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Effect of Dietary Protein and Peptide in Corn-Soy Diets on Hen Performance, Egg Solids, Egg Composition and Egg Quality of Hy-Line W- 36 Hens During Second Cycle Phase Three

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Abstract: A 5 × 2 factorial arrangement of five protein levels with and without Peptiva was conducted to evaluate the effect of Peptiva on performance, egg composition, egg solids, and egg quality of commercial Leghorns. Hy-line W-36 hens (n=1200, 98 weeks old) were randomly divided into 10 dietary treatments (8 replicates of 15 hens per treatment). The experiment lasted 12 weeks. Protein had a significant effect on feed consumption, egg weight, egg production, egg mass, egg specific gravity, egg albumen solids, and percent egg components. As dietary protein increased from 13.53 to 15.62%, egg production, feed consumption and egg weight increased by 6.14%, 8.2% and 5.18% respectively. Feed consumption of hens fed the diets supplemented with Peptiva was significantly lower than that of hens fed the diets without Peptiva. Peptiva supplementation also significantly increased egg production of hens during week 98 and numerically higher in week 99, 103, 105, 106, 107, 109 and overall egg production. There was also a significant effect of peptiva on egg mass and feed conversion during first week but the significant effects were lost after second week. Peptiva significantly decreased feed intake without causing any adverse effects on egg weight and egg production. Peptiva might be more beneficial for young hens. More research is needed with young hens to evaluate performance and profits of commercial layers at different egg and ingredient prices.

Key words: Peptiva, protein utilization, hen

INTRODUCTION

Protein is a critical nutrient for laying hen growth, production and health. The quality of protein depends on AA balance as well as digestion and absorption in the small intestine. Soy protein is the most commonly used protein source in the laying hen industry. However, layers are not well adapted to soy protein because of the presence of anti-nutritional factors, such as glycinin and beta - conglycinin (Sissons and Smith, 1976). Laying hen nutritionists are interesting in finding appropriate substitutes for soy protein, due to the high cost of animal protein, and public concerns about feeding animal products back to animals. Since the identification of peptide transporters and increased efforts directed at peptide research, utilization of small peptides as a protein source in the animal industry becomes a reality; however, limited information regarding their effects on laying hen performance is available.

It was thought that all dietary proteins need to be hydrolyzed to free amino acids in order to be absorbed. This theory changed when the first intestinal oligo-peptide transporter, PepT1, was identified by two separate groups in rats and rabbits (Boll *et al.*, 1994; Fei *et al.*, 1994). The peptide transporter was then identified and characterized in domestic animals like pigs (Klang *et al.*, 2005), chickens (Chen *et al.*, 1999), turkey (Van *et al.*, 2005), and ruminant animals (Chen *et al.*, 1999). The PepT1 protein is located at the brush border membrane of intestinal epithelial cells (Leibach and Ganapathy, 1996), and has been shown to have rather broad substrate specificity, compared to the relatively narrow substrate specificity of most free amino acids transporters.

Peptide absorption from the lumen is faster and more efficient than amino acids transportation (Johnson, 1997). Hypothetically, it is therefore possible that incorporation of small peptides or hydrolyzed protein into the diet would be beneficial for layers. However, very limited information is available using peptides as protein sources in laying hen diets because of the lack of manufacturing and the difficulty of peptide detection methods. There are few commercial peptide products available for use in the laying hen diets. Peptiva® is produced by Vitech BioChem Corporation (San Fernando, CA), by blending appropriate amount of porcine mucosa peptides, fish peptides, and microbial peptides. It is claimed to contain feed stimulating peptides, small intestine activity peptides, exorphine peptides, immune modulating peptides and anti-microbial peptides (www.vitechusa.com).

Protein is a major nutrient representing a high percentage of total cost of the laying hen diets. Liu *et al.* (2004; 2005) and Wu *et al.* (2005) reported that increasing protein level significantly improved egg production, egg weight, feed consumption, feed conversion, egg specific gravity, and body w eight of

Table 1: Ingredients and nutrient content of the experimental diets

Ingredients (%)	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	Diet 7	Diet 8	Diet 9	Diet 10
Corn (8.6%)	66.30	66.30	68.05	68.05	69.76	69.76	71.40	71.40	72.90	72.90
Soy bean meal 48%	20.59	20.59	19.14	19.14	17.72	17.72	16.38	16.38	15.12	15.12
Limestone	7.24	7.24	7.25	7.25	7.25	7.25	7.26	7.26	7.30	7.30
Hard shell[1]	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Dicalcium Phosphate	1.30	1.30	1.30	1.30	1.32	1.32	1.32	1.32	1.33	1.33
Poultry oil	1.62	1.62	1.33	1.33	1.04	1.04	0.77	0.77	0.51	0.51
NaCl	0.38	0.38	0.38	0.38	0.38	0.38	0.38	0.38	0.38	0.38
Vitamin Premix[2]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Mineral Premix[3]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
DL-Methionine	0.06	0.06	0.04	0.04	0.03	0.03	0.01	0.01	0.00	0.00
Peptiva[4]	0.00	0.20	0.00	0.20	0.00	0.20	0.00	0.20	0.00	0.20
Total	100	100	100	100	100	100	100	100	100	100
Calculated analysis (%)										
Crude protein	15.62	15.62	15.06	15.06	14.52	14.52	14.01	14.01	13.53	13.53
ME (Kcal/kg)	1310	1310	1310	1310	1310	1310	1310	1310	1310	1310
Ca	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Avilable phosphorus	0.34	0.34	0.34	0.34	0.34	0.34	0.34	0.34	0.34	0.34
Methionine	0.32	0.32	0.30	0.30	0.27	0.27	0.25	0.25	0.24	0.24
Methionine +Cystine	0.59	0.59	0.56	0.56	0.53	0.53	0.51	0.51	0.48	0.48
Lysine	0.79	0.79	0.75	0.75	0.71	0.71	0.68	0.68	0.64	0.64

[1]Hard shell = large particle limestone (passing US mesh #4 and retained by US mesh #6) $CaCo_3$ supplied by Franklin Industrial Minerals, Lowell ,FL.
[2]Provided per kilogram of diet: Vitamin A (as retinyl acetate), 8,000 IU; cholecalciferol, 2,200ICU, vitamin E (as DL – alpha - tocopheryl acetate), 8 IU; vitamin B12, 0.02mg; riboflavin, 5.5mg; D-calcium pentothenic acid, 13mg; niacin, 36mg; choline, 500mg; folic acid, 0.5mg; vitamin B1(thiamin mononitrate), 1mg; pyridoxine, 2.2mg; biotin, 0.05mg; vitamin K (menadione sodium bisulfate complex), 2mg.
[3]Provided per kilogram of diet: manganese, 65mg; iodine,1mg; iron, 55mg;copper, 6mg;zinc, 55mg; selenium, 0.3mg.
[4]Vitech Bio-Chem Corporation California, USA recommended addition rate=0.2%

hens. Even though feeding high protein diets can optimize performance, the cost of high protein diets and egg prices determine the level needed for optimal profits. As protein requirement decrease as hens age, normally, hens during second cycle are fed lower protein diets ranging from 14 to 16%.

The liquid and breaker egg industry have grown during last 10 years. Egg components and egg solids might be altered by manipulation of protein. Increasing protein significantly increased percent albumen (Shafter *et al.*, 1998 and Novak *et al.*, 2004). More research is needed to investigate the effect of protein on egg solids to improve the profits of egg further processing industry. Therefore the objective of this study was to determine the effect of dietary protein and Peptide on performance, egg solids, egg composition and egg quality of Hy-line W - 36 hens during second cycle phase three (98 to 109wk).

MATERIAL AND METHODS

A 5 × 2 factorial arrangement with five protein levels (13.56, 14.01, 14.52 15.06 15.62%) with and without Peptiva was used in this experiment. Ingredients and nutrient composition of the experimental diets are shown in Table 1. Hy-Line W-36 hens (n=1200, molted at 66wk) in their second cycle (98wk old) were randomly divided among 10 treatments (8 replicates of 15 hens per treatment). Replicates were equally distributed into upper and lower cage levels to minimize cage level effect. Three hens were housed in a 40.6 X 45.7cm cage, and 5 adjoining cages consisted of a replicate. Replicates were equally distributed into upper and lower cage levels to minimize cage level effect. All hens were housed in an environmentally controlled house with temperature maintained at approximately 26°C. The house had controlled ventilation and lighting (16L:8D). All hens were supplied with feed and water *ad libitum*. Animal housing and handling procedures during experimentation were in accordance with guidelines of Auburn University's Institutional Animal Care and Use Committee (IACUC).

Feed consumption was recorded weekly for calculation of average daily feed consumption. Egg production was recorded daily and egg weight and specific gravity were recorded once every two weeks. Egg weight and egg specific gravity were measured using all eggs produced during two consecutive days. Egg specific gravity was determined using 9 gradient saline solutions varying in a specific gravity from 1.060 to 1.100 in 0.005 unit increments (Holder and Bradford, 1979). Mortality was determined daily, and feed consumption was adjusted accordingly. Body weight was obtained by randomly weighing three hens (1 of 5 cages) per replicate at the end of the experiment. Egg mass (g of egg/hen per day) and feed conversion (g of feed/g of egg) were calculated from egg production, egg weight, and feed consumption. Feed samples were sent for Peptide activity analysis[1].

Egg components were measured using 3 eggs from each treatment replicate at the middle (103 wk of age) and end (109 wk of age) of the experiment. Eggs were weighed and the broken. The yolks were separated from the albumen. Before the yolk weight was determined, the chalaza was removed by forceps. Each yolk was rolled on a blotting paper towel to remove adhering albumen.

Table 2: Influence of Peptiva and protein, on feed consumption, egg specific gravity, body weight, egg weight, and mortality of Hy-line W-36 hens during second cycle phase three (98wk to 109wk of age)

Protein (%)	Feed consumption (g/hen per day)	Egg Specific Gravity (Unit)	Body Weight (Kg)	Egg weight (g)	Mortality (%)
13.56	86.98	1.0754	1.76	61.33	0.31
14.01	88.58	1.0751	1.82	62.45	0.21
14.52	92.07	1.0777	1.77	63.31	0.16
15.06	93.73	1.0751	1.78	64.74	0.11
15.62	94.12	1.0755	1.75	64.51	0.21
Peptiva -	91.78	1.0755	1.79	63.33	0.15
+	90.41	1.0760	1.76	63.21	0.25
Pooled SEM	0.77	0.00076	0.057	0.78	0.12
	Probability				
Protein	<0.0001	0.0045	NS	<0.0001	NS
Peptiva	0.05	NS	NS	NS	NS
Protein×Peptiva	NS	NS	NS	NS	NS

The shells were cleaned of any adhering albumen and dried for 5 days. Albumen weight was calculated by subtracting the weight of yolk and shell from the whole egg weight.

Three eggs from each treatment replicate were collected at the middle (103 wk of age) and at the end (109 wk of age) of the experiment for measuring whole solid. The yolk and albumen were mixed and 5 to 6g of homogenate was pipetted into aluminum dish with weight recorded to 0.001g. The sample was dried in an oven for 24h at 40.5°C (AOAC, 1990) and then weighed. Three eggs per treatment replicate were used to analyze yolk and albumen solid. After yolk was separated from albumen, three yolks and albumen per treatment replicate were mixed separately. The procedure for analyzing albumen and yolk solid was the same as the procedure for whole egg solid content. Yolk color and haugh unit were measured (3 eggs of each treatment replicate) at the middle (103 wk of age) and at the end (109 wk of age) of the experiment by a egg multitester EMT-5200 (Robotmation,co,Ltd.Tokyo,Japan). Haugh units were calculated from the records of albumen height and egg weight using the formula: $HU=100 \log_{10} (H-1.7 W^{0..37}+7.56)$, where HU=Haugh unit, H=height of the albumen (mm) and W = egg weight (g).

Data were analyzed by proc ANOVA using proc mixed of Statistical Analysis System (SAS institute, 2000) for a randomized complete block with factorial arrangement of treatments. Dietary protein and Petiva were fixed, whereas blocks were random. The factorial treatment arrangement consisted of five protein levels with and without Peptiva. The following model was used to analyze the data:

$$Y_{ijk} = \mu + P_j + R_k + PR_{ik} + B_1 + e_{jk}$$

Where Y_{ijk} = individual observation, μ = experimental mean, P_j = protein effect, R_k = Peptiva effect, Pr_{ik} = interaction between protein and Peptiva, B_1 = effect of block, e_{ijk} = error component.

If differences in treatment means were detected by ANOVA, Duncan's multiple range test was applied to separate means. A significance level of p= 0.05 was used during analysis.

RESULTS AND DISCUSSION

There were no interactions between dietary protein and Peptiva on any factor. Protein had a significant linear effect on feed consumption (Table 2). Hens fed 15.62% protein level had the highest feed consumption. Similarly, Wu *et al.* (2005) and Liu *et al.* (2004 and 2005) reported that feed consumption significantly increased with increased dietary protein levels. Increased feed consumption of laying hens fed the high dietary protein might be due to improved performances and low dietary energy content in the high protein diets. Increasing dietary protein significantly increased egg weight (Table 2). The influence of dietary protein on egg weight in this study was consistent with that of Parsons *et al.* (1993); Keshavarz (1995); Leeson (1989); Wu *et al.* (2005) and Sohail *et al.* (2003) who reported that egg weight of hens fed higher dietary protein had a higher egg weight than the hens fed lower protein diets.

Increasing dietary protein intake from 11.8 to 14.7 g/hen/day, increased egg production from 63.5 to 68.6% (Table 3). Similarly, Liu *et al.* (2004) and Wu *et al.* (2005) reported that increasing dietary protein improved egg production. Zou and Wu (2005) reported that increasing dietary protein intake from 15.3 to 16.3 g/hen/day, increased egg production by 3.2%. Keshavarz (1995) indicated that with an increase of dietary protein intake from 17.4 to 21.4 g/hen/day, egg production increased by 1.9%. Increasing dietary protein intake from 11.8 to 14.7 g/hen/day, increased egg mass 4.97g/hen day. (Table 4).

As dietary protein increased from 13.56 to 15.62%, percent albumen, percent albumen solids linearly increased and percent yolk linearly decreased (Table 6). Similarly, Shafter *et al.* (1998) and Novak *et al.* (2004) reported that increasing amino acids such as methionine and lysine significantly increased percent albumen. These results are important for the egg breaker industry.

Peptiva significantly decreased feed consumption from

Table 3: Influence of Peptiva and protein, on egg production of Hy-line W-36 hens during second cycle phase three (98wk to 109wk of age)

Protein (%)	98 wk	99 wk	100 wk	101 wk	102 wk	103 wk	104 wk	105wk	106wk	107wk	108wk	109wk	AEP*
13.56	66.96	67.92	65.83	64.17	62.41	61.91	57.62	60.95	61.74	63.58	64.82	64.20	63.51
14.01	69.64	68.99	65.95	64.46	64.58	63.51	64.20	63.72	61.46	63.26	65.73	64.58	65.01
14.52	64.82	65.51	66.43	68.93	67.08	64.49	67.08	65.66	62.57	63.66	68.35	68.48	66.09
15.06	71.73	69.94	71.07	70.74	69.17	65.98	68.12	68.19	63.47	64.47	69.75	69.61	68.52
15.62	70.54	68.99	69.76	69.61	69.76	68.48	69.82	69.94	62.71	64.01	68.24	70.74	68.55
Peptiva -	66.95	66.84	67.90	67.75	66.62	64.30	65.46	65.43	62.28	63.19	67.41	66.26	65.87
+	70.52	69.69	67.71	67.42	66.58	65.45	65.27	65.95	62.50	64.40	67.35	68.79	66.80
Pooled SEM	2.62	2.45	2.32	2.76	2.29	2.41	2.45	2.04	2.80	2.64	2.24	2.21	1.82
	Probability												
Protein	0.06	NS	0.07	0.05	0.04	0.05	0.0001	0.0001	NS	NS	0.04	0.001	0.004
Peptiva	0.03	0.07	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Protein×Peptiva	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

AEP : Average Egg Production

Table 4. Influence of Peptiva and protein, on egg mass of Hy-line W-36 hens during second cycle phase three (98wk to 109wk of age)

Protein (%)	98 wk	99 wk	100 wk	101 wk	102 wk	103 wk	104 wk	105wk	106wk	107wk	108wk	109wk	AEM*
13.56	42.16	42.41	41.16	39.18	37.72	37.55	34.83	37.26	38.87	39.39	39.56	39.39	39.12
14.01	44.09	43.47	41.73	39.69	40.21	39.39	40.24	39.40	38.88	39.53	41.18	41.91	40.81
14.52	41.15	41.37	42.85	42.82	42.86	40.79	42.32	41.49	40.19	40.96	43.39	44.35	42.05
15.06	46.56	46.06	46.48	45.09	44.69	42.89	43.73	43.83	41.24	42.14	44.80	42.84	44.20
15.62	44.92	45.25	45.15	44.23	45.24	44.26	44.98	45.28	40.63	41.65	43.88	43.59	44.09
Peptiva -	42.59	42.96	43.63	42.75	42.02	40.53	41.31	41.22	39.78	40.22	42.62	42.08	41.81
+	44.97	44.47	43.32	41.66	42.28	41.43	41.13	41.68	40.15	41.25	42.51	42.75	42.30
Pooled SEM	1.86	1.71	1.67	1.92	1.72	1.71	1.81	1.46	1.86	1.72	1.70	1.52	1.38
	Probability												
Protein	0.03	0.04	0.009	0.007	0.0001	0.002	0.0001	0.0001	0.05	NS	0.01	0.01	0.0001
Peptiva	0.04	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Protein×Peptiva	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

*AEM : Average Egg Mass

Table 5. Influence of Peptiva and protein, on feed conversion of Hy-line W-36 hens during second cycle phase three (98wk to 109wk of age)

Protein (%)	98 wk	99 wk	100 wk	101 wk	102 wk	103 wk	104 wk	105wk	106wk	107wk	108wk	109wk	AFC*
13.56	2.18	2.12	2.12	2.19	2.30	2.29	2.43	2.40	2.40	2.24	2.24	2.28	2.27
14.01	2.04	2.02	2.11	2.17	2.19	2.21	2.26	2.36	2.35	2.18	2.16	2.21	2.19
14.52	2.26	2.25	2.16	2.11	2.16	2.30	2.18	2.26	2.30	2.14	2.14	2.12	2.20
15.06	1.99	2.05	2.03	2.03	2.14	2.24	2.17	2.14	2.32	2.19	2.11	2.22	2.14
15.62	2.07	2.09	2.07	2.09	2.12	2.14	2.14	2.13	2.38	2.20	2.16	2.19	2.15
Peptiva -	2.18	2.15	2.11	2.10	2.21	2.27	2.24	2.30	2.34	2.24	2.17	2.24	2.21
+	2.03	2.06	2.08	2.13	2.15	2.21	2.23	2.21	2.36	2.14	2.15	2.16	2.16
Pooled SEM	0.07	0.06	0.05	0.06	0.06	0.07	0.07	0.07	0.09	0.07	0.07	0.07	0.05
	Probability												
Protein	0.08	NS	NS	NS	NS	NS	0.04	0.03	NS	NS	NS	NS	NS
Peptiva	0.03	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Protein×Peptiva	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

*AFC : Average Feed Conversion

Table 6: Influence of Peptiva and protein, on egg components, egg solids, and egg quality of Hy-line W-36 hens during second cycle phase three (98wk to 109wk of age)

	Egg components (%)			Egg solids (%)			Egg Quality	
Protein (%)	Yolk	Albumen	Shell	Whole egg	Albumen	Yolk	Haugh Unit	Yolk color
13.56	29.71	61.71	8.58	25.35	11.20	54.36	71.29	5.69
14.01	28.95	63.06	7.99	25.75	11.49	53.72	72.93	5.50
14.52	28.33	63.58	8.09	25.56	11.57	54.34	70.36	5.47
15.06	27.99	63.95	8.06	26.05	11.72	54.72	68.79	5.53
15.62	27.80	64.35	7.85	25.45	11.95	54.06	68.23	5.25
Peptiva -	28.58	63.41	8.02	25.64	11.63	54.51	71.61	5.55
+	28.53	63.26	8.21	25.62	11.54	53.97	69.03	5.43
Pooled SEM	0.71	0.54	0.23	0.42	0.24	0.53	2.37	0.17
	Probability							
Protein	0.05	0.006	0.03	NS	0.04	NS	NS	NS
Peptiva	NS	NS	NS	NS	NS	NS	NS	NS
Protein × Peptiva	NS	NS	NS	NS	NS	NS	NS	NS

91.8 to 90.4 g/hen per day or by 1.49% (Table 2). The mechanism on how peptiva influences feed consumption without adversely affecting performance is not known. Peptiva supplementation also significantly increased egg production of hens during week 98 and numerically higher in week 99, 103, 105, 106, 107, 109 and overall egg production (Table 3). There was also a significant effect of peptiva on egg mass (Table 4) and feed conversion (Table 5) during first week.

In conclusion, Protein had a significant effect on feed consumption, egg weight, egg production, egg mass, egg specific gravity, egg albumen solids, and percent egg components. As dietary protein increased from 13.53 to 15.62%, egg production, feed consumption and egg weight increased by 6.14%, 8.2% and 5.18% respectively. Feed consumption of hens fed the diets supplemented with Peptiva was significantly lower than that of hens fed the diets without Peptiva. Peptiva supplementation also significantly increased egg production of hens during week 98 and numerically higher in week 99, 103, 105, 106, 107, 109 and overall egg production. There was also a significant effect of peptiva on egg mass and feed conversion during first week but the significant effects were lost after second week.

Peptiva significantly decreased feed intake without causing any adverse effects on egg weight and egg production. Peptiva might be more beneficial for young hens. More research is needed with young hens to evaluate performance and profits of commercial layers at different egg and ingredient prices.

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