EFFECT OF PROBIOTIC (LACTOBACILLUS SACCAROMYCES) ON THE IMMUNOLOGICAL, BIOCHEMICAL AND HAEMATOLOGICAL CHANGES OF BROILER CHICKEN FED ON OCHRATOXICATED RATION.

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SUMMARY

Eighty one day old Hubbard chicks were divided into four equal groups . First group fed on ration containing only ochratoxin A 2.5mg / 1kg , Second Group fed on ration containing probiotic and ochratoxin A 2.5 mg /1kg ; third Group fed on ration containing Probiotic only an fourth group remains as a control fed on plain ration. The experiment continued for 45 day. The chicks vaccinated at 7^{th} day with Hitchner B_1 by occular route and at 18^{th}, 28^{th} day vaccinated by Lasota vaccine via of drinking water. Every week the birds (weighted with calculation of the amount of ration which have been consumed.

Whole blood was collected at 14, 28, and 42 day of age from all groups. The blood serum collected for evaluation of immune response to ND virus vaccine. Liver and kidney function assays were applied and the collected whole blood for differential leucocytic count. Immunosuppression was observed in chickens fed diets containing ochratoxin A for 4 weeks, when compared with controls,in which the treated birds showed reduction in total serum protein, lymphocyte precentage weights of the thymus, bursa of Fabricius, and spleen. Urea, creatinine, cholesterol, also Alkaline phosphatase, AST, and ALT exhibited a significant increase in ochratoxicated group by comparison to the control group. The addition of probiotic into the ochratoxicated ration improve both the total body weight and organ weight in comparing with the ochratoxicated ration which showed increasing the relative weight of livers and kidney,on the other hand there were a decreasein the relative weights of bursa of Fabricius and spleen.The obtained results concluded that, the addition of probiotic into the ochratoxicated ration improve the immune response to NDvirus vaccine, total body weight and organ weight and also improve most biochemical and hematological parameters .

INTRODUCTION

Mycotoxins are among the most common contaminants in poultry feed. It causes great economic losses. Elimination or reduction of mycotoxin producing fungi in grains is not always successful. Ochratoxins are one of the most important toxins produced by moulds and affect human and animal heath and cause economic losses in animals and poultry. Mycotoxines has been found as a natural contaminants of feed stuff in many countries *(Purwoko et al., 1991)*. Ochratoxins A may leads to what is called vaccination failure or vaccinal immunity and may lead to occurrence of disease even in properly vaccinated flacks *(Lesson et al.. 1995)*.

There are different systems for protection against the effects of mycotoxins in poultry industries; one of these systems is the use of some chemicals additives to the food of the poultry to minimize the effect of mycotoxines. There are varieties of physical, chemical and biological approaches employed to counteract the mycotoxins problems such as Probiotic, which are used for chickens to replace organisms that are not present in the alimentary tract or to provide the chickens with the effect of beneficial bacteria. *Smith (1970)* found that the most obvious changes in the flora of the chickens induced by dietary changes and occurred at the anterior end of the digestive tract. *Churchil et al. (2001)* reported that addition of live yeast culture (Saccharomyces cerevisiae)0.1% or 0.2% to diet of chickens contain aflatoxin(1ppm)resulted in counter acting the toxic effect of aflatoxin on live body weight, feed conversion and mortality rate. *Khalaf-Allah et al. (2002)* they studied the effect of some detoxifying compounds on broilers fed on ration containing ochratoxin with addition of Saccharomyces cerevisiae (dried yeast powder using of these compounds in addition to ochratoxicated ration leads to improvement of body weights, body weights gain. Recent approaches for detoxification is through dietary modifications, involving the use of lipotopes as choline, and sulpher containing amino acids *(Nahm 1991)*., vitamins *(Kim and Combs 1992)*.

The objects of the present study:

1-Studies on different types of toxins produced by Asperagillus ochraceus
2- Experimintal studying the effect of ochratoxin in broiler chickens.
3- Study the effect of probiotics as feed additive on chickens fed ochratoxicated ration:
   a-Immunological changed of broiler chicks.
   b- Biochemical and hematological changes.

MATERIAL AND METHODS

1. Materials:

1.1. Experimental chicks: 80 one day (unsexed) old Hubbared chicks were obtained from Alarabia company for poultry production.

1.2. Basal diet:

a. Commercial broiler starter grower ration from Cairo company for poultry production.
b. Probiotic antitoxin-mold clear from Ascopharme added to some groups of chicks in a dose of: 1 g/ kg ration along the period of the experiment from one day old up to 43 day of age.

Each 1 kg probiotic contain :

Lacto bacillus + saccaromyces	50 g
Calcium propionate	200g
Copper sulphate	8 g
Aluminium, calcium and Magnisium silicate up to	1000g

1.3. Media:

1.3.1 Sabouraud's dextrose agar medium: was prepared according to *Cruickshank et al. (1975)*

1.3.2. Yeast exteact sucrose broth: It used for testing the toxicity and production of ochratoxins and was prepared according to *Davis et al. (1969)*

Yeast extract	20 g
Sucrose	40 g
Dist water	up to 1000 mL

Then autoclaved at 121C° for 15 minutes

1.3.3. New castle disease virus veccines: Hitchner B_1 produced by Izovac Company and Lasota vaccine produced by Lohman animal health GmbH.

3.1.4. Chemicals for thin layer chromatography: Kit. for ochratoxin obtained from Biochemical Hrt No. 70040 1 mg each.

***3.1.5..* Kits for determination of total serum protein,** albumin and globulin according to *Henry(1964).*

2. Methods:

2.1: Testing the *toxicity of A. ochraceus isolates* which obtained from the department of Bacteriology,Mycology and Immunology; Faculty of veterinary Medicine Sadat City, Minufiya University: according to *(Davis et al, 1966)*

2.2 Extraction of ochratoxin from liquid medium:

The culture filtrate through whatman filter paper into culture filtrate and mycellial mats. The filtrate was acidified by adding 0.1 N hydrochloric acid and the ochratoxin extracted from the acidifed filtrate by adding chloroform twicely and separate the chloroform layer by seperatory funnel. Equal amount of 0.1 M sodium bicarbonate solution was added to the acidified chloroform extract and shaken in separatory funnel. Thus the chloroform contained no ochratoxins. The sodium bicarbonate extract was acidified with 0.1 N hydrochloric acid and retracted by chloroform again. The purified chloroform extract was evaporated to dryness to remove chloroform using evaporator or water bath then the residuse taken to detamine the ochratoxin.

2.3. Qualitative determination of the ochratoxin:According to (Scott and Hand, 1970)

2.4. Confirmatory tests for the presence of ochratoxin :*(according to scott and Hand (1967)*

2.5. Quantitative determination of and ochratoxin A: according to *Shnnon et al. (1983).*

2.6. Preparation of ochratoxicated ration:

A known amount of prepared ochratoxin A were dissolved in 95% ethanol and added to small part of the feed. and dried to evaporate ethanol than added to the remained of the feed.

Experimental design:

80 one day old Hubbard chicks were divided into 4 groups each group contain 20 birds.

Group No 1: fed on ration contain only ochratoxin A 2.5mg / 1kg

Group No.2: fed on ration contain probiotic and ochratoxin A 2.5 mg /1kg

Group No 3: fed on ration containing Probiotic only

Group No 4: control fed on ration with out any additives.

The experiment continued for 45 day. The chicks vaccinated at 7^{th} day with Hitchner B_1 by occular route and at 18^{th}, 28^{th} day vaccinated by Lasota vaccine via of drinking water. Every week the birds (weighted with calculation of the amount of ration which have been consumed.

2.7. Collection of blood: Whole blood was collected from shank and /or wing vein at 14, 28, and 42 day of age from all groups. The blood serum collected for evaluation of immune response to ND virus vaccine and liver and kidney function assaysand collected whole blood with neparin as anti coagulant for differential leucocytic count.

2.8. Methods for evaluation of immune response

2.9. Haemagglutineation test (HA) *according to (Anon, 1971) and Haemayglutination inhibition test (HI) according to (Takatsy 1956 and Gough 1974)*

3.2.8 Evaluation of cell mediated immune response *(Differential leucoytic count)*

3.2.9 Serum Biochemical Analysis:

3.2.9.1 Total protein:

According to *Henary (1974).* (Kits were obtained from Biomereux co.)

3.2.9.2 Albumin:-

Was colorimetrically determined in serum according to *Domas et al. (1971)*

3.2.9.3 Globulines:

According to *Mancini et al. (1965)* Immunoglobulin G. A and M (IgG, I g A , I g M ,) were measured in serum by using Endoplate immunoglobulin test kit obtained from BioMereux co.

3.2.10. Nutritional parameters:

3.2.10.1 Relative organ weight:- According to Huff etal. (1986)

3.2.10.2 Growth rate: According to Broody (1945

RESULTS AND DISCUSSION

The probiotics act as organisms and substances which contribute to intestinal microbial balance and can be used to stimulate microbial growth. Also, Fuller (1973) defined probiotics as a live microbial feed supplement which beneficially affects the host by improving its intestinal microbial balance. Martin, (1996) the addition of probiotics to poultry ration improves the production of vitamin and short chain fatty acids from the food substances, beside keeping the integrity of the intestinal epithelium, stimulation of the immune response and protection against entero-pathogenic microorganisms. The obtained results for the detection of ochratoxin production by Aspergillus ochraceus using HPLC techniques, the same results was developed by Soares and Rodriguez Amaya. (1985) they developed a simple, economical and rapid method for screening and quantitetion of OA in cereals. Screening was carried out by using a silica gel aluminum oxide minicolumn with adetection limit of 80 ug / kg. the detection limit was lo ug/kg for TLC quantitation. There are two groups of probiotic preparations: those which are primarily intended to be effective in the crop and the anterior regions of the alimentary tract. Among the first group are various lactobacillus cultures and preparations which are thought to colonize the crop and small intestine. Homma and Hamaoka (1998) reported that birds supplements with Bacillus subtilis culture as probiotic feed additive improved health, productivity and management problems.

In chickens fed diets containing ochratoxin A at a concentration of 2–4 mg/kg for 20 days, the lymphoid cell population of immune organs was decreased (Dwivedi & Burns, 1984a).

The results obtained in table (1) cocluded that the addition of probiotic into the ochratoxicated ration improve both the total body weight and organ weight in comparing with the ochratoxicated ration without any probiotic which showed increasing the relative weight of livers. decrease the relative weights of bursa of Fabricius and spleen and increase the relative weights of kidneys.

Immunosuppression was observed in chickens fed diets containing ochratoxin A at 0.5 or 2 mg/kg for 21 days. When compared with controls, the treated animals had reduced total serum protein, lymphocyte counts, and weights of the thymus, bursa of Fabricius. and spleen (Singh *et al.*, 1990). Ochratoxins A has carcinogenic. teratogenic. mutagenic and immuno-

suppresive effect (*Krough 1992 and Kuipr and Scott 1998*). Also the same findings were noticed by (Gentles et al . 1999) Groups of 20 Peterson x Hubbard broiler chickens were fed diets containing ochratoxin A alone at 0 or 2.5 mg/kg of diet or in combination with cyclopiazonic acid for 3 weeks. A significant reduction in body-weight gain was seen by the second week of feeding and was still present at the third week (by 19%). And also the relative kidney weight was increased in the group given ochratoxin A.

Table (2) showed the effect of ochratoxinA and probiotics on total serum protein, albumin and globulines were higher in their levels in comparing with the chicks fed ochratoxicated diets only noticed that, the levels of total serum protein; albumin and globulines. These results agree *with those of Tung et al. (1975), Huff (1986), Kubena (1993), El Shewey (1999), Salwa et al . (2000) and Flourage (2005).*The reduction of levels of total serum protein, albumin and globulines during ochratoxication are indicators of impaired protein synthesis. The reduction of serum immunoglobulins meight is due to the significant depression immunoglobulin containing cells in all lymphoid organs, atrophy of lymphoid organs Dwivedi and Burns (1984). Also the same results of blood parmeters noticed by Chang et al. (1979) who found that ochratoxin A caused leucocytopenia in chickens which were characterized by lymphocytopenia and monocytopenia with normal heterophils count.

Also, Effat (1989) and El Kady (1993) studied the effect of ochratoxin A on chicken immune response and found that dietary ochratoxin leads to decrease antibody titre against NewCastle disease virus vaccine and decrease differential leucocytic count: decrease number of lymphocytes, monocytes, basophiles and eosinophils. Florage (2000 and 20005) reported that ochratoxin A a lone and in combination with aflatoxin B_1 in chickens leads to immuno suppression effect which appear in decrease in serum immunoglobulins and decrease in leucocytic count. Khalaf-Allah et al. (2002) They found that using of these compounds in addition to ochratoxicated ration leads to improvement of body weights, body weights gain. Weights of liver, spleen, bursa and proventriculus became near to control values prevented ochratoxin residues in various organs of chickens also the level of total proteins and globulins were restored to nearly the normal ranges. Ramadevi et al.(1998)found that broilers fed on ochratoxin A contaminated ration were suffered from anemia and decrease in total erythrocyte count, packed cell volume and hemoglobin concentration values. Gentles et al.(1999)noticed that ochratoxin A reduced body weight serum total albumin, protein and cholesterol levels in broilers fed on OA contaminated ration. Awaad et al. (2005) they used probiotic in prevention of chicken ochratoxicosis immune dysfunction and found that using of this probiotic in a dose of 100g /ration with broiler basal diet containing 5 ppb ochratoxin A resulted in decrease mortality rate in broiler chickens ,improved mean body weights, decrease histopathologicl changes in lymphoid organs(bursa and thymus)and increase heamagglutinetin inhibition geometric mean titer against Newcastle and Gumboro .

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Table (1): Body weight and relative body weight ratio of liver, bursa, kidney, spleen and pancreas of the different treated groups.

groups	N	B.Wt	Liver Wt	Bursa Wt	Kidney Wt	Spleen Wt	Pancreas Wt
		Mean Std. Error	Mean Std. Error	Mean Std. Error	Mean Std. Error	Mean Std. Error	Mean Std. Error
1 (ochratoxinA2.5mg/kgm)	5	CD 1570.00±53.85	AB 2.04±0.14	BC 0.25±0.02	A 0.72±0.04	BC 0.11±0.01	A 0.29±0.01
2(Probiotic+ochratoxinA2.5mg/kgm)	5	BC 1620.00±71.76	B 1.68±0.36	ABC 0.28±0.03	AB 0.64±0.03	C 0.10±0.01	AB 0.25±0.02
3 Probiotic only)	3	A 2025.00±38.19	AB 2.21±0.00	A 0.35±0.03	B 0.58±0.00	A 0.14±0.00	B 0.23±0.01
4 (fed ration only without any additives)	3	A 1958.33±8.33	AB 2.17±0.01	AB 0.29±0.01	B 0.54±0.01	A 0.14±0.00	B 0.21±0.00

Table No(2) Biochemical changes on the different serum parameters.

	Group1 (2.5 mg ochratoxin A)			Group2 (2.5 ochratoxin A+probiotic)			Group3 (probiotic)			Group4 (Control)		
	After 2weeks	After 4weeks	After 6weeks	After 2weeks	After 4weeks	After 6weeks	After 2weeks	After 4weeks	After 6weeks	After 2weeks	After 4weeks	After 6weeks
Total protein	3.9 ^{bc} ±0.62	5.5 ^{bc} ±0.56	4.8 ^{bc} ±0.78	4.96 ^{b} ±0.16	5.4 ^{b} ±0.25	4.96 ^{b} ±0.62	5.32 ^{a} ±0.42	6.00 ^{a} ±0.67	6.01 ^{a} ±0.74	5.37 ^{a} ±0.80	6.51 ^{a} ±0.12	6.2. ^{a} ±1.06
Albumin	2.9 ^{bc} ±0.92	2.42 ^{bc} ±0.80	2.93 ^{bc} 0.75	3.22 ^{b} 0.98	2.12 ^{b} 0.61	2.22 ^{b} 2.12±	2.7 ^{a} ±0.52	2.22 ^{a} ±0.49	2.93 ^{a} ±0.41	2.89 ^{a} ±0.12	2.45 ^{a} ±0.02	3.16 ^{a} ±0.58
Globulin	1.33 ^{bc} ±0.59	1.62 ^{bc} ±0.78	1.74 ^{bc} ±0.58	1.78 ^{b} ±0.52	1.82 ^{b} ±0.47	1.24 ^{b} ±0.79	1.78 ±0.30	1.52 ^{a} ±0.22	1.65 ^{a} ±0.33	1.81 ^{a} ±0.48	1.46 ^{a} ±0.88	1.78 ^{a} ±0.49
Urea	53.15 ^{bc} ±2.82	49.52 ^{bc} ±1.63	51.16 ^{bc} ±2.85	58.10 ^{b} ±2.52	50.32 ^{b} ±1.67	52.26 ^{b} ±1.42	20.42 ^{a} ±1.62	26.45 ^{a} ±2.00	23.12 ^{a} ±1.76	25.12 ^{a} ±2.82	24.42 ^{a} ±2.00	26.42 ^{a} ±2.85
Creatinine	2.24 ^{bc} ±0.66	1.96 ^{bc} ±0.62	2.4 ^{bc} ±0.69	2.34 ^{b} ±0.62	2.10 ^{b} ±0.671	2.00 ^{b} ±0.412	2.12 ^{a} ±0.45	2.003 ^{a} ±0.50	1.98 ^{a} ±0.21	1.81 ^{a} ±0.22	1.80 ^{a} ±0.32	1.86 ^{a} ±0.41
cholesterol	380.85 ^{bc} ±1.76	379.45 ^{bc} ±1.78	364.32 ±1.45	395.33 ^{b} ±1.72	383.41 ^{b} ±1.032	382.54 ^{b} ±1.45	339.25 ^{a} ±3.5	345.12 ^{a} ±2.962	364.00 ^{a} ±2.61	338.6 ^{a} ±2.52	357.0 ^{a} ±2.14	352.72 ^{a} ±1.98

_Means followed by different letters as superscript in the same row was significantly differed.
-Means followed by the same letters as superscript in the same row was non significantly differed.
-The significance change at P≤0.05.

Table No(3)Effect of ochratoxin A on the serum liver functions enzymes(means ± stander error)

	Group1 (2.5 mg ochratoxin A)			Group2 (2.5 ochratoxin A+probiotic)			Group3 (probiotic)			Group4 (Control)		
	After 2weeks	After 4weeks	After 6weeks	After 2weeks	After 4weeks	After 6weeks	After 2weeks	After 4weeks	After 6weeks	After 2weeks	After 4weeks	After 6weeks
ALT	36.80 bc ±1.92	34.15 bc ±1.30	39.02 bc ±2.00	29.12 b ±2.71	37.15 b ±2.22	39.00 b ±2.52	19.5 a ±2.12	21.35 a ±1.09	20.89 a ±2.02	17.06 a ±1.02	23.0 a ±0.12	19.023 a ±2.01
AST	97.22 bc ±1.00	99.12 bc ±2.012	97.03 bc ±2.65	89.12 b ±2.01	90.08 b ±1.96	92.08 b ±1.63	79.94 a ±1.085	82.00 a ±2.65	86.26 a ±3.42	81.89 a ±2.12	84.15 a ±3.32	84.12 a ±2.55
Alkaline phosphatase	88.12 bc ±1.01	86.12 bc ±1.78	82.14 bc ±2.11	76.12 b ±2.32	74.15 b ±1.44	72.12 b ±1.22	67.86 a ±3.00	64.86 a ±1.45	55.12 a ±2.11	63.86 a ±2.02	57.15 a ±1.14	52.41 a ±1.02

_Means followed by different letters as superscript in the same row was significantly differed.
-Means followed by the same letters as superscript in the same row was non significantly differed.
-The significance change at $P \leq 0.05$.

Table (4) Effect of Probiotics combination with Ochratoxins, Ochratoxins, and probiotics on differential leukocytic count in Broilers at different ages

	Lymphocytes %			Heterophils %			Eosinophils %			Basophils %			Monocytes %		
Age(week)	2weeks	4weeks	6weeks	2weeks	4weeks	6weeks	2weeks	4weeks	6weeks	2weeks	4weeks	6weeks	2weeks	4weeks	6weeks
Group1	61.33a ± 0.88	60.33a ± 0.88	60.00a ± 1.00	28.33d ± 0.88	29.33d ± 0.88	29.67d ± 1.20	2.33g ± 0.33	2.33g ± 0.33	3.67g ± 0.33	1.67h ± 0.33	1.67h ± 0.33	0.67h ± 0.33	6.33i ± 0.33	6.33i ± 0.33	6.00i ± 0.00
Group2	56.33b ± 0.88	54.33b ± 0.88	53.00b ± 1.15	33.33e ± 0.88	35.33e ± 0.88	36.67e ± 1.20	2.67g ± 0.33	2.67g ± 0.33	3.33g ± 0.33	1.67h ± 0.33	0.67h ± 0.33	0.33h ± 0.00	6.00i ± 0.00	6.33i ± 0.33	6.00i ± 0.00
Group3	70.33c ± 0.88	69.00c ± 0.58	68.67c ± 1.20	19.33f ± 0.33	21.00f ± 0.58	21.00f ± 0.58	2.67g ± 0.33	2.67g ± 0.33	3.33g ± 0.33	2.00h ± 0.00	1.33h ± 0.67	1.33h ± 0.33	5.67i ± 0.33	6.00i ± 0.58	5.67i ± 0.88
Group4	69.33c ± 1.26	68.33c ± 0.88	67.67c ± 1.26	20.00f ± 1.15	21.33f ± 1.45	22.00f ± 1.15	2.33g ± 0.33	3.00g ± 0.00	3.67g ± 0.67	1.67h ± 0.33	1.33h ± 0.67	0.67h ± 0.33	6.33i ± 0.33	6.00i ± 0.00	6.00i ± 0.58

Each value represents mean ± SE

Means with the same letter are not significantly different (P<0.05)

الملخص العربى

تأثير البروبايوتك(لاكتوباسيلس السكارومايسس) على التغيرات المناعية والكيميائية والدموية فى كتاكيت التسمين المغذاة على عليقة تحتوى على الاوكراتوكسين.

ا.د/عبد العزيز عبد الخالق مسعد .. استاذ الميكروبيولوجيا المساعد- كلية الطب البيطرى-جامعة المنوفية-السادات.
ا.د/سعيد ابراهيم فتح الله .. استاذ الفسيولوجيا المساعد- كلية الطب البيطرى-جامعة المنوفية-السادات.
د/ أحمد فرج الكرداسى..مدرس الكيمياء الحيوية - كلية الطب البيطرى-جامعة المنوفية-السادات.

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أجريت هذة الدراسه على 80 كتكوت هبرد قسمت الى اربع مجموعات متساوية وقد اعطت المجموعة الاولى عليقة تحتوى على الاوكراتوكسين بتركيز 2.5ملليجرام/كجم كما أعطت المجموعة الثانية عليقة تحتوى على البروبايوتك و الاوكراتوكسين.أما المجموعة الثالثة فتم اضافة البوبابايوتك فقط بنفس النسب السابقة.أما المجموعة الرابعة فقد اعتبرت كمجموعة ضابطة .استمرت التجربة لمدة 45 يوما وتم وزن الطيور كل اسبوع. ثم تم اخذ عينات من الدم فى عمر 14و28و 42يوم من جميع المجموعات وتم تجميع المصل لقياس الاستجابة المناعية لتحصين النيوكاسل ولقياس وظائف الكبد والكلى كماتم اخذ عينات من الدم لعمل عد نوعى لخلايا الدم البيضاء.وقد لوحظ انخفاض مناعى فى المجموعة المغذاه على عليقة محتوية على على الاوكراتوكسين لمدة اربع اسابيع. وعند مقارنة المجموعة الضابطة بباقى المجموعات تبين وجود نقص معنوى فى بروتينات الدم ونسبة الخلايا الليمفاوية ووزن غدة التوثةو غدة كيس فابريشيس وكذا وزن الطحال.بيما لوحظ زيادة معنوية فى مستوى كل من اليوريا والكرياتينين والكوليسترول و انزيمات الالنين أمينوترانسفيريزوالاسبارتك أمينوترانسفيريز والالكالين فوسفاتيز فى المجموعة المغذاة على عليقة محتوية على على الاوكراتوكسين.وعند اضافة البروبايوتك الى العليقة المحتوية على الاوكراتوكسين ظهر تحسن فى الوزن الكلى للطائر ووزن الاعضاء منفصلة وذلك بالمقارنة بالمجموعة المغذاة على عليقة محتوية على الاوكراتوكسين فقط.كما حدث نقص نسبى فى وزن الطحال و غدة كيس فابريشيس وزيادة فى الوزن النسبى للكلى. ومن النتائج السابقة يتضح ان اضافة البروبايوتك الى العليقة قد حسن من الاستجابة المناعية للطيور وقلل من التاثير المثبط المناعى للاوكراتوكسين وكذلك حسن من أوزان الطيور والوزن النسبى للاعضاء كمالوحظ تحسين واضح فى القياسات البيوكيميائية والهيماتولوجية.