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EFFECT OF USING BIOTIC PRODUCTS AS ALTERNATIVES TO ANTIBIOTICS IN BROILER CHICKENS

Amal, H. T. Abdelnaser[a], Suzan, H. Tolba[b] and Marwa, I. Abd El-Haleem[a].

[a] Department of Poultry Diseases, Faculty of Veterinary Medicine, Benha University. [b] Vet. Serum and Vaccine Res. Inst.. Abbasia, Cairo, Egypt.

A B S T R A C T

An experiment was conducted on 198 one-day-old broiler chicks to evaluate the influence of supplementation of probiotic (PRO), prebiotic (PRE) and synbiotic (SYN) for 42 days as alternatives to antibiotics on performance, immunity and histopathological examination of immune organs. Our study revealed that the body weight (BW), the weight gain (WG) and the feed conversion ratio (FCR) in the biotic products supplemented birds did not show significant differences from the control group, also there were no significant changes in the mortality rate. On the other hand, we recorded a significant improvement of these parameters in the antibiotic (AB) treated group in comparison with the control group. The T lymphocyte activity against infectious bursal disease (IBD) vaccination in the birds supplemented with the biotic products was improved at day (d) 14-post vaccination in comparison with both the antibiotic and the control groups. The humoral immune response against Newcastle disease (ND) vaccination in the PRO and PRE supplemented birds was significantly improved at d 42 of age in comparison with the vaccinated non-treated control group. The PRE significantly improved the weight of BF at d 42. The SYN and PRE treatments caused increase in the villar length of the small intestine in comparison with the control group.

KEYWORDS. Broiler, Probiotic, Prebiotic, Synbiotic, Immunity, Performance, Histopathology

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1.INTRODUCTION

Poultry are raised nowadays under intensive production systems in densely populated colonies or flocks to achieve high levels of economic efficiency. During this process, chickens may get stress from a number of factors such as overcrowding, unfavorable ambient medium, feed-intake and vaccination, etc. [1]. Antibiotics have been a common feed additive in poultry rations as a growth promoter to improve performance by reducing the burden of pathogens [2]. Also, they are frequently used therapeutically and prophylactically for the treatment of diseases in poultry [3]. However, the continued feeding of antibiotics at subtherapeutic levels has created concerns about the extent to which usage increases the possibilities of antibiotic residue, the development of drug-resistant bacteria and reduction in the ability to cure these bacterial diseases in humans [4], also imbalance of normal microflora [5]. Due to this, the Europe Union Commission (EUC) decided to phase out and ultimately ban the marketing and including of antibiotics as growth promoters in animal diets. This ban became effective on January 1, 2006 [6]. The use of dietary additives as probiotics and prebiotics individually or in combination (synbiotics) is gaining momentum and paid an attention to be used as an alternatives to antibiotics because of their beneficial effects

on growth rate and feed efficiency [7,8], their prevention of intestinal infection [9] and their anticarcinogenic effect [10]. The use of probiotics in the modern era evolved from a theory proposed by Elie Metchnikoff in 1907, who suggested that the prolonged life span of Bulgarian peasants was a result of their consumption of fermented milk products [11]. In contrast to the use of antibiotics as nutritional modifiers, which destroy beneficial bacteria, the inclusion of probiotics in foods is designed to encourage certain strains of bacteria in the gut at the expense of less desirable ones [12]. The probiotics are applied in farm animal nutrition to improve feed conversion and increase weight gains [13] and to influence functional digestive system development in young animals [14]. Furthermore, they are used in the preventive therapy of animal diseases [15] as they have inhibition effect on pathogens and stimulating effect on the immune system [16, 17]. The prebiotic substances have been defined as a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and or activity of limited number of bacteria in the colon. Therefore, compared to probiotics which introduce exogenous bacteria into the colonic microflora, a prebiotic aims at stimulating the growth of one or a limited number of the potentially health promoting indigenous microorganisms thus modulating the composition of the natural ecosystem [12]. The synbiotics are a mixture of probiotics and prebiotics that beneficially affect the host by improving the survival and implantation of live microbial dietary supplements in the gastrointestinal tract by selectively stimulating the growth and / or activating the metabolism of one or a limited number of health promoting bacteria, and improving host welfare [18]. The aim of this research is to evaluate the influence of supplementation of commercial probiotic (PRO), prebiotic (PRE) products and synbiotic (SYN), combination of both, for 42 days as alternatives to antibiotics on performance, immunity against vaccination, immune organs weight and histopathology of immune organs.

2. MATERIALS AND METHODS

2.1- Experimental chicks

The experiment was conducted on 198, healthy one- day- old broiler (Cobb breed) chicks purchased from El-Nile Company for poultry and rations. Birds were fed on well-balanced diet [19], starter diet (1 d-14 d), grower diet (15 d - 28 d) and finisher diet (29 d – 42 d).

2.2- Management and housing

The broiler chicks were housed in a clean well ventilated room (5m×7m) previously fumigated with formaldehyde gas. The room was divided into 5 equal space areas for the first 5 groups (G1 - G5) and the 6th control group (G6) was housed in an adjacent separate place. Each compartment was provided by suitable feeders and waterers.

2.3- Experimental design

198 chicks were randomly divided into 6 groups (33 bird / group) and were treated as follows, 1) AB group treated by antibiotics in feed (500 g Zinc bacitracin / tone feed /42 d) and in water (Enrofloxacine (1-4 d), Neomycin 20% (5-8 d), Amoxicillin (25-28 d), Colistine and Doxycycline (34-37 d)), 2) PRO group treated by probiotic in water (Bio BC® :2 g / liter) for the first 3 days of life and probiotic in feed (AD-farm® :250 g / tone / 42 d), 3) PRE group treated by prebiotic in feed (Bio Mos ® : 1 g / tone / 42 d), 4) SYN group treated by combination of programs given to groups 2 and 3, 5) VNTC group: vaccinated not treated control and 6) NVNTC group: negative control. The first five groups were vaccinated against ND (Abbasia) at 7, 18 and 28 days, infectious bursal disease (Intervet) at 14 d, infectious bronchitis disease (Abbasia) at 7 d in DW and avian influenza (Intervet) at

10 d by S/C injection. All groups received a course of anticoccidial drugs in water.

2.4- Performance parameters

The feed intake (FI), body weight (BW), weight gain (WG), feed conversion ratio (FCR) and mortality % were measured weekly [20, 21].

2.5- Immunological studies

2.5.1- Lymphocyte blastogenesis assay was performed in order to evaluate the cellular immunity (T lymphocyte activity) against Infectious bursal disease (IBD) vaccination. The assay is based on the ability of metabolically active cells to reduce the tetrazolium salt XTT to the orange colored compounds of formazan. The dye is water soluble and the dye intensity can be read at a given wave length in a spectrophotometer. The intensity of the dye is proportional to the number of metabolically active cells [22]. Blood samples were collected from Jugular vein from vaccinated and non-vaccinated chicks (two samples / group), with anticoagulant (Heparin 20-40 IU/ml) at 7, 14 and 21 days post vaccinations. Separation of lymphocytes was applied [23, 24]. Viability of separated lymphocytes was determined [25]. Viable lymphocytes were adjusted to a concentration of 5 x 10^6 cells/ ml suspended in 1 ml Roswer Park Memorial Institute (RPMI-1640) medium containing 10% fetal calf serum. Setting up of lymphocyte and using cell proliferation kit XTT [26].

*2.5.2-Haemagglutination (HA) and haemagglutination inhibition (HI) test*s were performed in order to evaluate the humoral immune response against Newcastle disease (ND) vaccination. The HA test procedures [27] was done in order to obtain 8 haemagglutination unit (HAU).The procedure of HI test was done according to the standard microplate system [28].

2.5.3- Weighting of the immune organs, three birds were randomly chosen from each treatment group and slaughtered at 21 d and 42 d. BF, thymus and spleen were removed from each bird and weighted separately. All weights were recorded.

2.6- Histopathological studies

Specimens from different parts of the small intestine (duodenum, jejunum and ileum.), bursa of Fabricius, thymus and spleen were collected at 21 d and 42 d and preserved in 10% formalin solution and examined [29].

2.7- Statistical analysis

Data obtained in this study were statistically analyzed for variance ANOVA with confidence limits set at 95 % (Significance at $P \leq 0.05$ probability levels) and critical difference as described by LSD, SPSS 16 Student Version 10.0.7, June 2000. The results were reported as the mean ± standard error (SE) and least significant difference (LSD).

3. RESULTS

3.1- Performance parameters

The performance parameters were calculated weekly and at the end of the experiment (Table 1). The results revealed that the PRE treated group showed insignificant low total FI (4831.03 g) in comparison to control group (NVNTC) (5104.9 g) while, the PRO, SYN, and AB groups recorded the least TFI at the end of the experiment. The TBW of the AB treated birds was significantly higher (2417.30 g) than the PRO and the SYN groups (2081.7 g and 2139.7 g respectively). The TWG in the AB treated group was significantly higher (2380.93 g) than the PRO and SYN groups. The TFCR at the end of the experiment was significantly improved in AB group (2.01) in comparison to NVNTC group (2.28). The highest total mortality rate was recorded in the PRE group (12.12%).

3.2- Immunological parameters

Regarding to the cellular immunity, at 7 days post vaccination (p.v) the SYN group recorded the highest level of the T-lymphocyte activity (cellular immunity)

Table 1. Effect of antibiotic, probiotics, prebiotics and synbiotics on the total performance parameters at the end of the experiment (42 d). (Means ± SE).

	AB	PRO	PRE	SYN	VNTC	NVNTC
TFI	4777.80±114.82^{bcd}	4512.10±83.59^{d}	4831.03±170.28^{abc}	4684.0±10.41^{cd}	5046.1±33.81^{ab}	5104.9±51.17^{a}
TBW	2417.30±55.11^{a}	2081.7±60.01^{b}	2230.0±43.10^{ab}	2139.7±61.33^{b}	2209.3±136.84^{ab}	2278.3±8.97^{ab}
TWG	2380.93±55.11^{a}	2045.57±60.11^{b}	2195.73±44.27^{ab}	2103.27±61.33^{b}	2172.93±136.84^{ab}	2242.23±9.18^{ab}
TFCR	2.01±0.01^{b}	2.21±0.04^{ab}	2.20±0.08^{ab}	2.23 ±0.07^{ab}	2.34±0.17^{a}	2.28±0.03^{a}
T mort. %	3.03%	6.06%	12.12%	9.09%	6.06%	6.06%

LSD represents least significant differences between different groups at probability $P< 0.05$. Means with different superscripts (a, b, c, d) within a row are significantly different at $P < 0.05$.

Table 2. Effect of antibiotic, probiotics, prebiotics and synbiotic on lymphocyte blastogenesis assay (means ±SE).

group	AB	(PRO	PRE	SYN	VNTC	NVNTC
d 7 p.v	1.04	0.74	0.65	1.19	0.78	0.06
d 14 p.v	0.98	1.64	1.19	1.75	0.99	0.43
d 21 p.v	0.39	0.43	0.45	0.42	0.45	0.35

Table 3. Effect of antibiotic, probiotics, prebiotics and synbiotic on HI antibody titer (means ±SE).

Age	AB	PRO	PRE	SYN	VNTC	NVNT)
D 0			3.311±0.00			
D 7	2.61±0.36^{a}	2.21±0.10^{a}	2.21±0.10^{a}	2.21±0.10^{a}	2.31±0.10^{a}	2.11±0.00^{a}
D 14	1.81±0.00^{ab}	2.11±0.0^{a}	1.81±0.00^{ab}	2.11±0.00^{a}	1.51±0.17^{b}	2.11±0.17^{a}
D 21	1.81±0.17^{ab}	2.21±0.10^{a}	1.91±0.10^{ab}	1.51±0.30^{b}	2.11±0.00^{ab}	1.81±0.30^{ab}
D 28	1.00±0.36^{b}	2.41±0.0^{a}	1.81±0.17^{a}	2.01±0.10^{a}	2.21±0.20^{a}	0.50±0.10^{b}
D 35	1.91±0.10^{ab}	2.01±0.10^{ab}	2.01±0.10^{ab}	1.81±0.00^{b}	1.91±0.10^{ab}	2.11±0.00^{a}
D 42	2.11±0.0^{ab}	2.41±0.0^{a}	2.31±0.20^{a}	1.71±0.20^{c}	1.91±0.10^{bc}	1.71±0.10c

LSD represents least significant differences between different groups at probability $P < 0.05$.Means with different superscripts (a, b, c, d) within a row are significantly different at $P < 0.05$.

Table 4. Effect of antibiotic, probiotics, prebiotics and synbiotic on weight of immune organs/ gm (means ±SE).

Age	Organ	Group					
		AB	PRO	PRE	SYN	VNTC	NVNTC
3rd w	BF	0.92±0.18^{c}	1.35±0.05^{bc}	2.04±0.41^{a}	1.72±0.10^{ab}	1.54±0.06^{abc}	1.83±0.16^{ab}
	Thymus	2.88±0.90^{b}	4.48±0.55^{ab}	4.31±0.90^{ab}	4.31±0.44^{a}	4.71±1.30^{ab}	5.08± 0.64^{ab}
	Spleen	0.88±.31^{a}	0.51±.04^{a}	0.64±0.03^{a}	0.58±0.10^{a}	0.52±0.26^{a}	0.64±0.14^{a}
6th	BF	2.82±0.75^{ab}	1.16±0.14^{c}	3.50±0.57^{a}	1.62±0.52^{bc}	1.83±0.24^{bc}	2.79±0.37^{a}
week	Thymus	11.29±0.44^{a}	14.19±2.88^{a}	12.01±2.57^{a}	10.72±0.30^{a}	13.68±1.43^{a}	14.50±0.29^{a}
	Spleen	2.18±0.41^{a}	2.31±.56^{a}	2.8847±0.66^{a}	2.47±0.40^{a}	1.78±0.28^{a}	2.37±0.32^{a}

LSD represents least significant differences between different groups at probability $P < 0.05$. Means with different superscripts (a, b, c, d) within a row are significantly different at $P < 0.05$.

(1.19) in comparison with VNTC group (0.78). At d 14 p.v there was an increase in the level of the T-lymphocyte activity in the SYN group (1.75) while it decreased in the AB group (0.98) as well as the VNTC group (0.99) as in table 2. Concerning to the humoral immunity, the results of HI test in different groups from zero days until the end of the experiment were transformed into $\log_{10}$ and listed in table 3. At d 14, the PRO and the SYN groups recorded significant high antibody titer (2.11 for both groups) in comparison to the VNTC group (1.51). At d 28, the PRO, the SYN and the PRE groups showed significant higher titer (2.41, 2.01 and 1.81 respectively) than the AB group (1.0). At d 42 of age, both the PRO and the PRE groups recorded significant high titer (2.41 and 2.31 respectively) in comparison with VNTC group (1.91). Concerning to the immune organs weight as in table 4, at the 3rd week of age, the PRE group recorded significant high weight of BF (2.04 g) in comparison to the PRO and the AB groups (1.35 g and 0.92 g respectively). For the thymus, the SYN group recorded significantly higher weight (4.31 g) than the AB group (2.88 g), but it was in significant in comparison to VNTC group (4.71 g). At 6th week of age, the PRE group recorded the highest weight of BF (3.50 g) which was significant in comparison to the VNTC, the SYN and the PRO groups (1.83 g, 1.62 g and 1.16 g respectively). The spleen did not show significant difference in weight between different groups at both 3rd and 6th weeks.

3.3- Histopathological findings

There was no histopathological alteration but normal histological structures observed in the BF, thymus, spleen and small intestine of NVNTC birds as illustrated in table 5**.** Concerning to the effect of biotic products (probiotic, prebiotic and synbiotic) on histopathological lesions of immune organs (Table 5), the present results showed that

Table 5. The different histopathological lesions that observed in groups treated with probiotic, prebiotic, synbiotic and antibiotics.

organ	Lesions	Groups										
		AB		PRO		PRE		SYN		VNTC		NVNTC
		D 21	D 42	D 21	D 42	D 21	D 42	D 21	D 42	D 21	D 42	-
BF	Lymphocytic depletion	++	+	+	++	+	++	+	+++	+	++	-
	Lymphocytic degeneration	+	+	+	+++	-	+	-	+++	+	++	-
	Heterophilic infiltration	-	-	-	+	-	-	-	-	-	+	-
	Cystic formation	-	+	+	++	-	-	-	+	-	++	-
	Fibrosis	-	-	-	++	-	-	-	++	-	+	-
	Epithelial hyperplasia	+	++	+	+	+	+	-	++	-	+++	-
Thymus	Hemorrhage	+	-	+	+	+	+	-	-	+	-	-
	Lymphocytic depletion	+	-	+	+	+	++	+	-	+	+	-
	Lymphocytic degeneration	++	-	+	+	+	++	+	-	+	+	-
Spleen	Hemorrhage	+	-	++	-	+	+++	++	+++	+	+	-
	Lymphocytic depletion	-	+	+	-	++	+++	+++	++	-	+++	-
	Capillary sheath activation	-	+	+	-	++	+++	+++	+++	-	+++	-
	Lymphocytic activation	-	+	-	-	-	-	-	-	-	++	-
Small intestine	Villus length	-	+	-	-	-	+	+	++	-	+	-
	Hemorrhage	+	+	+	-	+	-	-	+	-	+	-

Nil= - Mild= + Moderate= ++ Severe= +++

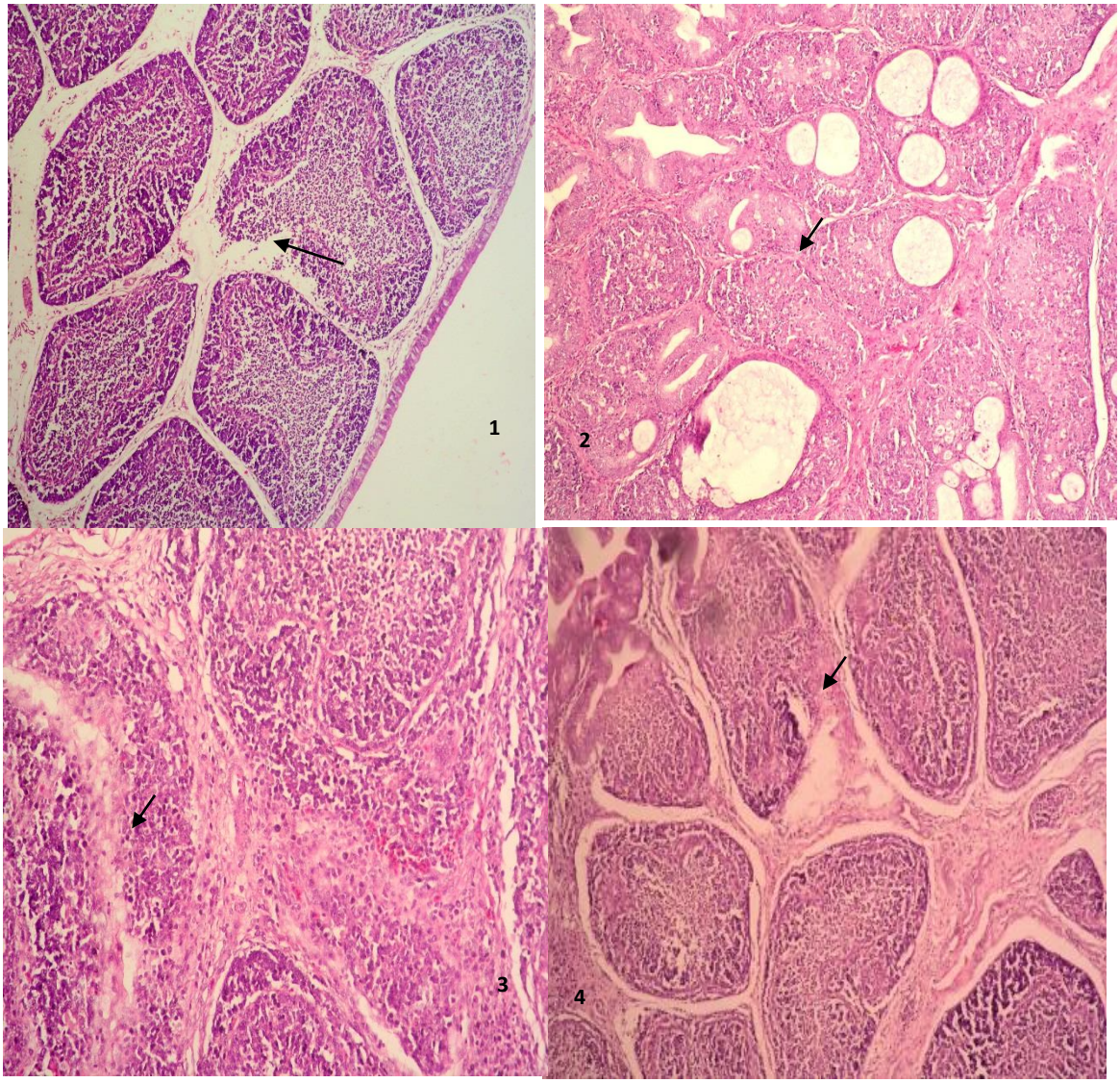

Plate 1.
1) Bursa showed mild depletion of lymphocytes (H&E stain × 100). 2) Bursa showed moderate cystic formation (H&E stain × 100). 3) Bursa showed moderate lymphocytic depletion (H&E stain × 100). 4) Bursa showed severe degeneration of lymphocytes (H&E stain × 200).

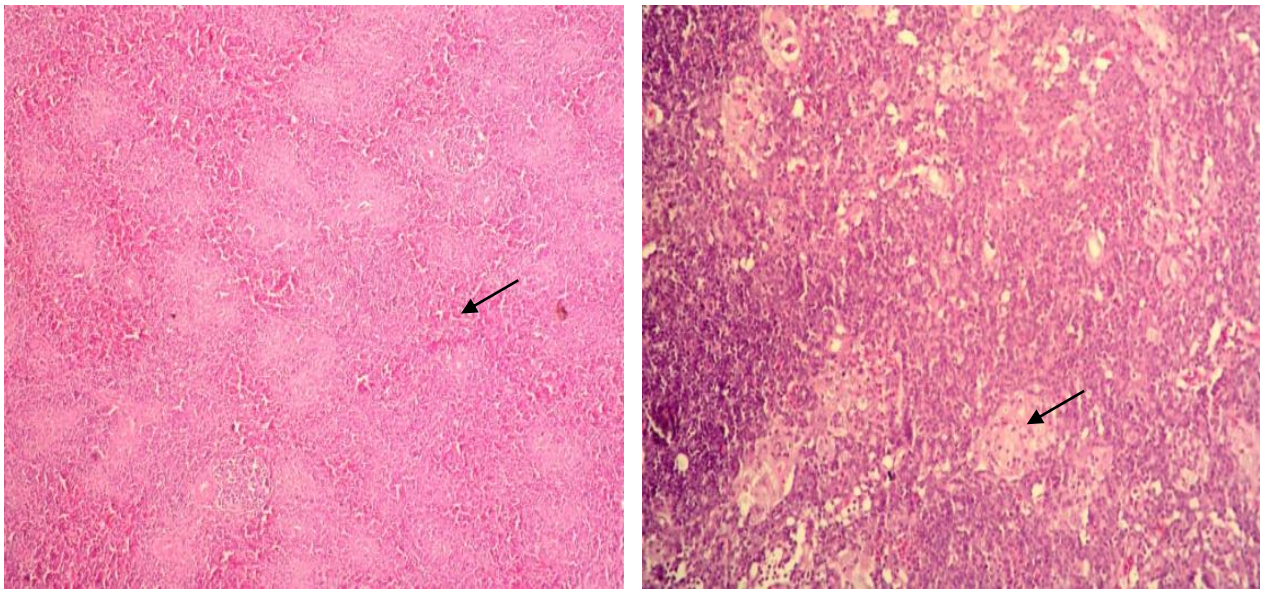

Plate 2. Left, Thymus showed mild hemorrhage (H&E stain × 200). Right, Thymus showed mild degeneration (H&E stain × 200).

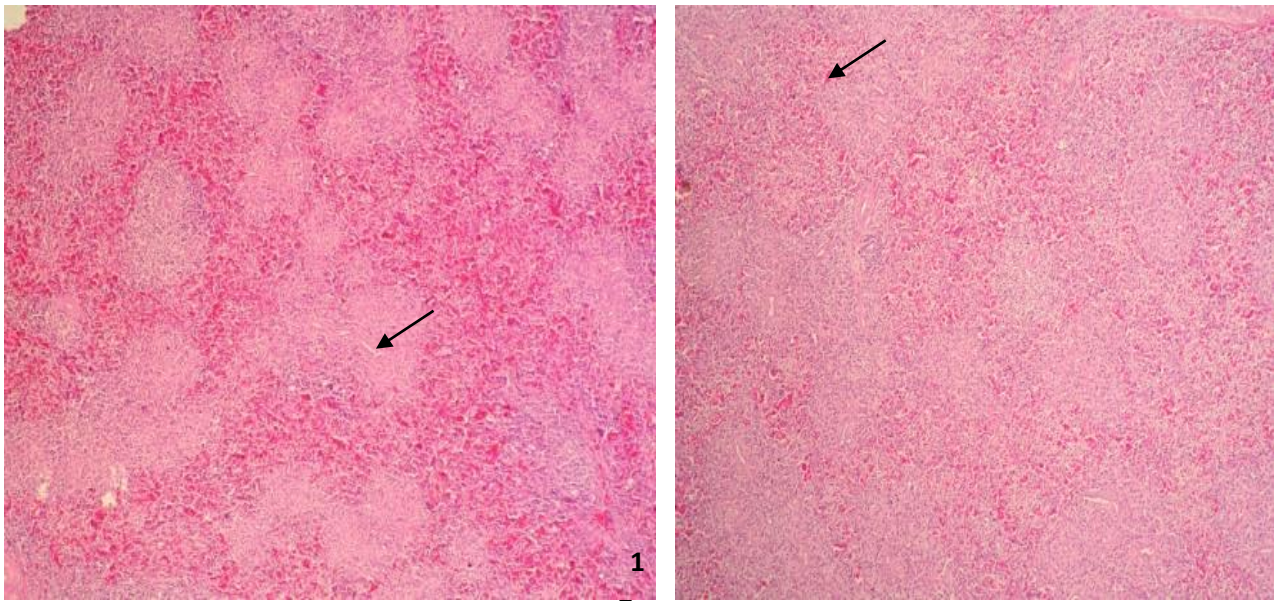

Plate 3. 1) Spleen showed severe depletion and capillary sheath activation (H&E stain × 100). 2) Spleen showed severe hemorrhage (H&E stain × 100).

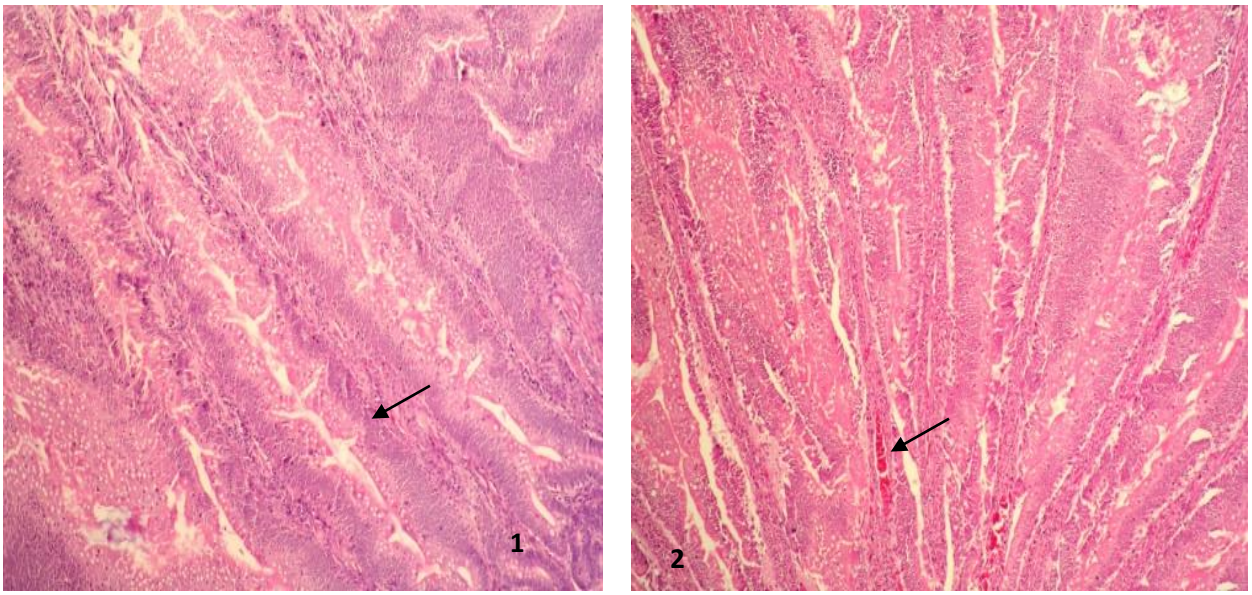

Plate 4. 1) Intestine showed increase of villar length (H&E stain × 100). 2) Intestine showed hemorrhage in lamina propria (H&E stain × 100).

these products did not assist in alleviation of histopathological alterations caused by vaccination when compared to NVNTC group while the antibiotic treated group showed mild histopathological lesions in the immune organs, lesions of BF, thymus and spleen showed in plates 1, 2, 3 respectively. Concerning to the effect of probiotic on the cells of the small intestine histopathology (Table 5), the results showed that the probiotic did not affect the villus height but alleviate the other histopathological alterations in small intestine. Regarding to the effect of prebiotic on small intestine histopathology, the results showed that the prebiotic caused mild improvements in villus height and alleviates the other histopathological alterations in small intestine in comparison to NVNTC group. Concerning to the effect of synbiotic on the small intestine histopathology, our results revealed that synbiotic treated birds showed improvement in villus height with mild histopathological alterations in comparison with the untreated groups, intestinal lesions showed in plate 4.

4. DISCUSSION

The use of dietary additives as prebiotics and probiotics individually or in combination (synbiotics) is gaining momentum and paid an attention to be used as an alternative to antibiotics because of their beneficial effects on health of poultry. In this study, broilers fed probiotic, prebiotic and synbiotic recorded insignificant improvements in performance parameters. The supplementation of the probiotic and / or prebiotic to the broilers did not have any effect on body weight, weight gain and feed conversion ratio [30, 31, 32]. However, the present study disagreed with other authors [33] as they found that the combination of probiotics and prebiotics or each additive alone in broiler diets resulted in higher body weight, higher daily weight gain, better-feed conversion ratio and lower mortality rate than the control group. The recorded results may be attributed to that well-nourished healthy chicks do not

positively respond to growth promoters when they are housed under clean conditions and at a moderate stoking density [34].

Regarding to the effect of these additives on broiler immunity, we found that the biotic additives improved the cellular immunity in comparison to the AB and the control groups. In addition, the probiotic significantly improved the humoral immunity at d 14 and d 42 of age, the prebiotic significantly improved the humoral immunity at d 42 of age and the synbiotic significantly improved the humoral immunity at d 14 of age. However, these additives did not have significant effect on weight of the immune organs except in case of prebiotic treated group which showed significant improvement in weight of BF at 6^{th} week. Concomitant to the finding of the present investigation, previous study reported also that the HI antibody titers against ND virus were high in broiler chicks fed diets supplemented with probiotic compared to control groups [35]. Moreover, Fayoumi hens treated by probiotics in feed under hot climate showed significant increase in HI antibody titter against ND vaccination with relative increase in weight of thymus and spleen [36]. The consumption of prebiotic fibers could modulate immune parameters in gut-associated lymphoid tissues (GALT), secondary lymphoid tissues and peripheral circulation [37]. Furthermore, the synbiotic enhanced the humoral immune response to live ND vaccines in immunosuppressed broilers but it did not decrease the post virulent NDV challenge mortality [38]. Our results can be attributed to that the probiotic bacteria cause increase of the lysozyme activity in serum and the spleen, the peripheral blood mononuclear cell (PBMC) proliferation, the CD4+.CD8+ T lymphocyte ratio in the spleen and reduction in prostaglandin E2 (endogenous inhibitor of immune response) synthesis in the serum [39]. Also, the prebiotics selectively increase the levels of beneficial microbes, including species *lactobacillus and Bifidobacteria* in the cecum of broilers and prevent its colonization by pathogenic bacteria [40]. Our results are dissimilar to previous investigation [32] who found that the dietary probiotic and/or prebiotic supplementation to one-day old Ross-308 broiler chicks of mixed sex did not significantly affect concentration of immunoglobulin (IgG) in the serum. Also, the probiotic supplementation did not affect the specific antibody synthesis to ND vaccine antigen administered to layer birds via drinking water [38].

Concerning to the effect of biotic products on histopathology of immune organs, these products did not assist in alleviation of histopathological alterations caused by vaccination when compared to the VNTC and NVNTC groups. These results are dissimilar to those reported that the biotic product (probiotic) had the ability to alleviate the severe histopathological alterations caused by ochratoxin A in lymphoid organs of treated groups when compared with non-treated one [41]. In the small intestine, the probiotic did not affect the villus height while, the prebiotic and the synbiotic caused improvement in the length of the villi in comparison to the NVNTC group. Absence of change in the small intestinal morphology with increased goblet cell mucin storage was shown in broiler fed diet-containing probiotic [42]. Moreover, the jejunal villi were longer in chicks raised on diets with 5gm prebiotic/ kg diet [43]. The improvement of villus length by prebiotics can be attributed to that the oligosaccharides promote build-up of lactic acid, which induces the mucosal cell proliferation [44]. Furthermore, the villus height and crypt depth of intestinal mucosa of broilers increased when fed the synbiotic due to the increase in the intestinal epithelial turn over [33].

In conclusion, the biotic products in broiler feeds improved their immunity in comparison to the antibiotic treated groups however; they did not have effect on the performance of these birds.

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مجلة بنها للعلوم الطبية البيطرية

تأثير استخدام المنتجات الحيوية كبدائل للمضادات الحيوية في بداري التسمين

أمال حسن توفيق عبد ال ناصر[1] وسوزان حسن طلبة[2] ومروة إبراهيم عبد الحليم[1]

[1] قسم أمراض الدواجن-كلية الطب البيطري-جامعة بنها. [2] قسم النيوكسل - معهد بحوث الأمصال و اللقاحات البيطرية بالعباسية – القاهرة

الملخص العربي

أجريت هذه الدراسة على 198 طائر من كتاكيت التسمين (كب) عمر يوم لتقييم تأثير استخدام مركز البكتيريا الحيوية و بادئات البكتيريا الحيوية و تأثيرهم معا لمدة 42 يوم كبدائل للمضادات الحيوية على الأداء الإنتاجي و المناعة و التغيرات الهستوباثولوجية في الأعضاء المناعية. وقد اظهرت دراستنا أن وزن الجسم والزيادة في وزن الجسم ومعامل التحويل الغذائي في مجاميع الطيور المعالجة بالإضافات الحيوية لم تظهر اختلافات معنوية بالمقارنة بمجموعة عدم الإضافة، كما لم نجد اختلاف معنوي في معدل النفوق للمجاميع. ومن جانب أخر سجلنا تحسنات معنوية في هذه المعايير في مجموعة المضاد الحيوي بالمقارنة بمجموعة عدم الإضافة. كما وجدنا أن نشاط الخلايا اللمفاوية (T cells) ضد تحصين مرض التهاب كيس فابريشى المعدي في المجاميع المعالجة بالإضافات الحيوية قد تحسن في اليوم 14 بعد التحصين بالمقارنة بمجموعتي المضاد الحيوي وعدم الإضافة المحصنة. كما ظهر أن مستوى الأجسام المناعية في هذه المجاميع ضد تحصين النيوكسل قد تحسن بصورة معنوية في اليوم 42 مقارنة بمجموعة عدم الإضافة المحصنة. كما ظهرت زيادة معنوية في وزن كيس فابريشى في المجموعة المعالجة بالبريبيوتك عند اليوم 42. بينما أظهرت مجموعتي السنبيوتك والبريبيوتك زيادة في طول الزغب في الأمعاء الدقيقة مقارنة بمجموعة عدم الإضافة.

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