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INFLUENCE OF A SYNBIOTIC ON SOME HEMATOLOGICAL AND IMMUNOLOGICAL PARAMETERS IN BROILERS

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ABSTRACT

One hundred and fifty one day old chicks were grouped into five groups each of 30 chicks and kept under strict hygienic and isolation measures. The first group was kept as control .The 2nd group was given synbiotic by a dose 1g/5 Litres of drinking water for the first 3 days of age and 2 days every week during the whole experimental period . The 3rd group was given synbiotic by a dose 2g/5 Litres of drinking water for the first 3 successive days and 2 days every week during the whole experimental period . The 4th group was given synbiotic by a dose of 1g/5 Litres of drinking water for the first 5 successive days and 2 days every week during the whole period of experiment while the 5th group was given synbiotic by a dose 2g /5 Littre of drinking water for the first 5 successive days and 2 days every week all the period of experiment . On the days 28 ,35 and 42 of age ,blood samples were collected from all groups for haematological and immunological examinations .The result of this experiment showed non significant changes in RBCs count and Hb in all groups. Total leucocytic count, basophil ,esinophil phagocytic assay were significantly increased in groups 3,4 and 5 beside significant increase in heterophil in group 5 only . Synbiotic administration elicited significant increase in body weight . The results also showed significant increase in bursal weight in all groups while thymus weight was increased in group 4 only It could be concluded that synbiotic administration produced a favorable effects on immunological parameters and growth performance of broiler chickens.

INTRODUCTION

In poultry industry, antibiotics are used worldwide to prevent poultry pathogens and disease so as to improve meat and egg production. However, the use of dietary antibiotics resulted in common problems such as development of drug-resistant bacteria ***(Sorum and Sunde, 2001)***, drug residues in the body of the birds ***(Burgat, 1999)***, and imbalance of normal microflora ***(Andremont, 2000).*** As a consequence, it has become necessary to develop alternatives using either beneficial micro-organsims or non digestible ingredients that enhance usefull microbial growth .A probiotic was defined as a live microbial feed supplement that beneficially affects the host animal by improving its microbial intestinal balance ***(Fuller, 1989)***.On the other hand, a prebiotic was defined as non digestible food ingredient that beneficially affects the host, selectively stimulating the growth or activity, or both, of one or a limited number of bacteria in the colon ***(Gibson and Roberfroid, 1995)***.. Efficacy of probiotics may be potentiated by several methods: the selection of more efficient strains, gene manipulation, the combination of several strains, and the combination of probiotics and synergistically acting components. This approach seems to be the best way of potentiating the efficacy of probiotics and is widely used in practice. A way of potentiating the efficacy of probiotic preparations may be the combination of both probiotics and prebiotics as synbiotics, which may be defined as a mixture of probiotics and prebiotics that beneficially affects the host by improving the survival and implantation of live

microbial dietary supplements in the gastrointestinal tract. Those effects are due to activating the metabolism of one or a limited number of health-promoting bacteria or by selectively stimulating their growth, which improved the welfare of the host, or both ***(Gibson and Roberfroid, 1995).*** In this respect, this study was designed to explore the possible effects of synbiotic on some hematological and immunological parameters and growth performance of broiler chickens.

MATERIAL AND METHODS

Synbiotic :-

- Mercopro+C is a synbiotic each kilogram contains

- Mannan oligosaccharides	50 g
- Ascorbic acid	100 g
- Enterococcus faecium DSM 7134"Live strain "	0.2gm (2 x10 9 cfu)
- Carrier :Medical lactose + colloidal silica	849.8 gm

Experimental design:-

One hundred and fifty one day old chicks were grouped into five groups each of 30 chicks were kept under strict hygienic and isolation measures

The first group was kept as control .The 2nd group was given synbiotic by a dose 1g/5 Litres of *drinking water for the first 3 successive days and 2 days ever*y week all the period of experiment. The 3rd group was given synbiotic by a dose 2g/5 Litres of *drinking*

*water for the first 3 successive days and 2 days ever*y week all the period of experiment . The 4th group was given synbiotic by a dose 1g/5 Litres of *drinking water for the first 5 successive days and 2 days ever*y week all the period of experiment . The 5th group given synbiotic by a dose 2g/5 Litres of *drinking water for the first 5 successive days and 2 days ever*y week all the period of experiment.

Vaccination:-

All groups were vaccinated against Newcastle disease with Hitchiner B1 at 7days and LaSota at 21 days of age. Also all groups were vaccinated against Gumboro disease with IBD at 13 day of age .

Sampling:-

Two blood samples were collected from all groups at the days 28,35,42 days of age. The first blood sample was collected on EDTD from wing vein for heamogram (RBCs count, Hb, PCV) and leukogram (TLC ,Differential leucocytic count). The 2nd blood sample was collected by heart puncture under strict aseptic condition on heparin for immunological examination.

Hematological examinations

Blood samples were coollectd from all groups for RBCs and TLC evaluation using Natt and Herrick solution as special avain diluent according to ***Coles (1986).*** Blood smear were stained with **Wright'**stain for differential leucocytic count and absolute values were calculated according to ***Schalm(1975).***

Immunological test:-

Phagocytic assay was estimated according to *(Woldenhiwet, 1987 and Woldenhiwet and Rowan 1990).*

Histomorophological study:

The whole intestinal tract was removed and before removal of the content, segments from the duodenum (the midpoint), and illeum (10 cm proximal to the illeocecal junction) were taken. Segments were fixed in 10% neutral buffered formalin solution and embedded in paraffin wax. All histological studies were performed on 5 μm sections (4 cross-sections for each sample), stained by haematoxylin and eosin, and examined by Olympus AX70 microscope (Olympus Cooperation, Tokyo, Japan) fitted with a digital video camera (Sony DXC-930P).

The total of the intact well-oriented, crypt-villus units were selected in triplicate for each intestinal cross-section for each sample. The criterion for villus selection was based on the presence of intact lamina propria. The villus length was measured from the villus tip to the villus-crypt junction, while crypt depth was defined as the depth of the invagination between 2 villi. The measurement was done with the stereological image software, Cast Image System (Version 2.3.1.3) (Visiopharm, Horsholm, Denmark). The mean villus heights and crypt depth from 10 birds were expressed as a mean villus height for each treatment group.

Statistical analysis:

The data obtained from thi investigation were statistically analysed by "T" test according to ***Fisher (1953).***

Feed Convertion rate:- was estimated according to equation FCR= $\frac{\text{Feed intake}}{\text{Weight gain}}$ ***Brown(2001).***

Results and discussion:

In the recent decades, deficiencies in feed formulation and management practices have been masked by the routine use of antibiotic growth promoters (AGP). However, the ban of AGP in Europe has driven the implementation of alternative strategies in order to maintain health and performance status and optimizing digestion in poultry production. Several feed additives have been used to manipulate microbial communities in the digestive tract. However, their efficacy has not always been proven and their modes of action require further research. The present study focused on the role and the efficacy of the synbiotic as potential modulators of gut health and growth performance in poultry production. Probiotics have been reported to improve microbial balance in the gastrointestinal tract through bacterial antagonisms, competitive exclusion and immune stimulation. Prebiotics which include non-digestible oligosaccharides may control or manipulate microbial composition and/or activity, thereby assisting to maintain a beneficial microflora that suppresses through different regulatory mechanisms the growth of pathogens. The combination of probiotics and prebiotics, also referred to as synbiotics, may improve the survival rate of probiotics during their passage through the digestive tract, thus contributing to the stabilisation and/or enhancement of the probiotic effects ***(Wageha et al .,2008).***

In this experiment the result of heamatology revealed non significant changes in RBCs count ,Hb,PCV at 28,35 and 42 days of age(table 1,2,3). Total leucocytic count(TLC) and defferential leucocytic count showed non significant changes in all groups but phagocytic assay (PA)showed significant increase in group 3and 4 at 28 days of age (table 4) .

At 35 days of age the result showed significant increase in heterophil count and significant decrease in PA in group 2 but there was significant decrease in TLC, lymphocytes, basophil and esinophil in group 3 and in group 4 the results showed significant decrease in monocyte count only (table 5). The results tabulated as follow, group 2 showed significant increase in basophil, esinophil counts and PA. Group 3 showed significant increase in TLC, lymphocytes, basophil, esinophil and PA.Group 4 revealed highly significant changes in TLC, lymphocytes, basophil, esinophil and PA. Group 5 showed highly significant increase in TLC,lymphocytes, monocyte basophil, esinophil, heterophil and PA (table 6).

The result also revealed significant increase in body weight of chickens of group 3 in 4^{th} ,5^{th} and 6^{th} week of age and significant increase in 4^{th} and 6^{th} week in group 4 but in group 5^{th} there was significant increase in the 6^{th} week only(table 7)and feed convertion rate (FCR) (table 8). The present study shows changes in the mucosal architecture in terms of increased ileal villus height to crypts depth in

birds fed with synbiotic supplemented diet(table 9). Interestingly, the weight of small intestine relative to BW showed a slight increase for birds fed either synbiotic or probiotic, which may explain the histological changes. It is assumed that an increased villus height is paralleled by an increased digestive and absorptive function of the intestine due to increased absorptive surface area, expression of brush border enzymes, and nutrient transport systems ***(Pluske et al., 1996)***. It is understood that greater villus height is an indicator that the function of intestinal villi is activated ***(Langhout et al., 1999; Yasar and Forbes, 1999; Shamoto and Yamauchi, 2000).*** This fact suggests that the villus function is activated after feeding of dietary symbiotic. Increased passive absorption of glucose and proline was reported in broiler chickens fed a probiotic containing lactobacilli, B. thermophilum, and E. faecium ***(Chichlowski et al., 2007)..*** The intestinal mucosal architecture can reveal useful information on the intestinal function. Increasing the villus height suggests an increased surface area capable of greater absorption of available nutrients. ***Caspary,1992. Samanya and Yamauchi 2002*** found that longer villi in the ileum of adult male layer with slight improvement in feed efficiency after dietary addition of Bacillus subtilis var. natto and in broilers after addition Enteroccocus faecium ***(samli etal.,2007)*** or Eubacterium sp. ***(Awad et.,2006).*** The depth of crypt and villus length of intestinal mucosa are measured at 42 days of age and the result showed significant increase in depth of crypt in all groups as compared to control. The histomorphological changes in the intestine of broiler chickens reported in the present study provide new information regarding

their potential for using synbiotics in broiler feed. Increasing the villus height suggests an increased surface area capable of greater absorption of available nutrients *(Caspary, 1992)* The intestinal microbiota plays a vital role in the normal nutritional, physiological, immunological, and protective functions of the host animals *(Vispo and Karasov 1997)*. The composition and metabolic activity of the intestinal microbiota can be influenced by the diet *(Netherwood et.,1999)*. There is a growing interest in the use of a variety of synbiotics to promote animal health by altering the intestinal microbial community. Although a marked proportion of the beneficial effects of synbiotic so far discussed seem to be attributable to certain epithelial function, there are relatively few experimental data to support this hypothesis.. The villus crypt is considered as the villus factory and deeper crypts indicate fast tissue turnover to permit renewal of the villus as needed in response to normal sloughing or inflammation from pathogens or their toxins and high demands for tissue *(Yason et al., 1987; Anonymous, 1999)*. The intestinal epithelial cells originating in the crypt migrate along the villus surface upward to the villus tip and are extruded into the intestinal lumen within 48 to 96 h *(Imondi and Bird, 1966; Potten, 1998)* improving absorpation and feed convertion rate (FCR). The weight of spleen ,bursa and thymus of all group of chickens were taken at 42 day of age and its result showed significant increase in bursal weight in all groups compared to control (table 10). However thymus weight was increased in group 4 only. It could be concluded that synbiotic administration produced a favorable effects on immunological parameters and performance of broiler chickens.

Table (1): Effect of synbiotic on RBCs,Hb, PCV at 28 days of age.

Parameters / Groups	RBCs $\times 10^6$/µl	Hb (g /dl)	PCV%
Group 1	2.05±0.08	8.4±0.33	26.3±0.89
Group 2	2.23±0.04	8.61±0.48	26.8±1.18
Group 3	1.96 ±0.06	9.2±0.38	27.4±0.99
Group 4	1.99±0.07	8.6±0.21	27.1±0.91
Group 5	1.97±0.06	8.4±0.37	27±0.85

Table (2): Effect of synbiotic on RBCs,Hb, PCV at 35 days of age.

Parameters / Groups	RBCs $\times 10^6$ /µl	Hb g/dl	PCV%
Group 1 (control)	1.96±0.1	8.4±0.65	26.9±1.27
Group 2	2.08±0.09	8.8±0.45	27.1±1.25
Group 3	1.99±0.1	9.9±0.33	29.8±0.29
Group 4	1.81±0.08	9.7±0.34	29.9±0.91
Group 5	1.9±0.12	9.4±0.27	28±0.5

Table (3): Effect of symbiotic on RBCs,Hb, PCV at 42 days of age.

Parameters / Groups	RBCs $\times 10^6$/µl	Hb g/dl	PCV%
Group 1	1.62±0.07	7.83±0.37	23.83±1.47
Group 2	1.58±0.01	7.83±0.71	25.5±1.24
Group 3	1.73±0.05	7.17±0.27	24.32±0.51
Group4	1.73±0.01	8.2±0.18	27.17±1.58
Group 5	1.73±0.04	7.67±0.37	28±1.69

Table (4): Effect of synbiotic on Total and differential leucocytic count and phagocytic assay at 28 days of age.

Parameter / group	TLCs ×10³ /µl	Lympho%	Mono %	Baso %	esino %	hetrophil %	Phagocytic assay
Group1	10.23±0.25	8.86±0.2	1.3±0.11	0.21±0.005	0.21±0.005	10.23±0.25	1.8±0.03
Group2	10.19±0.27	8.45±0.18	1.26±0.09	0.21±0.004	0.21±0.004	10.19±0.27	2.03±0.1
Group3	10.8±0.21	8.86±0.14	1.42±0.05	0.22±0.003	0.22±0.003	10. 8±0.21	2.04±0.07
Group4	10.59±0.24	8.74±0.19	1.35±0.08	0.21±0.004	0.21±0.004	10.59±0.24	2.05±0.09
Group5	10.31±0.37	9.2±0.24	1.27±0.08	0.22±0.003	0.22±0.003	10.31±0.37	1.88±0.11

Table (5): Effect of synbiotic on Total and differential leucocytic count and phagocytic assay at 35 days of age.

Parameter / group	TLCs ×10³ /µl	Lympho%	Mono %	Baso %	esino %	hetrophil %	Phagocytic assay
Group1control	20.4±0.4	10.99±0.37	1.41±0.07	0.21±0.004	0.21±0.004	8.35±0.08	2.01±0.04
Group2	20.6±0.11	9.64±0.1	1.28±0.09	0.21±0.003	0.21±0.003	9.27±0.23**	1.89±0.03*
Group3	18.8±0.34*	9.25±0.21*	1.21±0.06	0.19±0.004*	0.19±0.004*	7.97±0.19	2.01±0.11
Group4	19.2±0.91	10.2±0.4	1.14±0.004**	0.19±0.009	0.19±0.009	7.48±0.55	1.94±0.09
Group5	20.7±0.22	10.68±0.15	1.33±0.1	0.212±0.002	0.212±0.002	8.28±0.12	1.94±0.07

*P ≤ 0.05 ** P≤ 0.01

Table (6): Effect of synbiotic on Total and differential leucocytic count and phagocytic assay at 42 days of age.

Parameter / group	TLCs ×10³ /µl	Lympho%	Mono %	Baso %	esino %	hetrophil %	Phagocytic assay
Group1control	19.5±0.31	7.28±0.17	1.28±0.17	1.23±0.02	0.19±0.002	10.6±0.21	1.42±0.03
Group2	20.33±0.27	7.46±0.29	1.29±0.03	0.20±0.002**	0.20±0.002**	11.18±0.22	1.7±0.025***
Group3	21±0.18**	8.75±0.04***	1.26±0.06	0.213±0.003**	0.21±0.003**	10.58±0.2	1.68±0.03***
Group4	21±0.18**	8.13±0.3*	1.33±0,04	0.213±0.003**	0.213±0.003**	11.13±0.26	1.75±0.03***
Group5	21±0.18**	7.84±0.04*	1.33±0.1	1.4±0.05*	0.213±0.002***	11.34±0.1*	1.68±0.04***

*P ≤ 0.05 ** P≤ 0.01 *** P≤ 0.001

Table (7): Effect of synbiotic on weekly body weight of chickens in grams.

Weeks / Groups	1st week	2nd week	3rd week	4th week	5th week	6th week
Group 1	139.2±5.9	420±28.5	850±39.5	1380±45.4	1870±41.83	2280±8202
Group 2	134±4.3	450±30.6	910±44.7	1480±62.8	2060±103.7	2540±136.2
Group 3	136.4±4.6	390±20.9	810±32.6	1540±*44.7	2160±*97.5	2740±***32.6
Group 4	137.4±4.38	430±37.9	820±37.9	1520±*37.9	2100±127.5	2800±***46.8
Group 5	142.4±5.9	395±5.6	805±37.9	1410±45.2	1850±90.1	2610±**32.6

*P ≤ 0.05 ** P≤ 0.01 *** P≤ 0.001

Table (8): Effect of synbiotic on feed convertion rate (FCR) at 42 days of age.

Parameter / Group	Feed convertion rate (FCR)
Group 1	2.33
Group 2	1.68
Group 3	1.9
Group 4	1.79
Group 5	1.97

Table (9): Effect of synbiotic on depth and length of villi and depth of crypt at 42 day of age.

Parameters / groups	Depth (µml)	Length (µml)
Group 1	7.65±0.83	3.91±0.37
Group 2	17.17±1.3***	5.31±0.29*
Group 3	11.78±0.26**	5.36±0.13**
Group 4	15.96±0.43***	5.57±0.32**
Group 5	14.73±0.5***	6.35±0.59**

*P ≤ 0.05 ** P≤ 0.01 *** P≤ 0.001

Table (10): Effect of synbiotic on (spleen ,bursa and thymus) weight at 42 days of age.

Organs / Groups	Spleen (g)	Bursa (g)	Thymus (g)
Group 1	0. 11±0.01	0. 16±0. 08	0. 5±0.0 1
Group 2	0. 13±0.01	0. 09±**0. 01	0. 6±0.01
Group 3	015±0. 03	0.01±**0.00004	0. 6±0. 04
Group 4	0. 14±0. 02	0. 09±*0. 02	0. 3±**0. 04
Group 5	0. 14±0.01	0. 09±*0. 02	0. 5±0.01

*P ≤ 0.05 ** P≤ 0.01 *** P≤ 0.001

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تأثير إعطاء سينبيوتيك علي صورة الدم والمناعة والكفاءة الانتاجية في دجاج التسمين

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معهد بحوث صحة الحيوان – طنطا

أجريت هذه التجربة على عدد 150 كتكوت عمر يوم قسمت إلى 5مجموعات متساوية كل مجموعة 30 كتكوت وضعت تحت ظروف صحية وبيئية مناسبة. المجموعة الأولى اعتبرت مجموعة ضابطة والمجموعة الثانية أعطيت السنبيوتك بجرعة 1جم/5 لتر ماء شرب لمدة الثلا ثة أيام الأولى من عمر الطيور ثم يعطى لمدة يومين كل أسبوع حتى نهاية التجربة المجموعة الثالثة أعطيت مستحضرالسينبيوتك بجرعة 2 جم /5لتر ماء شرب خلال الثلاثة أيام الأولى من عمر الطيور ثم لمدة يومين متتاليين كل أسبوع حتى نهاية التجربة . المجموعة الرابعة أعطيت نفس المستحضر بجرعة 1 جم /5 لتر ماء شرب لمدة الخمسة أيام الأولى من عمر الطيور ثم لمدة يومين متتاليين كل أسبوع حتى النهاية .المجموعة الخامسة أعطيت المستحضر بجرعة 2 جم /5 لتر ماء شرب لمدة الخمسة أيام الأولى من عمر الطيور ثم لمدة يومين متتاليين كل أسبوع حتى نهاية التجربة. عند بلوغ الطيور عمر 28–35–42 تم تجميع عينات دم من جميع المجموعات لإجراء الإختبارات الهيماتولوجية و المناعية

ولقد أوضحت النتائج أنه لايوجد تغيير معنوى في عدد كرات الدم الحمراء ونسبة الهيموجلوبين ولكن وجد تغير معنوى فى العد الكلى والنوعى لخلاي الدم البيضاء وزيادة معنوية نشاط الخلايا الأكولة.

كذلك وجد تغير معنوى فى وزن الجسم الحى فى جميع المجموعات فى الأسبوع السادس وفى نهاية التجربة وجد أن هناك تغير معنوى فى وزن غدة فابريشيس فى جميع المجموعات. والخلاصة من هذا البحث أنه يمكن استخدام مستحضر السينبيوتك فى الدواجن حيث أنه محفز قوى للمناعة ويزيد من من معدل الأداء والكفاءة الإنتاجية للطيور.