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Exogenous Enzyme Improves Immunocompetence in Laying Hens Fed Diets Containing Safflower Meal

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Abstract

An experiment was conducted to study the effects of different levels of exogenous enzymes on egg quality characteristics and immunological responses of laying hens fed diets containing different levels of safflower meal. The 47-week old layers (Hy-Line W-36) were fed 10 experimental diets consisting five levels of safflower meal (0.0, 2.5, 5.0, 7.5 and 10.0%) and two levels of dietary exogenous enzyme (0.0 and 0.1% Bergazym P®) during the experimentation. The trial period lasted for a total of 10 weeks, and egg quality indices were measured as two 35 day experimental periods. After 48 and 55 days of experiment, birds were injected with 1 mL of 7% Sheep Red Blood Cells (SRBC) intramuscularly. At the end of experiment, the antibody titer against SRBC and differential circulating leukocyte count were measured for investigating immune status. Least squares mean analysis showed that although the inner egg quality characteristics (yolk index and Haugh unit) were not influenced by dietary treatments, using the highest level of safflower meal caused remarkable decrease in eggshell weight and strength during the first 35-day experimental period. Nonetheless, these parameters showed tendency to decrease during the second trial period. The heterophils to lymphocytes ratio were significantly increased after feeding different levels of safflower meal and reach to the highest level in hens fed diet containing 10% of this meal. This response was due to the increase in heterophils population and contemporaneous decrease in lymphocytes percentage. Inclusion of 0.1% dietary enzyme had remarkable modulatory effect on heterophils count and the heterophils to lymphocytes ratio. Following the administration of dietary enzymes, the number of lymphocytes and thereby, the primary humoral immunity response increased significantly, however, this alteration had tendency to increase during the secondary immunity response against SRBS. Therefore, our results indicated that using high level of safflower meal (10%) in laying hens diet could have aversive effects on eggshell quality. Also, administration of dietary exogenous enzyme to the diets of laying hens would have beneficial effects on immunological responses, especially in terms of heterophil to lymphocyte ratio depression or improvement of humoral immunological functions

Key words: safflower meal, exogenous enzyme, laying hens, egg quality, immune responses

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Introduction

Cereals and some oil seed meal are the main constituents of poultry diets (Yoruk et al., 2006). These feedstuffs have some factors called as non starch polysaccharides (NSP's) that can not be digested by poultry due to the resistance to hydrolysis in the digestive tract (Montanhini et al., 2013). NSP's mask protein and carbohydrate and reduce availability of nutrients for digestion and absorption (Yoruk et al., 2006). NSP-rich diets make a shift in microbial populations of intestinal lumen that can damage the mucosa and increase the infiltration of immune cells in the intestinal tract of poultry (Teirlynck et al., 2009). Several studies have shown that some NSP's stimulate the innate immune system and increase the proliferation of macrophages and monocytes at the site, by breaking cells of the intestinal mucosa. The nutritional and metabolic cost to support an immune response in broilers is high and reversely correlated with growth performance (Montanhini et al., 2013). On the other hand, modern broiler chickens have rapid growth rate that is mainly based on a high rate of feed intake and not due to an increased nutrient digestibility. It has been proven that increased body weight gain is negatively correlated with antibody response including total antibody response and specific anti-sheep red blood cell (SRBC) in broilers (Bao and Choct, 2010).

To reduce the mentioned adverse effects of NSP's and rapid growth rate on poultry, enzymes have been used widely in the last years. Using enzymes as a feed additive has become common since last four decades (Yoruk et al., 2006). Enzymes are supplemented to improve nutritive value by different mechanisms such as hydrolyzing the NSP's; for example some recent evidence has shown that exogenous xylanase majorly degrades NSP to oligosaccharides and release more phenolics to act as anti-oxidants (Yoruk et al., 2006; Bao and Choct, 2010). These enzymes caused to improve overall energy utilization and elimination of the anti-nutritive properties of certain NSP's by their enzymatic hydrolysis to prebiotic type components which may facilitate gut development and health in young chickens (Slominski, 2011).

Jackson et al. (2003) reported that using β-mananase in chickens infected with *Eimeria sp*. and *Clostridium perfringens* resulted in reduced severity of the challenge generated by these microorganisms. This effect was observed as increased body weight as well as reduction of intestinal lesions. Zou et al. (2006) used 1-day-old broilers to investigate the effects of β-mannanase (Hemicell) on growth performance and immunity. The chicks received the same basal diet based on corn-soybean meal and Hemicell was added to the basal diet at 0.00, 0.025, 0.05, and 0.075%, respectively. They indicated that Hemicell may improve growth performance and immunity of broilers based on increased serum IgM concentration, higher proliferation of T lymphocytes, and improved the relative weights of spleen and bursa compared with the control group. One possible reason why β-mannanase might improve immunity is that β-mannan is degraded to mannan oligosaccharide that could influence the immune system. Some previous studies reported that mannan oligosaccharide significantly increased maternal antibody levels of broilers. Furthermore, mannan oligosaccharide may also improve the intestinal absorption of some nutrients such as Zn, Cu, and Se (Zou et al., 2006). On the other hand, Mushtaq et al. (2007) added glucanase and xylanase in diets based on canola

meal, and did not record any benefits in terms of performance, nutrient digestibility, and immune response or carcass characteristics of broilers.

Additionally, Soltan (2009) investigated the effects of replacing soybean meal by palm kernel cake in broiler chicks' diets on an ideal protein basis without or with enzyme supplementation. According to his results, enzyme supplementation improved phagocytic activity, dressing percentage and immune organs relative weights (spleen, bursa and thymus gland) when compared with group fed on the same diets without enzyme supplementation. Also, Hassan Khan et al. (2011) concluded that the supplementation of enzyme and probiotic in layer diets did not appear to cause any adverse effects on egg production, egg quality and immunity compared with the negative control laying hens. However, Akbari Gharaei et al. (2012) indicated that 0.05% β-mannanase could improve humoral immunity responses against Infectious Bronchitis Virus (IBV) and SRBC in broiler chicks fed diets containing different levels of guar meal.

The objective of the present study is to evaluate the effects of adding an exogenous multi-enzyme complex (Bergazym P®) on egg quality traits and immune responses of laying hens fed diets containing safflower meal.

Materials and Methods

Birds, diets, and experimental design

In the present experiment, two hundred and fifty 47-week old white leghorn hens (Hy-Line, W-36) were randomly assigned to a 5 × 2 factorial arrangement of treatments based on a completely randomized design that consisted of 10 dietary treatments with 5 replicates of 5 birds each. Experimental diets were included five levels of safflower meal (0.0%, 2.5%, 5.0%, 7.5%, and 10%) and two levels of multi-enzyme (0.0% and 0.1% Bergazym P®). Bergazym P® **(**Berg + Schmidt (GmbH & Co.) KG. Ander Alster 81, Hamburg**,** Germany) is a multi-enzyme consisting of xylanase, β-glucanase, α-amylase and protease. Treatments were a sample of the population to which we could make inferences. Every experimental unit had the same probability of receiving any treatment. Randomization was performed using a random number table. Hens had free access to water and to the experimental diets during 10-week experimental period and were housed in the environmentally controlled cage system (45 × 50 cm) with 16-hours light to 8-hours dark lighting program. All treatment groups received the normal isocaloric and isonitrogenous diets formulated to meet or exceed the nutrient requirements of laying hens as recommended by Hy-Line W-36 manual (Table1).

Qualitative characteristics of egg

During the present study, laid eggs were collected daily (at 08:00) and immediately weighed. Three eggs (15 eggs per each treatment group) were collected from each replicate at days 35 and 70, and egg quality indices including egg shape index, yolk index, Haugh unit, eggshell weight, eggshell thickness and eggshell strength were measured. The yolk and egg shape indices were calculated by yolk height/yolk diameter and

Table 1. The composition and nutrient content of experimental diets (47 to 56 weeks of age)

Item	Dietary treatments[1]				
	A	B	C	D	E
Ingredient (%)					
Soybean meal	21.1	20.2	19.3	18.4	17.5
Soybean oil	2.50	2.50	2.50	2.50	2.50
Fat powder	2.50	2.50	2.50	2.50	2.50
Safflower meal	0.00	2.50	5.00	7.50	10.0
Wheat bran	5.36	4.04	2.72	1.31	0.00
Chip wood	2.00	1.50	1.00	0.50	0.00
Oyster shell	5.00	5.00	5.00	5.00	5.00
Monocalcium phosphate	1.50	1.49	1.48	1.46	1.45
Limestone	5.52	5.51	5.51	5.50	5.50
Common salt	0.22	0.22	0.22	0.21	0.21
L-Lysine HCL	0.03	0.04	0.05	0.06	0.07
DL- Methionine	0.17	0.17	0.16	0.16	0.15
Na bicarbonate	0.70	0.73	0.76	0.80	0.82
Mineral premix[2]	0.25	0.25	0.25	0.25	0.25
Vitaminepremix[3]	0.25	0.25	0.25	0.25	0.25
Crude fat (%)	7.34	7.34	7.33	7.33	7.32
Crude fiber (%)	5.24	5.28	5.32	5.36	5.41
Crude protein (%)	14.6	14.6	14.6	14.6	14.6
Lysine (%)	0.75	0.75	0.75	0.75	0.75
Methionine (%)	0.42	0.42	0.41	0.41	0.41
Methionine + cysteine (%)	0.65	0.65	0.65	0.65	0.65
Calcium (%)	4.17	4.17	4.17	4.17	4.17
Available phosphorus (%)	0.44	0.44	0.44	0.44	0.44
Na (%)	0.30	0.31	0.32	0.33	0.33
Electrolyte balance (mEq/Kg)	249.8	250.0	250.0	249.9	250.0

[1]A: control group, B: 2.5% safflower meal treatment, C: 5% safflower meal treatment, D: 7.5% safflower meal treatment, E: 10% safflower meal treatment.

[2]Mineral premix provided per kilogram of diet: manganese, 80 mg; copper, 10 mg; iodine [from Ca (IO3)2·H2O], 0.8 mg; cobalt, 0.25 mg; selenium, 0.3 mg; zinc, 80 mg; iron, 80 mg.

[3]Vitamin premix provided per kilogram of diet: vitamin A (from vitamin A acetate), 10000 IU; vitamin D3, 2500 IU; vitamin E (from dl-α-tocopheryl acetate), 10 IU; vitamin B_1, 2.2 mg; vitamin B_2, 4 mg; pantothenic acid, 8 mg; vitamin B_6, 2 mg; niacin, 30 mg; vitamin B_{12}, .015 mg; folic acid, 0.5 mg; biotin, 0.15 mg; choline chloride,200 mg.

egg width/egg length formulas, respectively. The following formula was used to calculate Haugh unit: $HU= 100 \log (H + 7.57 – 1.7 W^{0.37})$.

Blood differential leukocyte count

For analysis of blood variables, two birds per replicate were selected at the end of the experimental period and blood collection was performed from brachial vein. Blood samples were transferred immediately to tubes containing EDTA. Differential counts of leukocytes were made by screening a Giemsa-stained slide. The different subpopulations of leukocytes were counted and the heterophil: lymphocyte ratio was calculated as described by Stedman et al. (2001).

Humoral immune response

Sheep red blood cells were washed three times in phosphate buffered saline (PBS) and diluted in PBS to a final dilution rate of 7% (vol/vol). At days 48 and 58 of trial, two hens per replicate were injected with 1.0 mL of 7% SRBC suspension intramuscularly (thigh muscle). Heparinized blood samples were collected at days 6 and 12 after first and second immunizations, respectively. Plasma samples were stored at −20 °C until

further analysis. The hemagglutination assay was performed as described by Leshchinsky and Klasing (2001). Each well of a 96-well plate received 0.05 mL of diluents buffer containing PBS. The initial well received 0.05 mL of plasma sample which was serially doubly diluted by transferring 0.05 mL to the next wells. Then, 0.05 mL of 2% SRBC suspension in PBS was added to each well. The plates were shaken for 1 min, incubated for 1 h at room temperature, and then scored. The agglutination titer was expressed as the $\log_2$ of the highest titer with 50% agglutination.

Statistical analyses

All data were analyzed for variance (ANOVA) using the Generalized Linear Model procedure (PROC GLM) of SAS software (SAS Institute, 2001). In the present study, a threshold of significance was set at $P < 0.05$ and trends were declared at $0.05 < P < 0.10$.

Results and Discussion

Qualitative characteristics of egg

The effects of different levels of safflower meal and dietary enzyme on egg qualitative characteristics in the middle and the end of experimental period have been shown in Tables 2 and 3, respectively. Supplementation of diets with at least 5% of safflower meal for 70 days tended to decrease egg weight slightly ($P = 0.08$). Reinforced the results of Yu et al. (2007), using dietary exogenous enzymes had no significant effect on this parameter during experimental weeks; however, Jackson et al. (2003) reported that feeding dietary enzyme increased egg weight in low-energy diets of laying hens. Although, the inner egg quality characteristics were not influenced by dietary treatments, using the highest level of safflower meal caused remarkable decrease in eggshell weight and strength during the first 35-d experimental period ($P < 0.05$). Nonetheless, these parameters showed tendency to decrease during the second trial period ($P = 0.09$ and $P = 0.07$, respectively). It is probable that the present of higher content of NSPs and their cage effects on vital ions such as calcium or phosphorous decreased the bioavailability of these ions during the eggshell configuration. The present results are consistent with the findings of Nadeem et al. (2005).

Differential circulating leukocyte count

The effects of different levels of safflower meal and multi-enzyme on differential leukocyte counts and also primary and secondary humoral immune responses against SRBC have been shown in Table 4. The heterophils to lymphocytes ratio were significantly ($P < 0.05$) increased after feeding different levels of safflower meal and reached to the highest level in hens fed diet containing 10% of this meal. This response was due to the increase ($P < 0.05$) in heterophils population and contemporaneous decrease ($P < 0.01$) in lymphocytes percentage. Although, the numbers of monocytes and eosinophils were not affected by different levels of safflower meal, the birds fed 7.5% and 10% of this meal had lower level of basophils compared with another groups ($P < 0.05$). It has been shown that the avian heterophils, as the predominant phagocyte

Table 2. Effects of different levels of safflower meal and multi-enzyme on egg quality traits of laying hen during the first 35-d experimental period

		Shape index	Yolk index	Haugh	Egg weight	Eggshell	Eggshell	Eggshell
Safflower meal								
0.0		73.2	0.43	75.8	64.2	9.66[a]	38.0	2.56[a]
2.5		74.4	0.43	72.6	63.9	9.71[a]	38.5	2.67[a]
5.0		73.8	0.42	71.1	63.2	9.26[ab]	38.0	2.38[a]
7.5		73.3	0.41	73.8	62.4	9.38[a]	36.0	2.44[a]
10.0		74.6	0.42	73.7	62.7	8.90[b]	38.5	2.06[b]
SE		0.77	0.01	1.41	0.55	0.19	2.67	0.13
0.0		73.9	0.43	74.1	63.4	9.37	37.5	2.44
0.1		73.8	0.43	72.7	63.2	9.39	38.1	2.40
SE		0.47	0.01	0.98	0.40	0.13	0.49	0.08
0.0	0.0	43.1	0.42	75.0	65.0	9.80	37.6	2.50
0.0	0.1	73.3	0.42	76.5	63.5	9.51	38.5	2.62
2.5	0.0	74.6	0.43	73.5	63.7	9.64	38.1	2.60
2.5	0.1	74.2	0.42	71.8	64.1	9.79	38.8	2.74
5.0	0.0	73.5	0.41	71.5	63.5	9.10	37.7	2.35
5.0	0.1	74.1	0.43	70.7	63.0	9.42	38.3	2.40
7.5	0.0	73.8	0.43	76.8	62.1	9.46	34.4	2.74
7.5	0.1	72.9	0.42	70.8	62.8	9.30	37.5	2.13
10.0	0.0	74.8	0.42	73.7	62.8	8.87	39.4	2.01
10.0	0.1	74.5	0.41	73.6	62.7	8.94	37.5	2.11
SE		1.10	0.01	1.89	0.76	0.27	0.97	0.17
		P-value						
Safflower meal		0.68	0.30	0.34	0.14	0.04	0.29	0.03
Multi-enzyme		0.84	0.17	0.33	0.72	0.82	0.42	0.83
Interaction		0.97	0.10	0.54	0.65	0.83	0.43	0.09

[a,b]Means with no common superscript within each column are significantly different (*P<0.05*).

Table 3. Effects of different levels of safflower meal and multi-enzyme on egg quality traits of laying hen during the second 35-d experimental period

		Shape index	Yolk index	Haugh	Egg weight	Eggshell	Eggshell	Eggshell
Safflower meal								
0.0		71.8	0.43	73.7	65.9	9.61	37.4	2.79
2.5		72.0	0.43	71.4	66.0	9.37	37.8	2.91
5.0		72.7	0.43	73.7	64.6	9.53	37.6	2.78
7.5		71.4	0.42	70.8	64.1	9.31	35.5	2.76
10.0		72.3	0.39	75.1	64.7	9.08	37.9	2.14
SE		0.48	0.01	0.12	0.60	0.21	2.50	0.11
0.0		72.3	0.42	73.4	65.2	9.41	36.9	2.68
0.1		71.8	0.42	72.4	65.0	9.36	37.6	2.71
SE		0.31	0.01	0.66	0.42	0.14	0.42	0.07
0.0	0.0	71.8	0.44	73.1	66.7	10.0	37.0	2.76
0.0	0.1	71.8	0.43	74.3	65.2	9.18	37.9	2.82
2.5	0.0	72.2	0.44	71.1	65.9	9.29	37.5	2.99
2.5	0.1	71.8	0.43	71.8	66.2	9.45	38.1	2.83
5.0	0.0	72.6	0.43	73.1	65.0	9.28	37.2	2.68
5.0	0.1	72.8	0.42	74.3	64.2	9.79	38.0	2.88
7.5	0.0	71.3	0.42	72.5	63.3	9.19	33.9	2.89
7.5	0.1	71.5	0.42	69.2	64.9	9.42	37.1	2.64
10.0	0.0	73.7	0.38	77.5	65.1	9.23	38.8	2.11
10.0	0.1	70.9	0.41	72.6	64.4	8.94	37.0	2.17
SE		0.66	0.01	1.54	0.83	0.11	0.91	0.15
		P-value						
Safflower meal		0.40	0.09	0.13	0.08	0.09	0.25	0.07
Multi-enzyme		0.23	0.92	0.40	0.62	0.80	0.41	0.73
Interaction		0.17	0.64	0.30	0.31	0.23	0.41	0.64

polymorphonuclear leukocyte, form the first line of avian cellular defense against inflammatory substances such as pathogenic bacteria or some anti nutritional factors. (Mellata et al., 2003; Bimczok and Rothkötter, 2006; Teirlynck et al., 2009). Heterophils activity would restrict bacterial proliferation to a level permitting more efficient elimination of bacteria by the subsequent host defenses (Mellata et al., 2003).

T**able 4.** Effects of different levels of safflower meal and exogenous enzyme on differential circulating leukocyte count (%) and antibody titers against Sheep Red Blood Cells (SRBC)

		Basophils	Eosinophils	Monocytes	Heterophils	Lymphocytes	Heterophils/ Lymphocytes	Anti-SRBC	
								Primary	Secondar
Safflower									
0.0		0.25^{ab}	0.85	4.30	37.6^{b}	57.0^{a}	0.66^{b}	6.80	6.45
2.5		0.55^{a}	0.70	4.45	41.1^{ab}	53.1^{b}	0.77^{ab}	6.75	6.20
5.0		0.40^{ab}	0.85	4.65	42.7^{a}	51.3^{b}	0.83^{a}	6.50	6.35
7.5		0.05^{b}	0.90	4.25	42.5^{ab}	52.3^{b}	0.81^{a}	6.00	5.60
10.0		0.10^{b}	0.80	4.30	44.9^{a}	49.9^{bc}	0.90^{a}	5.95	5.70
SE		0.11	0.11	0.22	2.09	2.22	0.94	0.41	0.30
0.0		0.34	0.88	4.36	44.0^{a}	50.4^{b}	0.87^{a}	5.66^{a}	5.34
0.1		0.40	0.76	4.38	39.5^{b}	55.0^{a}	0.71^{b}	7.14^{b}	6.78
SE		0.08	0.07	0.13	1.30	1.41	0.92	0.26	0.40
0.0	0.0	0.30	0.70	4.20	42.1	52.6	0.80	6.00	5.20
0.0	0.1	0.20	1.00	4.30	33.1	61.4	0.53	7.60	7.70
2.5	0.0	0.60	0.80	4.20	45.5	50.1	0.90	5.70	5.30
2.5	0.1	0.50	0.60	4.70	37.8	57.2	0.66	7.80	7.10
5.0	0.0	0.60	1.00	4.80	43.7	50.1	0.87	6.10	5.90
5.0	0.1	0.20	0.70	4.10	41.8	52.6	0.79	6.90	6.80
7.5	0.0	0.10	1.00	4.20	43.0	51.6	0.83	5.20	5.20
7.5	0.1	0.00	0.80	4.10	42.0	53.0	0.79	6.80	6.00
10.0	0.0	0.10	0.90	4.40	45.8	47.6	0.96	5.30	5.10
10.0	0.1	0.10	0.70	4.20	42.6	50.8	0.83	6.60	6.30
SE		0.17	0.18	0.30	2.66	2.88	0.92	0.55	0.41
					P-value				
Safflower		0.03	0.76	0.86	0.02	0.04	0.03	0.38	0.17
Multi-		0.20	0.23	0.70	0.02	0.03	0.02	0.04	0.09
Interactio		0.81	0.34	0.47	0.57	0.73	0.46	0.31	0.26

[a-c]Means with no common superscript within each column are significantly different ($P < 0.05$).

Inclusion of 0.1% multi-enzyme had remarkable modulatory effect on heterophils count and the heterophils to lymphocytes ratio. Dietary multi-enzyme supplementation was not observed to have any significant effect on the enumeration of basophils, monocytes and/or eosinophils. Heterophils to lymphocytes ratio may be a good quantitative indicator for stress, and intestinal inflammatory factors such as bacterial lipopolysaccharide or anti nutritional agents could increase this proportion (Shini et al., 2008; Teirlynck et al., 2009). In the presence of 0.1% dietary enzyme, the ratio of heterophils to lymphocytes was significantly decreased ($P < 0.01$). We postulate two mechanisms for this depression. Firstly, the depression of intestinal microflora after feeding multi-enzyme might reduce the pathogen stimulatory effects on the leukocytes migration and heterophils proliferation. Because the previous reports indicated that using dietary enzymes could have beneficial effects on intestinal pathogenic bacteria; for example, Jackson et al. (2003) reported that using β-mananase in chickens infected with *Eimeria* sp. and *Clostridium perfringens* resulted in reduced severity of the challenge generated by these microorganisms. Otherwise, it is probable that the concentrations of intestinal secretory IgA and thereby serum IgA have been decreased. Because, Zou et al. (2006) indicated that dietary enzyme supplementation may improve growth performance and immunity of broilers based on increased serum immunoglobulins concentration, as well as higher proliferation of T lymphocytes. It has been demonstrated that although secretory IgA exerts the first line of defense by limiting invasion of pathogens, serum IgA may act as a second defensive line (Otten and van Egmond, 2004). In contrast to secretory IgA, serum IgA has been defined as an inflammatory immunoglobulin that induces local inflammatory reactions via the activation of complement system. These reactions include proliferation and influx of polymorphonuclear leukocytes and the release of cytokines (Snoeck et al., 2006).

Humoral immune responses

As shown in Table 4, using different levels of safflower meal did not have significant effects on primary and secondary humoral responses against SRBC. Al-Maya (2006) have pointed out that alternations of some nutrients including amino acids caused immunoregulatory effects because of involving the nutrient or its products in relationship within and between leukocytes. It seems that the improvement of nutrient digestibility after administrating of dietary multi-enzyme affect on differential leukocyte counts, resulting in optimal antibody production. Increased number of lymphocytes as antibody producers (B type lymphocyte) is closely related to serum antibody concentrations.

In the present study, following the increase in the number of lymphocytes, the primary humoral immunity response increased significantly ($P < 0.05$); however, this alteration showed a tendency to increase during the secondary immunity response against SRBS ($P = 0.09$). Increased number of lymphocytes in the multi-enzyme groups could be due to the greater availability of energy, amino acids and other nutrients that occurs by these enzymes. This causes effective reaction of the host when involved in pathogens. In agreement with our findings, Akbari Gharaei et al., (2012) indicated that using 0.05% β-mannanase may improve antibody titers against IBV and SRBC in broilers fed different levels of guar meal. Taken together, using multi-enzymes may improve humoral immune responses of laying hens.

Conclusion

In conclusion, our results indicate that using high level of safflower meal (10%) in laying hens diet may have adverse effects on eggshell quality. Also, administration of exogenous enzymes to the diets of laying hens may have beneficial effects on immunological responses, especially in terms of heterophil to lymphocyte ratio depression and/or improvement of humoral immunity functions.

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