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The study of using effective microorganisms (EM) on health and performance of broiler chicks

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Abstract

The objective of this study was to evaluate the effect of aqueous EM supplementation to broilers on the performance, immunological and histological status of broilers. A total of 60 unsexed day old broilers (Ross 308) were assigned randomly in two equal groups (treated and control groups 30 birds 15/each replicate) reared on controlled system for 5 weeks. Ten ml/ liter of EM solution was only added to drinking water of the treated group (T2). Blood and performance parameters included body weight gain, feed consumption and feed conversion ratio. As well as estimation of differential leucocyte count. Immunologic criteria involved measurement of relative weights of spleen, thymus and bursa of Fabricius of necropsied birds. The results showed a positive significant effect of EM on the body weight of the treated group which was clear during the 3rd to the 5th week of the trial. There was significant difference in feed consumption and feed conversion efficiency between the two groups. However, the latter parameter had the most notable significance. Increased lymphocyte percentage, increased jejunal villus height and crypt depth as well as increase in goblet cell count were observed in the treated group.

Keywords: Effective microorganism; Broiler performance; Immunological status; Jejunal histology.
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دراسة استخدام المتعضيات الفعالة EM عل صحة وانتاجية فروج اللحم

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الخلاصة

الهدف من هذه الدراسة هو لتقييم تأثير اضافة المتعضيات الفعالة EM على الاداء الإنتاجي والحالة المناعية والنسيجية لأفراخ فروج اللحم. استخدمت ٦٠ فرخة من نوع روز ٣٠٨ غير مجنسة وبعمر يوم واحد، حيث وزعت عشوائيا على مجموعتين متساويتين (مجموعة سيطرة T1 ومجموعة معاملة T2 بواقع ١٥ مكرر/ مجموعة) ولمدة خمسة اسابيع. حيث تم اضافة ١٠ مل/لتر من محلول ال EM الى ماء الشرب لمجموعة المعاملة (T2). تم حساب معاير الدم والمعايير الانتاجية التي تتضمن معدل الزيادة الوزنية ومعدل استهلاك العليقة ومعامل التحويل الغذائي، بالإضافة الى العد التفريقي لكريات الدم البيض والقياسات المناعية والمتضمنة حساب الاوزان النسبية للطحال وغدة التوثة والفايبريشيا للطيور بعد الذبح. اظهرت النتائج وجود تأثير معنوي موجب لل EM على معدل وزن الجسم لمجموعة المعاملة (T2) والذي كان واضحا خلال الاسبوع الثالث والخامس من التجربة. وكان هناك اختلافا معنويا في معدل استهلاك العليقة ومعامل التحويل الغذائي بين المجموعتين. كما لوحظ اختلاف معنوي بزيادة النسبة المئوية للخلايا اللمفاوية وزيادة ارتفاع وسمك الزغابات المعوية للصائم وزيادة اعداد خلايا كوبلت في مجموعة المعاملة.

Introduction

Increased bacterial resistance to antibiotics in patients had caused an augmented public and governmental interest in eliminating sub-therapeutic use of antibiotics in livestock. Such practice had urged to find alternatives to administration of antibiotics for poultry production, which have probiotic action in these animals. (1). Initiately, probiotics are live microorganisms, which when consumed in adequate amounts; confer supporting healthy effects on the host (2). Recently, there are several researches on feeding of *Lactobacillus* spp. to livestock (3). Consequently, probiotics have different protective mechanisms, it may increase resistance to infection (4), or promote the growth (5-7), or having prophylactic effect (6). It has a role in promoting growth rates by improving feed efficiency (7) with subsequent of animal health improvements (8). Furthermore, probiotics have positive effects on the main physiological functions of the gastrointestinal tract, reflected by better digestion, absorption and metabolism (9). Effective using of probiotics were widely investigated on the mucosal immune system (10,11), on the immune organs (12), on the intestinal epithelium (13-16), on the increased lymphocyte (17), on the increase of the phagocytic activity of leukocytes and the phagocytic index in broilers. (18).

On the histological section of GIT of broiler chicks probiotics act as crypt cells proliferation of small intestine (19), increased the jejunal villus height (20), ileal villus height (21), and the number and depth of crypts (22,23). Many reports had supported the idea that the use of prebiotics can lengthen villi within the gut as well as their influence on the length of the gut (24).

However, there is still lack of information regarding the efficacy and beneficial effects of EM in poultry.

EM were innovated in Japan as a new technological advance constituting of 70 to 80 of different types of beneficial microorganisms contributing to the wide range of applications. The principal organisms of EM are usually five; photosynthetic bacteria (phototrophic bacteria), lactic acid bacteria, yeasts, actinomycets and fermenting fungi (25).

There are several defensive proposed mechanisms of EM actions (26).

The objectives of the current study was to evaluate the effects of supplementing broiler′s diet with EM as water probiotic additive on the performance, immunological and histological changes of intestinal linings of broilers.

Materials and methods

The study was conducted at the farms of the College of Veterinary Medicine/ Mosul University, during the period 1st/10 till 5th/11 2012. A total of 60 unsexed one day old chicks (Ross 308) were assigned randomly in two equal groups (30 birds, 15/ each replicate) as control T1 and treatment groups T2. The birds were reared on deep litter system. Feed and water were provided *ad libitum* throughout the experimental 5 weeks period. The chicks were fed on standard rations supplied by a local factory which met their feed requirements table (1) (27).

Table 1: Feed composition (Kg).

	Starter	Grower	Finisher
Corn	26.8	54.3	50.6
Wheat	20.0	17.4	27.0
Barley	20.0	-	-
Soybean Meal (48%)	27.0	23.0	17.0
Fat	1.7	1.0	1.0
Ground Limestone	1.6	1.5	1.5
Calcium Phosphate (20% P)	1.5	1.5	1.5
Iodized Salt	0.3	0.22	0.3
Vitamin: mineral[1] premix	1.0	1.0	1.0
Methionine	0.10	0.08	0.10
Calculated analysis			
Crude Protein	22.0	18.0	16.1
Digestible protein (%)	17.7	14.4	12.9
Crude Fat (%)	5.9	3.4	3.4
Metabolized Energy (kcal/kg)	3060	3022	3050
Calcium (%)	1.00	0.95	0.96
Av. Phosphorus (%)	0.42	0.41	0.41
Sodium (%)	0.17	0.17	0.18
Methionine (%)	0.48	0.38	0.37
Methionine &Cysteine (%)	0.82	0.65	0.61
Tryptophan (%)	0.31	0.25	0.22
Lysine (%)	1.25	0.93	0.78
Threonine (%)	0.94	0.75	0.65

Liquid form of EM product (Alannam company for natural agriculture, Tortuous–Syria under the supervision of EMRO Japanese institute - Okinawa-Japan) was used. EM stock solution is formed from Lactic acid bacilli: *Lactobacillus plantarum; L. casei Streptococcus Lactis*.; Photosynthetic bacteria *Rhodopseudomonas palustris*; *Rhodobacter sphaeroides,* Yeast; *Saccharomyces cerevisiae*; *Candida utilis torula*, *Pichia jadinii*; Actinomycetes; *Streptomyces albus*; *S. griseus* and Fermenting fungi; *Aspergillus oryzae*; *Mucor hiemalis*.as described by the manufacturer.

EM was administered at a rate of 10 ml/liter of drinking water as shown in T2.

The birds were reared on typical controlled environment. The birds were vaccinated against Newcastle disease at the 8th day and Gumboro disease at the 15th day of the experiments.

Performance criteria included weekly estimation of body weight gain, feed consumption and feed conversion ratio. At the end of the experiment all birds were necropsied and blood samples were obtained for differential leucocyte count as well as determination of relative weights of spleen, thymus and bursa of Fabricious.

Jejunal samples were cut 4 cm length and were fixed in 10% buffered formalin saline and were prepared for routine histological study as mentioned by (28).

Statistical analysis

Data were presented as means ± S.E. and were analyzed using two way analysis of variance (ANOVA) using significant level of (P <0.05). Specific group differences were determined using Duncan's multiple range test as described by (29).

Results

Body weight

A significant increase (P<0.05) in the body weight gain at the end of the experimental period was observed in the treated group T2 comparing with the control group T1 as mentioned in table (2).

Feed consumption and conversion

Feed consumption was significantly (P<0.05) different in T2 as compared with T1 which was seen over all periods of the experiment (table 3). There was a significant difference (P<0.05) in feed consumption between the two groups in favor of the second group T2 (table 3). Among all productive parameters studded, feed conversion efficiency had the most notable significance.

Table 2: Weekly body weight and body weight gain in the two groups of broiler chicks.

week	weekly body weight (g)		body weight gain (g)		%from T1
	T1	T2	T1	T2	
1st	127.1 ± 3.33	129.1 ± 2.24	81.652 ± 3.07	82.94 ± 2.1	10.1
2nd	346.5 ± 7.99	347.18 ± 7.35	216.0 ± 7.11	218.1 ± 7.95	10
3rd	691.8 ± 22.1	779.8 ± 13.21 *	347.8 ± 23.83	437.3 ± 16.34*	12.5
4th	1260.5 ± 36.87	1444.5 ± 18.6 *	568.8 ± 38.28	664.8 ± 24.02	11.6
5th	1758 ± 34.52	2019.8 ± 63.7 *	497.5 ± 47.89	575.3 ± 70.59	11.5

M±SE for 15 bird/group, * significant at (P<0.05)

Table 3: Weekly feed intake and feed conversion of the two groups of broilers.

week	Feed intake (g)		Feed conversion(g/g)	
	T1	T2	T1	T2
1st	113.2 ± 4.03	113 ± 4.62	1.40 ± 0.003	1.37 ± 0.003 *
2nd	394.3 ± 24.25	378.8 ± 10.48 *	1.81 ± 0.003	1.73 ± 0.003*
3rd	638.14 ± 15.3	593.1 ± 7.80 *	1.85 ± 0.003	1.37 ± 0.003 *
4th	845.71 ± 5.81	965.8 ± 25.6 *	1.49 ± 0.003	1.46 ± 0.003 *
5th	754.29 ± 9.32	844.3 ± 20.66 *	1.52 ± 0.003	1.47 ± 0.003 *

M±SE for 15 bird/group, * significant at (P<0.05).

Differential leucocyte count

There was only a significant increase (P<0.05) in the lymphocytes percentage of the treated group T2 as compared with the control group T1 (Table 4).

Relative weight of immunity organs

There was an increase in the mean relative weights of bursa of faibricios and thymus of the treated group. However no difference was noted in the relative weight of spleen of the two groups (Table 5).

Table 4: Differential leucocyte count in two groups of broiler chicks.

Groups	Basophil %	Eosinophil %	Heterophil %	Monocyte %	Lymphocyte %
T1	2 ± 0.43	0.28 ± 0.18	25 ± 1.6	15.85 ± 1.2	56.85 ± 2.6 *
T2	1.3 ± 0.3	0.23 ± 0.2	22.1 ± 1.29	13.66 ± 1.02	62.55 ± 1.46

M±SE for 15 bird/group, * significant at (P<0.05)

Table 5: Mean relative weight of immunity organs of the two groups of broilers.

groups	organs (weight, gm/100gm)		
	spleen	Bursa	Thymus
T1	0.0800	0.183	0.351
T2	0.0600	0.193	0.357

Villus height and Crypt depth

Significant changes (P<0.05) in the mucosal architecture in terms of increased jejunal villus height and crypts depth as well as increased goblet cell counts were observed in the T2 as compared with T1 (Table 6) and (Fig.1 and 2).

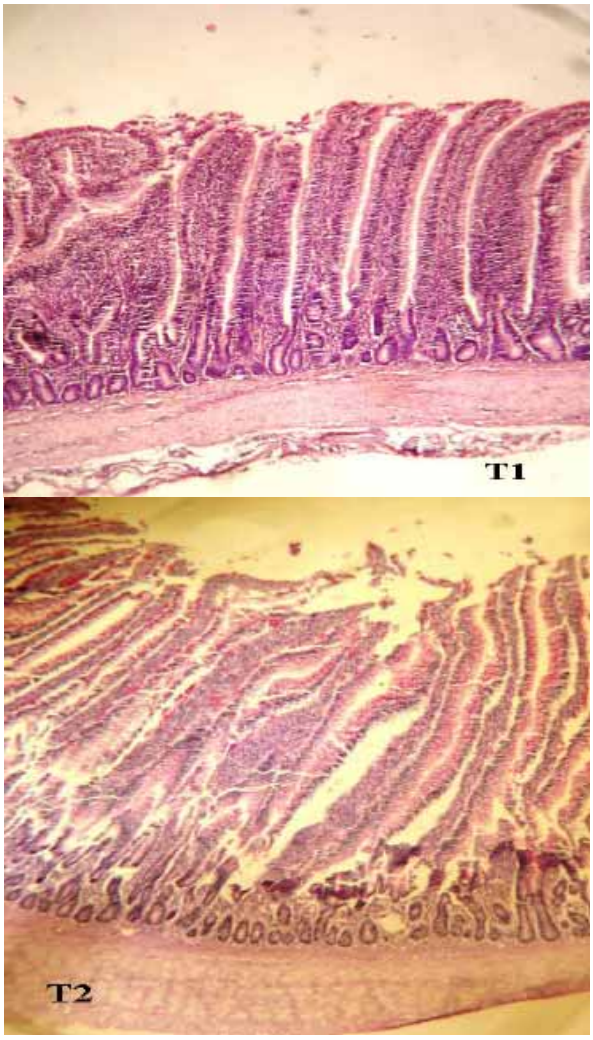

Fig. 1: cross section in the jejunum showing the difference in villi height and crypt depth in treated group T2 and control group T1. H&E stain. 145 X.

Table 6: Villi height, crypts depth and goblet cells count in the two groups of broiler chicks.

Parameters	Group	
	T1	T2
Villi height/µm	395 ± 29.72	449 ± 3.9 *
Crypts depth/µm	42.9 ± 0.4 63	52 ± 0.63 *
Goblet cell count/mm²	43.5 ± 0.45	50 ± 0.33 *

M±SE for 15 bird/group, * significant at (P<0.05).

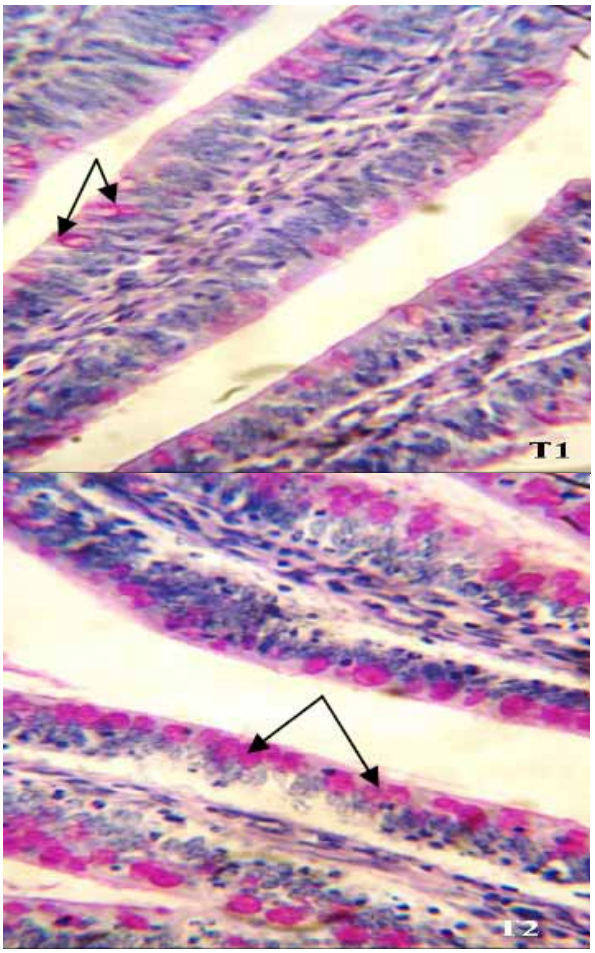

Fig. 2: Cross section in the jejunum showing the difference the goblet cells number (arrows) and the severity of reaction in control group T1and treated group T2. PAS stain. 350 X.

Discussion

It was found that body weight gain was greater in broiler treated with EM than those consumed water free of EM at (P<0.05) significant level. Such increase may be attributed to enhancement of feed utilization efficiency as

reflected by improved in feed conversion ratio observed in the study. These findings conformed with those of (30-34) who showed that live body weight was significantly greater in all groups treated with different types of probiotics compared with the control group. The best weight gain in broiler treated with specific type of probiotics could be related to better digestibility of crude protein and crude fiber found by (31).

Feed intake was observed to be more in T2 group. There was more efficient utilization of feed in T2 indicating that EM provoked assimilation. It is worthy to mention that probiotics have great effect on the main physiological functions of the gastrointestinal tract, including digestion, absorption and propulsion as well as a reinforcement of the intestinal mucosal barrier against various deleterious agents (9).

It was found that feed conversion ratio was better in T2, referring the efficiency of EM to improve food utilization. These results were in agreement with those of (35,36). On the other hand, our results were in discrepancy with those of (37-39) who suggested no such effects of certain levels of probiotics on feed conversion ratio.

Concerning the relative weights of lymphoid organs (spleen, bursa and thymus) as an indicator of the immune system condition. Consequently no significant weight changes in these organs with slight decrease of the spleen weight was noted on the second group T2. The obtained results were in accordance with those of (40) who found no significant differences in the relative weights of the spleen in broilers fed diet containing certain probiotic compared with control groups fed routine ration. The Increase in the relative weight of bursa may possibly due to increase in the number of lymphocytes in the primary lymphoid organs (15).

On the other hand there was significant increase in lymphocyte percentage, which could be ascribed to the diathesis of the body to challenge the increased number of bacteria that administered through EM supplementation. That could be explained to the immune stimulatory activity of EM leading to increase lymphocytes percentage and enhance the immune system. Similar observations were recorded by (16). Also, probiotic supplementation revealed significant leukocytosis and lymphocytosis, and immune response in broilers. Probiotics as feed additives can be considered as immune potentiators due to their stimulation of immune system in broiler chicks (16).

The positive effect of feeding diet containing probiotics on the immune response could be due to their direct effect which may be related to stimulate the lymphatic tissue (41), Nonetheless, the indirect effect may occur via changing the normal microbial population flora of the lumen of gastrointestinal tract. (42) reported that the bursa of probiotic treated chickens showed an increase in the number of follicles with high plasma cell reaction in the medulla. Additionally, (43) suggested that some of these effects were mediated by cytokines secreted by immune system cells stimulated with probiotic bacteria. Notably, It has been concluded that EM is immune modulator in broilers since treated birds had significantly more serum antibodies than those served as control birds (19,44).

The intestinal mucosal architecture can reveal useful information on the intestinal function. The histological changes found in small intestines of the treated group probably had increased the intestinal surface area, facilitating the nutrient absorption to a greater extent and, thus boosted the promoting growth effect of certain probiotic (EM) supplementation. (45) found that longer villi in the ileum of adult male birds with slight improvement in feed efficiency after dietary addition of *Bacillus subtilis var. natto* and in broilers after addition *Enteroccocus faecium*. Moreover, the increase releasing of mucin by goblet cells inhibit the reproduction of harmful bacteria in the intestine (46) Increasing the villus height suggests an increased surface area capable of greater absorption of available nutrients.

The intestinal microbiota, epithelium, and immune system provide resistance to enteric pathogens, some species of non-pathogenic intestinal microbiota also communicate with the epithelium and immune system, modulating tissue physiology and ability to respond to infection (1,47).

The present study showed that EM had beneficial effect on the broiler performance, immunological and intestinal articherial parameters. However, further investigation is required for the explanation of EM mechanism in poultry.

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