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Dietary Inclusion of Probiotics, Prebiotics and Synbiotic and Evaluating Performance of Laying Hens

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Abstract: Problem Statement: This study was conduct to compare effects of various commercial feed additives on performance of laying hens. **Approach:** To evaluate effects of dietary inclusion of feed additives (Yeasturer, A-Max, Thepax, Fermacto and Biomin) on performance of laying hens, 216 Lohmann LSL-Lite hens were divided in 36 cages (n = 6). Approach Hens in 6 cages (replicates) were assigned to feed on one of the 6 iso-caloric and iso-nitrogenous experimental diets (ME = 2720 Kcal Kg^{-1} and CP = 145 g kg^{-1}) including control and diets with 0.5 g kg^{-1} of feed additives. Collected data of Feed Intake (FI), Egg Production (EP), Egg Weight (EW), Egg Mass (EM) and calculated Feed Conversion Ratio (FCR) during 6-week trial period was analyzed based on completely randomized design using GLM procedure of SAS. **Results and Conclusions:** Dietary additive inclusion significantly affected on EW on 1-3 and 3-6 weeks. Feed additive did not have significant effect on EP, FCR, FI and EM. There was no significant difference in EP, EM, FI and FCR among the experimental groups. Hens received Yeasturer or A-Max showed improved EW compared to hens fed the control diet during weeks 1-3. Hens fed diets included additives showed improved egg shell weight and thickness compared to hens fed the control diet. There was no significant effect of dietary treatment on blood levels of cholesterol, TG and HDL. Adding Thepax or Biomin to diet significantly reduced blood levels of LDL compared to hens fed the other experimental diets. There was no significant effect of dietary treatment on diacritical counts of white blood cells. **Recommendations**: According to the results of the present study, probiotic Yesture and A-Max can be included in laying hens diets to improve EM. In addition, the commercial feed additives (Yeasturer, A-Max, Thepax, Fermacto and Biomin) used in this study had beneficial effects on egg shell quality characteristics in terms of shell weight and thickness and to decrease egg abnormalities due to poor shell, these feed additives could be recommendable.

Key words: Probiotics, laying hens, eggs quality, Feed Intake (FI), Egg Production (EP), Egg Weight (EW), Egg Mass (EM) Feed Conversion Ratio (FCR), intestinal microflora, diacritical counts, white blood cells, significant effect, experimental diets

INTRODUCTION

Gibson and Roberfroid (1995) first defined prebiotics as ‘non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one, or a limited number of, bacteria in the colon’. Prebiotics must be indigestible to the animal host while remaining available to the probiotic bacteria. Futhermore, a prebiotic should be included at low quantities in the animal diet so that there is negligible effect on the inclusion of other necessary dietary ingredients (Roberfroid, 2001). A probiotic is a culture of a single bacteria strain, or mixture of different strains, that can be fed to animals to improve some aspect of their health. Probiotics are also referred to as direct fed microbial (La Ragione *et al*, 2001). Numerous studies in humans and animals have been conducted to assess the ability of probiotics to change the type and number of the microflora in the digestive tract (Gibson and Fuller, 2000). Some investigations on probiotics with laying hens indicated positive responses to dietary supplementation (Li *et al*., 2006; Kurtoglu *et al*., 2004). In addition, Gallazzi *et al*. (2008) and observed significant improvements in egg

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production in layers receiving probiotics. Prebiotics are nondigestible carbohydrates; many of these carbohydrates are short chains of monosaccharides, called oligosaccharides. Some oligosaccharides are thought to enhance the growth of beneficial organisms in the gut and others are thought to function as competitive attachment sites for pathogenic bacteria. Two of the most commonly studied prebiotic oligosaccharides are Fructo Oligo Saccharides (FOS) and Mannan Oligo Saccharides (MOS). Xu *et al*. (2003) studied the effect of FOS, at 4 levels of dietary inclusion, on growth performance and intestinal microflora in broilers. They reported that the diets containing 0.4% FOS resulted in significant improvements in average daily gain and feed efficiency compared with those fed the control diet. In piglets fed prebiotics, probiotics or synbiotics (combinations of probiotics and prebiotics) the population of bifidobacteria in the ileum increased and prebiotics and synbiotics increased their body weight gain (Shim *et al*., 2005). Furthermore, evidence suggests that synbiotics are more effective than are either probiotics or prebiotics alone and that a mixture of probiotic strains may be more effective than the individual strains (Timmerman *et al*., 2004). Supplementation of a diet with a mixture powder of garlic and thyme may assist in improving performance of laying hens and egg quality traits (Ghasemi *et al*., 2010). Supplementing corn-soybean or corn-soybean-guar meal diets by β-mannanase would have beneficial effects on performance of hens especially in terms of FCR and EP (Ehsani and Torki, 2010).

The objective of this study was to compare effects of diet addition of probiotics (Thepax and Yeasturer), prebiotics (Fermacto and A-Max) and synbiotic (Biomin) on performance of laying hens, egg quality characteristics, biochemical parameters of serum and diacritical counts of white blood cells.

MATERIALS AND METHODS

A total number of 216 Lohmann LSL-Lite hens were divided in 36 cages (n = 6) with almost equal distribution of average body weight and egg production among cages. Hens in 6 cages (replicates) were assigned to feed on one the 6 experimental diets. Based on a completely randomized design arrangement of treatments, 6 iso-caloric and iso-nitrogenous diets (ME = 2720 Kcal Kg^{-1} and CP = 145 g kg^{-1}) consisting control (with no additive) and 5 other diets with additives (Yeasturer (Y), A-Max (AM), Thepax (T), Fermacto (F) and Biomin (B) were formulated (Table 1). Characterizations of the feed additives used in the present experiment were mentioned below.

Yeasturer®, The commercial name of Yeasturer is composed of live yeast cultures selected from three strains Saccharomyces cerevisisiae (1×10^{11} cell) in combination with probiotic bacteria (Lactobacillus acidophilus and L. casei 5×10^{9} cfu, Sterptococcus faecium 5×10^{9} cfu and Bacillus subtilis 1×10^{10} cfu). A-Max®, A-Max is Saccharomyces cerevisiae (1×10^{11} cell) yeast grown media of sucrose cane and molasses and processed grain by-product. Thepax® is a commercial product containing yeast cells of Saccharomyces cerevisiae a commercial product containing yeast cells of Saccharomyces cerevisiae Var. ellipsoideus. Fermacto® is the commercially available fermentation product of Aspergillus orizae referred to as Aspergillus meal with no live cells or spores. Biomin® is a symbiotic with inulin (40%) and probiotic strain Enterococcus faecium. The colony forming unit per gram (cfu/g) of synbiotic was enumerate 5×10^{9}.

Collected data of Feed Intake (FI), Egg Production (EP), Egg Mass (EM) and calculated Feed Conversion Ratio (FCR) during 6-week trial period was analyzed based on completely randomized design using GLM procedure of SAS.

Table 1: Composition of the experimental diets

	Experimental diets	
Feed ingredients	Control g/100 g	Additives diet
Corn	68.67	68.67
Fish meal	3.000	3.000
Soybean meal	15.16	15.16
Dicalcium phosphate	1.250	1.250
Limestone	8.480	8.480
Common salt	0.250	0.250
Sand	2.580	2.520
Additives[1]	-	0.050
Vit. & Min. Premix[1]	0.500	0.500
DL-Methionine	0.110	0.110
Calculated analyses		
ME (Kcal/kg)	27200	27200
Crude protein (%)	14.500	14.500

[1]Yeasture, A-Max, Thepax, Fermacto and Biomin, [2]The vitamin and mineral premix provide the following quantities,per kilogram of diet: vitamin A, 10,000 IU (*all-trans*-retinal); cholecalciferol, 2,000 IU; vitamin E, 20 IU (*α*-tocopheryl); vitamin K3, 3.0 mg; riboflavin, 18.0 mg; niacin, 50 mg; D-calcium pantothenic acid, 24 mg; choline chloride, 450 mg; vitamin B12, 0.02 mg; folic acid, 3.0 mg; manganese, 110 mg; zinc, 100 mg; iron, 60 mg; copper, 10 mg; iodine, 100 mg; selenium, 0.2 mg; and antioxidant, 250 mg

RESULTS

Effects of probiotics, prebiotics and/or synbiotic on EP, FI, FCR, Egg Weight (EW) and EM during experimental period (6 weeks) are presented in Table 2-6, respectively. There was no significant difference in EP, EM, FI and FCR among the experimental groups. Diet inclusion of probiotics significantly affected on EW (weeks 1-3 and 3-6). Hens received Yeasturer or A-Max showed improved EW compared to hens fed the control diet during weeks 1-3. The effects of dietary treatment on the measured egg quality characteristics were shown in Table 7. Among the egg quality traits, only egg shell weight and shell thickness were significantly affected by dietary additive. Hens fed diets included additives showed improved egg shell weight and thickness compared to hens fed the control diet; however, regarding to egg shell weight the difference between eggs of hens fed Fermacto-included diet and control was not significant (P>0.05). Effects of adding probiotics, prebiotics and/or synbiotic on blood biochemical parameters (cholesterol, TG, LDL and HDL) are presented in Table 8. There was no significant effect of dietary treatment on blood levels of cholesterol, TG and HDL. There was no significant effect of dietary treatment on diacritical counts of white blood cells (Table 9).

DISCUSSION

In this study, EP, EM, FI and FCR were not significantly affected by dietary additive. This is in support with results obtained by Mutus *et al.* (2006) and Kalavathy *et al*. (2009) who reported that inclusion of probiotic had no significant effect on EP and EM. But Yoruk *et al*. (2004) and Panda *et al.* (2003) reported statistically significant increase of produced egg mass in ISA-Brown and Leghorn laying hens fed diet included probiotic during the whole laying period. Mahdavi *et al.* (2005) reported that addition of Bioplus 2B in diet of commercial layer hen had no positive effect on FI, EP, EW, EM and FCR. Based on some of studies changing in microbial ecology in layers' intestine might enhance their health and improve feed efficiency by the use of feeding probiotics (Aghaei *et al*., 2010; Chen *et al*. 2005). Also Sims *et al*. (2004) showed that turkeys fed diets with 0.1% MOS for the first 6 wk of life and then 0.05% for the remainder of the trial had significantly improved FCR compared with the turkeys in the un-supplemented control group at 12-15 wk of age. The reason of variable effect of biological additives may be confounded by variations in gut flora and environmental conditions (Mahdavi *et al*., 2005).

The inclusion of desirable microorganisms (probiotics) in the di*et al*lows the rapid development of beneficial bacteria in the digestive tract of the host, improving its performance (Edens, 2003). As a consequence, there is an improvement in the intestinal environment, increasing the efficiency of digestion and nutrient absorption processes (Pelicano *et al*., 2004), which may explain the improvement in egg weight observed in the present study. The efficiency of probiotics, however, will depend on the quantitative and qualitative characteristics of microorganisms used in the production, making it difficult to conduct comparative studies between different products. Balevi *et al.* (2001) who fed commercial multi strain probiotic to 40-week-old layers showed no statistically significant differences in EP and EW compared with the control.

Table 2: Egg production (%) of hens fed experimental diets

	Egg production (%)		
Weeks	1-3	3-6	1-6
Treatments			
Control	80.42	92.06	86.24
Yeasture	76.05	88.35	82.20
A-Max	83.86	92.32	88.09
Thepax	79.49	88.49	83.99
Fermacto	78.43	86.50	82.47
Biomin	85.79	89.35	87.57
P value	0.154	0.555	0.208
SEM	2.708	2.535	2.060

SEM = Standard Error of Means

Table 3: Feed intake (g hen^{-1} day^{-1}) of hens fed experimental diets

	Feed intake (g hen^{-1} day^{-1})		
Weeks	Wk 1-3	Wk 3-6	Wk 1-6
Treatments			
Control	110.73	114.39	112.56
Yeasture	109.21	116.14	112.67
A-Max	115.77	119.17	117.47
Thepax	109.81	114.65	112.23
Fermacto	113.48	111.81	112.64
Biomin	115.18	117.07	116.12
P value	0.0990	0.3030	0.2400
SEM	1.9720	2.2430	1.8790

SEM= Standard Error of Means

Table 4: Feed conversion ratio (g feed: g egg) of hens fed experimental diets

	Feed conversion ratio		
Weeks	Wk 1-3	Wk 3-6	Wk 1-6
Treatments			
Control	2.290	2.000	2.150
Yeasture	2.310	2.070	2.190
A-Max	2.190	2.040	2.120
Thepax	2.250	2.070	2.160
Fermacto	2.390	2.120	2.250
Biomin	2.180	2.120	2.150
P value	0.466	0.662	0.593
SEM	0.080	0.057	0.055

SEM = Standard Error of Means

Table 5: Egg weight (g) of hens fed experimental diets

	Egg weight (g)		
Weeks	Wk 1-3	Wk 3-6	Wk 1-6
Treatments			
Control	61.12^{b}	62.05^{ab}	61.58
Yeasture	62.95^{a}	63.47^{a}	63.21
A-Max	63.38^{a}	63.28^{a}	63.33
Thepax	62.56^{ab}	63.07^{a}	62.82
Fermacto	60.97^{b}	61.17^{b}	61.07
Biomin	62.05^{ab}	62.64^{ab}	62.35
P value	0.023	0.019	0.131
SEM	0.557	0.485	0.485

$^{a-b}$: Means within a column with no common superscript differ significantly (p<0.05), SEM = Standard error of means

Table 6: Egg mass (g) of hens fed experimental diets

	Egg mass (g hen^{-1} day^{-1})		
Weeks	Wk 1-3	Wk 3-6	Wk 1-6
Treatments			
Control	49.16	57.16	53.16
Yeasture	47.92	56.05	51.98
A-Max	53.25	58.41	55.83
Thepax	49.66	55.80	52.73
Biomin	53.20	55.96	54.58
P value	0.088	0.410	0.124
SEM	1.694	1.769	1.391

$^{a-b}$: Means within a column with no common superscript, differ significantly (p<0.05), SEM = Standard error of means

They were stated that the difference between their results and previous works may be related to differences in the ages of the hens. Responses to probiotics and prebiotics supplementation are inconsistent. This led to abundant investigations on possible factors that could influence the responses to these additives. In general these additives have proved most effective under conditions of stress, possibly the presence of un-favorable organisms, extremes in ambient temperature, diseases, crowding and poor management. In commercial layers production one or more of these conditions are invariably present. Further possible causes of variations in response to probiotics, prebiotics and/or synbiotic supplementation in layers could be differences between strains, hybrids, age, plane of nutrition, nutrient composition of the diet, microbial population of gastrointestinal tract, levels of inclusion of probiotics, prebiotics and/or synbiotic in the diet, duration of supplementation or other environmental conditions.

Hens fed diets included additives showed improved egg shell weight and thickness compared to hens fed the control diet. This result agreed with the previous report that egg shell weight and shell thickness were significantly higher due to dietary inclusion of Saccharomyces cerevisiae and Bioplus (Bageridizaj *et al*., 2006). The better results obtained for the eggshell quality parameters could be partly due to the fact that the probiotics and prebiotics influence on the metabolic activity of the beneficial bacteria colony within the layers' intestine, which positively influence mineral absorption rate, especially those of Ca^{2+} and Mg^{2+} (Roberfroid, 2000).The egg shell quality improvement is under the influence of the intestinal Ca^{2+} absorption rate improvement, phenomenon facilitated by the presence within feed of some fodder additives like prebiotics, as previously stated by other researches. This beneficial effect on eggshell quality due to probiotic feeding may be attributed to a favorable environment in the intestinal tract by feeding of L. sporogenes, which might have helped to assimilate more calcium, which was evident by increased concentration of Ca in serum (Panda *et al*., 2008).

In the present study, diet additives did not significantly affect plasma levels of cholesterol, triglyceride and HDL. This finding was in agreement with Kurtoglu *et al*. (2004) who showed that probiotic did not affect serum cholesterol in 30-days period of experiment. But Mahdavi *et al*. (2005) report that probiotics could depress serum and egg yolk cholesterol concentrations. Cholesterol depressing effect of probiotics, prebiotics and/or synbiotic in the serum and egg yolk in layers requires further investigation. Adding Thepax or Biomin to diet significantly reduced blood levels of LDL compared to hens fed the other experimental diets. In addition, in some study, probiotic supplementation reduced the serum LDL cholesterol (Kalavathy *et al*., 2003). However, Hens fed the Thepax and Biomin diets did have decreased blood levels of LDL compared to hens fed the control diet. Baillon *et al*. (2004) reported that administration of probiotic increased neutrophils and monocytes in adult dogs. It has been recognized that the gut-associated immune system can be modulated by nutritional means (Koenen *et al*., 2004).

CONCLUSION

This study provides evidence that adding Yeasturer, A-Max, Thepax, Fermacto and Biomin to layer diets did not cause any beneficial effects on hens' performance with the exception of EW, so that the hens received Yeasturer and A-Max did show better EW than hens fed the control diet. In addition, dietary supplementation by Thepax or Biomin reduced blood levels of LDL.

Table 7: Egg quality characteristics (egg index, yolk index, Haugh unit, egg shell weight and egg shell thickness) of hens fed experimental diets

	Egg quality characteristics				
	Egg index	Yolk index	Haugh unit Egg	shell weight	Shell thickness
Treatments					
Control	75.17	44.37	70.80	6.56[b]	39.00[c]
Yeasture	74.68	44.30	72.22	7.35[a]	43.16[ab]
A-Max	73.82	44.19	71.59	7.40[a]	43.66[a]
Thepax	78.79	44.47	71.03	7.23[a]	42.33[ab]
Fermacto	74.11	44.35	73.31	7.13[ab]	41.33[b]
Biomin	75.23	44.16	71.30	7.45[a]	43.83[a]
P value	0.174	0.999	0.073	0.039	0.0001
SEM	1.395	0.525	1.243	0.199	0.648

[a-c]: Means within a column with no common superscript differ significantly (p<0.05), SEM = Standard error of means

Table 8: White blood cell counts (heterophil, lymphocyte, monocyte, eosinophil and basophil) of hens fed experimental diets

	Heterophile	Lymphocyte	Monocyte	Eosinophile	Basophile
Treatments					
Control	24.75	73.75	0.250	0.000	1.000
Yeasture	26.75	65.25	0.000	0.000	2.500
A-Max	29.25	66.00	0.750	0.750	3.250
Thepax	37.50	57.50	0.750	0.250	4.000
Fermacto	30.75	65.25	0.500	0.250	3.250
Biomin	34.50	61.25	0.000	0.500	4.000
P value	0.186	0.081	0.488	0.738	0.091
SEM	4.227	4.476	0.413	0.456	0.866

[a-b]: Means within a column with no common superscript differ significantly (p<0.05), SEM= Standard error of means

Table 9: Blood lipids (Cholesterol, TG, HDL and LDL) of hens fed experimental diets

	Cholesterol	TG	HDL	LDL
Treatments				
Control	261.75	2448.75	80.50	79.25[a]
Yeasture	205.00	2532.50	69.50	82.00[a]
A-Max	332.75	3012.25	78.75	85.25[a]
Thepax	474.25	1621.25	55.50	65.25[b]
Fermacto	254.50	2822.50	67.50	84.50[a]
Biomin	146.75	1643.75	59.00	64.00[b]
P value	0.649	0.120	0.122	0.022
SEM	23.189	177.812	2.645	2.604

SEM= Standard error of means

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