Plant Archives Vol. 20 No. 1, 2020 pp. 1773-1781 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

EFFECT OF FEED SUPPLEMENTATION OF PROBIOTIC AND DIGESTIVE ENZYMES IN PRODUCTION PERFORMANCE AND INTESTINAL BIOMETRICS OF LAYING HENS

Mahdi Salih Jasim and Ghassan Yacoub Fadel*

*Department of Animal Production College of Agriculture - University of Diyala, Iraq.

Abstract

This study aimed to determine the effect of feed supplementation of the probiotic, digestive enzymes and their combination on productive performance, biometrics of small intestine and humoral immunity of laying hens. It was using 144 laying hens 23 week-old Lohmann Brown, be distributed in 12 ground pens, 12 hens/pen (Rep.), were randomly divided into four treatments (3 Rep./ treat.). Hens in the first treatment were fed a standard layer diet (control), hens in second treatment were fed a standard layer diet was added with 0.5g/kg probiotic, hens in third treatment were fed a standard layer diet was added with 0.75 g/kg digestive enzymes, hens in forth treatment were fed a standard layer diet was added with combination 0.5 g/kg probiotic and 0.75 g/kg digestive enzymes. The results showed a significant ($P \leq 0.05$) improved in egg production, egg weight, egg mass, accumulative egg production and feed conversion ratio of supplementation treatments of the probiotic, digestive enzymes and their combination, In addition, probiotic treatment and combination treatment led to a significant increase in weight, length, villi length and crypts depth of small intestine, as well as humoral immunity. The results suggested that adding the probiotic or digestive enzymes improved the productive performance of laying hens, the probiotic was the best in the results, while the combination of the probiotic and digestive enzymes did not have improved results compared to the probiotic.

Key words: Probiotic, Digestive enzymes, Production performance**,** laying hens.

Introduction

The poultry industry has played an important role in meeting the increasing global demand for animal protein resulting from the large population increase, due to the poultry growth and high efficiency of feed conversion and the possibility of breeding large numbers of them in relatively small areas with short duration of breeding, Therefore, this industry has experienced a great development in recent years and in all areas of poultry science, especially the production of modern breeds of Broiler Chickens elected on the basis of the Fast growth and from laying hens on the basis of egg production, As well as going towards the use of biological food supplementation of vitamins and minerals and organic acids and digestive enzymes and Probiotics to face the challenge of low immunity in these breeds and increase their nutritional requirements (Havenstein *et al.,* 2003; Bagal *et al.,* 2016; Moftakharzadeh *et al.,* 2017; Guo *et al.,* 2018). Probiotic is one of the most important biological supplementations for poultry, which consists of a group of bacteria Beneficial for the host, and its use in Poultry improves the balance of gut microbiota (Tiwari *et al.,* 2012; Wan *et al.,* 2016), It leads to increased production of vitamins K and B, volatile fatty acids and the activity of digestive enzymes in the intestinal cavity which improves intestinal cell nutrition, the shape and structural characteristics of intestinal tissue, and energize the tissue immune which Spread on the inner surface of the intestine, as well as improve digestion and absorption (Mazhari *et al.,* 2016; Sikandar *et al.,* 2017). Also digestive enzymes used as biological feed supplementation in poultry diets to help in digestion, due to the limitation of digestive channel in the excretion of these enzymes, especially cellulose (β- glucanase, Xylanase) digestion enzymes, Because these enzymes are characterized by traits that important in improving the health and environment of the intestine and the ability to break down the complex cellular structure of the feed material through which nutrients are released and increased its availability (Khattak *et al.*, 2006; Abd El-Hack *et al.*, 2018), Also that plant sources, which constitute the majority of poultry diets, contain 10% non-starch polysaccharides (NSP) Represented by cellulose fibers, hemicellulose and pectin, which poultry cannot digest and benefit from (Choct and Hughes, 2000), Adding these enzymes to their diets will reduce the loss percentage of the components, And what it carries with it from nutrients with the fecal, and prevent the viscosity in the intestines and reduce the moisture of the floor, Thus reducing the ammonia formed in the poultry farm, this leads in improving the health and production of birds (Bao *et al.*, 2013; Alagawany *et al.*, 2018). Based on the above, this study aimed to add Probiotic, digestive enzymes and their combination to the diet of laying hens to know the effect of each of these additives on productive performance and biological traits of the small intestine and immune response then compare the effect of these three additives.

Materials and methods

Birds management and experiment treatments

One hundred and forty-four Lohmann brown chickens aged 23 weeks, was used, they were obtained from a local company in the region. Be distributed in 12 ground pens (1. 5 × 2 m/pen), 12 hens/pen (Rep.), every pen equipped with an automatic plastic hanging water, plastic cylindrical hanging water, and two eggs nests. The chicken was randomly distributed among four treatments with three pens (rep.) per treatment, and the treatments were as follows:

- T1: were fed on a standard diet without additives (control).
- T2: were fed on a standard diet with 0.5 g/kg of probiotic.
- T3: were fed on a standard diet with 0.75 g/kg of digestive enzymes.
- T4: were fed on a standard diet with a combination of 0.5 g/ kg probiotic and 0.75 g/ kg of digestive enzymes.

Probiotic and the digestive enzyme mixture were added to the experimental poultry diet as recommended by the manufacturer. Probiotic was obtained as Poultry Star® me, from the Australian company BIOMIN GmbH, prepared from the following bacteria: *Enterococcus* sp., *Bifidobacterium* sp., *Pediococcus* sp. and *Lactobacillus* spp. 2 × 1011 CFU/g (Colony Forming Unit per gram) each in the product. While the mixture of digestive enzymes was obtained as KEZYME®, from the American company KEMIN®, containing the following enzymes:

- Endo-1, 4-β-Xylanase IUB 3.2.1.8. - 20000 U/g.
- α- amylase IUB 3.2.1.1. -400 U/g.
- Endo- 1, 3(4)-β-glucanase IUB 3.2.1.6.-2350 U/g.
- Endo- 1, 4-β-glucanase IUB 3.2.1.4. -4000 U/g.
- Bacilloysin IUB 3.4.24.28. -450 U/g.

The birds provided with 14 hours of light every day, and with proper ventilation and heat, and were fed on a standard diet as crushed fodder (Mash) and by a limited amount of 115 g/bird/day, as recommended by the management manual of Lohmann Company (Lohmann tierzucht, 2015) Table 1 shows the components of the standard diet system used in the experiment and their chemical analysis.

Productive traits

The duration of the 84-day trial was divided into three equal periods of 28 days/duration. To calculate the productive traits for each period, the egg production rate was calculated based on Hen Day Product (Fayad and Naji, 1989), according to the following equation:

$$\%H.D = \frac{X}{Y \times Z} \times 100$$

Where:

X = Number of eggs produced during the specified period (28 days).

Y = The period in days.

Z = The number of chickens at the end of the period.

The weight of the eggs produced was recorded at the end of each period of the experiment for three consecutive days collectively for each Rep. and extracted the average weight of eggs, using a sensitive electronic balance that can read to the nearest two decimals, After extraction of eggs production rate and eggs weight, the mass of produced eggs was calculated (Fayad and Naji, 1989), according to the following equation:

Egg mass (g /chicken/day) = egg production rate during the period (H.D %) X average weight of eggs during the period (g).

And to calculate the cumulative number of eggs (Naji *et al.*, 2007), we can use the following equation:

Cumulative number of eggs (egg / chicken / 28 days) = egg production rate during the period X period in days.

The feed conversion ratio was calculated at the end of each period to produce one gram of eggs (Ibrahim, 2000), according to the following equation:

$$FCR = \frac{Daily\ feed\ consumption\ ration\left(\frac{g}{bird}\right)}{Daily\ egg\ mass\ ration\left(\frac{g}{bird}\right)} \times 100$$

Intestinal biometrics

After four hours of not feeding the birds, 16 chickens were slaughtered randomly and four chickens from each treatment at the age of 28 and 34 weeks (middle and end of the experiment) and after the extraction of the intestines from the birds and weighed the small intestine by a sensitive balance read to the nearest two decimals, Then measuring their length by metric tape, as well as a histological examination was performed to 2 cm sample of this intestine and washed with brine, The samples were placed in the neutral formalin diluted to 10% concentration, The preparation of the tissue slides was carried out depending on the Bancroft and Steven (1982) method, All prepared slides were examined using an optical microscope at a magnification of 40 ×. Measurements were recorded using the exact scale of the Ocular Micrometer, after calibrated it to the exact scale micrometer stage then identify the villi length and the crypts depth Note that the length of villi extends from the top of the villi until its linked to the crypt, and the depth of the crypt is the distance from the base of the villi to the end of the crypt (Gao *et al.,* 2008).

Table 1: Ingredients and chemical composition of feed used in chicken nutrition.

Components	%
yellow corn	52
Soybean Meal *	22
wheat	15
Premixes **	2.5
Limestone	8
Calcium diphosphate	0.5
Total	100
Calculated chemical composition***	
Crude protein,%	16
ME Kcal / kg	1726
Methionine%	0.39
Lysine %	0.82
Calcium %	3.59
Available phosphorus %	0.48

*Argentine soybean meal contains 44% raw protein and 2230 kcal/kg of metabolizable energy ** PREMIX INTRACO produced by INTRACO company contains 6.80% raw protein, lysine 1.95%,methionine 5.60%, methionine and cysteine 5.60%, calcium 20.20, available phosphorus 9.50% and metabolizable energy 1000 kcal/kg *** the chemical composition of the diets according to the analysis of food in the reports of the US National Research Council (NRC, 1994).

Humoral immunity

Blood samples were collected directly from the jugular vein of each bird slaughtered in the middle and end of the experiment, Using a blood collection tubes without anticoagulant placed in the centrifuge at 3000 r / min for 15 min to separate the serum, and measure the volumetric standard of antibodies against Newcastle disease using an enzyme-linked immunosorbent assay (ELISA) technique by using the Standard kit from the US company SYNBIOTICS.

Statistical analysis

Statistical analysis was performed using complete random design (CRD) in data analysis of treatments for each production period in the experiment, the randomized complete block design (RCBD) was used to analyze data for the treatments at the end of the experiment to eliminate the effect of the duration of the experiment, to test the significance of differences between treatments means, the Duncan test was used (Duncan, 1955) at ($P<0.05$), by using a statistical analysis program SAS (2001) to analyze the data.

Results and Discussion

Productive traits

It is noticed from the results in Table 2 that there is significant effect of treatments means on the ratio of egg production at all periods of the experiment, and for the effect of the experimental treatments on the overall rate of egg production during the whole experiment period (23-34 weeks) there is a significant means effect of the experimental treatments In egg production ratios, it improved significantly ($P \leq 0.05$) in all additive treatments compared to the control treatment, Probiotic treatment, and combination treatment recorded the highest values of production ratios compared to the control treatment. Table 3 shows that there were no significant differences ($P \leq 0.05$) between the experimental treatments in the of eggs weight ratio during the first production period (23-26 weeks), but during the second production period (27-30 weeks) and the third (30-34 weeks) there was a significant mean ($P \leq 0.05$) for all additive treatments compared to the control treatment. The probiotic, the enzymes and the combination treatment significantly improved ($P \leq 0.05$) in the egg weight rate compared to the control treatment. The results in Table 4 show that there were significant differences ($P \leq 0.05$) between the produced egg mass rates (g/hen/day) for the experimental treatments during all productive periods. From the same table, there were also significant differences between the experimental treatments in the overall rate of egg mass Probiotic treatment and combination treatment recorded the highest values in the overall rate of egg mass compared with the control treatment. The results of Table 5 show a significantly increased (P ≤ 0.05) for the additive treatments in the cumulative number of eggs compared to the control treatment during the first productive period (23-26 weeks) and the second (27-30 weeks), while there was no significant difference in the number of cumulative eggs produced among the three additive treatments in the two periods, while during the third production period (31-34 weeks), the performance was similar between the probiotic treatment and the digestive enzymes and these treatments differs from the combination treatment the , for the overall rate of the cumulative number of eggs at complete experiment period (23 -34 week) notes from the same table that the three additive treatments were significantly increased compared to the control treatment, and there is no significant difference between the probiotic treatment and the combination treatment. It is noticed from the results in Table 6 that there is a significant effect of the additive treatments, either individually or in combination in the feed conversion ratio compared to the control treatment and at all productive periods in the experiment, from the same table, the complete period of the experiment (23-34 weeks) showed significant increase in the overall feed conversion ratio of the birds fed on the probiotic or digestive enzymes diets or their combination compared to the birds fed on the diets without addition. The results of the productive traits in this study showed that the probiotic treatment or digestive enzymes treatment or their combination in the diet had a significant effect on the improvement of these traits, especially the probiotic treatment and the combination treatment, which did not differ significantly in effect between each other but were significantly increased in improving most productive traits compared to digestive enzymes treatment. The significant increase of the probiotic treatment is due to its role in improving the internal environment of the digestive system by increasing the beneficial bacteria that already present in the gut microbiota and maintaining the small intestine of harmful bacteria by competitive exclusion through the sticking of beneficial bacteria in the lining gut tissues, and block receptors in them to inhibit pathogens from sticking to the intestinal tissue and displace them out of

Table 2: Effect of the probiotic and enzymes in the diet on egg production (H.D %) (Mean ± standard error) during productive periods (23-34 weeks) from the life of the laying hens.

Treatments			Productivity durations/age per week			Overall Rate
Treatment	Level of addition(g / kg)		1	2	3	
	Probiotic	Enzymes	(23-26)	(27-30)	(31-34)	(23-34)
T1Treatment of control	0	0	83.93b±0.30	89.15b±1.54	90.57c±0.34	87.88c ±2.02
T2	0.5	0	89.78a±1.38	93.29a±0.44	93.64b±0.10	92.24a ± 1.23
T3	0	0.75	87.10a ± 1.10	92.10a±0.78	93.10b± 0.24	90.77b ±1.86
T4	0.5	0.75	88.99a±0.17	93.83a±0.17	94.52a±0.15	92.45a ±1.74
significance			**	*	**	**

The different letters within a single column indicate that there are significant differences between the treatments. * Mean significant effects found of treatment at P< 0.05 in the variance analysis table. ** Mean significant effects found of treatment at the probability level P<0.01 in the variance analysis table.

Table 3: Effect of the probiotic and enzymes in the diet on egg weight (g) (Mean ± standard error) during productive periods (23-34 weeks) from the life of the laying hens.

Treatments			Productivity durations/age per week			Overall Rate
Treatment	Level of addition(g / kg)		1	2	3	
	Probiotic	Enzymes	(23-26)	(27-30)	(31-34)	(23-34)
T1	0	0	50.64b±0.53	55.15b±1.16	56.05c±0.51	53.87 c±1.63
T2	0.5	0	55.21a±1.16	59.10a±0.30	59.75ab ±0.26	58.02a± 1.42
T3	0	0.75	53.85a ± 0.41	58.23a±0.87	59.05b± 0.18	57.04b±1.61
T4	0.5	0.75	54.93a±0.19	59.27a±0.21	60.62a±0.31	58.27a±1.71
significance			N.S	*	**	**

The different letters within a single column indicate that there are significant differences between the treatments. N.S mean that there are no significant effects in the Variance Analysis table. * Mean significant effects found of treatment at P< 0.05 in the variance analysis table. ** Mean significant effects found of treatment at the probability level P<0.01 in the variance analysis table.

body (Peric *et al.,* 2009; Yenice *et al.,* 2014), the increase of beneficial bacteria enhances the production of vitamins K and B and volatile fatty acids and, increase the activity of digestive enzymes in the intestinal cavity (Filho *et al.,* 2006; Awad, *et al.,* 2009), therefore improve the shape and structural characteristics of Intestinal tissue (Beski and Al-Sardary, 2015; Wang *et al.,* 2017), as shown by the results of this study (Table 7 and 8), this slows down the passage of food and improves digestion and absorption of the intestines, nutrients become more ready-made thus increasing the number of uptake nutrients (Yenice et al., 2014), this leads to improving the productive performance of probiotic treatment (Abd El-Hack *et al.,* 2017; Guo *et al.,* 2018). As for the effect of the digestive enzyme treatment is due to their role in improving the digestion of nutrients, through their ability to release them from the complexes and compounds in the feed and the passage of enzymes available internally in these compounds makes the process of absorption easier (Francesch and Geraert, 2009; Alagawany *et al.,* 2017; Abd El-Hack *et al.,* 2018).

Table 6: Effect of the probiotic and enzymes in the diet on egg mass (g/hen/ day) (Mean ± standard error) during productive periods (23-34 weeks) from the life of the laying hens.

Treatments			Productivity durations/age per week			Overall
Treatment	Level of addition(g / kg)		1	2	3	Rate
	Probiotic	Enzymes	(23-26)	(27-30)	(31-34)	(23-34)
T1	0	0	50.64b±0.53	55.15b±1.16	56.05c±0.51	53.87 c±1.63
T2	0.5	0	55.21a ±1.16	59.10a±0.30	59.75ab ±0.26	58.02a± 1.42
T3	0	0.75	53.85a ± 0.41	58.23a±0.87	59.05b± 0.18	57.04b±1.61
T4	0.5	0.75	54.93a ±0.19	59.27a±0.21	60.62a±0.31	58.27a±1.71
Significance			**	*	**	*

The different letters within a single column indicate that there are significant differences between the treatments. * Mean significant effects found of treatment at P< 0.05 in the variance analysis table. ** Mean significant effects found of treatment at the probability level P<0.01 in the variance analysis table.

Table 5: Effect of the probiotic and enzymes in the diet on the cumulative number of eggs (egg /chick/28 days) (Mean ± standard error) during productive periods (23-34 weeks) from the life of the laying hens.

Treatments			Productivity durations/age per week			Overall
Treatment	Level of addition(g / kg)		1	2	3	Rate
	Probiotic	Enzymes	(23-26)	(27-30)	(31-34)	(23-34)
T1	0	0	23.5b±0.08	24.96b ±0.43	25.36c ±0.09	24.58c ±0.55
T2	0.5	0	25.14a±0.39	26.12a±0.009	26.22b±0.03	25.83a± 0.34
T3	0	0.75	24.39a ± 0.31	25.78a±0.21	26.07b± 0.07	25.41b±0.52
T4	0.5	0.75	24.92a ±0.05	26.27a±0.05	26.47a±0.04	25.89a±0.49
Significance			**	*	**	**

The different letters within a single column indicate that there are significant differences between the treatments. * Mean significant effects found of treatment at P< 0.05 in the variance analysis table. ** Mean significant effects found of treatment at the probability level P<0.01 in the variance analysis table.

Table 6: Effect of the probiotic and enzymes in the diet on feed conversion ratio (g feed / g eggs) (Mean ± standard error) during productive periods (23-34 weeks) from the life of the laying hens.

Treatments			Productivity durations/age per week			Overall
Treatment	Level of addition(g / kg)		1	2	3	Rate
	Probiotic	Enzymes	(23-26)	(27-30)	(31-34)	(23-34)
T1	0	0	2.27a ± 0.02	2.08a ± 0.04	2.05a ± 0.01	2.14a ± 0.07
T2	0.5	0	2.08b ± 0.04	1.95b ± 0.009	1.92bc ± 0.008	1.98c ± 0.05
T3	0	0.75	2.14b ± 0.02	1.98b ± 0.03	1.95b ± 0.005	2.02b ± 0.06
T4	0.5	0.75	2.09b ± 0.007	1.94b ± 0.006	1.90c ± 0.009	1.98c ± 0.06
significance			**	*	**	**

The different letters within a single column indicate that there are significant differences between the treatments. * Mean significant effects found of treatment at P< 0.05 in the variance analysis table. ** Mean significant effects found of treatment at the probability level P<0.01 in the variance analysis table.

Biometrics of the small intestine

Table 7 shows that there was a significant effect of the probiotic and Combination treatments on the weight and the length of the small intestine for the brown chicken Lohmann at weeks 28 and 34 of the age of the chickens, where we notice at this age there are significant differences (0.05 ≥ P) in the weight of the small intestine and its length between the treatments. While the treatment of digestive enzymes did not record a significant effect on the weight of the intestine, this effect of the experimental treatment was reflected in the overall rate of intestinal weight and length during the total duration of the experiment**.** Note from Table 8 that the probiotic treatment and the combination treatment leads to a significant increase in the length of bird villi at the age of 28 weeks, while the digestive enzymes treatment did not have a significant effect on the length of villi, and at the age of 34 weeks also had the same effect of experimental treatments in the length of villi for the intestines of birds, but the treatment of digestive enzymes did not record a significant difference compared to the control treatment. From the same table 8 we find that the effect of the experimental treatments in the depth of the crypts was the same as that of the villus length of the intestines, this increase was in the same direction in the overall rate of crypts depth during the total experiment duration, while the treatment of digestive enzymes had no significant effect in crypts depth at the age of 28 and 34 weeks as well as in the overall rate of crypts depth during the total experiment duration compared to the control treatment, the improvement in the results of the small intestine traits (weight, length and some of their histological properties) in the birds of the probiotic treatment and combination treatment (probiotic and digestive enzymes), is due to the role of the probiotic in both treatments, but the role of digestive enzymes did not have any significant effect on intestinal traits (Table 7 and 8), the probiotic has the potential to improve the internal environment of the small intestine by improving the balance of the gut microbiota by increasing the numbers of beneficial bacteria in the gut and decreasing the number of harmful bacteria, which in turn lead to increased secretion of vitamins, mineral elements, organic acids, hydrogen peroxide, Bacteroicins and volatile fatty acids, all of these secretions consider as a source of food to intestinal cells for the purpose of growth, maintenance and renewal as well as increase the secretion of the Mucin layer that protects it from external and internal toxins, in addition, secreted organic acids in the intestines lower the pH, which improves digestion and absorption of nutrients (Cho and Finocchiaro, 2010; Saminathan *et al.*, 2011;

Table 7: Effect of the probiotic and enzymes in the diet on Intestinal weight and length (Mean ± standard error) during productive periods (23-34 weeks) from the life of the laying hens.

Sig.	Treatments				Age (week)
	T4	T3	T2	T1	
	(g) Weight of small intestine				
**	80.02^{a} ±0.3	75.78^{b} ± 1.45	82.47^{a}±1.01	76.44^{b}±1.1	28
**	80.99^{b} ± 0.65	77.29^{c} ± 0.98	84.28^{a}±1.23	77.69^{c} ±0.75	34
**	80.51^{b}± 0.49	76.54^{c} ±0.76	83.38^{a}±0.91	77.07^{c}±0.63	Overall Rate
(cm) Length of small intestine					
*	188.25^{a} ± 0.85	175^{b}±2.27	189^{a} ± 5.28	178.75^{b}±0.48	28
**	194.25^{a} ± 4.07	181.25^{b} ± 3.2	200.5^{a} ±3.77	182^{b}±3.74	34
*	191.25^{a} ±3.00	178.13^{b}± 3.13	194.75^{a}±5.75	180.37^{b}±1.63	Overall Rate

T1: were fed on a standard diet without additives (control).T2: were fed on a standard diet with 0.5 g/kg of probiotic.T3: were fed on a standard diet with 0.75 g/kg of digestive enzymes.T4: were fed on a standard diet with a combination of 0.5 g/ kg probiotic and 0.75 g/ kg of digestive enzymes. The different letters within a single row indicate that there are significant differences between the treatments. * Mean significant effects found of treatment at P< 0.05 in the variance analysis table. ** Mean significant effects found of treatment at the probability level P <0.01 in the variance analysis table.

Table 8: Effect of the probiotic and enzymes in the diet on villus length and crypts depth (Mean ± standard error) during productive periods (23-34 weeks) from the life of the laying hens.

Sig.	Treatments				Age (week)
	T4	T3	T2	T1	
	(g) Weight of small intestine				
*	1170^{a}±55.15	898.95^{b}±65.92	1184.3^{a}±66.55	911.3^{b}± 80.28	28
**	1280.5^{a}±11.36	993.2^{b} ±42.91	1285.7^{a} ±29.61	±25.50 997.75^{b}	34
**	1225.25^{a}± 55.25	946.08^{b}± 47.13	1235^{a}±50.7	954.52^{b}±43.23	Overall Rate
	crypts depth (Micrometer)				
*	239.85^{a} ±15.5	192.4^{b} ±12.06	243.75^{a} ±6.04	195.65^{b}±13.77	28
*	263.25^{a}±14.98	219.05^{b}±9.09	265.2^{a}±13.96	220.35^{b}±12.66	34
**	251.55^{a}± 11.7	205.73^{b}±13.33	254.48^{a}±10.73	208^{b}±12.35	Overall Rate

T1: were fed on a standard diet without additives (control).T2: were fed on a standard diet with 0.5 g/kg of probiotic.T3: were fed on a standard diet with 0.75 g/kg of digestive enzymes.T4: were fed on a standard diet with a combination of 0.5 g/ kg probiotic and 0.75 g/ kg of digestive enzymes. The different letters within a single row indicate that there are significant differences between the treatments. * Mean significant effects found of treatment at P< 0.05 in the variance analysis table. ** Mean significant effects found of treatment at the probability level P <0.01 in the variance analysis table.

Beski and Al-Sardary, 2015), this leads to improved traits of the intestine biometrics by increasing the weight of the intestine, its length, the length of its villus and the depth of its crypts (Ashayerizadeh *et al.*, 2016; Sikandar

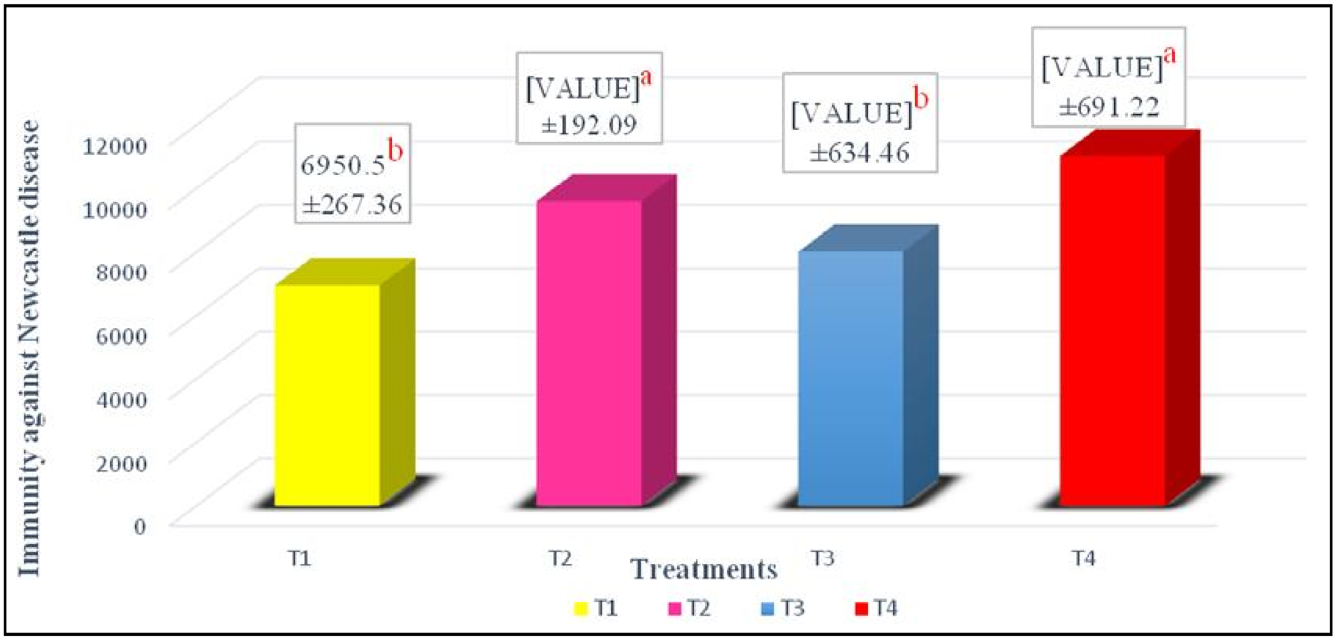

Fig. 1: Effect of the probiotic and enzymes in the diet on the volumetric standard of antibodies directed against Newcastle disease (Mean ± standard error) at week 28 of the laying hens. T1: were fed on a standard diet without additives (control).T2: were fed on a standard diet with 0.5 g/kg of probiotic.T3: were fed on a standard diet with 0.75 g/kg of digestive enzymes.T4: were fed on a standard diet with a combination of 0.5 g/ kg probiotic and 0.75 g/ kg of digestive enzymes. The different letters indicate that there are significant differences mean between the treatments at P< 0.05 according to the Dunkin' test.

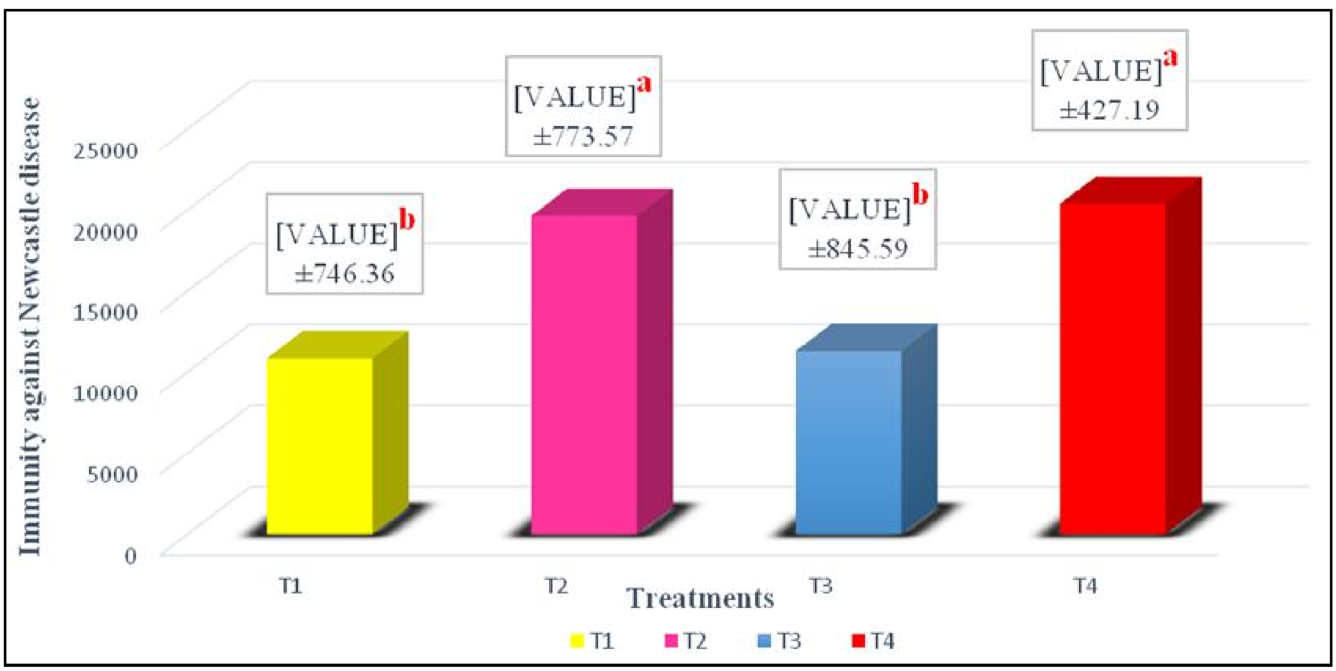

Fig. 2: Effect of the probiotic and enzymes in the diet on the volumetric standard of antibodies directed against Newcastle disease (Mean ± standard error) at week 34 of the laying hens. T1: were fed on a standard diet without additives (control).T2: were fed on a standard diet with 0.5 g/kg of probiotic.T3: were fed on a standard diet with 0.75 g/kg of digestive enzymes.T4: were fed on a standard diet with a combination of 0.5 g/ kg probiotic and 0.75 g/ kg of digestive enzymes. The different letters indicate that there are significant differences mean between the treatments at P< 0.05 according to the Dunkin' test.

et al., 2017).

Immune response

Figs. 1 and 2 show the effect of adding the probiotic, the digestive enzymes and their combination to the diet in the volumetric standard of antibodies directed against Newcastle disease of brown laying hens, this effect was similar at 28 and 34 weeks of age, the probiotic treatment and the combination treatment showed a significant increase in the volumetric standard of antibodies directed against Newcastle disease at 28 and 34 weeks of age, while the treatment of enzymes showed no significant effect in immunity values compared with control treatment, the results of this experiment show an improvement in the immune response of birds by increasing the antibodies directed against Newcastle disease in the birds of the probiotic treatment and the combination treatment, and this is due to the effect of the probiotic in both treatments, the treatment of digestive enzymes showed no significant effect on antibodies directed against Newcastle disease as shown by this study (Fig. 1, 2), this effect is due to its role in improving the balance of the gut microbiota by increasing the number of beneficial bacteria and decreasing the number of harmful bacteria, which will improve the histological and health properties of the intestines and activate the lymphatic organs spread along the intestines represented by the Payers patches, the cecal tonsil and the Fabricia gland, Moreover, beneficial bacteria induce B lymphocytes in the lymphatic tissue of the intestine to produce antibodies in the blood (Lorenzo *et al.,* 2014; Abdel-Tawab *et al.,* 2015; Talebi *et al.,* 2015).

Conclusion

The results suggested that adding the probiotic or digestive enzymes improved the productive performance of laying hens, the probiotic was the best in the results, while the combination of the probiotic and digestive enzymes did not have improved results compared to the probiotic.

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