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Orijinal Article

Influence of Enzyme and/or Lysolecithin Supplementation on Performance, Nutrient Digestibility and Egg Quality for Laying Hens

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Abstract

The aim of the present study was to further evaluate the effects of lysolecithin supplementation on laying hen performance and to determine whether or not there are any synergistic effects when a combination of enzyme and lysolecithin are fed to laying hens. Eighty, 32-week-old, Lohmann Brown-Lite laying hens were randomly allocated to one of five diets based on corn, wheat and animal fat as energy sources with soybean meal, canola meal and corn gluten meal providing the supplementary protein. The experimental diets comprised a high energy diet formulated to provide 11.81 MJ/kg of ME (positive control) as well as four moderate energy diets formulated to provide approximately 11.38 MJ/kg of ME. The moderate energy diets were fed either unsupplemented (negative control), or supplemented with 0.1% enzyme (7 U/g of α -1-6 galactosidase and 22 U/g of β 1-4 mannanase), 0.1% lysolecithin or the two additives in combination at the same levels as those used separately. There were eight replicates per treatment and two hens per replicate. There were no differences in egg production or egg weight between treatments (P>0.05). However, feed conversion was best for birds fed the diet supplemented with enzyme alone and poorest for birds fed the two additives in combination (P=0.04). The improved feed conversion for hens fed the enzyme supplemented diet appeared to be mediated by an increase in amino acid digestibility as digestibility coefficients for histidine, leucine, isoleucine, lysine, methionine, phenylalanine and threonine were significantly (P<0.01) higher for birds fed the moderate energy diet supplemented with enzyme than for the unsupplemented moderate energy diet. The improvement in feed conversion for birds fed the enzyme supplemented diet was accompanied by an increase in albumen weight and percentage and a decrease in the percentage of yolk and the ratio of yolk to albumin (P<0.01) in the eggs. There did not appear to be any synergistic effects between lysolecithin and enzyme on laying hen performance.

Keywords: Laying hen, Lysolecithin, Enzyme, Egg quality, Egg production, Digestibility

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INTRODUCTION

Soybean meal contains approximately 23% of its carbohydrates in the form of non-starch polysaccharides (Jackson et al. 2004). These include acidic polysaccharides (8 to 10%), arabinogalactins (5%) and cellulose (1 to 2%) as well as approximately 1.3% β -mannans (Hsiao et al. 2006). β -mannans are linear polysaccharides composed of repeating β 1-4 mannose and α 1-6 galactose and glucose units attached to the β -mannan backbone (Jackson et al. 2004). Monogastric animals lack endogenous enzymes targeting β 1-4 mannosyl bonds. As a result, the presence of β -mannan

in the diet has been shown to diminish the growth of broilers (Ray et al. 1982). Soybean meal also contains galactosidic oligosaccharides including 0.67-0.94% raffinose, 2.9-4.1% stachyose and trace amounts of verbascose (Wang et al. 2005). There is also about 0.3% raffinose in corn. These α -galactosides have been implicated in reducing energy utilization, fiber digestion and feed retention in broilers fed soybean meal (Coon et al. 1990).

Over the past two decades, it has become a common practice to include fibrolytic enzymes in diets fed to poultry

(Thacker 2005; Meng and Slominski 2005; Cowieson and Adeola 2005; Ao et al. 2009). Basically, the mode of action of the fibrolytic enzymes is to disrupt solubilized fiber complexes reducing the viscous condition imparted by the solubilized fiber, thus improving digestion, growth and feed efficiency (Bedford 1993; Campbell and Bedford 1992; Jeroch et al. 1995).

Lysolecithins are an important metabolite produced by many cells and are widely distributed in a variety of tissues and are capable of increasing ion permeation in membranes (Lee and Chan 1977). Lysolecithin can function as a membrane transducer by diffusing rapidly through the lipid portion of cellular membranes to modify the activity of various membrane associated enzymes which may alter the general properties of the membrane such as increasing its fluidity and permeability (Shier et al. 1976). Lysolecithin may also alter mucosal barrier function and increase gut permeability to macromolecules such as proteins (Tagesson et al. 1985).

Although lysolecithins are a potent membrane transport modifier, their role in animal performance has not been widely studied and only a few studies have monitored their effects in laying hens. Calton et al. (1998) showed that the addition of lysolecithin to laying hen rations produced heavier eggs due largely to an increase in the weight of the egg yolk. These results were ascribed to a better assimilation of nutrients at the gut level. In addition, we recently reported that supplementation of lysolecithin in the diet of laying hens significantly increased egg weight and feed efficiency (Han et al. 2010).

It has not been established whether the effects of enzyme supplementation and lysolecithin are additive. Since enzymes facilitate nutrient digestion and lysolecithin may improve nutrient absorption, it is possible that enzymes and lysolecithin may act synergistically. Therefore, the aim of the present study was to further evaluate the effects of lysolecithin supplementation on laying hen performance and to determine whether or not there are any synergistic effects when a combination of enzyme and lysolecithin are fed to laying hens.

MATERIAL AND METHODS

Experimental Design, Birds and Housing

All procedures used in this nine week experiment were approved by the Animal Ethics Committee of Sungkyunkwan University (Suwon, Korea) and complied with the "Guidelines for the Care and Use of Animals in Research" published by the Korean Ministry for Food, Agriculture, Forestry and Fisheries (2008). Eighty, 32-week-old, Lohmann Brown-Lite layers, with an average laying rate

of 96.3 ± 3.2%, were obtained from a commercial supplier (Join Farm, Songtan, Korea). After a week of adaptation, the hens were randomly allocated to one of five diets based on corn, wheat and animal fat as energy sources with soybean meal, canola meal and corn gluten meal providing the supplementary protein. There were eight replicates per treatment and two hens per replicate. The experimental diets comprised a high energy diet formulated to provide 11.81 MJ/kg of ME (positive control) as well as four moderate energy diets formulated to provide approximately 11.38 MJ/kg of ME (Table 1). The moderate energy diets were fed either unsupplemented (negative control), or supplemented with 0.1% enzyme, 0.1% lysolecithin or the two additives in combination at the same levels as those used separately.

The lysolecithin used in the present experiment was obtained from Devenish Nutrition (Belfast, North Ireland) and is marketed under the trade name Lipidol®. The lysolecithin was prepared from phospholipids present in soybean lecithin. Phospholipase enzymes were used to remove one of the fatty acid chains from the soybean lecithin resulting in a small charged lipid which encourages interaction with cell membranes. The charge connects water soluble nutrients with oil based nutrients thus facilitating nutrient absorption. The final product contained 50% lysolecithin along with an inert calcium silicate carrier

The carbohydrase enzyme (Endopower, EasyBio System Incorporated, Seoul, Korea) was composed of dehydrated fermentation products obtained from *Aspergillus niger* (PRL 2351), *Aspergillus oryzae* (ATCC 66222; 40% by weight), and dehydrated barley malt sprouts (60% by weight). The enzymatic potencies provided by the manufacturer were 7 U/g of α -1, 6-galactosidase and 22 U/g of β -1, 4-mannanase.

The experimental diets were fed in mash form and all nutrient levels (Table 2) with the exception of energy met or exceeded the nutrient requirements suggested in the Lohmann Brown Management Guide (Lohmann Tierzucht 2008). The reduction of energy for the moderate energy diets and the inclusion levels of lysolecithin and enzyme were those recommended by the respective suppliers and applied by commercial feed manufacturers in Korea.

The hens were housed in pairs in laying cages made from galvanized metal wire (approximately 25 x 35 x 50 cm) which provided approximately 430 cm²/hen. The cages were placed in double-decked rows and one upper deck of cages and one lower deck of cages were used for this experiment. The cages were located in a windowless and environmentally controlled room with the room temperature kept at 21-23°C and the photoperiod set at 16 h of light (incandescent lighting, 10 lux) and 8 h dark. Each cage had a nipple waterer.

Table 1 Ingredient composition of diets fed to determine the effects of lysolecithin and enzyme on the performance of laying hens (% as fed)

	High energy	Moderate Energy	Moderate energy + lysolecithin	Moderate energy + enzyme	Moderate energy + combination
Corn	50.02	52.50	52.40	52.40	52.30
Wheat	10.00	10.00	10.00	10.00	10.00
Canola meal	3.00	3.00	3.00	3.00	3.00
Soybean meal	15.34	14.95	14.95	14.95	14.95
Soybean meal, dehulled	7.00	7.00	7.00	7.00	7.00
Corn gluten meal	1.00	1.00	1.00	1.00	1.00
Animal fat	2.98	0.90	0.90	0.90	0.90
Sodium bicarbonate	0.04	0.04	0.04	0.04	0.04
L-Lysine HCl	0.04	0.04	0.04	0.04	0.04
Methionine	0.09	0.08	0.08	0.08	0.08
Limestone	9.98	9.98	9.98	9.98	9.98
Phytase	0.05	0.05	0.05	0.05	0.05
Premix ¹	0.23	0.23	0.23	0.23	0.23
Salt	0.23	0.23	0.23	0.23	0.23
Lysolecithin (50%)	0.00	0.00	0.10	0.00	0.10
Enzyme, carbohydrase	0.00	0.00	0.00	0.10	0.10

¹Provided the following nutrients per kg diet: vitamin A, 12,000 IU; vitamin D, 3,500 IU; vitamin E, 30 IU; vitamin K₃, 3.0 mg; thiamin, 3.0 mg; riboflavin, 7.0 mg; pyridoxine, 5.0 mg; vitamin B₁₂, 0.025 mg; niacin, 40.0 mg; pantothenic acid, 10 mg; folic acid, 1.0 mg; biotin, 0.15 mg; Fe, 75.0 mg; Zn, 97.5 mg; Mn, 97.5 mg; Cu, 7.5 mg; I, 1.5 mg; Se, 0.2 mg.

Table 2 Chemical composition of diets fed to determine fed to determine the effects of lysolecithin and enzyme on the performance of laying hens (% as fed)¹

	High energy	Moderate energy	Moderate energy + lysolecithin	Moderate energy + enzyme	Moderate energy + combination
Dry matter	91.76	91.66	91.76	91.76	91.42
Crude protein	17.08	17.01	16.95	17.02	17.05
Ether extract	5.68	3.64	3.59	3.78	4.34
Ash	14.44	11.84	12.19	12.18	11.33
Calcium	4.15	4.19	4.09	4.07	4.26
Total phosphorus	0.51	0.48	0.48	0.47	0.49
MEn, MJ/kg	11.81	11.38	11.38	11.38	11.38
Arginine	1.14	1.06	1.02	0.98	1.05
Histidine	0.42	0.40	0.39	0.38	0.39
Isoleucine	0.66	0.61	0.58	0.58	0.59
Leucine	1.44	1.38	1.33	1.32	1.34
Lysine	0.86	0.79	0.77	0.74	0.79
Methionine + cysteine	0.59	0.59	0.56	0.52	0.60
Phenylalanine	0.74	0.70	0.68	0.67	0.68
Threonine	0.66	0.62	0.61	0.59	0.62
Valine	0.78	0.75	0.73	0.71	0.74

¹With the exception of ME, all data are the results of a chemical analysis conducted in duplicate.

Feed and water were available ad libitum throughout the experiment. A continuous, plastic feed trough was divided by replicate to ensure that the hens were not able to consume

feed assigned to the adjoining replicate. Feed consumption was measured on a weekly basis.

Sampling and Analyses

A wire egg collector was installed in the front of each cage to prevent eggs from separate replicates from being mixed. Eggs were collected and weighed every day. Egg production was calculated on a replicate basis. Egg weight (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. Egg components (percentages of albumin, yolk and shell) were measured using two eggs of average weight from each cage on the last two days of each week. Yolk color and Haugh units were measured using an Egg Multi-Tester EMT-5200 (Robotmation Co. Ltd., Tokyo, Japan). Haugh units (HU) were calculated from the records of egg weight and albumen height using the formula: $HU = 100 \log_{10} (H 1.7 \text{ W}^{0.37} + 7.56$), where HU = Haugh unit, H = height of the albumen (mm), and W = egg weight (g). The yolks were separated from the tester tray (yolk, albumen and tray) using a Teflon spoon. Before the yolk weight was determined, the chalaza was removed by spatula. Albumen weight was calculated by subtracting the weight of tester tray from the remaining egg contents. The egg shells were weighed without drying. Daily egg content was calculated (% egg production x (daily yolk weight + daily albumen weight) from egg production, yolk weight and albumen weight. Eggs from each cage were sorted by four size groups (very large, large, medium, and small) according to the European Union Marketing Standards (European Commission 2008).

At the end of the 9-week experiment, all feed was removed from the feeders and replaced with similar diets containing 0.4% chromic oxide. Seven days later, the birds were weighed and euthanized using CO₂. The gastrointestinal tract, proventriculus, gizzard, ceca, liver and the pancreas were removed aseptically and any digesta present was removed. The ileal digesta between the yolk sac and the terminal ileum was collected. Digesta were freeze dried, and the digesta from two replicates were mixed together and ground through a 1.0 mm mesh screen before analyses. After digesta emptying, each organ was weighed. The weight of the organs was expressed relative to live body weight. Empty body weight was also determined.

Samples of feed and digesta were analyzed in duplicate according to the methods of the AOAC (1995). Analyses were conducted for moisture (method 930.15), ether extract (method 920.39), crude protein (method 984.13), ash (method 942.05) and ether extract (method 920.39). Calcium was determined by a Shimazu AA625 Atomic Absorption Spectrophotometer (Shimazu, Kyoto, Japan), and phosphorus was analyzed using a UV-vis. Spectrophotometer (Hitachi, Tokyo, Japan). An amino acid analysis of the feed was performed using a L8500-Hitachi Amino Acid Analyzer (Hitachi, Tokyo, Japan) after hydrolysis for 24 h in 6 N HCl.

Performic acid (85%) hydrolysis was performed for analysis of sulfur-containing amino acids.

Chromic oxide was determined by the method of Fenton and Fenton (1979). Digestibility coefficients for nutrients were calculated using the equations for the indicator method described by Schneider and Flatt (1975).

Statistical Analysis

Data were analyzed as a randomized block design (Snedecor and Cochran 1989), using the appropriate General Analysis of Variance procedures of Statistix (1996). Hens were blocked on the basis of hen-day egg production during the adaptation period and the cage was considered the experimental unit for all analyses. The model included the effects of replication (i.e., block), treatment, and replication x treatment (error). The significance of differences between means was determined by the Least Significant Difference (LSD) method at a level of α = 0.05.

RESULTS

There were no differences in egg production or egg weight between treatments (Table 3). However, feed intake and feed conversion were significantly influenced by dietary treatment. Feed intake was significantly higher (P<0.01) for hens fed diets supplemented with lysolecithin and enzyme when fed in combination than for the remaining treatments. Feed conversion was best for birds fed the diet supplemented with enzyme alone and poorest for birds fed the two additives in combination (P=0.04). Feed conversion for the remaining treatments was intermediate to the treatments involving enzyme alone and the combination diet.

No significant differences were detected concerning egg quality measurements as assessed by egg content, yolk weight, shell weight, yolk color and Haugh units (Table 4). However, albumin weight, percentage of albumen, yolk percentage, the ratio of yolk to albumen and egg shell percentage were significantly influenced by treatment. The albumen weight and percentage were highest (P<0.01) for hens fed the moderate energy diet supplemented with enzyme and lowest for hens fed the high energy diet. In contrast, the percentage of yolk and the ratio of yolk to albumin in the eggs were highest for birds fed the high energy diet and lowest for birds fed the moderate energy diet supplemented with enzyme.

The data on the ileal digestibility of nutrients are summarized in Table 5. Dietary supplementation with lysolecithin significantly increased (P<0.05) digestibility coefficients for nitrogen, fat, arginine, histidine, leucine, lysine, methionine, phenylalanine, and threonine compared with the values obtained for the high energy diet, the unsupplemented moderate energy diet and the moderate energy diet supplemented with the two additives in combination.

Table 3 Performance of laying hens fed diets supplemented with enzyme and/or lysolecithin ¹

	High energy	Moderate energy	Moderate energy + lysolecithin	Moderate energy + enzyme	Moderate energy + combination	SEM	P-value
Egg production (%)	97.5	97.3	96.7	95.9	98.7	0.83	0.19
Egg weight (g/hen/d)	58.6	58.1	59.6	60.3	59.1	0.66	0.17
Feed intake (g/hen/d)	111.7 ^b	111.8 ^b	111.9 ^b	111.5 ^b	115.6 ^a	0.91	< 0.01
Feed conversion (g feed/g egg)	1.92 ^{abc}	1.94 ^{ab}	1.90^{bc}	1.86 ^c	1.98 ^a	0.03	0.04

¹Means in the same row with same or no superscript do not differ (P>0.05).

Table 4 Egg components and egg quality of laying hens fed diets supplemented with enzyme and/or lysolecithin ¹

	High energy	Moderate energy	Moderate energy + lysolecithin	Moderate energy + enzyme	Moderate energy + combination	SEM	P-value
Egg content (g)	50.7	50.3	51.9	52.4	51.2	0.59	0.09
Egg content (g) Albumin weight (g)	35.0°	35.2°	36.8 ^{ab}	37.2 ^a	35.8 ^{bc}	0.39	< 0.09
Albumin (%)	59.7°	60.5 ^b	61.6 ^a	61.7 ^a	60.6 ^b	0.26	< 0.01
Yolk weight (g)	15.7	15.1	15.1	15.2	15.4	0.21	0.16
Yolk (%)	26.8^{a}	26.0^{b}	25.4°	25.2°	26.1 ^b	0.22	< 0.01
Yolk: albumin (%)	45.0^{a}	43.1 ^b	41.4 ^c	41.0^{c}	43.2 ^b	0.51	< 0.01
Egg shell weight (g)	7.8	7.8	7.7	7.9	7.9	0.11	0.74
Egg shell (%)	13.4 ^{ab}	13.5 ^a	13.0°	13.1 ^{bc}	13.3 ^{abc}	0.12	0.03
Yolk color	7.2	6.9	6.9	6.9	6.9	0.09	0.08
Haugh units	86.2	84.9	85.4	84.6	86.0	0.89	0.64

¹Means in the same row with same or no superscript do not differ (P>0.05).

Table 5 Ileal nutrient digestibility of laying hens fed diets supplemented with enzyme and/or lysolecithin ¹

	High	Moderate	Moderate energy	Moderate energy	Moderate energy	SEM	P-value
	energy	energy	+ lysolecithin	+ enzyme	+ combination	SEM	r -value
Organic matter	92.92	92.61	94.15	93.42	93.23	0.40	0.13
Nitrogen	91.00^{c}	91.71 ^{bc}	93.80^{a}	92.74^{ab}	91.88 ^{bc}	0.48	< 0.01
Fat	93.39 ^a	89.29°	92.04^{ab}	90.80^{bc}	90.42 ^{bc}	0.65	< 0.01
Amino acids	92.41 ^b	92.74 ^b	94.27 ^a	94.35 ^a	92.20^{b}	0.44	< 0.01
Energy	90.72	91.92	94.02	93.06	93.06	0.87	0.13
Essential amino acids							
Arginine	93.75 ^{bc}	93.82 ^{bc}	95.69 ^a	95.19 ^{ab}	93.22°	0.48	0.01
Histidine	92.68 ^b	92.72^{b}	94.33°	94.35 ^a	92.38^{b}	0.44	0.01
Isoleucine	91.67 ^{bc}	91.62 ^{bc}	93.03 ^{ab}	93.62 ^a	90.56°	0.57	0.01
Leucine	93.43 ^b	93.51 ^b	95.07^{a}	94.98^{a}	92.81°	0.46	0.01
Lysine	90.85^{b}	90.93 ^b	93.47 ^a	93.03 ^a	90.11 ^b	0.64	< 0.01
Methionine	91.24 ^c	93.91 ^b	95.54 ^a	95.58^{a}	92.50^{bc}	0.52	< 0.01
Cysteine	87.25	90.91	91.69	92.65	90.22	1.43	0.14
Phenylalanine	92.84 ^b	93.04 ^b	94.61 ^a	94.50 ^a	92.36 ^b	0.45	< 0.01
Threonine	89.14 ^b	89.21 ^b	91.22 ^a	91.67 ^a	88.74 ^b	0.64	0.01
Valine	89.03°	89.84 ^{bc}	91.15 ^{ab}	91.91 ^a	88.82°	0.57	< 0.01

¹Means in the same row with same or no superscript do not differ (P>0.05).

Digestibility coefficients for the moderate energy diet supplemented with enzyme were not significantly different to those obtained for the moderate energy diet supplemented with lysolecithin.

The percentage of normal eggs and egg grade were not affected by treatment (Table 6). The percentage of large and

very large eggs was numerically greater for birds fed the moderate energy level supplemented with either lysolecithin or enzyme with a corresponding decrease in the percentage of medium size eggs but these differences were not statistically significant. In addition, the relative weight of various organs were unaffected by dietary treatment(Table7).

DISCUSSION

Due to the widespread use of soybean meal as a protein source in poultry feeds (Britzman, 2006), and because soybean meal contains approximately 1.3% β -mannan (Hsiao et al. 2005), β -mannans are present in the vast majority of poultry rations currently used around the world. β -mannan is a polysaccharide with repeating units of mannose, with galactose and/or glucose often found attached to the β -mannan backbone. Several studies have shown that β -mannans are capable of stimulating the innate immune

system, with a resulting increase in proliferation of macrophages and monocytes, and increased cytokine production, leading to an increased severity of disease symptoms and a decrease in the efficiency of nutrient utilization (Peng et al. 1991; Ross et al. 2002; Zhang and Tizzard 1996). Soybean meal also contains α -1, 6 galactosidic oligosaccharides including raffinose, stachyose and verbascose (Wang et al. 2005). These α -galactosides have been implicated in reducing energy utilization, fiber digestion and feed retention in broilers fed soybean meal (Coon et al. 1990).

Table 6 Exterior egg quality of laying hens fed diets supplemented with enzyme and/or lysolecithin

	High energy	Moderate energy	Moderate energy + lysolecithin	Moderate energy + enzyme	Moderate energy + combination	SEM	P-value
Normal eggs (%) 1	47.80	51.20	39.75	47.37	42.07	7.12	0.79
Egg grade (%)							
Very large (above 70 g)	0.32	0.10	2.41	1.99	0.20	0.47	0.67
Large (63–72.9 g)	25.59	25.75	43.13	48.85	22.72	9.48	0.20
Medium (53-62.9 g)	69.95	61.85	44.50	48.23	74.97	9.24	0.11
Small (below 53 g)	4.14	12.30	9.96	0.93	2.11	4.63	0.34

¹Without stain, cage marks, pimples, abnormal shapes, sandpaper or rough shells, mottled shells, gross cracks, star cracks, or thin-shells.

Table 7 Effects of enzyme and/or lysolecithin supplementation on the relative weight (% BW) of the organs of laying hens

	High energy	Moderate energy	Moderate energy + lysolecithin	Moderate energy + enzyme	Moderate energy + combination	SEM	P-value
Empty body weight ¹	80.37	80.23	80.26	80.48	80.46	0.55	0.99
Gastrointestinal tract ²	6.53	6.67	6.99	6.41	6.33	0.24	0.35
Liver	2.53	2.52	2.58	2.64	2.74	0.16	0.85
Pancreas	0.13	0.14	0.14	0.15	0.15	0.01	0.62
Proventriculus	0.31	0.33	0.32	0.32	0.33	0.02	0.89
Gizzard	1.28	1.28	1.32	1.28	1.33	0.05	0.95
Ceca	0.35	0.37	0.39	0.35	0.34	0.02	0.33

Without the liver and the gastrointestinal tract and its contents.

Fibrolytic enzymes capable of degrading β -mannans and α -galactosides are commonly included in commercial poultry diets. In the present experiment, supplementation of a laying hen diet with an enzyme cocktail providing 7 U/g of α -1, 6-galactosidase and 22 U/g of β -1, 4-mannanse significantly improved feed conversion as measured by grams of feed per gram of egg produced. This finding supports previous work which has reported improved feed conversion for hens fed diets supplemented with enzyme (Jackson et al. 1999; Wu et al. 2005)

The improvement in feed conversion for birds fed the enzyme supplemented diet was accompanied by an increase in albumen weight and percentage and a decrease in the percentage of yolk and the ratio of yolk to albumin in the eggs. To the author's knowledge, this is a novel finding of

the present experiment as previous research has not focused on egg components.

The improved feed conversion for hens fed the enzyme supplemented diet appeared to be mediated by an increase in amino acid digestibility as digestibility coefficients for histidine. leucine, isoleucine, lysine, methionine, phenylalanine and threonine were significantly higher for birds fed the moderate energy diet supplemented with enzyme than for the unsupplemented moderate energy diet. The increase in amino acid digestibility with enzyme supplementation may be explained by an increase in the secretion of endogenous enzymes involved in protein digestion as Li et al. (2010) reported an increase in trypsin activity as a result of \beta-mannanase supplementation of broiler diets.

² From the end of the crop to the anus, including digesta content.

Although the intent of the current project was to improve the performance of laying hens fed diets containing oligosaccharides, it should be pointed out that the presence of oligosaccharides in the diet is not always negative. Certain oligosaccharides such as galacto-oligosaccharides and mannan-oligosaccharides can reach the colonic area and are preferentially fermented and utilized by Bifidobacteria as a source of carbon and energy. Bifidobacteria can improve animal health through mechanisms such as competitive exclusion of pathogenic and putrefactive bacteria and immune stimulation (Jung et al. 2008).

Dietary supplementation with lysolecithin alone had no effect on egg production, egg weight, feed intake or feed conversion compared with the unsupplemented moderate energy diet. These results contrast with those of Calton et al. (1998) who showed that the addition of lysolecithin to laying hen rations produced heavier egg weights. In addition, we recently reported that supplementation of lysolecithin (ranging from 0.0 to 0.15%) in the diet of laying hens significantly increased egg weight and feed efficiency (Han et al. 2010). In the present experiment, egg weight and feed efficiency were slightly improved as a result of lysolecithin supplementation but the differences did not reach statistical significance.

In our previous study (Han et al. 2010) the general increase in egg weight was associated with a dramatic increase in the number of large (63-72.9 g) size eggs with a concomitant reduction in the number of medium (53-62.9 g) size eggs. In the present study, there was a similar sized increase in the number of large size eggs and a reduction in the number of medium size eggs but only the change in medium sized eggs approached statistical significance. Further work should be conducted to clarify the effects of lysolecithin on egg size, as many countries pay a premium for larger sized eggs (Food and Agriculture Organization of the United Nations 2003), and there may be an economic incentive for producers to utilize lysolecithin as a means of increasing the percentage of large size eggs produced.

The improvements in feed conversion which we observed in our previous study (Han et al. 2010) may be partially explained by increases in nutrient digestibility. In the present experiment, dietary supplementation with lysolecithin significantly increased digestibility coefficients for nitrogen, fat, arginine, histidine, leucine, lysine, methionine, phenylalanine, and threonine compared with the values obtained for the unsupplemented moderate energy diet. These results may be explained by the effects of lysolecithin on the membranes lining the gastrointestinal tract. Lysolecithin can function as a membrane transducer by diffusing rapidly through the lipid portion of cellular membranes to increase fluidity and permeability (Shier et al. 1976). Lysolecithin may also alter mucosal barrier function

and increase gut permeability to macromolecules such as proteins (Tagesson et al. 1985). Although positive effects of lysolecithin were observed in the present study, lysolecithins are capable of lysing erythrocytes causing haemolysis (Hu and Kalkoff 1976). Therefore, care should be taken not to include lysolecithin at too high a level in the diet as there may be detrimental effects at high doses.

There did not appear to be any synergistic effects when enzymes and lysolecithin were fed in combination. In fact, several parameters measured appeared to be negatively influenced by the combination of feed additives compared with the results obtained when the additives were fed separately. Feed conversion as measured by grams of feed per gram of egg produced was significantly poorer for the combination treatment than for either additive fed alone. In addition, any improvement in the percentage of large size eggs or amino acid digestibility disappeared when the additives were fed in combination as opposed to separately. This affect was not expected and an explanation for the lack of synergy is not readily apparent.

A disappointing aspect of the present study was that none of the treatments increased the rate of egg production. However, since the rate of production exceeded 95% for all treatments, there was a very small window of opportunity for lysolecithin to increase the rate of egg production. In commercial poultry production, peak production usually occurs when birds reach 24 to 26 weeks of age and production steadily declines until the flock is taken out of production at approximately 76 weeks of age (Bell 2002). The present study was conducted relatively close to the period of peak production and it would be interesting to repeat the study to determine whether or not lysolecithin or enzyme supplementation have any beneficial effects on the productivity of laying hens during the later stages of the production cycle.

CONCLUSIONS

Dietary supplementation with lysolecithin or enzyme alone or in combination had no effect on egg production or egg weight. Supplementation with an enzyme cocktail providing 7 U/g of α -1, 6-galactosidase and 22 U/g of β -1, 4-mannanase significantly improved feed conversion as measured by grams of feed per gram of egg produced. The improved feed conversion for hens fed enzyme supplemented diets appeared to be mediated by an increase in amino acid digestibility as digestibility coefficients for histidine, leucine, isoleucine, lysine, methionine, phenylalanine and threonine were significantly higher for birds fed the moderate energy diet supplemented with enzyme than for the unsupplemented moderate energy diet. There did not appear to be any synergistic effects between lysolecithin and enzyme on laying hen performance.

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