Influence of Different Levels of Lysine, Methionine and Protein on the Performance of Laying Hens after Peak

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Abstract: This experiment was carried out to determine the effects of different levels of lysine methionine and protein on the performance of 240 Hy-Line W36 layers after peak of production. Ten experimental diets were tested in a 2×5 factorial arrangement with a completely randomized design. Two basal diets (13 and 14% protein) were tested at different levels of lysine and methionine include 10 and 20% lower than NRC, NRC and 10 and 20% above NRC recommendations (0.56, 0.62, 0.69, 0.76 and 0.83% of diet). Levels of Total Sulfur Amino Acids (TSAA) were constantly kept 85% of lysine level in each treatment. During the 3 months of experimental period, egg production, egg weight, egg output, feed intake and conversion and also lysine, TSAA and protein intake were determined. The results indicated that 0.76% lysine and more (10 and 20% above NRC recommendation) with 13% dietary protein led to significantly (p<0.05) higher egg production, egg output and better feed conversion but there was no significant difference regarding egg weight. The difference in performance with different levels of lysine was lower in the 14% dietary protein than that in the 13% one. In the 14% dietary protein, the lowest level (0.56%) of lysine led to significantly (p<0.05) lower egg production and egg output and higher feed conversion. The best performance with the 14% dietary protein belonged to 0.62% dietary lysine (10% below NRC recommendation). Regardless of lysine level, the 14% dietary protein had significantly (p<0.05) higher egg production, egg output and feed intake than 13% dietary protein group but there was no significant difference regarding egg weight and feed conversion totally. The best and most economical performance belonged to the 0.76% lysine of 13% protein group. The results of this experiment indicated that reducing dietary protein and addition of lysine and TSAA to the diets of post peak laying hens can obtain an equal performance to the higher dietary protein thus reducing production costs and some problems related to excess nitrogen in poultry diets.

Key words: Lysine, methionine, protein, laying hens, poultry diets, Iran

INTRODUCTION

Appropriate application of different components of poultry diet is of great importance in economical yield of the products. In the case of being unbalanced even in one component the feed will not only be consumed with low efficiency but it will also exert significant influence on the inappropriate consumption of other components of the diet. In this regard, protein percentage and the balance of amino acids of the diet due to high cost of protein products are of rather greater importance. Due to some limitations, particularly in some countries that high sums of currency are spent for the import of protein supplements like fish and soybean meal. Protein requirements in fact represent the need for amino acids as the constituents for the effective and economical production of proteins required for body tissues, vital functions and the bird products. The scientific feeding of

poultry is not in fact based on the raw protein of the diet but on the necessary amino acids content of the diet, their balance and intake together with some none protein nitrogen for the provision of the none essential amino acids (NRC, 1994). Therefore in the case of meeting the above said, it is of no importance whether the source of the amino acid is plant, animal protein or synthetic amino acids.

Based on the findings of some researches (Ayupov, 1985; Keshawarz and Jackson, 1992; Pens and Jensen, 1991), the best and most economical method of poultry feeding is to use the least amount of crude protein and provision of amino acids by synthetic amino acid supplements. In so doing more space will be opened for energizing components and converting protein into energy which has low efficiency will be minimized. The excess of the protein of the diet besides other problems will result in litter wetting and environmental pollutions

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due to uric acid discharge through urine (Lepkovsky, 1973; Shnfer, 1993). The research results reported by Parr and Summers (1991) and Blair *et al.* (1999) indicated that low protein diet supplemented by synthetic amino acids has higher efficiency than high protein diet. They believe that low protein diets lead to reduction of nitrogen and amino acids discharge through manure compared with high protein diets and consequently their feed conversion efficiency is higher. Keshawarz and Jackson (1992) have come to the same conclusions as well.

Amino acids level of plasma together with blood glucose level and body temperature are the factors that may affect feed consumption by hypothalamus stimulation (Lepkovsky, 1973; Musten *et al.*, 1974; Sturkie, 1986) so that with its increase feed consumption will decrease and vice versa. Murray *et al.* (1998) found that addition of synthetic amino acids like lysine, methionine at high levels to the diet can stimulate insulin secretion from pancreas by aggregating in plasma which in turn releases amino acids from the bodily saved sources and leads to protein synthesis. Han and Baker (1993) reported that the need for lysine to get to the best feed conversion efficiency is higher than the need for reaching to the peak production. Koon reported that a diet with 0.71% lysine in comparison with lower amounts have resulted in higher egg mass production (Muirhead, 1987). Furthermore, Karunajeswa *et al.* (1987) stated that an increase of the lysine level from 0.7-0.8% has led to higher egg mass production. Of course, it should be mentioned that the basal diet was contained sunflower meal. Other studies which have been conducted under specific conditions such as older age of laying hens (Muirhead, 1987), use of low quality protein (Fernandez and Parsons, 1996) and low temperature of the environment (Prochaska and Shafer, 1996) indicate the same positive effect of synthetic amino acids at higher level than NRC recommendation on the performance of laying hens. However, there are some reports that do not fully confirm such results. Koelkebeck *et al.* (1991) for example reported that no difference has been observed in production after increasing lysine to a level higher than NRC recommendation.

Regarding the effect of lysine and methionine on egg weight, the reports are more consistent and most of them have emphasized the positive effect of these two amino acids at proper level on the egg weight (Ayupov, 1985; Karunajeswa *et al.*, 1987; Keshawarz and Jackson, 1992; Prochaska and Shafer, 1996; Revingion *et al.*, 1991). Moreove, some reports have shown the positive effect of adding more lysine to the diet than required on the chickens suffering different stresses (Ayupov, 1985; Merch and Macmillan, 1987). The present study has

attempted to investigate the possibility of using low protein diet together with the proper level of lysine and methionine and to estimate the most appropriate level of the lysine added to the diet in order to have egg laying hens attain the best performance after peak production period.

MATERIALS AND METHODS

This study was performed in 2×5 factorial experiment with a completely randomized design. Two levels of protein (13 and 14%) and 5 lysine levels (10 and 20% lower than NRC recommendation, 10 and 20% higher than NRC recommendation) were compared. Each experimental diet applied to 8 replicate and there were 3 hens in each replicate, composition of basal diet contained 13 and 14% protein and the level of lysine and methionine amino acids were shown in Table 1 and 2, respectively, the methionine content of the diets has been taken to be 85% of lysine. The hens for each replicate were randomly placed in sequential cages (35.5×39.5 cm). The hens were

Table 1: Composision of basal diet (Per percentage of dried weather component)

Ingredients	Basal diet 1	Basal diet 2
Corn	72.370	70.560
Soybean meal	14.200	17.100
wheat	3.500	2.000
Corn oil	-	0.450
DCP	0.750	0.750
Oyster	8.000	8.000
Salt	0.400	0.400
Vitamin and mineral supplement	0.500	0.500
D-L-methionine	0.250	0.240
L-lysine	0.030	-
Total	100.000	100.000
Calculated ingredients		
Metabolizable energy (k cal kg^{-1})	2863.000	2860.000
Protein (%)	13.060	14.040
Calcium (%)	3.250	3.250
Available phosphorus (%)	0.250	0.250
Sodium (%)	0.150	0.150
TSAA (%)	0.473	0.476
Lysine (%)	0.557	0.560

Table 2: Lysine and methionine amount added to basal diets for experimental diets provision

Treatments	Lysine amount added (%)	Methionine amount added (%)	Lysine amount in final diet (%)	TSSA amount in final diet (%)
T1	Basal diet 1	0.000	0.557	0.473
T2	0.068	0.060	0.625	0.533
T3	0.138	0.116	0.695	0.589
T4	0.208	0.176	0.765	0.649
T5	0.278	0.235	0.835	0.708
T6	Basal diet 2	0.000	0.560	0.476
T7	0.061	0.052	0.621	0.528
T8	0.130	0.110	0.690	0.586
T9	0.200	0.170	0.760	0.646
T10	0.270	0.230	0.830	0.706

from commercial egg laying Hy-line w-36; they were 52 weeks old (after peak of egg production) at the beginning. The experiment was performed in three consequential periods for 28 days and the average temperature of the house was almost constant and 17°C to determine status of the flock, initial recording was done for a month before the experiment and after devoting the treatments it was found that there was no significant difference between the treatment groups in term of performance before the experiment. Feeding and watering systems were of traff kind. The feeding systems were completely separated by a partition so that the feeds of the neighboring cages were not mixed. Each unit or replicate had a space of 35.5 cm for both feeding and water systems. The hens were fed *ad libitum* and were under constant light for 18 h during day and night.

Data collecting during the experiment was include daily egg production, egg weight (were collected 3 days per each week and calculated egg mass), the feed consumption for each replicate (was estimated at the end of each period) feed converse ratio, daily death rate and body weight (were calculated at the end of the experiment) due to high amount of data and more importance. The data related to the final and whole period of the experiments were analyzed.

At the end, the collected data were turned in to hen-day and SAS software was used to perform statistical analysis. The means comparison was made by Duncan's test at the probability level of 0.05.

RESULTS AND DISCUSSION

Table 3 and 4 shows the analysis of variance of the different effects of treatments for whole and the third period of the experiment, respectively. As shown in Table 3 the effect of treatments and protein and lysine level of the diet on egg production was significant (p<0.01). The influences on egg mass was significant too although on egg weight was not. Although, the effect of lysine was not significant in the third period but the effect of different levels of lysine on egg production was

observed. Moreover, the effect of protein level of diet on feed consumption in the third period and in the whole period was significant (p<0.01). Similarly, the effect of lysine level on feed conversion ratio was significant as shown in Table 3 and 4 (p<0.05).

Table 5 shows the comparison for the traits mean of the whole period. The effect of different levels of lysine on egg production and egg mass particularly in the group with lower protein (13%) was more evident and with lysine level increase up to 0.76%, the egg production and egg mass have been increased.

In 14% protein group, only the lowest level of lysine (0.56%) has significantly lower egg and egg mass production and there were no other significant differences. Table 5 also shows that the lowest feed consumption belongs to the 0.76% lysine treatment from 13% protein group. In this group as the lysine level increased up to 0.76%, the feed conversion efficiency decreased and attained the lowest level at this level

Table 3: Analysis of variance of the different effects of the treatments during the whole period

Parameters	Model	Protein	Lysine	Lysine x protein interaction
Egg production	3.92**	11.00**	3.84**	2.24ns
Egg weight	1.43ns	1.25ns	1.14ns	1.77ns
Feed consumption	2.84**	14.34**	1.82ns	0.92ns
Protein consumption	5.23**	36.14**	1.77ns	0.96ns
Lysine consumption	8.75**	12.57**	15.54**	1.00ns
Egg mass	5.42**	15.24**	4.92**	3.52*
Feed conversion	7.57**	2.80ns	12.92**	3.40*

*,**and NS are significant at 0.05 level, 0.01 and non-significant, respectively

Table 4: Variance analysis of the different effects of the treatments during the third period

Parameters	Model	Protein	Lysine	Lysine x protein interaction
Egg production	2.57*	5.15*	2.03NS	2.33NS
Egg weight	1.47ns	3.06NS	1.02NS	1.54NS
Food consumption	1.31ns	8.52**	0.13NS	0.67NS
Protein consumption	2.74**	21.40**	0.12NS	0.67NS
Lysine consumption	5.55**	8.43**	9.64**	0.69NS
Egg mass	3.74**	8.42**	2.36ns	3.83**
Feed conversion	4.56**	2.28ns	2.56*	2.56*

*, ** and NS are significant at 0.05 level, 0.01 and non-significant, respectively

Table 5: Comparison of the mean of various traits during the whole period

Parameters	*Dietary lysine (%)					**Dietary lysine (%)					CV
	0.56	0.62c	0.69	0.76	0.828	0.56	0.62	0.69	0.76	0.828	
Egg production (%)	65.63c	65.65c	67.50bc	72.78ab	72.16ab	67.63bc	76.04a	73.04a	74.50a	72.57ab	7.65
Egg weight (g)1	59.08ab	58.60b	60.70ab	60.60ab	60.51ab	59.96ab	61.37a	59.16ab	59.60ab	61.74a	3.99
Feed consumption (g)1	105.70bc	93.70cd	98.70cd	91.45d	106.51bc	121.00a	108.46ab	112.96ab	110.73ab	106.60bc	12.17
Protein consumption (g)1	13.81cd	12.23d	12.89d	11.94d	13.95cd	16.99a	15.23abc	15.86abc	15.55abc	14.97bc	12.29
Lysine consumption (mg)1	588.00e	581.00e	681.00de	695.00cd	884.00a	677.00de	673.00de	780.00bc	841.00ab	882.00a	12.75
Egg mass (g)1	38.70d	38.40d	40.53cd	44.10ab	43.67abc	40.79cd	46.73a	43.31abc	44.38b	44.80ab	7.93
Feed conversion	2.90b	2.65bc	2.46cd	2.16d	2.50c	3.22a	2.35cd	2.67bc	2.54c	2.45cd	11.74

Dietary protein: 13.06*; 14.04**. 1Per hen-day. 2In each row the means that do not have the same letters their differences are significant (p<0.05)

Table 6: Comparison of the mean of various traits during the third period

Parameters	*Dietary lysine (%)					**Dietary lysine (%)					CV
	0.56	0.62	0.69	0.76	0.828	0.56	0.62	0.69	0.76	0.828	
Egg production (%)	67.68ᶜ	65.48ᶜ	69.47ᶜ	77.45ᵃ	70.53ᵇᶜ	72.53ᵃᵇᶜ	76.78ᵃᵇ	70.66ᵃᵇᶜ	74.53ᵃᵇᶜ	74.53ᵇᶜ	9.75
Egg weight (g)	59.87ᵃᵇ	59.00ᵇ	59.15ᵇ	61.36ᵃᵇ	60.79ᵃᵇ	61.41ᵃᵇ	62.51ᵃ	59.75ᵃᵇ	60.02ᵃᵇ	62.06ᵃᵇ	4.65
Feed consumption	97.86ᵇᶜ	102.30ᵃᵇ	89.60ᶜ	94.13ᵇᶜ	96.95ᵇᶜ	109.55ᵃ	103.5ᵃᵇ	112.84ᵃ	106.64ᵃᵇ	112.07ᵃ	15.81
Protein consumption (g)	12.78ᵇᶜ	13.35ᵇᶜ	11.70ᶜ	12.29ᶜ	12.66ᵇᶜ	15.38ᵃᵇ	14.54ᵃᵇ	15.85ᵃ	14.97ᵃᵇ	15.73ᵃ	15.74
Lysine consumption (mg)	545.00ᵈ	634.00ᶜᵈ	618.00ᶜᵈ	715.00ᵇᶜ	803.00ᵃᵇ	613.00ᶜᵈ	643.00ᵇᶜᵈ	781.00ᵃᵇ	810.00ᵃᵇ	927.00ᵃ	16.61
Egg mass (g)	40.55ᵈᵉ	38.69ᵉ	41.11ᵈᵉ	47.47ᵃᵇ	42.95ᵇᶜᵈᵉ	44.60ᵃᵇᶜᵈ	48.08ᵃ	42.39ᶜᵈᵉ	44.82ᵃᵇᶜᵈ	46.34ᵃᵇᶜ	10.23
Feed conversion	2.47ᵇᶜᵈ	2.96ᵃ	2.21ᵈᵉ	2.00ᵃᵉ	2.30ᶜᵈᵉ	2.70ᵃᵇᶜ	2.22ᵈᵉ	2.73ᵃᵇ	2.45ᵇᶜᵈ	2.49ᵇᶜᵈ	15.50

Dietary protein: 13.06*; 14.04**. ¹Per hen-day. ²In each row the means that do not have the same letters their differences are significant (p<0.05)

Table 7: Comparison of the protein groups during the whole period

Parameters	Protein level		CV
	13.06	14.04	
Egg production (%)	68.75ᵇ	72.75ᵃ	7.65
Egg weight (g)	59.77ᵃ	60.37ᵃ	3.99
Feed consumption (g)	99.27ᵇ	111.95ᵃ	12.17
Protein consumption (g)	12.96ᵇ	15.72ᵃ	12.29
Lysine consumption (mg)	686.00ᵇ	771.00ᵃ	12.75
Egg output (g)	41.08ᵇ	44.00ᵃ	7.93
Feed conversion	2.54ᵃ	2.65ᵃ	11.74

In each row the means that do not have the same letters their difference are significant (p<0.05)

Table 8: Comparison of the lysine groups during the whole period

Parameters	Lysine level (%)					CV
	0.56	0.62	0.69	0.76	0.828	
Egg production (%)	66.63ᵇ	70.85ᵃ	70.28ᵃ	73.64ᵃ	72.37ᵃ	7.65
Egg weight (g)	59.50ᵃ	59.97ᵃ	59.60ᵃᵇ	60.12ᵃ	61.12ᵃ	3.99
Food consumption (g)	113.30ᵃ	101.00ᵇ	105.80ᵃᵇ	101.10ᵇ	106.70ᵃᵇ	12.17
Protein consumption (g)	15.40ᵃ	13.73ᵇ	14.37ᵇ	13.74ᵇ	14.45ᵃᵇ	12.29
Lysine consumption	633.00ᶜ	627.00ᶜ	730.00ᵇ	768.00ᵇ	883.00ᵃ	12.75
Egg mass (g)	39.74ᵇ	42.57ᵃ	41.92ᵃᵇ	44.24ᵃ	44.24ᵃ	7.93
Feed conversion	3.06ᵃ	2.50ᵇᶜ	2.57ᵇ	2.35ᶜ	2.48ᵇᶜ	11.74

In each row the means that do not have the same letters their difference are significant (p<0.05)

among all treatments while egg production did not show significant differences compared with the highest average and even in the 3rd period (Table 6) attained the highest amount among all treatments. The same decline trend in feed consumption and feed conversion ratio was observed in 14% protein group (Table 6) but differences were significant (p<0.05).

As shown in Table 7, the comparison of the studied traits between two protein groups showed that the 14% protein group had more egg and egg mass production and feed consumption (p<0.05) whereas egg weight and feed conversion ratio did not show significant differences, although both of these traits in the 14% protein group had higher averages.

Table 8 shows the averages comparison of lysine groups without considering protein level. Egg production at lowest level of lysine (0.56%) was significantly (p<0.05) lesser than upper levels whereas the highest amount of feed consumption belonged to this group although, this difference was significant (p<0.05) with 0.62 and 0.76% lysine level group. In this group, feed conversion was significantly more than higher levels of lysine (p<0.05).

Correlation coefficients of different traits together with protein and lysine levels are shown in Table 9 and 10, respectively. As seen in this study, there are highly positive correlations between lysine level and egg weight in comparison with level of dietary protein and egg weight so that for lysine, the correlation was significant but not for protein.

Regarding egg production and egg mass, there was the same positive correlation between lysine and protein level. Between lysine level of diet and feed conversion ratio, there was negative correlation (p<0.01), regarding protein level, this correlation was positive but insignificant. According to Table 9, between egg production and body weight, there was negative and significant (p<0.05) correlation. Table 11 shows that the lowest body weight belonged to the treatment with highest level of lysine from 13% protein and the highest body weight to lowest level from 14% protein group.

With regard to the negative correlation between body weight and egg production and the existence of such a relation between lysine level of diet and feed conversion ratio which mentioned above, it can be found that the

Table 9: Correlation between different traits (Agulera *et al.*, 1990)

Parameters	Protein level	Lysine level	Egg production	Egg weight	Consumed feed
Protein level	1.000	0.000	0.221*	0.123[NS]	0.387**
Lysine level	0.000	1.000	0.220*	0.222*	-0.109[NS]
Egg production	0.221*	0.220*	1.000	0.104[NS]	0.095[NS]
Egg weight	0.123[NS]	0.222*	0.104[NS]	1.000	0.034[NS]
Consumed feed	0.387**	-0.109[NS]	0.095[NS]	0.034[NS]	1.000
Consumed protein	0.555**	-0.102[NS]	0.122[NS]	0.058[NS]	0.981**
Consumed lysine	0.290**	0.622**	0.225*	0.142[NS]	0.702**
Egg mass	0.221*	0.227*	0.967**	0.349*	0.106[NS]
Feed conversion	0.089[NS]	-0.285**	-0.711**	-0.219*	0.578**
Body weight	119[NS]	0.088[NS]	-0.269*	0.298**	0.174[NS]

*, ** and [NS] are significant at 0.05 level, 0.01 and non-significant, respectively

Table 10: Correlation between different traits

Parameters	Protein	Lysine	Egg mass	Feed converstion	Body weight
Protein level	0.555**	0.290**	0.221*	0.089[NS]	0.119[NS]
Lysine level	-0.102[NS]	0.622**	0.227*	-0.285**	-0.088[NS]
Egg production	0.122[NS]	0.225*	0.967**	-0.711**	-0.269*
Egg weight	0.057[NS]	0.142[NS]	0.349**	-0.219*	0.298**
Consumed feed	0.981**	0.702**	0.106[NS]	0.578**	0.174[NS]
Consumed protein	1.000	0.691**	0.138[NS]	0.540**	0.180[NS]
Consumed lysine	0.691**	1.000	0.230*	0.251*	0.062[NS]
Egg mass	0.138[NS]	0.230*	1.000	-0.715**	-0.172[NS]
Feed conversion	0.540**	0.251*	0.715**	1.000	0.326**
Body weight	0.180[NS]	0.062[NS]	-0.172[NS]	0.326**	1.000

*, ** and [NS] are significant at 0.05 level, 0.01 and non-significant, respectively

Table 11: Variance analysis and comparison of the averages related to body weight at the end of the experiment

Protein level (%)	Lysine level (%)	Body weight (g)
13.06	0.560	1689[ab]
	0.620	1683[ab]
	0.690	1648[ab]
	0.760	1728[ab]
	0.828	1610[b]
14.04	0.560	1785[a]
	0.620	1667[ab]
	0.690	1652[ab]
	0.760	1691[ab]
	0.828	1735[ab]

The means that do not have the same letters their differences are significant (p<0.05)

increase of the lysine level of diet and its concentration level increase in plasma as reported by some researchers (Fernandez and Parsons, 1996; Lepkovsky, 1973; Musten *et al.*, 1974; Sibbald and Wolyne, 1986) will stimulate pancreas insulin secretion and consequently results in other amino acids pick up from body reserves in order to synthesis of protein for egg production and leads to lower feed conversion ratio. The effect of percentage of lysine was here much more important than the consumption lysine, since the groups that have consumed more feeds and mostly belonged to the 14% protein group, more lysine has received due to high consumption of crude protein which could have resulted in amino acids unbalance (Summers and Leeson, 1985). The signification effect of lysine and protein interaction shows the same fact furthermore, according to some researchers, protein consumption efficiency in diets containing lower protein is usually higher (Keshawarz and

Jackson, 1992; Lepkovsky, 1973; Parrand Summers, 1991; Blair *et al.*, 1999). The results of this study about egg production, egg weight and egg mass are conform to the findings of Keshawarz and Jackson (1992) and Agulera *et al.* (1990). Regarding egg weight, reasons for insignificance of lysine level of diet in variance analysis can be attributed to different lysine effects in the treatments of 2 protein groups and somehow to low differences of lysine levels in treatments. Concerning feed consumption, the results of this study are consistent with the findings of some other researches like Summers and Leeson (1985), Lipstein and Bornstein (1975) and Koelkebeck *et al.* (1991).

CONCLUSION

It can be said that laying hens will attain a performance by the application of low protein diet in a way that amino acids shortage be compensated by synthetic amino acids as that of high protein diets and in so doing, feed conversion ratio will usually be improved. Furthermore, it appears that laying hens to relate a level of lysine and methionine in excess of their need more conveniently than lower level.

It may be so due to their abilities to discharge lysine and methionine in surplus through eggs (Ayupov, 1985). As a result, since common protein resources in poultry diets are extremely unstable and sensitive, it is suggested that a percentage higher than the estimated need for amino acids is included in diets for laying hens in order to increase certainty.

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