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Effect of Three Protein Levels and an Enzyme Blend on Egg Quality of Laying Hens

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Abstract: One approach to enhance the Crude Protein (CP) value of laying hens diets it is the use of enzymes. The objective of this experiment was to evaluate different CP levels and an enzyme blend (Avizyme; AZ) in laying hens diets on egg quality. Two hundred and eighty eight ISA Babcock B-300 hens were used. The diets were: (A) 18.8% CP, (B) 18.8% CP+AZ, (C) 16.6% CP, (D) 16.6% CP+AZ, (E) 16.0% CP and (F) 15.4% CP. Data were analyzed using analyses of variance for factorial designs (Diets A, B, C and D) and a complete randomized design (Diets C, E and F). CP or AZ had no effect on egg quality ($p>0.05$). Reduction of CP in laying hen diets tended ($p<0.08$) to increase feed intake (90.8, 93.6 and 96.5 g day^{-1}) and feed conversion ($p<0.05$) (2.1, 2.2 and 2.3) for 16.6, 16.0 y 15.4% of CP, respectively. Reduction of CP in laying diets increased ($p<0.05$) albumen height (11.4, 11.9 y 11.9 mm). Significant ($p<0.05$) reductions of feed intake (96.2 vs 89.9 g day^{-1}) and feed conversion (2.1 vs 2.0) in diets supplemented AZ were observed. Significant ($p<0.05$) interactions of PC and AZ on feed intake and feed conversion were found. The reduction of CP in the diets did not affect egg quality, but has an effect on feed intake and feed conversion. Avizyme supplementation reduced feed intake and improve feed efficiency in high CP diets.

Key words: Egg quality, enzyme blend, crude protein

INTRODUCTION

Protein is the nutrient of major cost in laying hen diets for optimum egg production. Appropriate crude protein level in the poultry diet improves feed utilization and reduces environmental pollution due to decrease output of nitrogen in manure (Novak *et al.*, 2006, 2007).

Various approaches have been studied to optimize crude protein utilization in laying hen diets. One approach is to reduce the CP content in the diets of laying hen. Blair *et al.* (1999) found that layer performance could be maintained with low protein diets (13.5% CP) supplemented with essential aminoacids. Junqueira *et al.* (2006) reported similar results between second cycle laying hens fed 16 and 20% CP diets. Khajali *et al.* (2008) found that layer performance remains satisfactory on reduced CP diets for short periods, but long term feeding of reduced CP diets may not be advisable because it reduce performance in the late stage of production. Recently, Latshaw and Zhao (2011) reported that feed intake reduction from 17 to 15 g of CP/day in laying hens did not affect laying performance. In other studies, reduction of sulphur amino acids from 0.71 to 0.65%, caused a reduction of egg weight (Correa *et al.*, 2007).

Other approach is to use enzymes to enhance the feeding value of the diets. The inclusion of an enzyme cocktail (xylanase, protease and amylase) in a corn-soybean meal diet improved nutrient digestibility and performance of broilers fed low metabolizable energy diets (Cowieson and Adeola, 2005). Cowieson and Ravindran (2008) concluded that the energy and amino acid values of maize based diets for broilers can be enhanced by supplementation with above enzyme cocktail. Novak *et al.* (2007) reported that supplementing a commercial corn, soybean meal and wheat middling diet with an enzyme blending (protease, xylanase and amylase) did not affect growth of White Leghorn pullets, but resulted in a significant improvement in cumulative Feed Conversion Rate (FCR), due to reduced feed intake. Furthermore, protease, amylase and xylanase supplementation (Troche *et al.*, 2007) improved significantly energy and protein ileal digestibility in turkeys (Troche *et al.*, 2007). Silversides *et al.* (2006) concluded that the inclusion of xylanase in wheat based diets increased egg and albumen weight and albumen height. Mirzaie *et al.* (2012) found that diets supplemented with xylanase wheat up to 69% had no effect in laying hens. Few studies on the use of protease, xylanase and amylase in sorghum-soya bean meal based diets for laying hens has been done under tropical conditions.

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The aim of this experiment was to evaluate the effects of different CP levels and Avizyme® (amylase, xylanase and protease) supplementation on egg quality.

MATERIALS AND METHODS

Materials: The experimental work was carried out at the Faculty of Veterinary and Animal Science of the Universidad Autonoma de Yucatán. The six diets utilized in this experiment are showed in Table 1. Diets A, C E and F had 18.8, 16.6, 16 and 15.4% CP, respectively; whereas diets B and D had two crude protein levels (18.8 and 16.6 %, respectively) and 370 g ton^{-1} of Avizyme® (AZ). The experimental diets were formulated according to recommendations of the manual of ISA Babcock B-300. Total Sulphur Aminoacids (TSAA) and lysine was according to CP level in the diets. The diets were based on sorghum and soya bean meal. Avizyme® (Danisco Animal Nutrition) includes a minimum of 1,000 units of xylanase/g, 4,000 units of protease/g and 2,000 units of α-amylase/g (according to the manufacturer).

Methods: Two hundred and eighty-eight Isa-Babcock B-300 laying hens of 56 weeks of age were monitored during 8 weeks. Hens and treatments were randomly distributed to 96, 40×40 cm cages (Three hens per cage) and every cage had one feeder and one drinker. The hens were fed *ad libitum* at 8:00 h and the eggs collected at 14:00 h every day. Feed offered and refused was measured also daily to estimate feed intake. Twice a week two eggs from each cage were weighed, as were the albumen and yolk utilizing a scale (0.1 g). Furthermore the albumen and yolk length and height and the albumen width were obtained. The eggs were broken on a plate and the albumen length, height and width was measured using a micrometer. Afterwards, the yolk was separated from the albumen and its weight width and height was taken using a micrometer. Feed intake was estimated as average intake/hen/day throughout the experimental trial. Total weekly egg weight and feed intake data were combined to calculate feed conversion.

Statistical analysis: Data of the six diets were analyzed as if two experimental designs had been used. Data from Diets A, B, C and D were analyzed according to a 2×2 factorial design and data from diets C, E and F were analyzed according to a completely randomized design. Egg quality trait data were analyzed using the MIXED procedure for repeated measures (SAS, 2006). Also, the linear and quadratic effects of diets C, E and F were evaluated. Data on feed consumption and conversion were analyzed using GLM procedures without repeated measures.

RESULTS

Egg weight and egg quality trait means by diets (A, B, C and D) are showed in Table 2. There were no differences (p>0.05) between treatments for any of the egg traits evaluated. Also, no difference between 18.8 and 16.6% CP in the diet was observed for feed intake (p>0.05). However, TSAA and lysine consumption was

Table 1: Diets utilized and chemical composition

Item	Diet[1] A and B 18.8% CP	Diet[2] C and D 16.6% CP	Diet E 16.0% CP	Diet F 15.4% CP
Sorghum	53.1	61.2	63.6	65.9
Soybean Meal	30.7	24.0	22.3	20.5
$CaCO_3$	9.7	9.6	9.6	9.6
Phosphate dicalcicum	1.9	2.0	2.1	2.1
Soybean oil	3.9	1.9	1.5	1.1
NaCl	0.30	0.30	0.30	0.30
Minerals premix[3]	0.10	0.10	0.10	0.10
Vitamins premix[4]	0.25	0.25	0.25	0.25
DL-Methionine	0.11	0.13	0.12	0.11
L-Lysine HCL			0.11	0.11
Calculate analysis				
ME (kcal kg^{-1})	2701	2701	2699	2701
Calcium (%)	4.09	4.09	4.10	4.10
Phosphorous available (%)	0.46	0.47	0.49	0.49
Calculate analysis of CP and aminoacids (%)				
Crude protein	18.8	16.6	16.0	15.4
Methionine	0.38	0.38	0.36	0.34
Methionine+Cysteine	0.68	0.64	0.61	0.59
Lysine	0.94	0.86	0.82	0.78
Calculate analysis of digestible aminoacids (%)				
Methionine	0.36	0.36	0.34	0.32
Methionine+Cysteine	0.64	0.60	0.58	0.55
Lysine	0.88	0.81	0.77	0.73

[1] Diet B was added 370 g ton^{-1} of AZ. [2] Diet D was added 370 g ton^{-1} of AZ. [3] Concentration/kg of diet: vitamin A, 8000 UI; vitamin D, 2500 UI; vitamin E, 8 UI; vitamin K, 2 mg; vitamin B_{12}, 0.002 mg; riboflavin, 5.5 mg; calcium pantothenic 13 mg; niacin, 36 mg; Choline, 500 mg; folic acid, 0.5 mg; tiamine, 1 mg; pyridoxine, 2.2 mg; biotin, 0.05 mg, [4] Concentration/kg of diet: manganese, 65 mg; iodine, 1 mg; iron, 55 mg, copper, 6 mg; zinc, 55 mg; selenium, 0.3 mg

Table 2: Egg traits means from laying hens fed diets with different crude protein levels and supplemented Avyzime

		Weight (g)			Height(mm)		Width (mm)		Length (mm)
Factor		Egg	Albumen	Yolk	Albumen	Yolk	Albumen	Yolk	Albumen
CP (%)	18.8	62.4	36.5	15.6	11.6	15.0	61.5	39.0	76.1
	16.6	61.2	35.7	15.3	11.5	14.8	60.4	38.8	74.7
Enzyme (g t^{-1})	0	62.6	36.5	15.6	11.6	14.8	61.2	39.0	75.3
	370	62.0	35.7	15.2	11.5	15.0	60.6	38.8	75.5
EEM		0.66	0.40	0.18	0.14	0.14	0.68	0.26	0.66
Significance (p-values)									
CP		0.2126	0.2041	0.2061	0.5340	0.1878	0.2410	0.5421	0.1517
Enzyme		0.6812	0.7627	0.6100	0.9022	0.2409	0.5538	0.6848	0.8104
CP × Enzyme		0.8453	0.6682	0.7819	0.1102	0.3879	0.4317	0.3441	0.9163

Table 3: Feed intake and feed conversion in laying hens fed different crude protein levels and supplemented Avizyme

		Intake/day						
Factor		Feed (g)	CP (g)	Energy (kcal)	Methionine (g)	Methionine+Cistine (g)	Lysine (g)	Feed conversion
CP (%)	18.8	95.0	17.9	25.7	0.36	0.64	0.89	2.1
	16.6	91.1	15.1	24.1	0.34	0.58	0.79	2.1
Enzyme (g t^{-1})	0	96.2	17.1	26.0	0.37	0.63	0.87	2.1
	370	89.9	15.9	24.3	0.34	0.59	0.80	2.0
EEM		1.69	0.30	0.45	0.006	0.011	0.015	0.06
Significance (p-values)								
Diet		0.1107	0.0000	0.1107	0.0231	0.0003	0.0000	0.0577
Enzyme		0.0105	0.0070	0.0105	0.0099	0.0087	0.0080	0.6249
Diet×Enzyme		0.0055	0.0038	0.0055	0.0052	0.0046	0.0043	0.0039

Table 4: Egg traits means from laying hens fed three levels of crude protein

		Weight (g)			Height (mm)		Width (mm)		Length (mm)
Factor		Egg	Albumen	Yolk	Albumen	Yolk	Albumen	Yolk	Albumen
CP (%)	16.6	61.6	36.1	15.3	11.4	14.8	60.9	39.0	74.9
	16.0	61.3	35.2	15.4	11.9	15.2	60.0	39.5	74.6
	15.4	61.8	35.5	15.4	11.9	15.1	60.6	39.3	74.2
EEM		0.54		0.18	0.17	0.17	0.70	0.31	0.66
Significance (p-values)									
CP		0.8492	0.4376	0.7657	0.0386	0.2713	0.6633	0.4461	0.7124
Lineal		0.8293	0.3862	0.5417	0.0254	0.2671	0.7635	0.4217	0.4114
Quadratic		0.5936	0.3531	0.6994	0.2358	0.2510	0.3974	0.3347	0.9506

lower ($p<0.05$) for the hens fed the 16.6% CP diet than for hens fed 18.8% CP diet (Table 3). Significant effect of AZ on feed intake was observed ($p<0.05$). Inclusion of AZ in the diets reduced the feed intake in 6 g. Therefore, CP, energy and amino acids intake were lower in the diets supplemented with AZ ($p<0.05$). However, significant interaction of CP and AZ was found for feed intake ($p<0.05$). This interaction was due to a similar feed intake of the hens under the low PC, with or without AZ (92.1 vs 90.8 g, respectively) and to the significant ($p<0.05$) reduction of feed intake in the high PC plus AZ diet (88.3 g) as compared to the high PC without AZ diet (101.5 g). However, the reduction in feed intake did not affect egg quality traits ($p>0.05$). Feed efficiency improved slightly ($p<0.06$) in laying hens fed the lower CP diets. Nonetheless, significant interaction ($p<0.05$) show that feed conversion improvement was greater in laying hens fed high protein diets (2.22 vs 1.94 for 0 and 370 g t^{-1} of AZ, respectively) than for hens fed low CP (2.03 vs 2.08 for diets with or without AZ, respectively). Non statistical differences for egg traits were found ($p>0.05$) when diets with 16.6, 16.0 and 15.4% CP were compared (Table 4). However, feed intake, feed conversion and energy intake increased lineally as CP was reduced in laying hen diets ($p<0.08$) (Table 5).

It is remarkable that TSAA amino acids and lysine intake were similar between diets ($p>0.05$). This result suggests that hens tended to increase their feed consumption to reach similar amino acids intake.

DISCUSSION

The lack of statistical differences between crude protein treatments (18.8 vs 16.6%) agree with other experiments where different crude protein levels, in laying hens were evaluated (Novak *et al*., 2006; Perez-Bonilla *et al*., 2012). However, the small decrease of 4 g of food per day observed in the hens fed 16.6% CP was enough to reduce TSAA and lysine consumption in comparison to the hens fed 18.8% CP (Table 3). This

Table 5: Feed intake of laying hens fed three levels of crude protein

Factor		Intake/day						
		Feed (g)	CP (g)	Energy (kcal)	Methionine (g)	Methionine+Cysteine (g)	Lysine (g)	Feed conversion
CP (%)	16.6	90.8	15.1	24.5	0.35	0.58	0.78	2.1
	16.0	93.6	15.0	25.2	0.34	0.57	0.77	2.2
	15.4	96.5	14.9	26.1	0.33	0.57	0.75	2.3
EEM		2.27	0.36	0.61	0.01	0.01	0.02	0.06
Significance (p-values)								
CP		0.2100	0.9282	0.2052	0.3649	0.8067	0.5793	0.1007
Lineal		0.0777	0.7011	0.0756	0.1566	0.5619	0.2969	0.0423
Quadratic		0.9742	0.9781	0.9396	0.9757	0.7720	0.9999	0.5265

result agrees with that of Junqueira *et al.* (2006) who reported that the consumption is reduced quadratically with decreasing protein levels in laying hens. Similarly, feed consumption in broilers was reduced when dietary CP decreased from 25 to 21%, even when limiting amino acids were maintained (Blair *et al.*, 1999). Other studies did not report effect of CP reduction in laying hens diets on feed intake and amino acids consumption, because regardless of the level of CP in the diet, amino acid content was the same (Roberts *et al.*, 2007). Contrary, in this experiment CP and amino acid content in the diet was reduced. Thus, although in this experiment there were no statistical differences between treatments in feed intake, lower intake of TSAA and lysine were expected.

In the present study, utilization of Avyzime reduced markedly the feed intake of hens, mainly of those fed the high CP diets. Contrary to our results, several studies that examimate the use of exogenous enzymes in poultry diets, did not reporte a reduction in feed intake (Meng *et al.*, 2005; Jalal *et al.*, 2007; Adeola *et al.*, 2008). However, AZ contain side effects of xylanase, amylase and protease and was expected to improve energy and N utilization by increasing the digestion of starch and nonstarch polysaccharides, thus releasing entrapped nutrients (Adeola *et al.*, 2008). Angel *et al.* (2011) reported an increase in amino acid digestibility in broilers supplemented with a protease in the diets. Similarly Adeola *et al.*, (2010) found that carbohydrate supplementation (xylanase and amylase) improved 5.7% the metabolizable energy in practical corn soybean meal based diets. Therefore, it is possible to assume that the additional energy and the amino acids released in the diets supplemented with AZ caused a feed intake reduction.

In the current study feed efficiency was improved noticeably in laying hens fed the high CP plus AZ diets. An improvement in feed efficiency was expected as a result of the decrease in feed intake and differences in egg weight between treatments. As explained in the preceding paragraph, the enzyme probably released more nutrients from the diet, which caused a reduction in consumption and thus improved the feed efficiency. No statistical differences between treatments were found for the egg traits, which suggest that the consumption of nutrients in all treatments was enough to support egg production.

The increase in feed intake (90.8, 93.6 and 96.5 g day^{-1}) and energy intake (24.5 to 25.2 and 26.6 kcal day^{-1}) and similar TSAA (0.58, 0.57 and 0.57) and lysine intakes (0.78, 0.77 and 0.75) when CP was reduced (16.6 to 15.4 and 15% of CP), indicates that feed intake increased as CP is reduced in the laying diets to meet constant TSAA and lysine intake. These results agree with that of Panda *et al.* (2012) who reported that, when nutrient density is decreased, the hen increases their feed intake from 98.9 to 109.6 g hen^{-1} per day to achieve similar energy and aminoacids intake. Rama Rao *et al.* (2011) found that feed intake decreased linearly with increasing dietary concentration of methionine in hens to achieve a similar methionine intake. Other researchers reported that the level of dietary energy regulates food intake. To that respect, Wu *et al.* (2005) mentioned that hens adjusted their feed intake per day to achieve a constant energy intake, when the dietary energy increased from 2,719 to 2,956 kcal of ME kg^{-1}. However, in our experiment it seems that energy consumption did not limit feed intake; it appears that poultry feed consumption was adjusted to have a constant TSAA and lysine intakes. Thus, it is recommendable to keep equal daily amounts of essential amino acids, when reduced CP diets are used (Latshaw and Zhao, 2011). Nevertheless, feed efficiency was reduced as a consequence of the increase in feed intake, which may have an important economic impact.

In the present study, it was observed that the decrease of CP from 16.6 to 15.4 and 15.0% in the laying hen diets did not affect the egg, albumen and yolk traits. Thus, the intakes of nutrients in the diets were adequate to support the egg quality traits here studied, except for a slight increase in the height of the albumen.

In conclusion, the addition of AZ in the diet reduced feed intake and improve feed efficiency, although its effect was more marked in the diets with high CP. The proportional reduction of CP and amino acids in the diet caused an increase in feed intake and reduced feed efficiency.

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