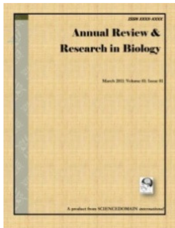

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Effects of Commercial Prebiotic and Probiotics of Diet on Performance of Laying Hens, Egg Traits and Some Blood Parameters

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

Research Article

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ABSTRACT

Aims: This study was conducted to evaluate the influence of adding commercial probiotics and prebiotic to diet on performance of laying hens, egg traits and some blood parameters.
Study Design: Data of feed intake (FI), egg production (EP), egg mass (EM), and calculated feed conversion ratio (FCR) were analyzed based on completely randomized design using GLM procedure of SAS.
Place and Duration of Study: All procedures used in this 7-week experiment were approved by the "Animal Ethics Committee of Razi University" and complied with the "Guidelines for the Care and Use of Animals in Research".
Methodology: Five iso-caloric and iso-nitrogenous diets (ME =2720 Kcal/Kg and CP=150 g/kg) including basal diet (control) and basal diet supplemented by probiotics (PrimaLac®, A-Max and Yeasture) and prebiotic (Fermacto) were formulated. A total number of 90 Lohmann LSL-Lite laying hens were randomly divided in 15 cages (n=6). The experimental hens were 56-wk old with an average egg production rate of 90.6 ±4.8% and 1,460±24 g live body weight. Hens in every 3 cages (replicates) were assigned to feed on one of the 5 experimental diets.
Results: FI, FCR, EM, EP, egg weight, egg traits (egg index, yolk index, Haugh unit, yolk color, shell weight and thickness) and blood parameters were not affected by adding

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probiotics or prebiotic to diets.
Conclusion: Using Primalac, Fermacto, A-Max and Yeasture did not have beneficial effects on performance of laying hens. However, the effects of probiotics and prebiotic on suboptimal circumstances should be investigated in future.

Keywords: Prebiotic; probiotic; laying hens; performance; egg traits; blood parameters.

1. INTRODUCTION

There is the need to look for viable alternatives that could enhance the natural defense mechanisms of animals and reduce the massive use of antibiotics [1]. One way is to use specific feed additives or dietary raw materials to favorably affect animal performance and welfare, particularly through the modulation of the gut microbiota which plays a critical role in maintaining host health [2]. A balanced gut microbiota constitutes an efficient barrier against pathogen colonization, produces metabolic substrates (e.g. vitamins and short-chain fatty acids) and stimulates the immune system in a non-inflammatory manner. In this context, probiotics, prebiotics and synbiotics could be possible solutions. The main effects of these feed additives are improved resistance to pathogenic bacteria colonization and enhanced host mucosa immunity; thus resulting in a reduced pathogen load, an improved health status of the animals [3,4] and a reduced risk of food-borne pathogens in foods. Nowadays, after the antibiotics prohibition as growth stimulators in animal nutrition, probiotics as biologically active compounds have been used [5-7]. The first goal of the livestock production is the delivery of safe foods for human consumption taking into account the welfare of the animal and respect for the environment. An important field of zootechnical research is the improvement of the quality and safety of the meat. It is well recognized that pathogens, such as Campylobacter and Salmonella can be transmitted along the food chain and can be the source of human illness. Prebiotics and probiotics are being tested under different experimental conditions to study the pathways used by these substances to assist in the prevention of carcass contamination and in the elimination of pathogens present in the birds' organisms [8,9]. Gibson and Roberfroid [10] defined a prebiotic as a non-digestible food ingredient which beneficially affects the host by selectively stimulating the growth of and/or activating the metabolism of one or a limited number of health-promoting bacteria in the intestinal tract, thus improving the host's microbial balance. The growth of endogenous microbial population groups such as Bifidobacteria and Lactobacilli is specifically stimulated and these bacteria species are perceived as beneficial to animal health. Prebiotics have the advantage, compared with probiotics, that bacteria are stimulated which are normally present in the GIT of that individual animal and therefore already adapted to that environment [11]. The dominant prebiotics are fructo-oligosaccharide products (FOS, oligofructose, inulin) [12] gluco-oligosaccharides, stachyose, malto-oligosaccharides, and oligochitosan have also been investigated in broiler chickens [13,14]. Probiotics are live micro-organisms, generally bacteria but also yeasts than, when ingested alive in sufficient amount, they have a positive effect on the health going beyond the nutritional ones commonly known. Their use was linked with a proven efficacy on the gut micro flora resulted in improved health status. Two main mechanism of action have been suggested and are summarized as follows: (a) nutritional effect, characterized by reduction of metabolic reactions that produce toxic substances, stimulation of indigenous enzymes and production of vitamins and antimicrobial substances; and (b) health or sanitary effect, distinguished by increase in colonization resistance, competition for gut surface adhesion and stimulation of the immune response [15]. In poultry, benefits of probiotic supplementation (live yeast or bacteria) are reported in broilers' performance and health, with evidence of increased resistance of chickens to Salmonella, *E. coli* or *C. perfringens* infections [16-18]. Probiotics can increase feed efficiency and productivity of laying hens [19,20] and an improvement in egg quality (decreased yolk cholesterol level, improved shell thickness, egg weight) has also been reported [19,21]. Studies on the beneficial impact on poultry performance have indicated that probiotic supplementation can have positive effects. Kabir et al. [22] for example, conducted a 6-wk growth performance study with broilers and found that live weight gain and carcass yields were significantly higher in broilers fed probiotic supplementation. They also found significant differences among spleen and bursa weights. Davis and Anderson [23] reported that PrimaLac as a direct-fed microbiotic improved egg size and lowered feed cost in laying hens. Capcarová et al. [24] recorded that preventive application of probiotic preparations achieved better utilization of nutrients and feed and they had a positive effect on environment. Gong et al. [25] define probiotics as health-promoting bacteria inhabiting the gastrointestinal tract of humans and animals. Recently, Chichlowski et al. [26] reported that a probiotic containing lactobacilli *Bifidobacterium thermophilum* and *Enterococcus faecium* increased the jejuna villus height and decreased the villus crypt depth compared with salinomycin and control. Moreover, shorter and thinner villi were associated with toxins [27,28]. In contrast, longer villi were found in the ileum of adult male layers with slight improvement in feed efficiency after dietary addition of *Bacillus subtilis* var. natto [29] and in broilers after addition of *E. faecium* [30] or Eubacterium sp. [28].

This study was conducted to evaluate the influence of adding commercial probiotics and prebiotics to diet on performance of laying hens, egg traits and some blood parameters.

2. MATERIALS AND METHODS

All procedures used in this seven-week experiment were approved by the Animal Ethics Committee of Razi University and complied with the "Guidelines for the Care and Use of Animals in Research".

2.1 Birds and Experimental Diets

As it is presented in the Table 1, Five iso-caloric and iso-nitrogenous diets (ME =2720 Kcal/Kg and CP=150 g/kg) including basal diet (control), which was based on Lohman recommended catalogue, and basal diet supplemented by probiotics or prebiotics (Primalac, Fermacto, A-Max and Yeasture) were formulated. Probiotic preparation Yeasture®, made from live *Saccharomyces cerevisiae* yeast strains grown on a medium of ground yellow corn, corn syrup, diastatic malt, and cane molasses dried to preserve its fermenting activity. *Aspergillus oryzae* fermentation extract, *Lactobacillus acidophilus*, *Streptococcus faecium*, b 1,3-b 1,6 D-Glucan, hemicellulase, protease, cellulase, alpha amylase, flavoring and sweetener. A-Max® is a yeast culture feed ingredient with high levels of metabolites, vitamin B, and amino acids that have been shown to increase milk production in dairy. Premalac® is a dried fermentation product of *Lactobacillus acidophilus*, *Aspergillus oryzae* extract, *Bifedobacterium bifedum*, *Streptococcus faecium*, Torula yeast, skim milk, vegetable oil and CaCO3. Prebiotic (Fermacto) was supplemented at the rate of 0 and 0.5 kg/ton of diets. Fermacto® is a natural feed supplement based upon a primary fermentation (Aspergillus).

A total number of 90 Lohmann LSL-Lite hens were divided in 15 cages (n=6). Hens in 3 cages (replicates) were assigned to feed on one of the 5 experimental diets. The hens were placed in wire-floored cages (0.3 m wide×0.4 m length×0.4 m height) arranged in a single tier within a conventional open-sided house. The cages were located in a windowless and environmentally controlled room with the room temperature kept at 2l-23ºC and the photoperiod set at 16 h of light (incandescent lighting, 10 lux) and 8 h dark. Each cage had a nipple watered. Water was available ad libitum throughout the experiment. Feed consumption and egg production was measured and feed conversion efficiency was calculated on a weekly basis. Data were analyzed in completely randomized design using GLM procedure of SAS [31]. All statements of significance are based a probability of less than 0.05.

Table 1. Ingredients and composition of experimental diets[1]

Feed ingredients	**C**	**G1**	**G2**	**G3**	**G4**
	Control	**A-Max**	**Fermacto**	**Primalac**	**Yeasture**
	g / 100 g diet				
Corn	64.06	64.11	64.04	64.04	64.04
Fish meal	4.60	4.60	4.60	4.60	4.60
Soybean meal	13.70	13.71	13.70	13.70	13.70
Date pits[2]	7.48	7.21	7.34	7.34	7.34
Dicalcium phosphate	1.29	1.29	1.29	1.29	1.29
Lime stone	8.15	8.15	8.15	8.15	8.15
Common salt	0.22	0.22	0.22	0.22	0.22
Premalac	-	-	-	0.05	-
Fermacto	-	-	0.05	-	-
A-Max	-	0.10	-	-	-
Yeasture	-	-	-	-	0.05
Vit. & Min. Premix[3]	0.50	0.50	0.50	0.50	0.50
DL-Methionine	-	0.10	0.10	0.10	0.10
Calculated analyses					
ME (Kcal/kg)	2720	2720	2720	2720	2720
Crude protein (%)	15	15	15	15	15
Calcium (%)	3.67	3.67	3.67	3.67	3.67
Available P (%)	0.33	0.33	0.33	0.33	0.33
Lys (%)	0.76	0.76	0.76	0.76	0.76
Met (%)	0.38	0.38	0.38	0.38	0.38
Met & Cys (%)	0.62	0.62	0.62	0.62	0.62

[1]Primalac: Star-Labs/Forage Research,Inc. P.O. Box 77. Clarksdale, MO 64430; Fermacto: 1051 Marion St, Winnipeg, MB, Canada, R2J 0L1; A-Max: Probiotic Application in Chickens, Bailey, J. Stan, USDA, ARS, BEAR, Athens, GA; Yeasture: CENZONE TECH-EUROPE , Ltd., 10475 Pinion Trail, Escondido, CA 92026, USA

[2]The chemical composition (nutrients contents) of used date pits: ME= 2000 kcal/kg, Crude protein= 7.03%,Ether Extract= 7.10%, Crude fiber= 48.2%, Calcium= 0.865%, Available Phosphorous= 0.03%.

[3]Mineral mix supplied the following per kg of diet: Cu, 20 mg; Fe, 100 mg; Mn, 100 mg; Se, 0.4; Zn, 169.4 mg. 'Vitamins mix supplied the following per kg of diet: Vitamin A, 18,000 IU; vitamin D3, 4,000 IU; vitamin E, 36mg; vitarnin K; 4 mg; vitamin B\2, 0.03 mg; thiamine, 1.8 mg; riboflavin, 13.2 mg; pyridoxine, 6 mg; niacin, 60 mg; calcium pantothenate, 20 mg; folic acid, 2 mg; biotin, 0.2 mg; choline chloride, 500 mg

2.2 Egg Traits and Blood Parameters

Egg traits were measured twice on wk 3 and 7 of experiment and each time all eggs during three frequent days were used. At the end of the experiment (7 wk) four hens were selected randomly from each treatment (one hen per replicate) and blood samples were collected from the wing vein into a 5-ml syringe. Part of the blood which had been obtained having been centrifuged (3000×g for 15 min) immediately and serum collected for subsequent analysis, the rest was placed in tubes with heparin as anticoagulant for in order to diacritical counts of white blood cells based on the procedures of [32]. Briefly, two drops of blood were placed on a slide, spin prepared and stained with May-Grünwald-Giemsa stain. All slides were coded and one hundred leukocytes, including granular (heterophils, eosinophils, and basophils) and nongranular (lymphocytes and monocytes) were counted on one slide per each bird, and the heterophil to lymphocyte (H/L) ratio was calculated. Serum triglycerides, high density lipoprotein (HDL), low density lipoprotein (LDL), and total cholesterol were analyzed using the diagnostic kit (Pars Azmun, Iran), and enzymatic methods.

2.3 Body Weight Change and Collection of Excreta Samples

Individual body weight was recorded at the beginning and end of the experiment. Body weight change (%) = body weight change (g)/initial bird body weight (g). The excreta were collected on galvanized zinc trays lined with plastic sheets. Dropped feathers, feed particles or foreign materials were removed to prevent contamination. Approximately 200g samples were collected daily. Each of the excreta samples was mixed and homogenized individually. The pH of 1 g of excreta in 10 mL of distilled water was measured using a digital pH meter (model 632 equipped with the electrode 6.0202.000 containing 3 *M* KCl electrolyte; Metrohm, Herisau, Switzerland).

3. RESULTS AND DISCUSSION

3.1 Productive Performance and Egg Quality Traits of Laying Hens

Based on the results of the present study, feed intake (FI), feed conversion ratio (FCR), egg mass (EM), egg production (EP) and egg traits (egg weight, egg index, yolk index, Haugh unit, yolk color, shell weight and shell thickness) were not significantly affected by adding probiotics or prebiotic to diets (Table 2,3 and 4). In agreement with our result, it has been reported by Panda et al. [33] that probiotics did have no significant effect on FCR and EW but increased EM, EW, thickness of eggshell. Balevi et al. [34] who fed commercial multi strain probiotic to 40-week-old layers showed no statistically significant differences in EP and EW compared with the control. Hosseini et al. [35] reported that addition of yeast in commercial laying hens' diet have no positive effect on egg shell thickness, Haugh unit, egg breaking strength and egg shell quality. Mahdavi et al. [36] realized that using different levels of probiotic had no significant effects on EP, EW, EM, FI, FCR, shell thickness, shell hardness and Haugh unit. Haugh unit is major indicator determining egg quality and does not change by dietary regimen [37]. In addition, [38] did not find any significant difference in bird performance among treatments with flavomycin and phosphorylated mannanoligosaccharides (MOS) used individually or in combination with organic acids, between 1 and 28 days of age. The reason for the variable effects of prebiotic and probiotics on poultry might be attributed to variations in gut flora, environmental conditions [36], ability or inability of probiotics to colonize in gastrointestinal tract and competitively exclude the pathogenic bacteria [39], amount of stress in flock [40], strain and concentration of probiotic, time and method of use [41]. Angel et al. [42] reported that supplementation of a commercial probiotic in broiler diets significantly improved FCR. [43] reported that FI was significantly reduced in broilers supplemented with probiotics, cecal cultures and Primalac did not have a cholesterol lowering effect in broiler chickens. Kurtoglu et al. [19] showed that probiotic effect on EP was not specific until day 60, but significant increase in EP by probiotic supplementation were seen on days 60-90 of their experiment.

3.2 Blood Parameters

As shown in the Tables 5 and 6, dietary treatments had no significant effects on the blood parameters. These findings were in disagreement with [36] found that using different levels of probiotic caused significant decrease in plasma cholesterol, plasma triglyceride and egg cholesterol. The difference between their results and previous works may be related to the strain of bacteria, concentration and the form of bacteria used (viability, dryness or their products) and differences in the ages of hens. No significant difference was observed in serum total cholesterol, high density lipoprotein (HDL) cholesterol, low density lipoprotein (LDL) cholesterol, very low density lipoprotein (VLDL) cholesterol or triglycerides and white blood cell count between treatments (Tables 5-6). These results are in good agreement, more or less, with Kalavathy et al [44] who showed that cholesterol content was not significantly reduced by probiotic supplementation; however, serum total cholesterol, LDL and triglycerides were significantly decreased in lactobacillus culture-fed compared to control broilers. Kannan et al. [45] have reported that the use of 0.5 g kg-1 mannanoligosaccharide obtained from yeast in the ration of broiler chickens, significantly reduced the serum cholesterol level on day 35 as compared with the control. Tizard et al. [46] reported that mannans and other similar carbohydrates (fructans) prevent cholesterol absorption in gastrointestinal tract. In contrast, Yalcinkaya et al. [47] reported that the use of MOS in broilers diet could not significantly reduce the serum cholesterol and triglycerides levels as compared with the control group. Synthesis of bile acids from cholesterol in the liver is the most important way of cholesterol excretion [48]. The use of probiotics and prebiotics can disintegrating bile salts and de-conjugate production of enzymes by the activity of lactic acid bacteria, as well as reduction of the pH in the intestinal tract can be effective in reducing the cholesterol concentration. Solubility of non-conjugate bile acids is lowered at a low pH and consequently, they are absorbed less from the intestine and are excreted more in the feaces [49]. Consequently, the liver, for re-establishment of the hepatic cycle of bile acids, coverts more cholesterol concentration into the tissues and therefore their concentrations in the blood is reduced [50]. In the growing birds, VLDL is the most important triglycerides carrier. A reduction in the serum triglycerides level may be due to an increase in the population of lactic acid bacteria in the gastrointestinal tract. Santose et al. [51] have reported that supplementation of *Bacillus subtilis* to the ration of broiler chickens, in addition to reducing the carcass fat, reduces the triglycerides concentration in the serum, the liver and the carcass and suggest that this bacterium can be effective in reducing the activity of acetyl coenzyme A carboxylase (the enzyme limiting the synthesis rate of fatty acids). Kabir et al. [22] evaluated the dynamics of probiotics on immune response of broilers and they reported significantly higher antibody production in experimental birds as compared to control ones. They also demonstrated that the differences in the weight of spleen and bursa of probiotics and conventional fed broilers could be attributed to different level of antibody production in response to SRBC. In addition, Haghighi et al. [52] demonstrated that administration of probiotics enhances serum and intestinal natural antibodies to several foreign antigens in chickens. Dalloul et al. [53] examined the effects of feeding a Lactobacillus-based probiotic on the intestinal immune responses of broiler chickens over the course of an *E. acervulina* infection and they demonstrated that the probiotic continued to afford some measure of protection through immune modulation despite a fairly overwhelming dose of E. acervulina. They also suggested a positive impact of the probiotic in stimulating some of the early immune responses against *E. acervulina*, as characterized by early IFN-γ and IL-2 secretions, resulting in improved local immune defenses against coccidiosis. Brisbin et al. [54] investigated spatial and temporal expression of immune system genes in chicken cecal tonsil and spleen mononuclear cells in response to structural constituents of *L. acidophilus* and they found that cecal tonsil cells responded more rapidly than spleen cells to the bacterial stimuli, with the most potent stimulus for cecal tonsil cells being DNA and for splenocytes being the bacterial cell wall components.

3.3 Excreta pH and Body Weight Changes

Effects of dietary inclusion of probiotic and probiotic on excreta pH and body weight changes (BWC) of laying hens are presented in the Table 7. Neither the dietary inclusion of prebiotics nor dietary supplementation of probiotics significantly affected BWC of the laying hens (*P*=.05). This is in support with result obtained by Kurtoglu et a1 [55]. Several studies have reported that prebiotics [56, 57] can improve the body weight in broilers. However, little or no reference to an effect of a prebiotic on layers body weight change was available. Dietary supplementation of prebiotics and probiotics performed no change on excreta pH compared with the control group. There is no record in the literature presenting the effects of dietary supplementation of prebiotics and probiotics on excreta pH of laying hens. However, further investigations are needed to clarify the effect of dietary supplementation of prebiotics and probiotics on excreta pH in laying hens.

Table 2. Effects of adding probiotics and prebiotics with commercial names of Primalac, Fermacto, A-Max and Yeasture to diet on productive performance of Lohmann LSL-lite laying hens (weeks 58-65 of age)[a]

Groups	Parameters			
	Feed intake (g/hen/day)	**Feed conversion ratio (g feed : g egg)**	**Hen-day egg production (%)**	**Egg mass (g/hen/day)**
Control	112±4.78	2.13±0.14	87.6±2.39	53.2±0.54
A-Max	116±2.13	2.20±0.15	85.4±3.44	55.0±5.13
Fermacto	115±4.21	2.13±0.11	89.3±3.35	54.5±3.29
Primalac	112±3.10	2.17±0.14	84.0±9.58	51.9±4.58
Yeasture	110±3.13	2.32±0.13	79.4±1.93	48.0±3.73
Pooled SEM	1.10	0.035	1.41	1.06
CV	3.71	6.18	6.06	7.23
P values				
Parameters	0.39	0.46	0.22	0.24

[a]Means±SD, abMeans within column (main effects) with different superscripts are significantly different ($P<0.05$)

Table 3. Effects of adding probiotics and prebiotics with commercial names of Primalac, Fermacto, A-Max and Yeasture to diet on egg quality traits (first sampling on wk 3)[a]

Groups	Egg quality traits (wk 3)							
	Parameters							
	Egg weight	**Egg index**	**Yolk index**	**Haugh unit**	**Yolk color (Roch)**	**Specific gravity ($g{\cdot}cm^{-3}$)**	**Shell weight (g)**	**Shell thickness ($mm10^{-2}$)**
Control	61.5±2.51	77.6±1.89	40.0±0.30	68.2±1.41	6.33±0.00	1.09±0.00	5.95±0.27	36.6±0.67
A-Max	60.0±1.39	75.9±0.68	39.4±0.61	69.9±5.48	6.78±0.19	1.09±0.00	5.84±0.24	37.0±1.45
Fermacto	61.7±1.22	76.7±0.53	39.0±1.60	69.6±2.08	6.33±0.33	1.09±0.00	5.99±0.32	36.8±1.17
Primalac	62.3±1.87	77.7±2.22	39.0±0.91	68.2±1.75	6.55±0.19	1.09±0.00	5.93±0.27	35.6±1.67
Yeasture	60.0±3.24	76.8±0.58	34.2±7.71	69.6±3.65	6.55±0.69	1.09±0.00	6.10±0.05	37.4±0.38
Pooled SEM	0.538	0.351	0.957	0.735	0.091	0.001	0.059	0.300
CV	3.57	1.80	9.28	7.88	5.61	0.32	4.19	3.19
P values								
Parameters	0.62	0.52	0.33	0.94	0.56	0.74	0.77	0.47

[a]Means±SD, [ab]Means within a column showing different superscripts are significantly different (P< 0.05), Duncan's multiple-range test were applied to compare means

Table 4. Effects of adding probiotics and prebiotics with commercial names of Primalac, Fermacto, A-Max and Yeasture to diet on egg quality traits (second sampling on wk 7)[a]

	Egg quality traits (wk 7)							
Groups	**Parameters**							
	Egg weight	**Egg index**	**Yolk index**	**Haugh unit**	**Yolk color (Roch)**	**Specific gravity ($g{\cdot}cm^{-3}$)**	**Shell weight (g)**	**Shell thickness ($mm10^{-2}$)**
Control	62.6±4.61	75.5±0.90	43.7±0.66	66.8±6.67	6.55±0.51	1.08±0.00	5.53±0.63	35.7±2.41
A-Max	64.0±3.05	73.8±1.34	42.5±1.26	69.8±2.91	6.78±0.51	1.08±0.00	5.67±0.36	36.2±1.07
Fermacto	62.0±1.81	74.9±1.58	44.1±1.88	65.8±1.91	6.78±0.84	1.08±0.00	4.92±1.16	36.0±2.52
Primalac	62.6±2.26	74.6±1.78	39.1±8.97	65.8±2.60	6.67±0.33	1.08±0.00	5.31±0.31	34.8±1.39
Yeasture	62.5±1.11	74.7±1.43	44.0±0.48	69.2±1.27	6.33±0.33	1.08±0.00	5.73±0.17	36.1±1.17
Pooled SEM	0.643	0.347	1.035	0.909	0.125	0.001	0.159	0.418
CV	4.51	1.92	9.73	5.34	8.12	0.36	11.69	5.09
P values								
Parameters	0.93	0.70	0.57	0.53	0.83	0.18	0.55	0.90

[a]Means±SD, [ab]Means within a column showing different superscripts are significantly different (P< 0.05), Duncan's multiple-range test were applied to compare means

Table 5. Effects of adding probiotics and prebiotics with commercial names of Primalac, Fermacto, A-Max and Yeasture to diet on productive performance of laying hens on white blood cell counts (heterophil-H, lymphocyte-L, monocyte-M, eosinophil-E, basophil-B and Heterophil to Lymphocyte ratio- H/L)[a]

Groups	White blood cell counts (%)					
	H[1]	L	M	E	B	H/L
Control	32.3±13.3	65.6±14.1	0.67±0.57	1.00±1.73	0.33±0.58	0.53±0.28
A-Max	24.6±4.51	72.0±6.00	1.67±1.53	0.00±0.00	1.67±0.58	0.35±0.09
Fermacto	22.6±8.02	72.6±10.1	2.00±2.00	1.00±1.00	1.67±2.08	0.33±0.16
Primalac	8.02±3.05	71.0±3.46	3.00±4.36	0.00±0.00	1.67±1.53	0.34±0.04
Yeasture	29.3±0.58	65.0±1.73	3.67±1.15	0.00±0.00	1.67±1.53	0.45±0.01
SEM	1.87	2.03	0.579	0.235	0.335	0.039
CV	27.6	12.1	105	223	99.3	38.1
P values						
Parameters	0.50	0.69	0.57	0.40	0.70	0.44

[a]Means±SD, ab Means within a column showing different superscripts are significantly different (P< 0.05), Duncan's multiple-range test were applied to compare means

Table 6. Effects of adding probiotics and prebiotics with commercial names of Primalac, Fermacto,A-Max and Yeasture to diet on productive performance of laying hens on serum biochemical metabolites (cholesterol-CHOL, triglycerides-TG, high density lipoprotein-HDL, low density lipoprotein-LDL)[a]

	Serum biochemical metabolites (mg/dL)			
Groups	**CHOL(mmol/L)[1]**	**T.G (mg/dL)**	**HDL (mmol/L)**	**LDL**
Control	163±32.7	1641±306	39.6±5.69	105±11.9
A-Max	206±45.0	2768±669	47.6±7.51	125±19.0
Fermacto	173±59.4	1983±1222	41.0±11.1	109±30.0
Primalac	147±29.3	1550±739	37.3±7.09	97.0±18.7
Yeasture	251±106	2715±770	57.6±20.6	145±39.3
SEM	16.5	222	3.22	7.21
CV	32.5	37.4	26.2	22.1
P values				
Parameters	0.32	0.25	0.28	0.24

[a]Means±SD, [ab]Means within a column showing different superscripts are significantly different (P< 0.05), Duncan's multiple-range test were applied to compare means

Table 7. Effects of adding probiotics and prebiotics with commercial names of Primalac, Fermacto,A-Max and Yeasture to diet on productive performance of laying hens on excreta pH and body weight changes(BWC)[a]

Parameter	Excreta pH	BWC (g)
Control	7.43±0.07	-38.8±19.1
A-Max	6.99±1.02	16.6±13.7
Fermacto	7.31±0.16	8.05±38.4
Primalac	6.74±0.65	-20.8±45.5
Yeasture	7.26±0.28	-61.1±34.9
SEM	0.378	10.5
CV	10.5	-169
P values		
Parameters	0.08	0.08

[a]Means±SD, [ab]Means within a column showing different superscripts are significantly different (P< 0.05), Duncan's multiple-range test were applied to compare means

4. CONCLUSION

In the present study adding four various commercial brands of probiotics or prebiotic including Premalac, Fermacto, A-Max and Yeasture to diets of laying hens did not have beneficial effects on performance, egg quality characteristics and the measured blood parameters. Using probiotics and prebiotics on suboptimal circumstances (such as stress, diseases, mal-nutrition, etc) may have beneficial effects on performance of laying hens that appreciates more research in future.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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