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Full Length Research Paper

Immune response and ileal microflora in broilers fed wheat-based diet with or without enzyme Endofeed W and supplementation of thyme essential oil or probiotic PrimaLac®

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An experiment was carried out to study the immune response and ileal microflora in broilers fed wheat-based diet with or without enzyme Endofeed W (EEW) and supplementation of thyme essential oil (TEO) or probiotic PrimaLac® (PP). Hypersensitivity, antibody production, haematology and immune organ weights were considered as the immune response and *Lactobacillus, Bifidobacterium,* and *Escherichia coli* were enumerated in ileum. A 2×3 factorial arrangement with two levels of EEW (0.0 and 0.05%) and two types of growth promoters (unsupplemented or supplemented with TEO or PP) was designed. Each of the six dietary treatments was fed to four groups with 12 chicks each from 0 to 42 days. Addition of EEW, TEO or PP to the wheat-based diet improved (P<0.05) immunity in terms of hypersensitivity response and heterophil to lymphocyte ratio as compared to the control group. Antibody production against sheep red blood cells (SRBC) antigen and other haematological analysis were numerically (P > 0.05) enhanced by the addition of EEW or TEO to diet, whereas total and IgG anti-SRBC altered significantly with administration of PP and white blood cells count was increased (P < 0.05) by the inclusion of growth promoters (TEO or PP) to the control diet. All feed additives induced competitive exclusion efficiently, in terms of increase (P< 0.05) in *Lactobacillus* and *Bifidobacterium* and reduction (P < 0.05) in *E. coli* counts.

Key words: Enzyme, essential oil, probiotic, wheat, broiler.

INTRODUCTION

One of the most viable alternatives to the corn is wheat. However, the use of wheat is limited due to its negative characteristics: varying nutrient contents, lower metabolizable energy than corn and non starch polysaccharides (NSPs; xylan and β-glucan). The NSP presents in cereal lower animal performance by raising intestinal viscosity and stimulating growth of microflora (Preston et al., 2001). NSP-degrading enzymes and growth promoters have been shown to alleviate adverse effects in poultry diets containing grains such as wheat, rye, barley and oats (Basmacioglu et al., 2010).

NSP-degrading enzymes supplementation to the wheat-based diet enhanced the immune system (Gao et al., 2007) and microflora in the intestine (Danicke et al., 1997). Recently, most of the antibiotics have been banned because the feeding of antibiotics is risky (Sorum and Snude, 2011). Essential oils and probiotics claimed to be alternatives to antibiotics have effects on the ecosystem of gastrointestinal microflora either directly or indirectly (Taylor, 2001). It is clear that controlling the microflora could positively influence birds' performance. Thyme essential oil appears to be a high potential tool in

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Table 1. Composition of starter, grower, and finisher wheat-based diets.

Ingredient (%)	Starter (1 to 10 days)	Grower (11 to 24 days)	Finisher (25 to 42 days)
Wheat	57.49	59.94	61.47
Soybean meal, 44% CP	34.05	30.70	29.00
Wheat bran	0.15	0.15	0.15
Vegetable oil	4.00	5.60	6.14
Limestone	1.45	1.25	1.20
Dicalcium phosphate	1.35	1.10	1.00
Salt (NaCl)	0.37	0.36	0.34
DL-Methionine	0.19	0.15	0.11
L-Lysine	0.33	0.20	0.08
Threonine	0.12	0.05	0.01
Vitamin mix[1]	0.25	0.25	0.25
Mineral mix[2]	0.25	0.25	0.25
Calculated nutrients			
ME (kcal/kg)	2850	2970	3020
Crude protein (%)	22.14	20.74	19.82
Calcium (%)	1.00	0.85	0.80
Available phosphorus (%)	0.47	0.42	0.40
Lysine (%)	1.35	1.17	1.03
Methionine (%)	0.48	0.42	0.39
Threonine (%)	0.89	0.78	0.70

[1]Vitamin mix provided the following per kilogram of diet: vitamin A (*trans*-retinyl acetate), 10,000 IU; vitamin D_3 (cholecalciferol), 3500 IU; vitamin E (DL-α-tocopheryl acetate), 60 mg; vitamin K (menadione), 3 mg; thiamine, 3 mg; riboflavin, 6 mg; pyridoxine, 5 mg; vitamin B_{12} (cyanocobalamin), 0.01 mg; niacin, 45 mg; pantothenic acid (D-calcium pantothenate), 11 mg; folic acid, 1 mg; biotin, 0.15 mg; choline chloride, 500 mg; ethoxyquin (antioxidant), 150 mg. [2]Mineral mix provided the following per kilogram of diet: Fe, 60 mg; Mn, 100 mg; Zn, 60 mg; Cu, 10 mg; I, 1 mg; Co, 0.2 mg; Se, 0.15 mg.

combating pathogens and stimulating immunity in poultry that improve performance (Acamovic and Broker, 2005; Akyurek and Yel, 2011).

Great amounts of active components can also be found in essential oils. Once the active components of thyme essential oil (thymol and carvacrol) crosses the bacterial cell wall, it may interact with periplasmic enzymes and, after penetration into the lipid-rich interior of the bacterial cytoplasmic membrane, may interact with membrane proteins and cause a back-flow of protons across the membrane thus affecting the cellular activities powered by the proton motive force (Lambert et al., 2001).

Probiotic is a culture of a single bacteria strain, or mixture of different strains, that can be fed to an animal to improve some aspect of its health and one commonly studied genus is *Lactobacillus* (Ehrmann et al., 2002). Proposed mechanisms for the beneficial effect of probiotic are: (1) to help in maintaining a beneficial microflora in the gastrointestinal tract by inhibiting the growth of pathogenic microorganisms (Jin et al., 1996) and (2) to increase nutrient utilization through improved intestinal health resulting in greater intestinal enzyme activities and nutrient availability (Nahashon et al., 1994). *Lactobacillus*-based probiotic showed an immunoregulatory effect (Dalloul at al., 2003).

Therefore, the aim of this study was to determine the immune response and ileal microflora in broilers fed wheat-based diet with or without enzyme Endofeed W and supplementation of thyme essential oil or probiotic PrimaLac®.

MATERIALS AND METHODS

Birds, diets and management

288 day-old male Ross-308 broilers chicks were randomly housed on floor pens with wood shavings and allocated to six diets in a completely randomized design. The six diets were arranged factorially with two levels of enzyme Endofeed W (0.0 and 0.05%) and two types of growth promoters (unsupplemented or supplemented with thyme essential oil or probiotic PrimaLac®). Each diet was fed to four groups with 12 chicks each. The composition of the starter, grower and finisher basal diets are shown in Table 1 and were calculated to meet the nutrient requirements of Ross-308 (Aviagen, 2007).

Enzyme Endofeed W produced from *Aspergillus niger* fermentation product contains the arabinoxylanase and β-glucanase activity of 2250 and 700 units per gram, respectively as reported by the manufacturer with barley malt sprouts dehydrated as carrier and standardize (Endofeed W, GNC Bioferm Inc.,

Saskatoon, Saskatchewan, Canada). *Zataria*, an important genus of the family *Lamiaceae* (previously called *Labiatae*), is widely distributed in Iran, Afghanistan and Pakistan and *Zataria multiflora* (thyme), an aromatic member of genus *Zataria*; its essential oil was considered in this study. The thyme essential oil was first dissolved in the vegetable oil component of the ration and homogenized by mixer and then the mixture was blended with wheat bran. Wheat bran with essential oil was added to pre-mixture. Finally, the pre-mixture was gently mixed with the basal diet. This experimental diet was prepared weekly and stored in airtight containers. Thyme essential oil was added at the rate of 0.1% to the diet.

PrimaLac® is a commercial microbial culture including a minimum presence of 1.0×10^8 CFU of friendly bacteria *Lactobacillus acidophilus*, *Lactobacillus casei*, *Bifidobacterium thermophilus* and *Enterococcus faecium* per gram (PrimaLac®, Star Labs, St. Joseph, Clarksdale,MO). Unique formulation of PrimaLac® ensures that a large portion of what is ingested actually gets into the bowel or gastrointestinal tract and is not destroyed in the crop or stomach. Therefore, its desired effect is achieved. The probiotic PrimaLac® was added to the starter (0 to 10 days), grower (11 to 24 days) and finisher (25 to 42 days) diets at the rate of 0.09, 0.045 and 0.0225%, respectively.

The temperature was 33°C on arrival of the chicks, was gradually decreased to 21°C after 4 week, and kept constant thereafter. The lighting was 24 h throughout the first week and afterwards the light (L): dark (D) cycle was 23L: 1D. Feed and water were provided *ad libitum*.

Extraction of thyme essential oil and gas chromatography - mass spectrometry (GC-MS) analysis

The fresh plants were collected at the flowering stage and processed immediately after harvest. Essential oil was distilled from the ground plant material using Clevenger distillation apparatus (Herbal Exir Co., Mashhad, Iran). The samples were distilled for 2 h and the oils obtained, were dried with anhydrous sodium sulphate, and stored in dark sealed glass vials at +4°C until required. The main active compounds of the thyme essential oil were determined by GC/MS and it contained thymol and its isomer, carvacrol, at the rate of 21.9 and 31.9% of oil, respectively.

Immune response evaluations

Hypersensitivity

At 9 day of age, a cutaneous basophil hypersensitivity test was administered (Corrier, 1990) to two birds per replicate. Phytohaemagglutinin-P (PHA-P) was injected intradermally [100 µg of PHA-P suspended in 0.1 ml of phosphate buffered saline (PBS) per bird] into the web between the third and fourth toes of the left foot and as a control, an equal volume of PBS was injected into the same toes web of the right foot. The thickness of the two toes webs was measured initially and 24 h afterwards with a pressure-sensitive caliper. The swelling response was measured by subtracting the preinjection measurement (initially) from the postinjection measurement (24 h afterwards) of the PHA-P-injected toe web. Because no change in toe web thickness occurred due to PBS injection, these data did not need to be reported.

Antibody production

Two birds per replicate were selected and were intramuscularly injected with 1 ml/chick sheep red blood cells (SRBC); 15% suspension in PBS at day 28. Sheep red blood cells (SRBC) were used as an antigen to quantify the antibody response. Blood samples were collected at 7 and 14 days after the injection. The serum from each sample was collected, heat inactivated at 56°C for 30 min and then analyzed for total, IgG (mercaptoethanol-resistant) and IgM (mercaptoethanol-sensitive) anti-SRBC antibodies as described by Cheema et al. (2003). The SRBCs used for inoculation and antibody titration were obtained from the same donor sheep.

Haematological analysis

At 40 days of age, two birds per replicate were selected and their blood samples were collected using heparin containing syringes to avoid blood clot formation for haematological analysis. Blood smears were prepared on slides and painted by Gimsa method. 100 leukocytes per sample were counted by heterophil to lymphocyte separation under an optical microscope then heterophil to lymphocyte ratio was calculated and recorded. The white blood cells and red blood cells counts were determined by an improved Neubauer haemocytometer method (Jain, 1986). The haematocrit and haemoglobin values were measured by micro-haematocrit and colorimetry cyanomethaemoglobin methods, respectively (Baker and Silverton, 1985).

Immune organs

At the end of trial, two birds per replicate were selected and euthanized by cervical dislocation, and then thymus (all lobes on the left side of the neck), spleen, and bursa of Fabricius were removed and expressed as g/100 g of body weight.

Ileal microbial population

These birds were also considered for assessing the ileal microbial population. The ileum was assigned from Meckel's diverticulum to ileo-caecal junction. The ileums were excised and contents were collected by gently fingers into tubes. Digesta were pooled with a replicate, put on ice until they were transported to the laboratory for enumeration of microbial population. 1 g of ileal contents was homogenized in 9 ml sterile water. Each sample was serially diluted. Using these diluted subsamples, *Lactobacillus* was enumerated on De Man-Rogosa-Sharpe (MRS) agar and *E. coli* was counted on Mac Conkey (MC) agar after incubation at 37°C in an anaerobic chamber for 48 h and in an aerobic chamber for 24 h, respectively (Guban et al., 2006).

The population of *Bifidobacterium* in ileal samples was determined using the standard laboratory method (Ibrahim and Salameh, 2001). Briefly, ileal samples (10 g) were diluted with 90 ml sterilized 0.1% peptone water and homogenized using Stomacher 400 Lab System 4 (Seward, Norfolk, UK) for 2 min, and 100 ml of appropriate dilution was surface plated onto modified BIM 24 agar (Ibrahim and Salameh, 2001). The level was determined at the serial dilution of 10^{-5}. Plates were incubated at 37°C for at least three days.

The experiment was conducted under the guidelines of the Animal Care Committee of the Ferdowsi University of Mashhad.

Statistical analysis

All data were checked for normality before analysis. The data were subjected to one-way analysis of variance (ANOVA) using SAS (SAS institute, 2000).

The differences between treatment means (mean±SEM) were separated by Tukey's multiple range tests. A value of $P< 0.05$ was considered significant.

Table 2. Hypersensitivity and antibody production in broilers fed wheat-based diet with or without enzyme and supplementation of growth promoters[1].

Treatment	Hypersensitivity (mm), day 10	SRBC injection at 28 day of age					
		7 days after injection			14 days after injection		
		Total anti-SRBC	IgG	IgM	Total anti-SRBC	IgG	IgM
Enzyme Endofeed W (EEW) (%)							
0	0.9[b]	4.65	3.73	0.92	2.64	2.41	0.23
0.05	1.13[a]	4.73	3.75	0.98	2.65	2.35	0.30
SEM	0.080	0.184	0.201	0.238	0.120	0.148	0.111
Growth promoters (GP)							
Unsupplemented	0.94[b]	4.74[b]	3.70[b]	1.04	2.65[b]	2.50[b]	0.15
TEO	1.20[a]	5.31[ab]	4.21[ab]	1.10	2.72[b]	2.61[b]	0.11
PP	1.20[a]	5.70[a]	4.53[a]	1.17	3.28[a]	3.15[a]	0.13
SEM	0.091	0.191	0.243	0.240	0.109	0.215	0.110
P-value							
EEW	0.042	0.389	0.501	0.514	0.421	0.484	0.300
GP	0.044	0.038	0.044	0.291	0.020	0.018	0.322
EEW×GP	0.382	0.462	0.682	0.383	0.289	0.720	0.420

[a,b]Means within the same column with uncommon superscript differ significantly ($P < 0.05$). [1]TEO, thyme essential oil; PP, probiotic Primalac®.

RESULTS AND DISCUSSION

Hypersensitivity

The effect of enzyme Endofeed W (EEW) and growth promoters [thyme essential oil (TEO) or probiotic PrimaLac®(PP)] on hypersensitivity of broilers fed wheat-based diet at 9 day of age is shown in Table 2. Phytohaemagglutinin-P (PHA-P) injection increased ($P< 0.05$) toe web skin thickness within 24 h after injection in all experimental groups including EEW, TEO or PP when compared to the control groups. To our knowledge, this was the first report of these feed additives-mediated increases of hypersensitivity response in broilers. Hypersensitivity response to a mitogen such as PHA-P is one type of cell-mediated immune response in the chicken that involves the nonspecific attraction of basophils to the injection site, with subsequent swelling. Regnier and Kelley (1981) demonstrate that exposure to heat and cold stress reduces *in vitro* and *in vivo* cell-mediated immune responses to PHA-P in chicks. It could be from the present study that inclusion of enzyme or growth promoters to the wheat-based diet enhanced ($P< 0.05$) this type of cell-mediated immune response and lowered stress in broiler chicks.

Antibody production

The effect of enzyme and growth promoters on antibody production against SRBC antigen of broilers fed wheat-based diet at 35 and 42 days of age is shown in Table 2. Data show that antibody production against SRBC antigen was numerically ($P > 0.05$) increased by the addition of EEW or TEO, whereas total and IgG anti-SRBC antibody titers in broilers were altered significantly with administration of PP to the control wheat-based diet. This improvement in humoral response against SRBC was in agreement with other reports. Birds fed *L. acidophilus* and *B. bifidum* have significantly more serum antibody to SRBC (Haghighi et al., 2006).

Khaksefidi and Ghoorchi (2006) indicate that the antibody titer in birds fed 50 mg/kg probiotic supplemented diet is significantly higher at 5 and 10 days of post-immunization in comparison with the control group, when SRBC is injected at 7 and 14 days of age. Kabir et al. (2004) evaluated the dynamics of probiotics on immune response of broilers. They reported a significantly higher antibody production ($P < 0.01$) in experimental birds vs. control ones. Dietary probiotic shows an increased antibody IgA and IgG in broiler chickens (Perdigon et al., 1990). Gao et al. (2007) declare that xylanase supplementation of wheat-based diets for cockerels significantly increase serum antibody titres to NDV, suggesting that enzyme supplementation enhances the humoral response. But in another study, there was no significant effect on immune response (IgG and IgM) with NSP-degrading enzyme supplementation (Basmacioglu et al., 2010).

Haematological analysis

The effect of enzyme and growth promoters on

Table 3. Haematological analysis in broilers fed wheat-based diet with or without enzyme and supplementation of growth promoters[2] at 40 days of age.

Treatment	H/L[1]	WBC[1] (10^6/mm^3)	RBC[1] (10^6/mm^3)	HCT[1] (%)	Hb[1] (g/dl)
Enzyme Endofeed W (EEW) (%)					
0	0.80[a]	23.6	2.17	27	12.20
0.05	0.70[b]	23.8	2.19	28	12.81
SEM	0.021	0.422	0.075	0.910	0.604
Growth promoters (GP)					
Unsupplemented	0.81[a]	24.0[b]	2.16	28	12.60
TEO	0.70[b]	26.9[a]	2.20	29	12.65
PP	0.68[b]	27.5[a]	2.20	30	12.77
SEM	0.021	0.390	0.090	1.211	0.700
P-value					
EEW	0.010	0.516	0.218	0.418	0.329
GP	0.009	0.023	0.110	0.432	0.371
EEW×GP	0.180	0.613	0.309	0.584	0.784

[a,b]Means within the same column with uncommon superscript differ significantly ($P < 0.05$). [1]H/L, heterophil to lymphocyte ratio; WBC, white blood cells count; RBC, red blood cells count; HCT, haematocrit; Hb, haemoglobin. [2]TEO, thyme essential oil; PP, probiotic PrimaLac®.

haematological analysis of broilers fed wheat-based diet at 40 day of age is shown in Table 3. Heterophil to lymphocyte ratio (H/L) was significantly depressed in broilers fed wheat-based diet which contained EEW, TEO or PP. All circulating cells bearing the receptors for stress related hormones could be primary targets of stress. Consequently, H/L has been widely used as a physiological indicator for different forms of stress (Maxwell, 1993) and this reduction of H/L in the present study demonstrated a reduced stress in broilers that beneficially influences the ecosystem of gastrointestinal microflora and enhances the immunity system which justified better the other immune responses and/or improved microbial population in birds which were measured. The use of TEO or PP enhanced ($P< 0.05$) white blood cells count (WBC) in broilers as compared to the control group. However, other measured haematological parameters were not changed in the present study, which could be related to the age of birds, concentration and method of feed additives consumed. Some investigators demonstrate the potential effect of enzyme, phytobiotic and probiotic on haematological analysis of broilers. It is noted that enzyme supplementation of wheat-based diet can increase proliferation of macrophages and monocytes (Jamroz, 2005).

Chicks fed on thyme essential oil had significantly lower heterophil and higher lymphocyte numbers compared to the control diet (Najafi and Torki, 2010). Al-Kassie (2009) shows that feeding diets supplemented with oil extract derived from thyme and cinnamon to broilers significantly increased WBC, red blood cells count (RBC), haematocrit (HCT) and haemoglobin (Hb) values compared to the control group. Shoeib et al. (1997) demonstrated the effect of dietary supplementation of probiotic on immune response in broilers. Hematologic profile enforces an increase in WBC and RBC and marks increase in the percentage of lymphocytes and monocytes.

Immune organs

The effect of enzyme and growth promoters on relative weight of immune organs of broilers fed wheat-based diet at 42 day of age is shown in Table 4. Relative weight of immune organs was not influenced by EEW, TEO or PP. This is in agreement with Teo and Tan (2007), which shows no significant differences in the relative weight of spleen in broilers fed the diet containing probiotic compared to the control group. However, Teo and Tan (2007) observe that birds fed diet supplemented with *B. subtilis* have a significantly heavier bursa and thymus weights compared to the control groups. Willis et al. (2007) reported that feeding broilers with probiotic causes an increase in the relative weight of spleen. Over growth of these lymphoid organs may indicate an infection and may result in more mortality.

Ileal microflora population

The effect of enzyme and growth promoters on ileal

Table 4. Relative weight (g/100 g body weight) of immune organs in broilers fed wheat-based diet with or without enzyme and supplementation of growth promoters[1] at 42 day of age.

Treatment	Spleen	Bursa of Fabricius	Thymus
Enzyme Endofeed W (EEW), %			
0	0.12	0.18	0.33
0.05	0.12	0.18	0.34
SEM	0.016	0.059	0.073
Growth promoters (GP)			
Unsupplemented	0.11	0.19	0.34
TEO	0.13	0.18	0.34
PP	0.13	0.18	0.35
SEM	0.011	0.064	0.086
P-value			
EEW	0.151	0.233	0.840
GP	0.132	0.572	0.816
EEW×GP	0.421	0.631	0.934

[1]TEO, thyme essential oil; PP, probiotic PrimaLac®.

Table 5. Ileal microbial population (log CFU/g of digesta) in broilers fed wheat-based diet with or without enzyme and supplementationof growth promoters[1] at 42 day of age.

Treatment	*Lactobacillus*	*Bifidobacterium*	*E. coli*
Enzyme Endofeed W (EEW) (%)			
0	8.11^{b}	8.15^{b}	7.10^{a}
0.05	8.54^{a}	8.49^{a}	6.72^{b}
SEM	0.210	0.220	0.221
Growth promoters (GP)			
Unsupplemented	8.08^{b}	8.10^{b}	7.12^{a}
TEO	8.58^{a}	8.59^{a}	6.67^{b}
PP	8.63^{a}	8.55^{a}	6.59^{b}
SEM	0.258	0.279	0.287
P-value			
EEW	0.004	0.030	0.001
GP	0.004	0.005	0.009
EEW×GP	0.670	0.769	0.783

[a,b]Means within the same column with uncommon superscript differ significantly ($P < 0.05$). [1]TEO, thyme essential oil; PP, probiotic Primalac®.

microbial population of broilers fed wheat-based diet at 42 day of age is shown in Table 5. All feed additives induced competitive exclusion efficiently, in terms of increase ($P<0.05$) in *Lactobacillus* and *Bifidobacterium* and reduction ($P<0.05$) in *E. coli* counts. Our results are in consistent with the findings of many researchers who account an enhancement in intestinal microbial population of broilers fed antibiotic alternatives. A wheat-based diet supplemented with xylanase significantly increases the number of *Lactobacillus* in jejunum and ileum (Engberg et al., 2004). It is possible that carbohydrates derived from the activity of NSP-degrading enzymes are fermented in the small intestine by *Lactobacillus*. A mixture of thymol and carvacrol increases the population of *Lactobacillus* in ileum (Akyurek and Yel, 2011).

As the pH in the gastrointestinal tract may be decreased by the active components of the thyme essential oil (thymol and carvacrol), thus the components can prevent the growth of pathogenic bacteria and

promote the population of non-pathogenic ones like *Lactobacillus* and *bifidobacterium*. It is shown that phytogenics modulated the intestinal microflora composition via the reduction of coliforms at 14 day of age and the beneficial fortification of gut microflora with purportedly beneficial members such as the *Lactobacillus* and *bifidobacterium* at 42 day of age (Mountzouris et al., 2011).

Jamroz and Kamel (2002) reported that the dietary herbal treatment results in lower *Escherichia coli* counts comparing to the control group. In contrary, thyme powder or essential oil has no effect on the intestinal microflora populations (Cross et al., 2007). The use of PrimaLac® supplementation in poultry diets stabilizes the microflora environment of the avian digestive tract (Jin et al., 1996). It has been demonstrated that probiotics inhibit the *in vitro* growth of many enteric pathogens (Fioramonti et al., 2003). Grimes et al. (2008) reported the significant reduction of intestinal levels of *Salmonella* spp., in turkeys or other livestock by the use of PrimaLac®.

Conclusion

In general, the immune response and ileal microflora were improved in broilers fed wheat-based diet with or without enzyme Endofeed W and supplementation of thyme essential oil or probiotic PrimaLac®. The probiotic PrimaLac® as a growth promoter was more efficient when compared to thyme essential oil.

Addition of EEW, TEO or PP to the wheat-based diet improved ($P< 0.05$) immunity in terms of hypersensitivity response and heterophil to lymphocyte ratio as compared to the control group. Antibody production against SRBC antigen (total and IgG anti-SRBC) altered significantly with administration of PP and white blood cells count was increased ($P< 0.05$) by the inclusion of growth promoters (TEO or PP) to control diet. All feed additives induced competitive exclusion efficiently, in terms of increase ($P< 0.05$) in *Lactobacillus* and *Bifidobacterium* and reduction ($P < 0.05$) in *E. coli* counts.

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