a simultaneous gain in BW.

Return over the feed cost was more in LCP when compared to MCP and control. There was $1 and $ 0.67 per bird gain over traditional control in LCP and MCP groups respectively (over a period of 20 weeks). These findings are in agreement with the findings of Novak et al. (2008), who reported feeding of layers with low protein diets from 18-34 wks of age was most economical, in addition to reduced cost, low protein feeding resulted in higher nutrient retention without affecting the performance of birds.

Nitrogen excretion was nearer to 64.18, 84.60 and 92.42% of the percent absorbed nitrogen into the body. However, nitrogen excretion was higher (28%) in control followed by medium protein (20%) diet when compared to low protein diet. A concomitant decrease in faecal nitrogen (Table 3) was observed with decrease in level of protein in diet. Similarly, Wu-Hann et al. (2007) observed 39 percent reduction in ammonia emission from rooms where laying hens were offered a diet containing low protein diets supplemented with 6.9 percent of gypsum-Zeolite mixture.

Reduction in CP or Nitrogen percent in poultry diets results in reduction of nitrogen excretion in faeces followed by reduced emission of ammonia into the environment (Liang et al., 2005). The combined N retention in the egg and the body amounted to about 42 percent of the N intake.

Gates et al. (2002) inferred that Nitrogen (N) content in faces is an important factor influencing NH_3 generation. Formulation of rations with reduced dietary crude protein (CP) and supplemented with limiting amino acids (AA) to match bird dietary requirements in turn helps to reduce the N content in faeces.

Excreta nitrogen levels were less in LCP fed birds than control and MCP diets. These findings were similar to Novak et al. (2008), who observed decreased excreta N in low CP fed (15%) birds at 38 weeks of age when compared with high protein (18%) groups. Reduction in CP levels in broiler diets by less than 2% units or 13% reduction in N intake resulted in greater than 18% decrease in litter N content (Keshavraz and Austic, 2004). Latshaw and Zhao (2011) reported increase in manure nitrogen (P<0.01) (N excretion)

Table 3: Percent Nitrogen, Calcium and Phosphorus intake and excretion in WLH fed with varying levels of protein in diet from 25 to 44 wks of age (Basing on % intake)

Treatment	Nitrogen			Calcium		Phosphorus	
Protein levels In diets (%)	N intake (g/b/d)	% absorbed	% voided	% absorbed	% voided	% absorbed	% voided
13.38 (LCP)	2.374	60.91	39.09	68.55	31.45	58.81	41.19
15.58 (MCP)	2.921	54.17	45.83	56.08	43.2	66.56	33.44
17.00 (Control)	3.163	51.97	48.03	54.54	45.46	70.84	29.16

with increase in dietary protein (13 to 17%) in laying hens of 29 to 57 weeks.

In supporting to these findings Wu-Hann et al. (2007) reported that reduced protein in combination with acidifying agent reduces the ammonia emission from the layers when fed with 18.30 to 16.50 % CP at 21-24 weeks of age , 17.80 to 15.80 % CP at 39-41 weeks of age and 17 to 15.30% at 59-62 weeks of age likely. Keshavraz and Austic (2004) reported that apparent retention of N was 48.8% when hens fed with 16.5% protein. Liang et al. (2005) inferred that ammonia emission rates were reduced (0.90 to 0.81) in birds by reducing the level of protein (1% lower protein) in diets. Blair et al. (1999) reported that a reduction of dietary CP level in layer diet from 17% to 13.5% resulted in a 30% to 35% reduction in N output in manure, with higher dry matter content in the excreta.

Phosphorus excretion was more from low protein fed group when compared to medium and high protein groups. This might be due to incorporation of more maize and bajra in LCP diet when compared to MCP and control diets. These ingredients are rich in phytate phosphorus even though the total phosphorus requirement was met the available phosphorus in LCP diet is less. This may lead to excretion of more P in LCP group when compared to high protein and control groups.

Feeding the layers closer to requirements, with addition of phytase to improve P availability (Selle and Ravindran, 2007) and thus dietary P used by poultry can substantially decrease P excreted (Keshavaraz and Austic, 2004; Angel et al., 2005).

Nitrogen excretion through faeces is influenced by the level of Nitrogen/protein in diet (Alagawany et al., 2014; Zeweil et al., 2011), but level of protein in diet of layers can be minimized by supplementation of amino acids to layer diets (Koreleski and Swiatkiewicz, 2010). Reduction in nitrogen excretion plays a crucial role in mitigating ammonia emission and environmental pollution from poultry manure in poultry farms, in addition to decreasing uric acid in litter.

CONCLUSION

Development of an ideal amino acid pattern is needed to reduce the protein intake further, and implementatinof such diets may lead to reduction in cost of the diets to make such an approach economically feasible.

It can be concluded that protein level at 13.38% in the diet of WLH layers by balancing the essential amino acids on basis of lysine may be sufficient at 25-44 weeks of age. Research is needed with increased supplementation of phytase to the low protein diets to minimise phosphorus excretion along with nitrogen excretion.

Substantial reductions in nitrogen emission and pollution can be achieved by decreasing the levels of CP in diets and balancing the requirements of digestible amino acid profiles with crystalline amino acid.

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CONFLICT OF INTEREST

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTION

Kallam Naga Raja Kumari planned and carried out research work in collaboration with Kondapalli Ravi Chandra. Dhulipalla Srinivas Kumar and Kondapalli Ravi Chandra helped in analysis of the samples for different parameters and preparation of feed. All authors participated in draft and revision of the manuscript. All authors read and approved the final manuscript.

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