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## Partial Explanation for Difference in Response of Hens Fed Diets Formulated Based on Lysine vs. Protein

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**Abstract:** Previous studies have indicated that hens fed diets formulated based on protein (FBP) produced more and heavier eggs compared with diets formulated based on lysine (FBL). It was hypothesized that this beneficial effect may be due to higher lysine or other amino acids rather than just protein per se. To test this hypothesis, fish meal (FM) which contains more lysine and has different amino acid ratios than soybean meal, was used to determine if the decline in performance associated with hens fed diets FBL could be prevented. Fish meal was used in diets FBL to supply the same quantity of TSAA and protein but more lysine (0.02 and 0.04%) than diets FBL. Three levels of FM (0, 2.5 and 5%) with three TSAA levels (0.65, 0.72 and 0.81%) were used to obtain the 9 dietary treatments. Three diets were also FBP to compare results. A total of 96 replicates of 16 hens each (n = 1536) were randomly assigned to 12 dietary treatments. Egg production (EP), feed consumption (FC), egg weight (EW) and egg specific gravity (ESG) were evaluated to determine hen performance. Hens fed diets FBP produced more (1.4%) and heavier (0.6g) eggs than hens fed diets FBL. Inclusion of FM (lysine) to diets FBL prevented the drop in EP and there was no significant difference ( $P > 0.05$ ) in EP of hens fed diets FBP (90.4%) and hens fed diets FBL using FM (90.4%). However, FM (lysine) did not improve EW equal to hens fed diets FBP. Although FC was not influenced by FM (lysine), a TSAA effect was observed. Hens fed the higher TSAA levels consumed more feed than those fed the lower level. These results indicate that the difference in performance of hens fed diets FBP and FBL were not due to protein per se but at least partially due to lysine and other non-sulfur containing amino acids.

**Key words:** Fish meal, hens, lysine, TSAA

### Introduction

There are two major methods of feed formulation used in the commercial egg production. Some breeding companies recommend formulating feed for commercial Leghorns based on protein to maximize performance, where minimum dietary protein specifications control the lysine level (Hy-Line, 2000). Diets are also formulated based on lysine, where minimum dietary lysine specifications control the protein level. Also, there is no minimum protein level specification and TSAA to lysine ratio is kept constant (Ahmad *et al.*, 1997). Diets formulated based on protein typically contain slightly higher energy levels, than diets formulated based on lysine. Previous research has indicated that energy intake had virtually no effect on egg size (Leeson, 1989), or hen performance when dietary energy was increased from 2783 to 2915 kcal ME/kg feed (Sohail *et al.*, 2002a). Roland *et al.* (1995) reported that hens fed diets formulated based on protein produced more and heavier eggs than hens fed diets formulated based on lysine. However, hens fed diets formulated based on lysine made two cents more per dozen eggs. Roland *et al.* (1998) published an econometric feeding and management program for commercial Leghorns to optimize profits by altering nutrient intake and environmental temperature as feed and egg prices

change. They formulated diets based on protein as well as based on lysine. All the respective diets from the two formulation methods had a similar methionine level and the difference between the higher and lower TSAA levels was identical. They reported that hens fed diets formulated based on protein produced 2.1% more and 0.8 g heavier eggs than hens fed diets formulated based on lysine. Harms and Ivey (1993) reported lysine as the second limiting amino acid after methionine in a corn soybean meal diet. Martin *et al.* (1998) reported improved performance of the ducklings fed fish meal. Sohail and Roland (1999) hypothesized that the beneficial effect of hens fed diets formulated based on protein vs. lysine may be due to higher lysine and/or other amino acids, rather than just protein per se. To test this hypothesis, fishmeal, which contains more lysine than soybean meal (NRC, 1994) was added as a source of lysine to diets formulated based on lysine, to supply the same TSAA and protein level but higher lysine levels than diets formulated based on lysine. The objective of this study was to determine if reduced performance associated with hens fed diets formulated based on lysine could be prevented using fishmeal.

### Materials and Methods

Fifteen hundred and thirty six Hy-Line W-36 hens (21 wk

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Table 1: Ingredients and calculated nutrient composition of experimental diets

Ingredients	Diets based on lysine			Based on lysine With 2.5% fish meal			Based on lysine With 5% fish meal			Based on protein		
	1	2	3	4	5	6	7	8	9	10	11	12
	----- % -----											
Corn 8.6% <sup>1</sup>	59.09	63.49	67.15	61.86	66.26	69.37	64.50	68.09	70.97	45.16	51.96	57.49
Soybean meal <sup>1</sup> (48% CP)	27.00	23.31	20.35	23.20	19.51	16.65	19.42	15.89	13.06	37.74	31.94	26.99
Fish meal <sup>1</sup>	...	...	...	2.50	2.50	2.50	5.00	5.00	5.00	...	...	...
Limestone	7.07	7.08	7.08	6.98	6.99	7.00	6.89	6.90	6.90	7.04	7.05	7.06
Hard shell	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Dicalcium phosphate <sup>2</sup>	1.84	1.86	1.88	1.49	1.51	1.52	1.13	1.15	1.17	1.79	1.82	1.85
Poultry oil	1.81	1.14	0.50	0.85	0.19	...	...	...	...	5.13	4.14	3.59
Salt (NaCl)	0.48	0.46	0.42	0.43	0.41	0.37	0.38	0.36	0.32	0.48	0.45	0.42
Vitamin premix <sup>3</sup>	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Mineral premix <sup>4</sup>	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
DL-methionine	0.22	0.16	0.12	0.20	0.14	0.10	0.18	0.12	0.08	0.17	0.13	0.10
Calculated analysis												
Crude protein, %	18.04	16.65	15.54	18.04	16.65	15.54	18.04	16.65	15.54	22.00	19.80	17.90
ME, kcal/kg	2,803	2,809	2,812	2,803	2,809	2,831	2,807	2,845	2,875	2,860	2,871	2,895
Calcium, %	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Total phosphorus, %	0.67	0.66	0.65	0.65	0.64	0.63	0.64	0.63	0.62	0.70	0.68	0.67
Nonphytatephosphorus, %	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45
Methionine, %	0.52	0.45	0.40	0.53	0.46	0.41	0.54	0.47	0.42	0.51	0.46	0.40
Met + cys, %	0.81	0.72	0.65	0.81	0.72	0.65	0.81	0.72	0.65	0.86	0.77	0.69
Lysine, %	0.97	0.87	0.79	0.99	0.89	0.81	1.01	0.91	0.83	1.26	1.10	0.97

<sup>1</sup>Protein and amino acid analysis were chemically determined for corn, soybean meal and fish meal. <sup>2</sup>Dynafos®, IMC-Agrico Feed Ingredients, Bannockburn, IL 60015. <sup>3</sup>Provided per kg of diet: vitamin A (as retinyl acetate), 8000 IU; cholecalciferol, 2,200 ICU; vitamin E (as dl-alpha tocopheryl acetate), 8 IU; vitamin B<sub>12</sub>, 0.02 mg; riboflavin, 5.5 mg; d-calcium pantothenic acid, 13 mg; niacin, 36 mg; choline, 500 mg; folic acid, 0.5 mg; vitamin B<sub>1</sub> (thiamin mononitrate), 1 mg; pyridoxine, 2.2 mg; d-biotin, 0.05mg; vitamin K (menadione sodium bisulfite complex), 2 mg. <sup>4</sup>Provided per kg of diet: manganous oxide, 65 mg; iodine (ethylene diamine dihydroiodide), 1 mg; iron (ferrous carbonate), 55 mg; copper oxide, 6 mg; zinc oxide, 55 mg; selenium (sodium selenite), 0.3 mg.

Table 2: Influence of feed formulation method and total sulfur amino acid (TSAA) level on egg production (EP) of Hy-Line W-36 hens

Dietary treatment	Weekly egg production (%)				
	4	8	12	16	Average <sup>1</sup>
Feed Formulation Method	NS <sup>2</sup>	NS	NS	NS	*
Lysine	89.2	90.1	90.1	84.5	89.0 <sup>b</sup>
Lysine + Fish meal (2.5%)	91.7	91.4	90.4	85.8	90.4 <sup>a</sup>
Lysine + Fish meal (5%)	89.8	90.9	91.8	86.0	90.1 <sup>ab</sup>
Protein	89.8	91.1	91.2	85.9	90.4 <sup>a</sup>
SEM	0.95	0.74	0.75	0.73	0.39
TSAA (%)	NS	NS	***	*	***
0.65	88.0	88.9	88.4 <sup>b</sup>	84.5 <sup>b</sup>	87.8 <sup>b</sup>
0.72	90.8	92.0	92.7 <sup>a</sup>	86.0 <sup>ab</sup>	91.0 <sup>a</sup>
0.81	91.5	92.3	92.4 <sup>a</sup>	86.9 <sup>a</sup>	91.5 <sup>a</sup>
SEM	0.82	0.64	0.65	0.65	0.34

<sup>1</sup>Average of wk 4 to 16. <sup>abc</sup>Values with different superscripts within a column differ at P < 0.05. \* P < 0.05. \*\*\* P < 0.001.

of age) were housed in a controlled environment for 16 weeks. Temperature was maintained at approximately 28.9 °C during the day and 20 °C during the night to maintain an average house temperature of 25.6 °C. At onset of production, light was increased from 12 h in increments of 15 min per wk to reach a constant photoperiod of 16 h light: 8 h dark. Hens were supplied feed and water *ad libitum*. Diets 1 to 9 were formulated based on lysine with three levels of fish meal (0, 2.5 and 5.0%) and three TSAA levels (0.65, 0.72 and 0.81%). In addition, diets (10 to 12) were formulated based on protein with methionine levels similar to those in diets formulated based on lysine to compare results (Table 1). Diets formulated based on protein typically have more protein and energy than diets formulated based on lysine. Hens were divided into 96 replicates comprising 16 hens per replicate (n = 1536). Each replicate comprised four adjacent cages housing four birds per cage (31 x 41 cm). Replicates were randomly assigned to the dietary treatments in a randomized complete block design with a factorial arrangement of 3 TSAA levels x 3 feed formulation methods. Response criteria used to measure hen performance were feed consumption, egg production, egg weight, egg specific gravity and mortality. Egg production and mortality were recorded daily. Feed consumption was measured weekly and egg weight and egg specific gravity values were determined biweekly using all eggs produced during 2 consecutive days of the wk. Egg specific gravity was determined using gradient saline solutions varying in specific gravity from 1.060 to 1.100 in 0.005 unit increments (Strong, 1989). Data were subjected to ANOVA (Steel and Torrie, 1980) using General Linear Model procedure of SAS (SAS Institute, 1989). Regression analysis was carried out to determine relationship between variables. Means were separated using Tukey's multiple range test (Steel and Torrie, 1980). The statistical model (Cochran and Cox, 1957) used to determine performance criteria was:

$$Y_{ijkl} = \mu + F_i + T_j + C_k + FT_{ij} + FC_{ik} + TC_{jk} + FTC_{ijk} + e_{ijkl}$$

where  $Y_{ijkl}$  is the individual observation,  $\mu$  is the overall mean,  $F_i$  is the formulation effect,  $T_j$  is the TSAA effect,  $C_k$  is the cage level effect,  $FT_{ij}$ ,  $FC_{ik}$ ,  $TC_{jk}$ , and  $FTC_{ijk}$  are interactions and  $e_{ijkl}$  is the random error.

## Results and Discussion

Results indicated no interactions between TSAA and lysine levels on the performance criteria evaluated. Therefore, all results are presented based on the main effects.

**Egg Production:** Egg production (EP) peaked at 93% and sustained at 90% for over 8 wk (not shown). Addition of fish meal (2.5 and 5%) to diets formulated based on lysine improved EP and there was no difference in EP of hens fed diets formulated based on protein and hens fed diets formulated based on lysine, with additional lysine supplied by fish meal (Table 2). As no extra protein was added to diets formulated based on lysine to improve EP, something other than protein improved EP. It is believed that the improvement in EP may be due to the extra lysine or a combination of other amino acids supplied by fish meal, which is in agreement with the report of Harms and Ivey (1993) that EP increased 6.9 to 10.5% when methionine and lysine were added to a protein deficient diet containing 13.8% protein. There was no significant difference in EP of hens fed 2.5 or 5.0% fish meal (Table 2). Reducing TSAA to 0.65% significantly reduced EP within 8 wk, which was in agreement with previous reports (Roland *et al.*, 1993; Sohail *et al.*, 2002b). This decline in EP indicated that diets containing 0.65% TSAA were inadequate to maintain optimum EP. Hens fed the lower level (0.65% TSAA) consumed 50 mg less TSAA than the minimum NRC (1994) recommendation of 580 mg/hen/d. However, no difference in EP was observed in hens fed the higher TSAA levels of 0.72 and 0.81%. Hens fed the

Table 3: Influence of feed formulation method and total sulfur amino acid (TSAA) level on egg weight (EW) of Hy-Line W-36 hens

Dietary treatment	Weekly egg weight (g)				
	4	8	12	16	Average <sup>1</sup>
Feed Formulation Method	NS <sup>2</sup>	NS	***	***	***
Lysine	50.00	53.23	55.76 <sup>b</sup>	57.66 <sup>b</sup>	53.46 <sup>b</sup>
Lysine + Fish meal 2.5%	50.15	53.48	54.76 <sup>c</sup>	56.82 <sup>c</sup>	53.29 <sup>bc</sup>
Lysine + Fish meal 5%	49.57	52.88	54.57 <sup>c</sup>	56.54 <sup>c</sup>	52.87 <sup>c</sup>
Protein	50.35	53.65	56.90 <sup>a</sup>	58.77 <sup>a</sup>	54.09 <sup>a</sup>
SEM	0.252	0.300	0.210	0.234	0.175
TSAA (%)	NS	NS	***	***	NS
0.81	49.78	52.87	55.89 <sup>a</sup>	57.71 <sup>a</sup>	53.36
0.72	50.20	53.46	55.50 <sup>a</sup>	57.71 <sup>a</sup>	53.55
0.65	50.11	53.50	54.81 <sup>b</sup>	56.66 <sup>b</sup>	53.15
SEM	0.218	0.260	0.182	0.203	0.152

<sup>1</sup>Average of wk 2 to 16. <sup>2</sup>Not significant at  $P > 0.05$ . <sup>abc</sup>Values with different superscripts within a column differ at  $P < 0.05$ . \*\*\* $P < 0.001$ .

Table 4: Influence of feed formulation method and total sulfur amino acids (TSAA) on egg specific gravity (ESG) of Hy-Line W-36 hens

Dietary treatment	Weekly egg specific gravity (ESG)			
	4	8	16	Average
Feed Formulation Method	***	NS <sup>1</sup>	***	***
Lysine	1.086 <sup>b</sup>	1.084	1.084 <sup>a</sup>	1.085 <sup>a</sup>
Lysine + Fish meal 2.5%	1.087 <sup>a</sup>	1.084	1.085 <sup>a</sup>	1.085 <sup>a</sup>
Lysine + Fish meal 5%	1.087 <sup>a</sup>	1.085	1.084 <sup>a</sup>	1.085 <sup>a</sup>
Protein	1.086 <sup>b</sup>	1.084	1.082 <sup>b</sup>	1.084 <sup>b</sup>
SEM	0.0002	0.0003	0.0003	0.0002
TSAA (%)	NS	NS	NS	NS
0.65	1.087	1.085	1.084	1.085
0.72	1.086	1.084	1.084	1.085
0.81	1.086	1.085	1.084	1.085
SEM	0.0002	0.0003	0.0003	0.0002

<sup>1</sup>Not significant at  $P > 0.05$ . <sup>abc</sup>Values with different superscripts within a column differ at  $P < 0.05$ . \*\*\* $P < 0.001$ .

higher levels consumed 583 and 680 mg TSAA/hen/d and exceeded the NRC recommendation of 580 mg TSAA/hen/d. This finding was in agreement with the report of Schutte *et al.* (1994) that no difference in EP was observed when hens consumed 685 to 820 mg TSAA/hen/d.

**Egg weight:** Influence of feed formulation method on egg weight (EW) was observed within 12 wk (Table 3). Average egg weights of hens fed diets formulated based on protein were heavier by approximately 0.6 g/hen/day than EW of hens fed diets formulated based on lysine. This result was in agreement with the previous report (Roland *et al.*, 1995). Addition of fishmeal to diets formulated based on lysine did not improve EW. Also, the TSAA levels used did not influence the average EW of hens; however, towards the end of the experiment, EW of hens fed the higher TSAA levels (0.72 and 0.81%)

were heavier than the lower TSAA level of 0.65%. These results agree with the previous reports that EW decreases when dietary TSAA level is reduced (Roland *et al.*, 1992a, b; Roland *et al.*, 1993; Sohail *et al.*, 2002b). Level of 0.65% TSAA was inadequate to maintain EW of hens consuming 80 to 85 g feed/hen/d.

**Egg specific gravity:** Inclusion of fish meal in diets formulated based on lysine influenced egg specific gravity (ESG) within 4 weeks (Table 4). On average, ESG was significantly higher ( $P < 0.001$ ) in hens fed diets formulated based on lysine than hens fed diets formulated based on protein. This result was probably due to the distribution of the same amount of calcium on a smaller surface area of eggs from hens fed diets formulated based on lysine compared to eggs from hens fed diets formulated based on protein. These results agree with the report of Calderon and Jensen

Table 5: Influence of feed formulation method and total sulfur amino acids (TSAA) on feed consumption (FC) of Hy-Line W-36 hens

Dietary treatment	Weekly feed consumption (g)				
	4	8	12	16	Average <sup>1</sup>
Feed formulation method	NS <sup>2</sup>	NS	NS	NS	NS
Lysine	77.1	82.1	88.3	87.7	83.7
Lysine + Fish meal 2.5%	76.3	81.9	85.6	87.4	82.9
Lysine + Fish meal 5%	76.6	81.0	86.1	87.9	82.9
Protein	76.2	81.3	87.2	85.7	83.2
SEM	0.52	0.50	0.76	0.88	0.45
TSAA (%)	*	***	*	*	***
0.65	75.6 <sup>b</sup>	80.0 <sup>b</sup>	85.2 <sup>b</sup>	85.4 <sup>b</sup>	81.6 <sup>b</sup>
0.72	77.4 <sup>a</sup>	82.4 <sup>a</sup>	87.8 <sup>a</sup>	88.8 <sup>a</sup>	84.0 <sup>a</sup>
0.81	76.7 <sup>ab</sup>	82.3 <sup>a</sup>	87.4 <sup>ab</sup>	87.5 <sup>ab</sup>	83.9 <sup>ab</sup>
SEM	0.47	0.43	0.66	0.79	0.39

<sup>1</sup>Average of wk 4 to 16. <sup>2</sup>Not significant at  $P > 0.05$ . <sup>abc</sup>Values with different superscripts within a column differ at  $P < 0.05$ . \*  $P < 0.05$ . \*\*\*  $P < 0.001$ .

(1990) that reduced EW resulted in an improvement in eggshell quality as measured by ESG. TSAA levels had no influence on ESG that was in agreement with Sohail *et al.* (2002b).

**Feed consumption:** Feed formulation method had no significant influence on average feed consumption (FC) of hens and there was no difference in FC of hens fed diets formulated based on lysine compared to diets formulated based on protein (Table 5). Addition of fish meal to diets formulated based on lysine did not improve FC and on average, hens consumed approximately 83 g feed/hen/d. The effect of TSAA level on FC was observed within four weeks ( $P < 0.05$ ). In agreement with Sohail *et al.* (2002b) FC was higher in hens fed the higher TSAA levels (0.72 and 0.81%) compared to hens fed the lower TSAA level (0.65%). This FC pattern was observed through out the study.

Results of this study confirm the previous report (Roland *et al.*, 1995) that hens fed diets formulated based on protein produce more and heavier eggs than hens fed diets formulated based on lysine. In this study inclusion of extra lysine using fish meal in corn-soy diets formulated based on lysine prevented the decline in EP due to lower protein. However, extra lysine from fish meal did not increase EW equal to hens fed diets formulated based on protein. Addition of 0.04% lysine using fish meal (5%), as a source of lysine did not give any additional benefit over 0.02% added lysine (from 2.5% fish meal). These results indicate that corn-soy diets formulated based on lysine were not just deficient in protein per se, but were deficient in amino acids. Because fish meal contains amino acids levels that are different from soybean meal, the reduced performance of hens fed corn-soy diets formulated based on lysine may be partially due to lysine and other amino acids and not due to protein per se.

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