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SOME BIOCHEMICAL AND IMMUNOLOGICAL STUDIES OF PROBIOTICS (BIONUTRA&DINAFERM) IN BROILERS

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ABSTRACT

The aim of the present work was to study some biochemical and immunological effects of two probiotics namely Bio nutra and Dinaferm on Hubbard broiler chickens. Birds were divided into three equal groups, each of 50 chicks. The 1st .group received only basal ration (control). The 2nd group received bionutra(1kg/ton) and the 3rd group received dinaferm (1kg/ton). All groups were routinely vaccinated against Newcastle and were challenged with a virulent strain of ND at the 35th day of age. The number of dead birds were recorded daily till the end of the experiment. Birds were sacrificed at 45 days and blood samples were collected from chickens. Moreover relative weights of bursa, spleen and thymus were also recorded. It was observed that administration of probiotics has no significant effects on the levels of serum AST, ALT, createnine and uric acid. Moreover, there was insignificant changes in the basophils ,eosinophils ,heterophils ,monocytes and lymphocytes percentagein treated groups. On the other hand, probiotics induced a significant increase of the number of RBCS and WBCS. Total serum protein and HI titer increased also in treated groups. Additionally, Bio- nutra increased the relative weights of bursa and thymus while Dinaferm increased the relative weight of bursa only .

INTRODUCTION

Medicated feeding is considered as one of the most effective means for growth promotion in poultry . Antibiotics and probiotics are effective in prevention of many animal diseases, decreasing mortality and playing important roles as growth promotors. The use of antibiotics as routine feed additives has been banned in some countries because of public concern or possible antibiotic residual effects and the development of drug resistant microorganisms in humans ***(Jin et al., 1997).***

The use of probiotics is one of the new alternatives for sustaining the growth of the poultry industry using recent developments in biotechnology ***(Kulkarian et al., 1997)***. They are 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 ***(Dilworth and Day, 1978, Jin et al., 2000 and Shehata et al., 2004).*** probiotics regulate microbial environment of intestines, decrease digestive disturbances, inhibit pathogenic intestinal microorganisms and improve feed conversion efficiency ***(Windschitl, 1992).***

This work was conducted to investigate some biochemical and immunological effects of probiotics (Bionutra&Dinaferm) on broilers.

MATERIALS AND METHODS

1- <u>probiotics</u>

a- Bionutra® (Dinatic American Company) contains 10^9 colony of yeast Sacharomyces cervisae per gram.

b- Dinaferm® (Ameco Bio-Company) contains Sacharomyces cervisae 220 billion CFU ,Asperigllus oryzae 15 gram, Lactobacillus acidophilus 1100 million CFU, Streptococcus faecium770 million CFU, Lactobacillus plantourum 330770 million CFU and Bacillus subtilius 1 billion CFU per kg.

2- Newcastle Challenge strain:

A local velogenic viscerotropic strain of New castle Disease Virus (NDV), was obtained from the Veterinary institute for biological products and vaccines (Abbasia,Cairo).

3- Experimental design

A total of 150 one day old Hubbard chicks were used in the present study. Prophylactic vaccinations were done against ND .The chicks were divided into three equal groups, each of 50 chick.. Group (1) was used as a control group. Group (2) received dinaferm at a level of 1kg/ton. Group (3) received bionutra at a level of 1kg/ton. All groups were challenged with a virulent strain of Newcastle at the 35^{th} day**.**

4- Sampling

Birds were sacrificed at 45 days. Blood samples were collected from chickens. Each blood sample was subdivided into two sub samples. The first sub sample was collected in clean dry Eppindorf tubes containing EDTA (1mg/ml fresh blood , ***Schalm et al .,1975***) for blood cell counts. The second sub sample was collected in a clean sterilized centrifuge tube without anticoagulant then allowed to coagulate. Serum was collect and stored at -20°c for other haematological and immunological studies.

5- Laboratory examinations

Total erythrocytic and leukocytic counts were done according to ***(Natt and Herrick , 1952)***. Blood film was prepared and stained with Giemsa stain for differential leukocytic count according to ***schalm et al., (1975).***

Determination of serum total protein was determined by Biuret test according to ***Weichselbaun (1946)***, albumin by ***Drupt, (1974)*** and serum globulin were calculated as the difference between serum total protein and albumin. HI titer determined according to ***Takatsy (1956).*** Serum AST, and ALT were measured according to ***(Reitman and Frankel, 1957),*** Createnine ***(Seeling and Wust,1969)*** and uric acid ***(Baraham and Trinder, 1972).***

6- Relative weights of lymphiod organs

The lymphoid organs (thymus, bursa and spleen)were carefully separated and weighted. Each organ relative weight was then determined.

7- Statistical analysis.

All obtained data were recorded and analyzed statistically by ***Snedecor and Cochran, (1967).***

RESULTS

The administration of dinaferm and bio nutra resulted in a significant increase of the number of RBCS and WBCS. Also total serum protein and HI titer increased in treated groups. There is no significant differences in serum AST, ALT, createnine and uric acid between treated groups and control one . The results of the study were illustrated in the following tables.

Table (1): The effect of probiotics on blood cell count.

parameters / Treatments	Total RBCs count (10^6/µl)	Total leukocytic count (X 1000 cells / cmm)
Control	2.76 ± 0.28	26.58 ± 1.547
Dinaferm	3.56 ± 0.40*	34.36 ± 2.31*
Bio nutra	3.71 ± 0.31*	30.64 ± 1.643*

* Significant at (P < 0.05)

Table (2): The effect of probiotics on differential leukocytic count.

Parameters / Treatments	Blood heterophils (%)	Blood lymphocytes (%)	Blood monocytes (%)	Blood esinophils (%)	Blood basiophils (%)
Control	24.3 ± 0.49	62.5 ± 0.385	10.2±0.23	1.2 ± 021	1.8 ± 0.36
Dinaferm	23.5 ±0.68 ns	63.6 ±0.52 ns	9.8 ±0.25 ns	1.5 ±0.23 ns	1.6 ±0.25 ns
Bio nutra	2.6 ±0.63 ns	63.4 ±0.54 ns	10.6 ±0.42 ns	1.4 ±0.26 ns	2.0 ±0.33 ns

Ns = non significant

Table (3): The effect of probiotics on the total serum protein ,albumin and globulin (gm/L).

Parameters / Treatments	Total serum protein	Serum albumin	Serum globulin
Control	40.31 ± 2.543	22.15 ± 1.692	18.16 ± 1.354
Dinaferm	44.72 ± 1.983*	23.07 ± 0.973 *	21.65 ± 1.651*
Bio nutra	46.74 ± 4.632*	24.36 ± 2.374 *	22.38 ± 2.323*

* Significant at (P < 0.05)

Table (4): The effect of probiotics on the haemagglutination inhibiting (HI) antibody titer (log 2).

parameters / Treatments	HI titer
Control	4.8 ± 0.886
Dinaferm	6.6* ± 0.512
Bio nutra	6.5 * ± 0.631

* Significant at (P < 0.05)

Table (5): The effect of probiotics on the protection percentage.

parameters / Treatments	Protection percentage
Control	70 **%**
Dinaferm	84 **%**
Bio nutra	86 **%**

Table (6): The effect of probiotics on the Serum AST, and ALT(U/L).

parameters / Treatments	AST	ALT
Control	122.70 ± 2.55	26.20 ±1.39
Dinaferm	125.77 ± 2.45 ns	28.20 ns ± 0.66
Bio nutra	126.79 ± 1.78 ns	26.60 ns ± 0.74

Ns =non significant

Table (7): The effect of probiotics on the Createnine and uric acid.

parameters / Treatments	Createnine (mg/dL)	uric acid (mg/dL)
Control	1.69 ± 0.14	14. 52 ± 0.57
Dinaferm	2.06 ± 0.17 ns	15.36 ± 0.78 ns
Bio nutra	2.14 ± 0.16 ns	14.87 ± 0.66 ns

NS=non significant

Table (8): The effect of probiotics on the relative weight of bursa, spleen and thymus.

Parameters / Treatments	Bursa relative weight	Spleen relative weight	Thymus relative weight
Control	1.623± 0.265	1.348 ± 0.142	1.735 ± 0.066
Dinaferm	2.391*± 0.148	1.841 ± 0.103	2.154 ± 0.278
Bio nutra	2.267*± 0.299	1.804 ± 0.151	2.435* ± 0.198

* Significant at ($P < 0.05$)

DISCUSSION

The data obtained from this study revealed that probiotics under the Egyptian environmental conditions significantly improved the immune response of birds where Bionutra and Dinaferm decreased the mortality. The total leukocytes count, serum total protein and globulin increased in treated groups. Moreover, Bio nutra increased the relative weight of bursa and thymus while Dinaferm increased the relative weight of bursa only. The previous results agree with ***Sato (1984)*** and ***Miake et al., (1985)*** who reported that probiotics have an immunostimulant effects. The increased values of total protein in treated groups could be attributed to nutritive biological value of the yeast, where the yeast contain numerous enzymes that improve digestion of feed ***(Kornegay et al., 1995)*** or to the ability of probiotics to suppress the pathogenic intestinal microorganisms which may cause enteritis and impairs nutrient absorption ***(Windschitl., 1992).***

protein is essential for cellular mitosis ***(Laurence and Bennet ,1985).*** So the high protein level in probiotics treated groups may be responsible for increasing the blood cell count.

There is an increase in HI antibody titer in probiotic treated groups this explanation is further in harmony with that reported by ***Haghighi et al., (2006).*** They stated that probiotics stimulate production of antibodies in chickens. According to ***Coles (1986)*** the hight level of serum globulin (especially gamma globulin) may be responsible for increasing the antibody titer in probiotics treated groups. The high antibody titer may be

due to increased the number of W.B.Cs which produce several cytokines important for antibody synthesis. Also B lymphocytes (the precursor of plasma cells) produce antibodies. Increasing the number of lymphocytes may be due to increasing the weights of some lymphoid organs as bursa and thymus ***(Tizard, 2000)***.

The obtained findings in this work showed that AST, AST, **c**reatenine and uric acid levels not significantly changed and this denoting neither hepatotoxic nor nephrotoxic effect on broilers. The findings reported by ***El-Banna et al., (2001)*** and ***El-Ramady*** *and* ***Doaa, (2003)***, are in agreement with the present result. They concluded that probiotics supplementation have no hepatotoxic or nephrotoxic effects in broiler chickens.

CONCLUSION

It could be concluded that, some probiotics as Bio nutra and Dinaferm can be used as alternatives to growth promoting antibiotics to overcome antibiotic residual effects and the development of drug-resistant microorganisms. These probiotics had also an immunostimulant effect and have no hepatotoxic or nephrotoxic effects.

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دراسات على التأثير البيوكيميائى والمناعى للخمائر (بيونترا و الدينافرم) على دجاج التسمين

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استهدف هذا البحث دراسة استخدام الخمائر(بيونترا والدينافيرم) على بعض القياسات البيوكيميائية والمناعية فى دجاج التسمين من نوع الهبرد قسمت الكتاكيت الى ثلاث مجموعات متساوية كل منها تحتوى على 50 كتكوت المجموعة الأولى أعطيت عليقه أساسيه فقط (مجموعه مقارنه بينما المجموعة الثانية أضيف إليها البيو نترا(1كيلو جرام/ طن) أما المجموعة ألا خيره فقد أضيف إليها الدينافيرم (1 كيلو جرام/ طن). وقد اتضح من هذه الدراسة أن استخدام بيونترا و الدينافيرم احدث زيادة معنوية فى عدد كرات الدم الحمراء والبيضاء ولم يحدث تغير معنوى فى النسبة المئوية للخلايا القلوية والخلايا الحامضية والخلايا الملتهمة وخلايا الهتروفيل والخلايا الليمفاوية.

مستوى البروتين الكلى والجلوبيولين لمصل الدم والأجسام المناعية لمنع التلازن الدموى ضد فيروس النيوكاسل زاد زيادة معنوية فى مجموعتى بيونترا والدينافيرم. بيونترا كذلك احدث زيادة معنوية فى مستوى مصل الزلال بينما لم يحدث تغير ملموس فى وظائف الكبد والكلى .وقد لوحظ أيضا أن استخدام بيونترا احدث زيادة معنوية فى الوزن النسبى للغدة الزعتريه وغدة البرسافابريش بينما استخدام الدينافرم احدث زيادة فى الوزن النسبى لغدة البرسافابريش فقط.