Some Biochemical and Immunological Changes in Holestein Cattle Administrated Some Probiotic Microorganisms

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ABSTRACT

Apparently healthy Holestein cattle in a private farm were used in the current study. The probiotics are beneficial microorganisms for the animal health, so the aim of the current study was for obtaining such positive effects after using a mixture of probiotics in Holestein cattle. The water diluted solution (0.5%) of probiotic mixture of 9-microorganisms was sprayed on the feed of one group of cattle, one time per week for three weeks, the other non-treated group was used as a control one, serum samples were obtained from all animals for determination of some elements, serum protein fractions by electrophoresis and some serum biochemical constituents. Results indicated that the probiotic treatment induced immunostimulant effect (through the elevation of gamma globulins), improved liver function (due to reduction of ALT, AST and AP - liver enzymes), induced some positive biochemical changes (reduced cholesterol and elevated total protein, total globulins, albumin, triglycerides and total lipids), elevated some macro-and micro-elements (calcium, phosphorus, sodium and cupper), in contrast, it reduced magnesium, zinc and iron (without deficiency signs), but the potassium, cobalt and selenium were non significantly changed., The probiotic treatment did not negatively affect the renal function. It could be recommended that the used probiotic mixture may be intermittently (not continually) used in cattle for inducing immunostimulant effects and improve the liver and other health functions. The magnesium, zinc and iron should be administered along with this probiotic mixture to avoid their deficiencies in cattle in case of long term administration.

INTRODUCTION

Many works reviewed that the probiotic bacteria have been successfully used to improve the health and growth parameters in the livestock.

The mucosal Alanine aminotransferase (ALT) and Aspatate aminotransferase (AST) and the serum AST enzyme levels were decreased in lambs treated with the probiotics (yea-sacc-1026 and Enterosan), but the liver enzyme (serum ALT) was not significantly changed by such treatment *(1)*.

The body weight, feed intake, feed efficiency, phosphorus, potassium, triglyceride and total bilirubin were increased in probiotic treated lambs, but the glucose, urea, ALT, AST and creatine kinase enzyme levels were decreased in the blood compared to non-treated control lambs *(2)*.

The serum total protein was increased and the serum glucose was decreased in dogs treated with Lactobacillus ADI probiotic for 7-days *(3)*.

In the probiotic (*Enterococcus faecium cornelle*- 68) treated broiler chicks for 49 days, the AST and ALT enzyme levels were decreased but the cholesterol level was decreased early at 14- days treatment. No significant haematological changes could be detected *(4)*.

Feeding of rats with lactobacilli probiotics for 3-days induced protection of gastrointestinal tract after E.coli infection, also the serum ALT enzyme and the Enterobacterial count were reduced *(5)*.

The Eterobacterial count was decreased in caecum and colon, the serum ALT enzyme and the bilirubin levels were decreased in the probiotic treated rats (which previousely had

liver damaged by D-galactosamine)compared with the non-probiotic treated animals *(6).*

In dairy cattle farm, the effective probiotic microorganisms solution was sprayed on the feed, manure and beding of cattle reduced the house fly population, total bacterial count of beding, malodours of stable and the frequencies of occurrence of both diarrhoea and pneumonia, Moreover improved the fertility of manure (through elevating nitrogen, phosphorus and potassium) they elevated the serum immunoglobulin compared to the non – probiotic treated cattle *(7)*.

The current work was conducted for evaluations of the efficacy of a mixture of probiotic microorganisms when used in dairy cattle farms, through studying the possible changes in some serum biochemical constituents,, serum micro and macro-elements and protein fractions including the immunoglobulin fractions, aiming finally for the useful and safety use of such probiotic treatment in large animal farms.

MATERIAL AND METHODS

Animals: Ten apparently healthy Holestein cattle in a private farm were divided into two equal groups of five -cattle each.

Probiotic Microorganisms :- Are used as solution of a mixtare of the following three groups:-

1. Lactic acid bacteria (5-species) as: *Lactobacillus plantarum, Lactobacillus casei, Lactobacillus fermentum, Lactobacillus salivarius and Lactobacillus delbruccki.*
2. Phototrophic bacteria (3-species) as: *Rhodopseudomonas palustris, Rhodobacter sphaeroides and Rhodobacter capsulatus.*
3. Yeast (one species) called *Saccharomyces cerevisiae.*

The mixture of these 9-species of the probiotic Effective Micro-organisms (EM) called EM-Bokashi for animals, which certified by the Organic Material Review Institute (OMRI), in March, 2003, and produced by Emrousa Company, USA as a product called "Emro-USA –Effective micro organisms" .

Methods

The two groups of cattle are reared on two yards, the Effective Probiotic Microolganisms (EM) were used in solution by a dilution of 1: 200 (stock : water) and sprayed on the animal feed of one group (the treated group). The other group of cattle not absolutely treated with the EM-probiotics and considered as the control group. The treatment by EM-spraying solution was conducted once a week for three consecutive weeks as described previously *(8).*

Blood samples (without anticoagulants) were collected from all cattle of the two groups after three weeks of treatment (for obtaining the serum samples). The following serum parameters were determined: The serum protein immunoelectrophoresis, using the cellulose acetate method *(9)*, serum macro- and micro–elements (calcium, phosphorus, magnesium, iron, potassium, sodium, cobalt, cupper, selenium and zinc)using the atomic absorption spectroscopy *(10)* and some serum biochemical constituents: the concentrations of total lipids *(11)*, cholesterol *(12)*, total bilirubin *(13),* triglycerides *(14)*, total proteins *(15),*creatinine *(16),* urea *(17)* and the activities of the following enzymes: alkaline phosphatase *(18)* alanine amino transferase (ALT) and aspartic aminotransferase (AST) *(19).*

The obtained data were statistically analyzed using F-test through the analysis of variance (ANOVA) *(20).*

RESULTS

a) Serum Elements

The serum calcium, phosphorus and sodium were significantly increased ($p \leq 0.01$) in the Effective Microorganisms (EM) treated Holestein cattle than that of the control cattle, also the serum cupper was significantly ($p \leq 0.05$) increased in the EM-treated cattle. The magnesium and zinc were significantly ($p \leq 0.01$) decreased in treated cattle. On the other hand, the serum potassium, cobalt and selenium were non-significantly changed in treated cattle (Table 1 and Fig. 1).

Table 1.The Concentrations of Serum Elements (ppm) in the Holestein Cattle treated with the Probiotic Microorganisms (EM).

Groups	Ca	P	Mg	Fe	K	Na	Co	Cu	Se	Zn
Control cattle	7.500 ± 0322	5.100 ± 0.236	2.600 ± 0.061	65.00 ± 3.405	161.33 ± 5.156	1266.1 ± 15.556	5.550 ± 0.640	2.050 ± 0.186	42.900 ± 1.194	2.050 ± 0.074
EM. Treated cattle	7.900** ± 0.330	5.800** ± 0.241	2.00** ± 0.081	59.500* ± 1.890	160.35 ± 4.278	2342.3** ± 22.436	5.550* ± 0.390	2.350* ± 0.269	43.00 ± 1.251	1.850 ** ± 0.102
LSD ($P \leq 0.05$)	0.187	0.188	0.089	4.945	-	12.592	-	0.261	-	0.087
LSD ($P \leq 0.01$)	0.310	0.312	0.149	8.201	-	35.811	-	0.432	-	0.144

N.B. LSD = least significant difference between means at $p \leq 0.05$ or 0.01, * = significant difference between means at $p < 0.05$, ** = highly significant difference at $p \leq 0.01$.

Table 2. Serum Protein Fractions by Electrophoresis of Holestein Cattle treated with the Probiotic Microorganisms (EM) (g/dl).

Groups	Alpha-1 (α_1) globulins	Alpha-2 (α_2) globulins	Beta (β) Globulins	Gamma (γ) Globulins	Total globulins	Albumin	Albumin/globulin ratio (A/G)
Control cattle	0.400 ± 0.027	0.330 ± 0.029	0.980 ± 0.062	3.030 ± 0.148	4.740 ± 0.397	1.946 ± 0.305	0.400 ± 0.034
EM. Treated cattle	0.400 ± 0.027	0.300 ± 0.032	1.770** ± 0.086	3.390** ± 0.211	5.860* ± 0.583	2.220* ± 0.305	0.360 ± 0.035
LSD ($P \leq 0.05$)	-	-	0.106	0.194	1.033	0.268	-
LSD ($P \leq 0.01$)	-	-	0.175	0.322	1.712	0.445	-

N.B. LSD = least significant difference between means at $p \leq 0.05$ or 0.01, * = significant difference between means at $p < 0.05$, ** = highly significant difference at $p \leq 0.01$.

b) Serum Protein Fractions (Serum Protein Electrophoresis)

Both the alpha -1(α_1) and alpha-2(α_2) globulin fractions non changed in the EM-treated cattle. Both the beta (β) and gamma (γ) globulin fractions highly significantly ($p \leq 0.01$) increased in EM- treated cattle, also the serum albumin and the total globulins significantly ($p \leq 0.05$) increased in serum of treated cattle. But the albumin/ globulin (A/G) ratio was non-significantly (Table 2 and Figs. 2 & 3).

c) **Serum Biochemical Constituents**

Alanine aminotransferase (ALT) enzyme activity significantly ($p \leq 0.05$) decreased in EM – treated cattle. Both Aspartic aminotransferse (AST) and Alkaline phosphatase (AP) enzyme activities significantly ($p \leq 0.01$) decreased in the serum of treated cattle. Serum cholesterol was significantly ($p \leq 0.05$) decreased in the EM-treated cattle, but both the serum triglycerides and the total lipids significantly ($p \leq 0.01$) increased in the treated cattle. The serum total protein significantly ($p \leq 0.01$) decreased in treated cattle. The serum urea significantly increased, but the serum creatinine showed no significant changes in EM– treated cattle (Table 3, and Fig. 4).

Table 3. Serum Biochemical Constituents of Holestein Cattle treated with the Probiotic Microorganisms (EM).

Group	ALT enzyme activity (U/L)	AST enzyme activity (U/L)	Alkaline phosphatase (AP) enz. activity (U/L)	Cholesterol conc. (mg/dl)	Triglyceride conc. (mg/dl)	Total lipid conc. (mg/dl)	Total protein conc. (g/dl)	Serum urea conc. (mg/dl)	Serum greatinine conc. (mg/dl)
Control cattle	10.00 ± 1.020	24.00 ± 1.811	55.00 ± 2.668	211.00 ± 3.932	115.00 ± 3.162	11.00 ± 1.105	6.680 ± 0.338	38.00 ± 2.280	1.200 ± 0.141
EM. Treated cattle	7.00* ± 0.447	20.00** ± 1.649	44.00** ± 2.280	110.00** ± 4.617	125.00** ± 3.622	** 13.600 ± 1.414	8.080** ± 0.416	46.00** ± 1.811	1.200 ± 0.063
LSD ($P \leq 0.05$)	2.238	0.878	3.876	6.098	4.727	0.982	0.247	2.483	-
LSD ($P \leq 0.01$)	3.712	1.456	4.937	10.113	7.840	1.628	0.410	4.118	-

N.B. LSD = least significant difference between means at $p \leq 0.05$ or 0.01, * = significant difference between means at $p < 0.05$, ** = highly significant difference at $p \leq 0.01$.

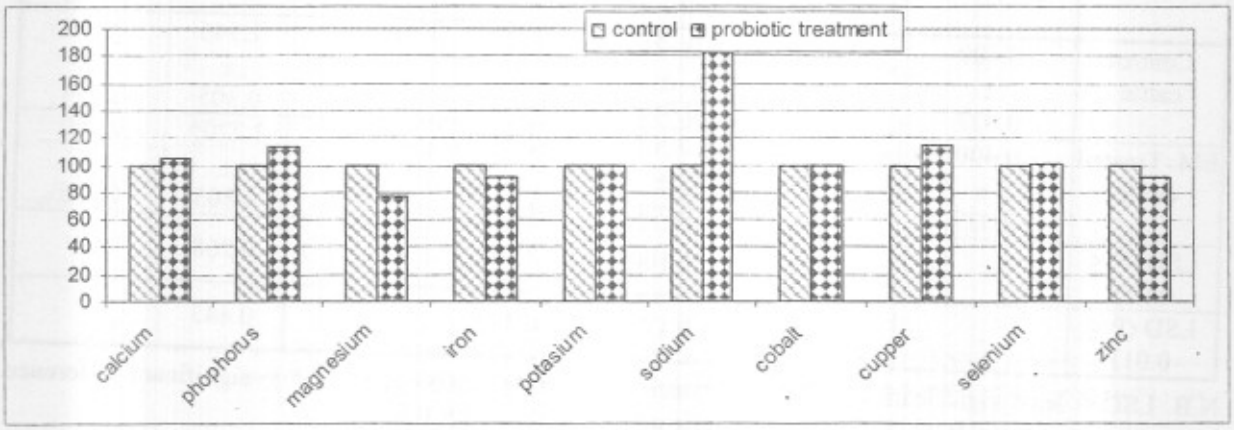


Fig. 1. The percentages of serum elements in the probiotic treated cattle

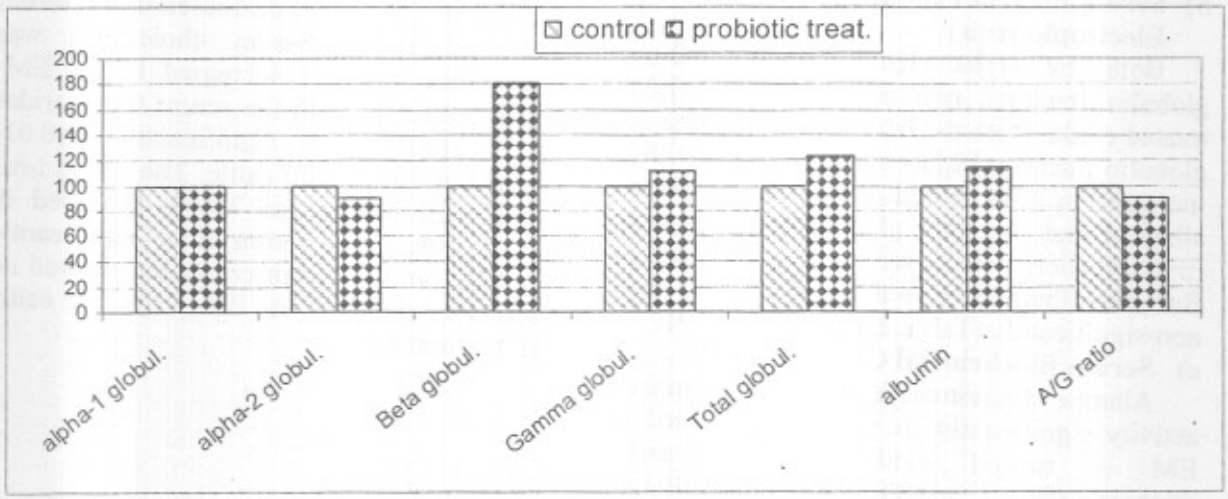
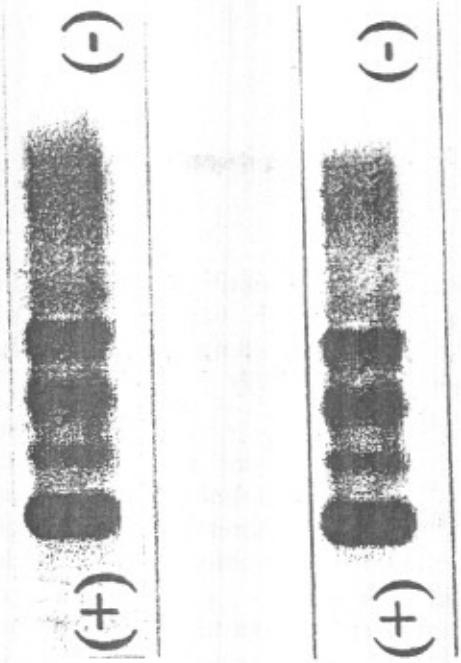


Fig. 2 The percentages of the different protein fractions (by electrophoresis) in probiotic treated cattle



Control **Probiotic treated**

Fig. 3. Electrophoregram of the different protein fractions of the probiotic treated and control Holestien cattle.

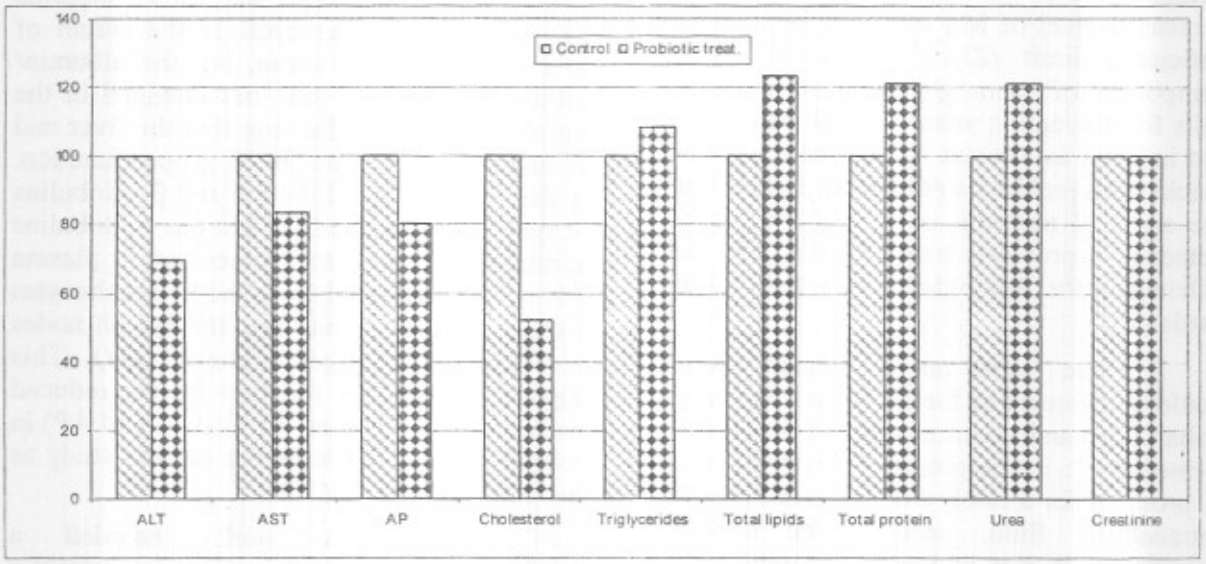


Fig. 4. The percentages of some serum biochemical constituents of probiotic treated cattle

DISCUSSION

The understanding of the function of the probiotics in maintenance of health and their importance in preventing disease serves to enhance the overall health of diseased animals, the probiotic microbial feed supplements are also gaining wide acceptance in livestock population.

The current study revealed that the serum sodium, calcium, phosphorus and cupper were significantly elevated in the probiotic treated cattle than that in the control cattle. The sodium maintains a stable osmotic pressure of the extracellular fluid, regulates acid-base balance and generates membrane and action potential *(21)*, but the excess sodium chloride may disturb the water balance as a result of reducing the ability of kidney and intestine to remove excess water from the blood coupled with the α_2 – globulins, ceruloplasmin, and 10% in the erythrocytes as erythrocupprein *(22)*. The increased levels of calcium and phophorus in the probiotic treated

cattle perhaps attributed to improvement the absorption of the two minerals as a result of protection of gastrointestinal tract of rats by lactobacillus probiotic *(5).* The Lactobacillus probiotic used in the current study as one of the mixture with 8-other probiotic microorganisms and perhaps the cause of increasing such minerals in treated cattle.

The present work revealed that there were a significant decrease in serum magnesium (Mg), Zinc (Zn) and Iron (Fe) in the probiotic treated cattle than that in the control animals. There was no signs of hypomagnesemic tetany in the probiotic treated cattle of the present study, so that the decreased level of Mg seems to be above the deficiency limit *(23).* The Fe is essential component of haemoglobin in the red blood cells for preventing anaemia *(24),* anaemia did not induced as a result of treatment of broiler chicks with probiotics *(4)*. In the present study, the signs of anaemia could not be clinically detected in probiotic treated cattle. The high calcium in the diet reduced Zn- absorption in cattle *(25)*.

There were no significant changes could be detected in the serum potassium (K), cobalt (Co) and selenium (Se) of the probiotic treated cattle in the present investigation. The K provides for a stable osmotic pressure of the intracellular fluid, acetylcholine synthesis, generation of rest and action potential *(21)*. The Co-deficiency is far to occur, due to its very small daily requirement which is around 0.08 ppm of feed dry matter *(23)*. The Se is required for the proper immune function *(26),* it is incorporated in WBCs, myoglobin, nucleoproteins and several enzymes *(27).* The present study revealed that the probiotic treatment could induce hypergammaglobulinaemia, that it induced immunostimulant activity. Many researches revealed the immunostimulant activities of probiotics, and the most commonly used genera of probiotic bacterial species are Lactobacillus, and some fungal strains *(28),* so that the probiotic microorganisms have a great potential for treating and preventing many diseases and disorders, and the future possibility of using Lactobacilli for production of vaccines for providing immunological defense against potentially harmful proteins *(29).*

The current study revealed that there were no significant changes of α_1 – and α_2 – globulin fractions, but there was a significant increase of the β-globulin fraction in the probiotic treated cattle. There are 13-types of α- globulins of diagnostic values, the increased β- globulins levels may attributed to some immunoglobulins (γ-fractions) which could migrate to the region of β-fraction in response to autoimmune diseases *(30)*.

The albumin and the total globulins were significantly increased in the serum of the probiotic treated cattle, but the albumin/ globulin (A/G) ratio did not changed in the treated cattle, this indicating that the liver and the immune system are in the proper function. As the albumin, α- globulins and β- globulins are synthesized in the liver but the γ- globulins (immunoglobulins) are synthesized in plasma cells which maturated from the B-lymphocytes in the spleen, bone marrow and lymph nodes in response to antigenic stimuli *(31)*. This suggestion was also fortified by the reduced levels of liver enzymes (ALT, AST and AP) in probiotic treated cattle in the current study as previously reviewed *(32).*

The present work recorded a significant increase in the serum triglycerides and total lipids, beside a significant decrease in the cholesterol level in the probiotic treated cattle. The hyperlipemia may be attributed to the regulatory disturbances in lipid metabolism. A similarly increased level of triglyceride in the present study was previousely recorded in lambs after probiotic treatment *(2).* Most cholesterol is biosynthesized from acetyl co-A which produced from the breakdown of fatty acids, amino acids, glycerol and carbohydrates *(33).* Hypocholesterolemia was reported with hyperthyroidism, hepatitis, heart disease and arteriosclerosis *(34),* and the cholesterol reduced also by carbohydrate diets, androgens, ascorbic acid, chloretetracycline , ethanol, monoamine oxidase inhibitors, niacin and

thiouracil *(35)*, also, a significant decrease of cholesterol level in the probiotic treated broiler chicks has been recorded *(4)*.

The serum urea showed a significant increase, but the serum creatinine did not show any significant change in the probiotic treated cattle. The creatinine usually does not elevated until the renal function is substantially impaired *(36)*, also the concentration of the serum urea depends partially on the amount of protein in the diet taken and partially to the renal and the hepatic functions, as the urea is formed in the liver and excreted by the kidney *(30)*.

Based on the present study, the used probiotic microorganisms could induced beneficial effects in treated cattle, as it improved the liver function through its reduction of ALT and AST and Alkaline phosphatase enzyme levels), it reduced the serum cholesterol concentration, also induced immunostimulant effects (elevating gamma globulins). It also elevated calcium, phosphorus and cupper levels. On the other side, the probiotic treatment has no effect the selenium, cobalt and potassium levels in the serum, but it reduced magnesium, iron and zinc levels (without a deficiency signs after 3weeks treatment). It could be recommended that the effective probiotic microorganisms should be intermittently administered to obtain the immunostimulant and other positive effects, but not administered contineously to avoid the possible occurrence of magnesium, iron and /or zinc deficiencies, which should be administered along with such probiotics.

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الملخص العربى

بعض التغيرات البيوكيميائية والمناعية فى أبقار الهولستين كنتيجة لاستخدام بعض ميكروبات البروبيوتيك

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ميكروبات البروبيوتيك لها تأثيرات إيجابية علي صحة الحيوانات، ولهذا كان الهدف من هذه الدراسة الحصول علي هذه التأثيرات النافعة من خلال إعطاء خليط من هذه الميكروبات في البقر الهولستين. ولهذا الغرض استخدم المحلول المخفف (٠,٥%) من ٩ أنواع من الميكروبات النافعة بالرش علي العلف ، مرة في الأسبوع ، ولمدة ثلاث أسابيع متتالية (المجموعة المعالجة)وبقيت مجموعة من البقر بدون علاج (المجموعة الضابطة) ، وتم أخذ عينات دم من جميع الحيوانات لتقدير بعض العناصر وقياس بعض المكونات البيوكيميائية وعمل الفصل الكهربي لبروتين المصل .أوضحت النتائج أن العلاج بميكروبات البروبيوتيك أدي إلي تنشيط المناعة (نتيجة لزيادة الجلوبيولينات المناعية) وأعطي تحسناً فى وظائف الكبد نتيجة خفض نشاط أنزيمات الكبد : الألانين-والأسبارتيك-أمينو ترانسفيريز والفوسفاتيز القاعدي)وإحداث تغيرات إيجابية لبعض المكونات البيوكيميائية (كخفض الكوليسترول وزيادة البروتين الكلي والجلوبيولينات الكلية والزلال وثلاثى الجليسريدات والدهون الكلية) ورفع مستوي بعض العناصر الأساسية والنادرة (مثل الكالسيوم والفسفور والصوديوم والنحاس) ولكن علي العكس انخفض مستوي بعض العناصر مثل المغنسيوم والزنك والحديد (ولكن ليس إلي حد ظهور أعراض)، ولم يحدث تغير ملحوظ في باقي العناصر(كالبوتاسيوم والكوبالت والسلينيوم)، وكذلك لم يؤثر العلاج علي وظائف الكلي سلبيا، ولهذا يمكن أن يوصي باستخدام مخلوط ميكروبات البروبيوتيك من أجل تنبيه جهاز المناعة وتحسين وظائف الكبد والصحة بوجه عام, ولكن يستخدم لفترات متقطعة وليست مستمرة لتجنب بعض الآثار السلبية من استخدام هذا العلاج وكذلك يوصى أيضا بإعطاء عناصر المغنسيوم والزنك والحديد بالتوازي مع استخدام ميكروبات البروبيوتيك لتجنب أعراض نقصها.