Effects Of Different Doses Of Probiotic (Biomin)® On Some Hematological And Biochemical Parameters In Chicken

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الخلاصة

أجري هذا البحث لمعرفة تأثير الجرع المختلفة (٠.١٥، ٠.٣، ١.٥، ٧.٥، ١٥ غم/كغم علف) من المعزز الحياتي البيومين ®(BIOMIN) على بعض المعايير الدموية والكيم وحيوية في أفراخ فروج اللحم. استعمل ٤٨ فرخ فروج لحم نوع روز بعمر يوم واحد . أظهرت النتائج أن الزيادة في وزن الجسم كانت معنوية ($p \leq 0.05$) ومرتبطة مع الزيادة في جرعة المعزز الحياتي، بينما لم يلاحظ تغيير معنوي على تركيز الهيموكلوبين وحجم الخلايا المرصوصة ضمن المجاميع المختلفة. الانخفاض في مستوى كولستيرول مصل الدم كان معنوياً وعكسياً مع زيادة جرعة المعزز الحياتي ، على العكس من مستوى البروتين واليوريا في مصل الدم حيث كانت الزيادة فيهما معنوية وطردية مع زيادة جرعة المعزز الحياتي، أما مستوى كلوكوز المصل فلم يتأثر بشكل واضح ضمن المجاميع المختلفة. وأخيراً فان جميع الأفراخ نفقت في مجموعة الجرعة العالية جداً (١٥ غم/كغم) وفي اليوم الثالث من التجربة.

ABSTRACT

This study was carried out to evaluate effects of probiotic (BIOMIN)® at different doses (0.15, 0.3, 1.5, 7.5, 15 g/kg diet) on some hematological and biochemical parameters in chickens. The number of broiler chickens are 48 at one day old Ross kind were used . Results show that body weight increased significantly ($p \leq 0.05$) as probiotic dose, while both Hb concentration and PCV% are not changed in relation to probiotic doses. Reduction of serum cholesterol level was clear and dependent on the contrary changes in both serum protein and urea, which inceased with

dose elevation, on the other hand, serum glucose level changes unclearly in different dose groups. Finally results show very high dose (15 g/kg diet) of probiotic (BIOMIN)® lead to death of all chickens in this group at the 3rd day of experiment.

Introduction

Probiotic in Greek means "for life" (1) and can be defined as a live microbial feed supplementations, which beneficially affects the host animal by improving its intestinal balance (2), or a mono- or defined mixed culture of live microorganism which, applied to animal or man, beneficially affected the host by improving the properties of indigenous gastrointestinal bacteriota (3), but restricted to products that these properties are: contain live microorganism (e.g.: freeze-dried cells or in fresh or fermented products), improve the health and wellbeing of animals or man (including growth promotion), can have their effect on all host mucosal surface including: the mouth and gastrointestinal tract, the upper respiratory tract, and the urogenital tract (4). However, its important to distinct more details about probiotc action, that can put in the following categories: First, production of inhibitory substances, which include: Hydrogen peroxide (H_2O_2) that protect host cell from pathogenic microorganisms by oxidation-reduction reactions (5), Antibacterial substances as Bacteriocin (protein produced by certain bacteria and act as antibacterial agent) (4), and Nicin and Reuterin (broad spectrum antibacterial agent in poultry produced by different types of bacteria) (6).

The second category is adhesion sites blocking, already microorganisms adhere on gastrointestinal mucosa by cell wall polysaccharide, probiotic prevent this adhesion by different mechanisms as: carbohydrate-binding specificities, preventing of microorganism to get to Mannan receptors, or lactobacillus competitive adhesion to epithelial cells of gastrointestinal mucosa blocking pathogenic bacteria touching these cells (4). The third category is competition for essential nutrients, for e.g.: abnormal balance of bacteria in the gastrointestinal tract is capable of utilizing all of the potential carbon sources in the environment (7). Also the fourth category is toxin receptor destruction, yeast containing probiotics act by this mechanism more than bacteria containing probiotics (8), briefly this action do by toxin binding to yeast cell receptor, so protecting epithelial cells from toxins (9). In addition also chick immunostimulation by bacteria containing probiotics is one of the most important mechanism to utilize the normal equilibrium of microflora in the gut (10).

From our survey on the past literatures we noted that most studies are directed away from probiotic effects on the body biochemical parameters, therefore, the present study was undertaken to study the variation in biochemical parameters (serum glucose, serum cholesterol, serum urea, and serum total protein) and Hb concentration, PCV % and body weight gain produced by different doses of probiotic (BIOMIN)® in broiler chicken, we chose these parameters to indicate general body condition from biochemical aspects, rather than revealing specific organ or system status.

Materials and Methods

Probiotic: BIOMIN® IMBO were used in our experiments, get from local market, its produced by Biomin G.T.I. Gmbh company, Ember AG-Austria, composed of spray dried *enterococcus faecium* 5×10^{11} CFU/kg (DSM 3530), also contain oligosaccharide as prebiotic, the recommended dose by manufacture was 1.5 g/kg diet.

Experimental animals: the present study was conducted during April and May 2008 at animal house section and department of physiology, college of veterinary medicine, university of mosul. A total of 48 chickens, one day old Ross kind broiler were used, they purchased from Al-Ameen Company (Mosul, Iraq). All birds were raised in floor cages, suitable environmental condition: continuous lighting, well humidity and ventilation, optimal temperature (38° C at 1st week, and reducing 2° C weekly), and have access to feed and water *ad libitum*.

Experimental design: chickens were randomly allotted to six groups (8 birds in each group) and given, from the 1st day to 28 day of age, the following diets:

1. control group: diet contained essential nutrients fit the requirements suggested by NRC (11).
2. Group of very low dose (VLD): control group diet + 0.15 g probiotic/ kg diet .
3. Group of low dose (LD): control group diet + 0.3 g probiotic/kg diet.
4. Group of recommended dose (RD): control group diet + 1.5 g probiotic / kg diet
5. Group of high dose (HD): control group diet + 7.5 g probiotic/kg diet.
6. Group of very high dose (VHD): control group diet +15 g probiotic/kg diet.

Each group birds are weight in 1st day and put in one cage, and birds observed daily along experimental time.

Sampling and analysis: Blood samples were collected from wing vein at the end of experiment (day 28) Hemoglobin (Hb) concentration (g/dl) and packed cell volume (PCV)% are determined immediately before serum separation by blood centrifugation 3000 xg for 15 minutes, and analysis was done as follow:

1. Hb concentration (g/dl): determined according to Drabkin method (12) by kit (syrbio) and using spectrophotometer at 540 nm.
2. PCV %: determined according to Hoffbrand method (13) using heparinized capillary tube and hematocrite centrifuge for 10 minutes.
3. Serum glucose level (mg/dl): determined according to Trinder method (14) by kit (syrbio) and using spectrophotometer at 505 nm.
4. Serum cholesterol level (mg/dl): determined according to Richmond method (15) by kit (syrbio) and using spectrophotometer at 505 nm.
5. Serum urea level (mg/dl): determined according to Kaplan method (16) by kit (syrbio) and using spectrophotometer at 560 nm .
6. Serum total protein (g/l): determined according to Reinhold (17) by Biuret method and using spectrophotometer at 505 nm.

And each chicken was weight befor killing.

Statistical analysis: Data were subjected to ANOVA test using SPSS computer program (Ver.10). Differences among groups were separated by Duncan's multiple range test. Probability values less than 0.05 ($p \leq 0.05$) were consider significant (18).

Results

The first important result that all chickens in very high dose group (VHD) were died at the 3rd day of experiment. Body weight of all chickens are approximately similar in the 1st day weighting (40g±7). From table (1) we noted that body weight gain are significantly ($p \leq 0.05$) increased in treated dose groups (except VLD group) in comparing with control group, and the highest increase in body weight was in (HD) group (figure 1), also from daily observation of chickens we were noted that probiotic in different doses cause more activity in chickens. From the same table (1) there was no significant ($p \leq 0.05$) changes in both Hb concentration and PCV% in different groups in comparing with control group (figure 2 and 3). Results in table (2) show biochemical parameters affected by different doses of probiotic as follows: serum glucose level (mg/dl) was changed but insignificantly between groups (except HD group and VLD group) which there was significant decline in serum glucose level when compared with control group (figure 4). In the case of serum cholesterol level (mg/dl), the results show a significant decrease in cholesterol level, with increasing probiotic doses (except VLD group) (figure 5), while serum urea level (mg/dl) was increased in all groups when compared with control, but these increasing are significantly in RD group and HD group only (figure 6), finally serum protein level (g/l) was increased significantly depending on dose of probiotic (BIOMIN)® in all groups (figure 7).

Table (1): Effect of different doses of probiotic (BIOMIN®) on body weight, Hb concentration and PCV% (Mean± S.E)*

Groups	Body weight (g)	Hb concentration (g/dl)	PCV%
Control Group	473±35 d	7.2±1.3 a	34±2.4 a
Very low dose (VLD)group (0.15 g probiotic / kg diet)	482±30 d	7.4±0.68 a	36±1.9 a
Low dose (LD)group (0.3 g probiotic / kg diet)	564±29 c	6.9±1.4 ab	31±2.0 a
Recommended dose (RD)group (1.5 g probiotic / kg diet)	670±41 b	7.1±0.89 a	33±1.8 a
High dose (HD)group (7.5 g probiotic / kg diet)	705±55 a	7.0±0.82 a	32±2.1 a
Very high dose (VHD)group (15 g probiotic / kg diet)	×	×	×

* (mean± S.E.) of 8 chickens in each group
Different letters in each column means significant differences at p≤0.05 .
× VHD group chickens died at 3rd day of experiment.

Table (2): Effect of different doses of probiotic (BIOMIN®) on Serum Glucose, Cholesterol, Urea, and Protein (Mean± S.E)*

Groups	Serum Glucose level (mg/dl)	Serum Cholesterol level (mg/dl)	Serum Urea level (mg/dl)	Serum Protein level (g/l)
Control Group	161±5.5 a	142±4.6 a	17.7±0.6 b	50.6±2.1 c
Very low dose (VLD)group (0.15 g probiotic / kg diet)	148±7.1 b	138±3.4 ab	18.1±1.0 b	54.4±3.4 b
Low dose (LD)group (0.3 g probiotic / kg diet)	151±2.3 a	128±1.3 b	18.5±0.9 b	55.9±2.6 b
Recommended dose (RD)group (1.5 g probiotic / kg diet)	155±9.3 a	109±2.7 d	20.9±07 a	68.2±1.9 a
High dose (HD)group (7.5 g probiotic / kg diet)	137±4.1 b	106±2.1 d	22.4±0.8 a	69.9±2.4 a
Very high dose (VHD)group (15 g probiotic / kg diet)	×	×	×	×

* (mean± S.E.) of 8 chickens in each group
Different letters in each column means significant differences at p≤0.05 .
× VHD group chickens died at 3rd day of experiment.

Figure (1): Effects of different doses of probiotic on body weight (g)

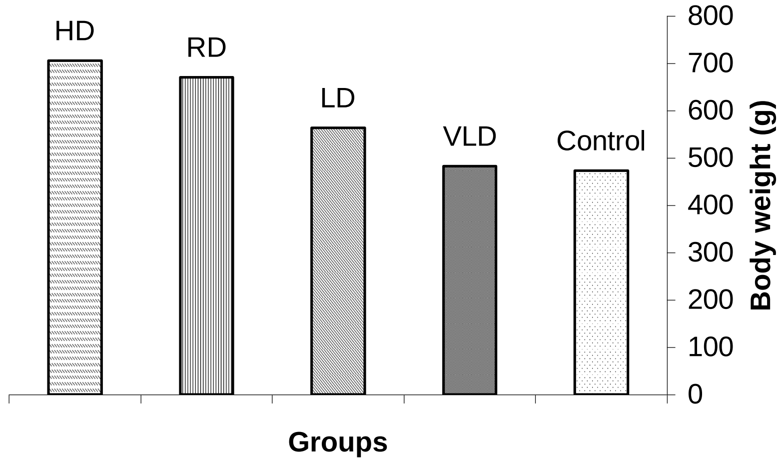


Figure (2): Effects of different doses of probiotic on Hb concentration (g/dl)

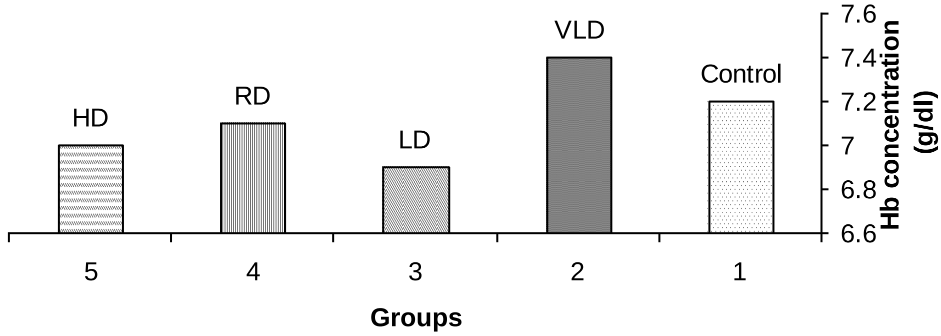


Figure (3): Effects of different doses of probiotic on PCV%

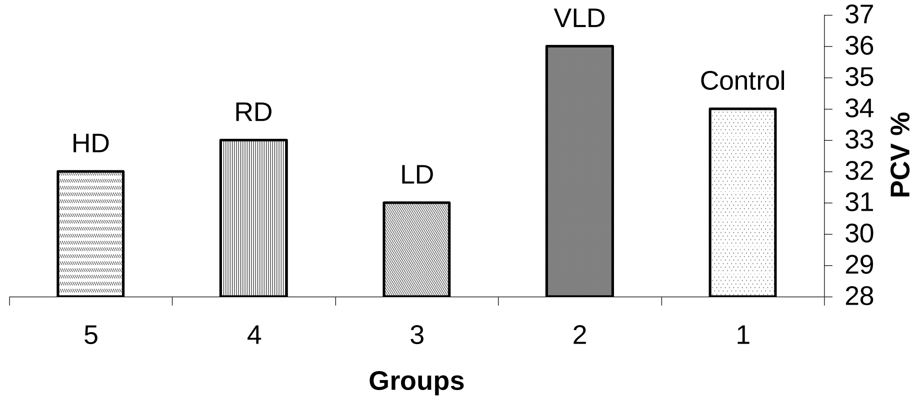


Figure (4): Effects of different doses of probiotic on serum glucose level (mg/dl)

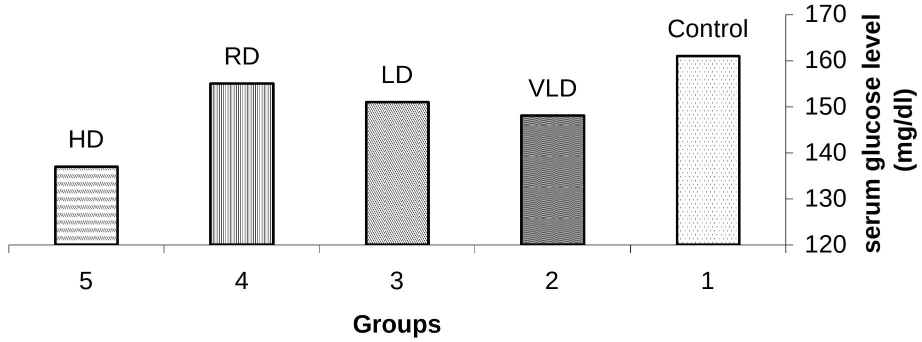


Figure (5): Effect of different doses of probiotic on serum cholesterol level (mg/dl)

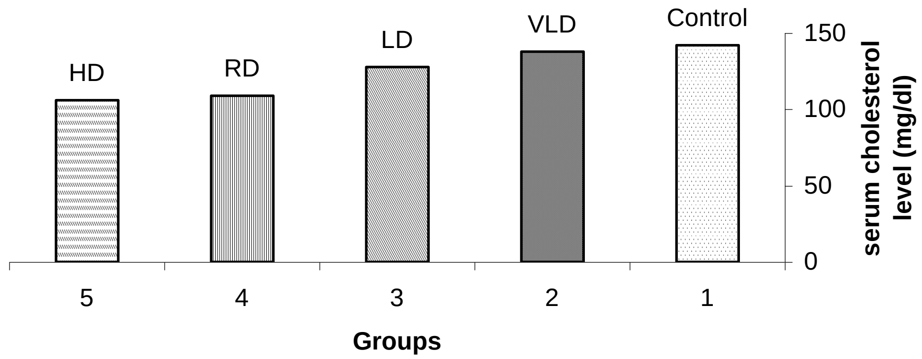


Figure (6): Effect of different doses of probiotic on serum urea level (mg/dl)

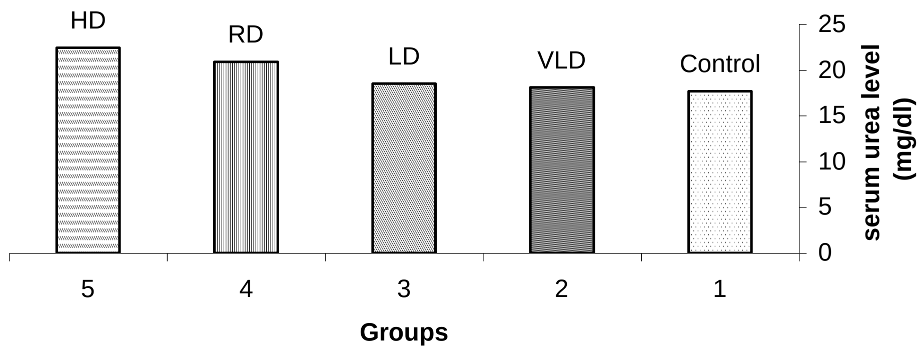
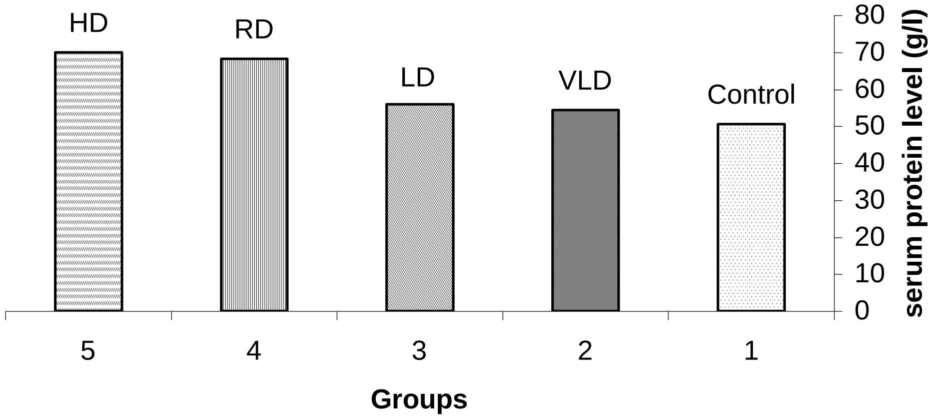


Figure (7): Effects of different doses of probiotic on serum protein level (g/l)



Discussion

Mortality of chickens at very high dose (VHD) group due to high doses of probiotic may be lethal. Results (table 1) explain that body weight gain was due to improving action of probiotic on body weight and performance due to growth promotion (3,19) and this agree with Yeo and Kim (20), they reported that average daily weight gain of chickens fed probiotics was significantly increased during the first 3 wk of growth, insignificant increase in body weight in (VLD) group may be due to insufficient dose to produce effect on body weight. Hematological parameters which were studied are not affected significantly by different doses of probiotic, these results concur with that of Kamruzzaman (21) and this may be due to that probiotic have no direct effect on both Hb concentration and PCV% (22). While biochemical parameters were dose dependent affected significantly but in different manner, serum glucose level changes unclearly, in different dose groups and this may be due to blood glucose in chicken have wide varieties (physiological variations) (23) also it has been recorded of spontaneous undulant glucose level in normal chickens (24). The decrease in serum cholesterol level which were dose dependent (except VLD group due to too small dose to produce effect) was agree with Mohan *et al.*(22) and Kalavathy *et al.*(25). Gilliland *et al.* (26) hypothesized that some Probiotic components (some bacteria) are capable to incorporate cholesterol into the cellular membrane of the organism, thus, cholesterol assimilation by these bacteria in turn reduce cholesterol absorption in the system. Another mechanism of hypocholesterolemic action is via bile acids, the cholic and deoxycholic bile acids are produced from cholesterol by hepatocytes and are conjugated with glycine and taurine, respectively, these acids enter the small intestine, where they are absorbed and directed to the liver, and a decrease in bile acid recycling would ultimately result in a lowering of serum cholesterol concentration because cholesterol is used for bile acid synthesis (27). Serum urea level is dose dependent, increased significantly in all groups except VLD group and LD group, which may be due to small dose of probiotic was insufficient to produce effect on serum urea level, our explanation is that high doses and long period of treatment (4 weeks) was stimulating to produce more urea by increasing body activity and also related to increasing in serum protein level, which urea is the main end product of protein degradation (28). Finally serum protein level was increased significantly with increasing probiotic doses in all groups and this may be due to probiotic improve gastrointestinal status (especially absorption) leading to increase protein absorption and blood protein level, similar improvements in feed efficiency have been reported for poultry receiving probiotics (24,29), its also recorded that probiotic greatly enhance immunostatus, immunoglobulin as apart, was important portion of blood protein (10).

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