Effect Of Bioplus Probiotic And Celmanax Prebiotic On Growth Rate And Immunity In Broiler Chickens

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ABSTRACT

Three hundred, one day old broiler chicks were divided into four equal groups (A, B, C and D). Group A control group (non medicated). Birds in group B was treated with probiotic (Bioples 2B) 1g/Kg feed for 6 weeks. Group C was treated with prebiotic (Celmanax) 0.5 ml/litre for 3 days before La Sota vaccination and 3 days post vaccination while group D was vaccinated with ND La Sota vaccine only. Broilers in all groups were weighted weekly, body gain and feed conversion rate were estimated. The obtained results revealed a significant ($p \leq 0.05$) improvement of body weight, body weight gain and feed conversion rate of broiler treated probiotic or prebiotic. A significant decrease in *E. coli* isolation from chicks treated with probiotic and prebiotic with high percentage of phagocytosis, high of ND HI titer and high percentage of lymphocyte was detected. The hematological pictures were improved at certain level of erthrogram and lymphocyte. It could be concluded that body weight gain and feed conversion rate as well as cellular and humeral immunity were improved by probiotic and prebiotic administration .

INTRODUCTION

The use of probiotics for growth promotion of poultry as substitute for antibiotics to avoid development of drug resistant microrganism and drug residues in poultry products has become a subject of interest. Probiotics, are a live microbial feed supplement such as bacteria or yeast which have been shown to be responsible for improved growth rate, feed conversion, fertility and hatchabilithy in poultry *(1-3)*. Probtotics act by increasing normal gut flora on the expense of pathogenic organism *(4)*.

Saccharomyces cerevisiae yeast cell wall (YCW) components have been used in animal feeding since the last decade *(5,6)*.

Their inclusion in broiler diets has resulted in improvements of animal productivity, which was attributed to physiological effects on intestinal digestive mucosa *(7-9)*.

In the digestive tract of animals, MOS present in YCW could act as high-affinity ligands, with the potential benefit of offering a competitive binding site for pathogenic bacteria mannose-specific type-1 fimbriae *(10)*.

The 3-1, 3/1, 6-glucans present in YCW acts also recognized as an immune modulator substance in animals and humans *(11,12)*; thus, dietary YCW might exert some benefits on the immune system of intestinal mucosa *(13)*.

MATERIAL AND METHODS

(A) Material

(1) Birds

Three hundred broiler chicks, one day old obtained from El-Baramoy Company from Sharkia province were used in this experiment.

(2) Probiotics (Bioplus 2B)

Highly concentrated probiotics *Bacillus licheniformis* DSM5749 and *Bacillus subtilis* DSM 5750 in ratio 1:1 produced by Biochem Co. were used.

(3) Prebiotics (Celmanex)

Obtained of B-glucan and mannooligo saccharide in ratio 1:1 approximately produced by Vicor Co were used.

(4) Media

Nutrient agar (Oxoid) and MacConky agar (Oxoid) were used.

(5) Nitro- blue tetrazolium (NBT) dye

NBT dye was obtained from Sigma Company (List No. 3780-34-0).

(6) Commercial Wright stain

It was obtained from Sigma Co. and used in heterophil function and leuckocytic count.

(7) Vaccines

(a) La Sota ND vaccine (Intervet).
(b) Hitchiner B1 vaccine (Intervet).
(c) Gumboro vaccine (Intervet).

A total three hundred, one day broiler chicks were brooded on deep litter and fed on balanced ration. Broilers were kept under good hygienic conditions. The birds were divided randomly into four equal groups. (A, B, C and D) each 75 chicks. All groups received Hitchinar vaccine at 6 days old age and Gumboro vaccine at 14 day age.

Group (A): was as control one (water was free from any growth promoter and not vaccinated.

Group (B): was fed on ration containing 1g/kg probiotic (Bioplus 2B) and continued up to the end of the experiment and vaccinated with La Sota at 20 days old.

Group (C): was fed on prebiotic (Celmanax), 0.5 ml/liter, 3 days before and after each vaccination and vaccinated with Lasota at 20 days old.

Group (D): was without treatment and vaccinated with Lasota at 20 day old.

Groups and treatment are shown in Table

Whole blood on heparin was collected form five birds in each group on 3 days pre-vaccination and post-vaccination with Lasota for total erythrocytic, differential leucocytic counts and herterophil function.

Serum sample were collected weekly for determination of ALT and AST.

Body weight was determined for all chicks from one day one old age till the end of experiment (8 weeks). At the end experiment two birds of each group were slaughtered for bacteriological isolation from internal organs.

(B) Methods

Nitro blue tetrazolium test (NBT): Heterophils stimulated function; ***(14)***, total erythrocytic count ***(15)***, differential leuckocytic count: the slide and cover slip method was used ***(16)***, haemagglutination inhibition (HI) test: The beta procedure of HI test was employed using microtitre plates***(17)*** and determination of ALT and AST ***(18)*** were carried out. Statistical analysis of data was carried out ***(19)***.

Table 1. Exp. design

Group	No.	Probiotic	Prebiotic	Lasota vaccine
Group (A) control (non-treated)	75	-	-	-
Group (B)	75	+	-	+
Group (C)	75	-	+	+
Group (D)	75	-	-	+

RESULTS

Effect of probiotic and prebiotic on bird performance

The obtained results revealed a significant ($P \leq 0.05$) improvement in live body weight, body weight gain and feed conversion of chicks fed on ration containing 1 gm/kg ration of probiotic and chicks treated with 0.5 ml prebiotic/ litre in drinking water compared with control groups (A and D) (Table 2).

Effect of probiotic and prebiotic on the phagocytic activity of heterophils (%):

As shown in Table 3, the heterophil positive formazan % in the use probiotic and prebiotic B

and D were significantly increased ($p \leq 0.05$) than the non treated groups at 3 and 7 days post treatment.

The effect of probiotic and prebiotic on total erythrocytic count

The obtained data revealed a significant increase ($p \leq 0.05$) in total erythrocytic count in groups B and C in comparison with the non treated groups.

Effect of probiotics and prebiotic on differential leuckocytic count

As shown in Table 4, the lymphocyte percentage on 3rd day and 7th day post treatment group B was significantly higher than in non treated with pro or prebiotic whereas the heterophil was significantly lower in probiotic and prebiotic treated B and C than untreated group. Eosinophils, basophils and monocyte percentage showed non significant differences between all groups.

Effect of probiotic and prebiotic on the response against La Sota Newcastle disease vaccine:

As shown in Table 4, the geometric means of HI titers were significantly increased ($p \leq 0.05$) 7 days post vaccination with La Sota vaccine in groups B and C, treated with pro and prebiotic. Similar results were obtained after 14 and 21 day post vaccination.

Effect of pro and prebiotic on determination of ALT, AST and uric acid

Pro, and prebiotic had no significant effect on serum ALT, AST and uric acid in treated broiler (Table 5).

Table 2. Effect of probiotic (Bioplus) and prebiotic (Celmanex) for six weeks on body gain and feed consumption (n = 10)

Group	Initial body weight gain (gm)	3 weeks live body weight (gm)	3 weeks body gain (gm)	6 weeks live body weight (gm)	6 weeks body gain (gm)	Feed conversion (FCR)
Group (A)	42 ± 3	708 ± 21^{c}	566 ± 20^{c}	1889 ± 100^{c}	1847 ± 81^{c}	1.025
Group (B)	43 ± 3	725 ± 20^{ab}	583 ± 23^{a}	2125 ± 80^{a}	2082± 60a	1.92
Group (C)	44 ± 3	740 ± 31^{a}	596 ± 15^{a}	2239 ± 55^{a}	2195± 61a	1.88
Group (D)	43 ± 2	720 ± 10^{b}	577 ± 2^{b}	2039 ± 101^{b}	1996 ± 97^{b}	1.22

Different letters in the same row indicated significant change ($P \leq 0.05$)

Table 3. Effect of different of probiotic and prebiotic on the phagocytic activity of heterophil%

Group	Heterophil phagocytic activity				
	3 days pre Lasota vaccine	3 days post Lasota vaccine	7 days post Lasota vaccine	14 days post Lasota vaccine	21 days post Lasota vaccine
Group (A)	24	25^{d}	26^{d}	25^{a}	26^{d}
Group (B)	52	76^{a}	74.2^{a}	70^{a}	69.2^{a}
Group (C)	24	78.1^{a}	75.2^{a}	72^{a}	70^{a}
Group (D)	23	35^{c}	36^{c}	34^{c}	34^{c}

Different letters in the same row indicated significant change ($P \leq 0.05$)

Table 4. Effect of different of probiotic and prebiotic on the differential leukocytic count in different treatments of broiler chicks

Group	*3 days post-vaccination*				
	Lymphocyte%	**Heterophil%**	**Basophil%**	**Eosinophil%**	**Monocyte%**
Group (A)	40.2^{b}	52^{a}	2	2	5
Group (B)	51.5^{a}	40.7^{b}	1.6	1.4	5
Group (C)	51.5^{a}	39.6^{b}	2	1	5
Group (D)	37.2^{b}	52^{a}	2.8	2	6
	7 days post-vaccination				
Group (A)	43^{b}	46^{a}	3	2	6
Group (B)	50^{a}	40^{b}	1.2	2	6
Group (C)	51^{a}	40^{b}	2	3	6
Group (D)	41^{b}	45^{a}	3	3	6
	21 days post-vaccination				
Group (A)	47^{b}	42	2	3	0
Group (B)	52^{a}	40	1.7	-	6
Group (C)	50^{a}	39	1.7	1	6
Group (D)	42^{b}	44	3	3	6

Table 5. Effect of different of probiotic and prebiotic on mean HI antibody response against NDV in sera of Exp. chicks

Group	*Geometric mean HI titres*			
	Before Lasota vaccine	**7^{th} post Lasota vaccine**	**21^{th} post Lasota vaccine**	**28^{th} post Lasota vaccine**
Group (A)	Zero	Zero	Zero	Zero
Group (B)	Zero	3.6^{a}	6.2^{a}	8.2^{a}
Group (C)	Zero	3.8^{a}	6.4^{a}	8.1^{a}
Group (D)	Zero	2.1^{b}	3.5^{c}	4.6^{c}

Table 6. Effect of different of probiotic and prebiotic on some biochemical values of 4 weeks old broilrs

Parameter / *Group*	**ALT**	**AST**	**Uric acid**
Group (A)	7.8 ± 0.12	9.7 ± 0.13	39 ± 0.07
Group (B)	7.9 ± 0.14	9.2 ± 0.14	42 ± 0.08
Group ©	7.8 ± 0.16	9.3 ± 0.16	41 ± 0.06
Group (D)	8.1 ± 0.12	9.1 ± 0.12	42 ± 0.02

DISCUSSION

The obtained result revealed that probiotic improved body weight gain and feed conversion of broilers. The same conclusion was suggested by previous reports which showed that supplementation of broiler chickens with probiotic for six weeks resulted in higher body weight and feed conversion efficiency than the non treated birds *(20,21)*.

Hopper and Mawer (22) noticed that probiotic improve the feed conversion rate in growing broiler. The growth promoting effect of the present probiotic product may be due to it's microbial constituent (*Bacillum Subtilis*) which produce natural lactic acid that helps in maintaining an optimum low pH to inhibit the growth of undesirable bacteria leading to optimal engeme activity***(23)***.

Prebiotic treated broilers showed a significant ($p \leq 0.05$) increase in body weight gain and feed conversion. The positive effects of prebiotic on growth might be attributed to induction of changes in the population and metabolic characteristic of gastrointestinal bacteria[24]. The same conclusion has been reported in previous study which showed that prebiotic in feed had significant effect on broiler performance ***(25)***.

Body weight gain and food conversion were improved in probiotic and prebiotic treated group. Similar results were detected in broiler chicken, when probiotic and prebiotie were used as growth promoters***(26)***.

The heterophil percentage in blood from chicks treated with probiotic and prebiotic were significantly higher ($p \leq 0.05$) than the non treated group (A and D), these findings proved previous studies ***(27, 28)*** which demonstrated that supplementation of broiler chickens with prebiotic resulting of phagocytic ability up to 18 to 25% and bactericidal killing.

A significant ($P \leq 0.05$) increase in total erythrocytic count and the hematological picture of lymphocytes of broilers received probiotic and prebiotic was recorded. This increase could explain on the base of improved bio-availability of essential nutrients ***(29)*** and increase of bacterial population enhancing Vit. B synthesis and/or absorption ***(30)***. This obtained result was reinforced with those recorded previously by ***(31)*** who detected improved erythrogram in chickens received probiotic.

Higher antibody response was observed in probiotic and prebiotic groups post vaccination with ND La Sota vaccine. This effect on antibody titers may be due to the influence of probiotic on immune system, and the improvement intestinal absorption of some nutrients such as Zn, Cu and Se. These obtained results were recorded previously by several authers ***(32,33)*** who reported improvement in antibody titers against IBDV and Newcastle disease virus in chicken fed on prebiotics and probiotics.

Results of sera analysis revealed non significant changes in activities of AST, ALT, and uric acid level which denoted neither hepatotoxic nor nephrotoxic effect on broilers, similar findings were recorded when using biogen , dry yeast ***(34),*** or other probiotic ***(35)*** for broiler chickens , had been resulted from the use of Bioplus or celmanax.

The isolation of *E. coli* from broiler chicks were 20% in non treated groups while in groups treated with probiotic and prebiotic, the isolation was 5%.

Supplementation of chickens with probiotic and prebiotic resulted in increase of anaerobic bacteria that were associated with decrease in number of facultative anaerobe including salmonella and coliforms ***(28, 36)***.

Therefore, it could be concluded that using probiotic and prebiotic for broiler chickens improve the body weight gain and feed conversion rate as well as increase of cellular and humoral immunity.

REFERENCES

1.Dilworth, B C and Day, E J (1978): Lactobacillus cultures in broiler diets. Poult. Sci. 57: 1101.

2.Jin, L Z , Abdullah, N and Jalaluelin, S (2000) Digestive and bacterial enzyme activities in broiler fed diets supplemented with lactobacillus cultures. Poult. Sci, 79: 886 981.

3.Shehata, M , Askar, A , Salwa, G K and Hassan, I (2004): Effect of flavomyein and some probiotic promotors on productive and reproductive troits of Mondar ah and salam Hens J. Agri. Sci. Mansoura. Univ. 24 (2): 613 – 629.

4.Kumaraj, R , Narahari D , Srinivasan, G and Rajini, R A (1997): Growth

performance and carcass characteristics by Japanese quail supplemented with probiotics. Indian J. of Poult. Sci.: 32 (1): 106 – 107.

5.Hooge (2004): Comparing antibiotic supplemented positive control oliets versus Mos diets fed at level stated during the entire study period. Int. J. Poult. Sci. 3 (3): 163 – 174.

6. Rosen, G D (2007): Halo-analysis of the efficacy of Biol.-Mos® in Broiler nutrition. Br. Poult. Sci. 48:21-26.

7. Santin, E , Maiorka, A and Macari, M (2001): Performance and intestinal mucosa development of broilers chickens fed diets containing *Saccharomyces cerevisiaes* cell wall. J. Appl. Poult. Res. 10: 236 - 244.

8. Zhang, A W , Lee, B D , Lee, S K , Lee, K W , An, G H , Song, K B and Lee, C H (2005): Effects of yeast *(Saccharomyces cerevisiae)* cell components on growth performance, meat quality and the ileal mucosa development of broilers chicks. Poult. Sci. 84: 1015 – 1021.

9. Baurhoo, B , Phillip, L and Ruiz-Feria, C A (2007): Effects of purified lignin and mannan oligosaccharides on intestinal integrity and microbial populations in ceca and litter of broiler chickens. Poult. Sci. 86:1070-1078.

10.Spring, P C , Wenk, K and Newman (2000): The effect of dietary Mos on cecal parameter and concentration of enteric bacteria in the ceca of Salmonella – challenged broiler chicks. Poult. Sci., 79: 205-211.

11.Abel, G and Czop, J K (1992): Stimulation of human monocyte β-glucan receptors by glucan particles induces production of TNF-α and IL-1. Int. J. Immunopharumacol. 14:1363-1373.

12.Raa, J (2003): The use of immune-stimulant to enhance disease resistance and growth performance of fish and shrimp. Pages 67-75 in Proc. XL Congreso Nacional de AMENA y I Congreso Latino-Americano de Nutricion Animal, Cancun, Qroo (Mexico). Asociacion Mexicana de Especialistas en Nutricion Animal, A.C. Queretaro, Qro. Mexico.

13.Ferket, P R , Parks, C W and Grimes, J L (2002): Benefits of dietary antibiotic and mannanoligosaccharide supplementation for poultry. In Proc. Multi-State Poultry Feeding and Nutrition Conference, Indianapolis, IN. http:// ag.ansc. purdue.edu/ poultry/ multistate/ Proceedings. Multi-State %20 Meeting Ferket. pdf Accessed June 2006.

14.Budny, J L , Grotke, N C and MacGilliary, M (1976): Nitro Blue Tetrazolium test. Med., 76: 877 - 881.

*15.***Wintrobe, M M (1976):** Clinical hematology (6th Ed.) Lea and Ferbirger, Philadelphia.

16.Coles, E H (1986): Veterinary Clinical Pathology. 4th ed., W.B. Saunders Company, Philadelphia and London.

17.Allan, W H and Gough, R E (1974): Standard haemagglutination inhibition test for ND. Vet. Rec., 95 (7): 120 - 123.

18.Reitman, S and Frankel, S (1957): A colorimetric method for determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. Am. J. Clin. Path., 28: 56 – 60.

19.Snedecor, G W and Cochran, W G (1967): Statistical method, 6lh ed. Iowa State Univ. Press. Ames Iowa.

20.Brroska, K , Grzybowski, R and Piezka, M (1999): Nutritive efficiency of selected probiotic microorganism in chicken broilers. Roeznik, Naukowe, Zootechniki; 26 (4): 291 - 301.

21.Kumaraj, R , Narahari, D , Srinivasan, G and Rajini, R A (1997): Growth performance and carcass characteristics in Japanese quail supplemented with probiotics. Indian J. of Poult. Sci. 32 (1): 106-107.

22.Hopper, S A and Mawer (1988): *Salmonella enteritidis* in a commercial layer flock. Vet. Rec. 123-350.

23.Homma, H and Ham Oaka, T (1998): Effect of commercial probiotics on the performance and some serum components in the Japanese quial . Proc. 8th world Conf. On Animal production. Soul National Univ. Seoul Korea 1: 794.

24.Buddington, K K , Donahoo, J B and Buddington, R K (2002): Dietary oligo fructose and inulin protect mice from enteric and systemic pathogens and tumor inducers. J. Nutr., 132: 472 - 477.

25.Hooge (2004): Comparing antibiotic supplemented positive control oliets versus Mos diets fed at level stated during the entire study periodc. Int. J. Poult. Sci. 3 (3): 163 – 174.

26.Aziz, M A , Zahara, A A and El-Dakroury, M F (2003): A comparative study on the antibiotics as growth promoters in broiler. The 46th Annual Conference of the Egyptian Society of Pharmacology & Experimental Therapeutic, Zagazig, Egypt. p.6.

27.Guo, Y , Ali, E A and Qureshi, M A (2003): The influence of B-glucan on immune responses in broiler chicks. Immunopharmacol. Immunotoxicol., 3: 461 – 472.

28.Lowery, V K , Farnell, M B , Ferro, P J , Swaggerty, C L , Bahl, A and Kogut, M H (2005): Purified B-glucan as an abiotic feed additive up-regulates the innate immune response in immature chickens against *Salmonella enterica* serovar Enteritidis. Int. J. Food Microbiol. 98: 309-318.

29.Haddadin, M S , Abd Ulrahim, S M , Odet Allah, N H and Robinso, R K (1997): A proposed protocol for checking the suitability of *Lactobacillus acidophilus* cultures of or use during feeding trails with chickens. Topical Science: 37: 16-20.

30. ***Jenkins, D J , Kendall, C W and Vuksan, V (1999):*** Inulin, oligo fructose and Intestinal function. J. Nutr.: 129:1431-1433.

31.El-Nabtity, S M , EL-Sayed, N A A , Gchan, N A and Gad (2000): Efficacy of probiotic and prebiotic in vaccinated chicken. 2nd Congress of Regional Lab A.H.R.T.

32.Cotter, P F , Malzone, B and Sefton, N (2000): Modulation of humoral immunity commercial laying hens by dietary prebiotic. Poultry Sci., 79 (Suppli) (Abstr.).

33.Cotter, B F , Sefton, A and Lilburn (2002): Manipulating the immune of layer and broilers. Novel application for Bio Mos Biotechnology in the feed and food industries. Pages 21-25 in Nutritional.

34.EL- Banna, R, Azza, M , Kamla , M and El Saied (2001) Effect of Biogen and dry yeast on performance of broiler chickens. J. Egypt. Vet Med Ass; 61 (6): 123 – 136.

35.Shoieb, I I and Madian, A (2002): A study effect on the of feeding diets containing probiotic on growth performance, intestinal flora and hematological picture of broiler chickens . Assiut Vet . Med . J, 47 (44): 110 – 128.

36.Spring, P C , Wenk, K and Newman (2000): The effect of dietary Mos on cecal parameter and concentration of enteric bacteria in the ceca of Salmonella – challenged broiler chicks. Poult. Sci., 79: 205-211.

الملخص العربي

تأثير البروبيوتك بيوبلس والبريبيوتك سامانيكس علي معدل النمو والمناعة في بداري التسمين

حسام علام
معهد بحوث صحة الحيوان بالزقازيق

تم تقسيم ٤٠٠ كتكوت تسمين أبيض عمر يوم إلي أربعة مجاميع (أ ، ب ، ج ، د) وفقاً لنوع المعاملة، (أ) مجموعة ضابطة ، (ب) مجموعة تم تغذيتها علي ١جم بروبيوتك/كجم عليقة لمدة ستة أسابيع والمجموعة (ج) تم إضافة البريبيوتك بمقدار ٠,٥ جم/لتر قبل ٣ أيام من تحصين اللاسوتا وثلاثة أيام بعد التحصين باللاسوتا والمجموعة (د) يتم تحصينها فقط باللاسوتا.

تم وزن الكتاكيت أسبوعياً وحساب متوسط استهلاك العليقة ومعدل التحويل الغذائي ، وتم أخذ عينات من الدم وتم فصل مصل الدم لدراسة بعض التغيرات الكيميائية وقياس مستوي المناعة. وأظهرت النتائج ما يلي:

(١) حدوث زيادة معنوية في وزن الجسم المكتسب في الطيور المعالجة بالبريبيوتك البيوليس أو البروبيوتك سلمانيكس.

(٢) حدوث كفائة خلايا الهتروفيل وزيادة في النسبة المئوية للخلايا الليمفاوية في الطيور المعالجة بالبريبيوتك أو البروبيوتك.

(٣) حدوث نقص في نسبة العزل لميكروب الإيشيريشيا كولاي مع زيادة في قياس مستوي المناعة في المجاميع المعالجة مع ظهور تحسن في عدد كرات الدم الحمراء.

ويستنتج من هذه الدراسة أن البريبيوتك والبروبيوتك يساعد علي رفع مستوي المناعة في الطيور وزيادة في الوزن المكتسب.