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# IMPACT OF SUPPLEMENTING DIETS WITH CHITOSAN AND PROBIOTICS ON CERTAIN BLOOD PROPERTIES OF BROILERS

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**Abstract**

This experiment was conducted at the poultry farm of the Animal Production Department, College of Agriculture, University of Anbar from 28 December 2023 to 8 February 2024 (42 days). It aimed to compare the effect of adding two different levels of chitosan and probiotics to the diet and their combinations in the physiological performance and some blood characteristics of broilers from the age of 6 weeks. The study used 10 one-day-old Ross chicks of 40 g average weight. The chicks were distributed into 7 treatments of 3 replicates each (10 chicks for each replicate). The first treatment (T1) was the control (without any addition), the T2 and T3 treatments included adding chitosan at 1 and 2 g/kg feed, respectively. The T4 and T5 treatments included adding probiotic at 1 and 2 g/kg of feed, respectively. The T6 treatment included the addition of chitosan and probiotics at 0.5 g/kg of feed each while T7 included the addition of chitosan and probiotics at 1 g/kg feed each. Results show that the T5 treatment had significant superiority ($P \leq 0.05$) in the PCV and hemoglobin values compared to T1, T2, and T4, and did not differ significantly from the other treatments. Treatment T5 also continued to be superior in the number of red blood cells compared

to T1, T2, T3, and T4 and did not differ significantly from the other treatments. There was also a significant decrease (P≤0.05) in the concentration of blood plasma glucose in T2 compared to T6, while treatment T2 recorded a significant increase in total protein concentration over T3, T4, and T6. Also, treatment T2 showed a significant increase in globulin concentration compared to T3 and T4 but was not significantly different from the other treatments. Lipid profiles recorded a significant decrease (P≤0.05) in the concentration of blood plasma cholesterol and low-density lipoproteins in treatment T6 compared to T2 and T4, but were not significantly different from the other treatments. Liver enzymes recorded a significant decrease (P≤0.05) in the AST enzyme level in treatment T2 over T4 and T5, but was not significantly at variance from the other treatments.

**Keywords:** Chitosan, Probiotics, Blood characteristics, Broilers.

# تأثير إضافة الكيتوزان والبروبايوتك إلى العليقة في بعض صفات الدم لفروج اللحم

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**الخلاصة**

أجريت هذه التجربة في قاعة الدواجن التابع لقسم الإنتاج الحيواني في كلية الزراعة/ جامعة الأنبار للفترة من 2023/12/28 الى 2024/2/8 (42 يوم). هدفت التجربة إلى مقارنة تأثير إضافة مستويين مختلفين من الكيتوزان والبروبيوتيك وخليطهما إلى العليقة في بعض الصفات الدمية لفروج اللحم في عمر 6 أسابيع. استخدم 210 فرخ سلالة روص 308 بعمر يوم واحد وبمتوسط وزن 40 غم. وزعت على 7 معاملات بواقع 3 مكررات لكل معاملة (10 فرخ لكل مكرر). أما بالنسبة لمعاملات التجربة فكانت المعاملة الأولى T1 سيطرة (بدون أي إضافة) والمعاملة الثانية T2 والمعاملة الثالثة T3 تضمنت إضافة الكيتوزان بمستوى 1 و2 غم/كغم علف على التوالي. تضمنت المعاملة الرابعة T4 والمعاملة الخامسة T5 إضافة البروبايوتك بمستوى 1 و2 جم/كجم علف على التوالي، وتضمنت المعاملة السادسة T6 إضافة الكيتوزان بمستوى 0.5 جم/كجم علف + البروبايوتك بمستوى

0.5 جم/كجم علف وتضمنت المعاملة السابعة T7 إضافة الكيتوزان بمستوى 1 جم/كجم علف + البروبايوتك بمستوى 1 جم/كجم علف. وأظهرت النتائج أن المعاملة T5 كان لها تفوق معنوي ($P\leq0.05$) في قيم PCV والهيموغلوبين مقارنة بالمعاملات T1 وT2 وT4 ولم تختلف بشكل كبير عن بقية المعاملات التجريبية. كما استمرت المعاملة T5 في التفوق في عدد خلايا الدم الحمراء مقارنة بالمعاملات T1 وT2 وT3 وT4 ولم تختلف بشكل كبير عن بقية المعاملات. كما أظهرت نتائج الصفات الكيماحيوية انخفاض معنوي ($P\leq0.05$) في تركيز جلوكوز بلازما الدم في المعاملة T2 مقارنة بالمعاملة T6 ولم تختلف معنويا عن باقي مجموعات التجربة، بينما سجلت المعاملة T2 ارتفاعا معنويا في تركيز البروتين الكلي مقارنة بالمعاملات T3 وT4 وT6 ولم تختلف معنويا عن باقي المعاملات، كما سجلت المعاملة T2 ارتفاعا معنويا في تركيز الكلوبيولين مقارنة بالمعاملات T3 وT4 ولم تختلف معنويا عن باقي المعاملات، وسجلت نتيجة صورة الدهن انخفاضا معنويا ($P\leq0.05$) في تركيز كولسترول بلازما الدم والبروتينات الدهنية منخفضة الكثافة في المعاملة T6 مقارنة بالمعاملتين T2 وT4 ولم تختلف معنويا عن باقي المعاملات التجريبية، وسجلت نتيجة تحليل أنزيمات الكبد انخفاضا معنويا ($P\leq0.05$) في مستوى أنزيم AST في المعاملة T2 مقارنة بالمعاملتين T4 وT5 ولم تختلف معنويا عن باقي المعاملات التجريبية.

**كلمات مفتاحية:** كيتوزان، بروبايوتك، صفات الدم، فروج اللحم.

## Introduction

Some European Union nations and the United States have banned the use of antibiotics as growth boosters in chicken feed because they harm both the health of the bird and the consumer by contaminating the leftover carcass tissues (6). Genetic alterations in the gene map are among the adverse consequences of these antibiotics (3) prompting the search for alternatives from natural sources that are safe, raise body immunity, and reduce the incidence of bacterial and fungal diseases (29). A non-nutritional additive to chicken feed, such as chitosan—a material made from chitin that is safe for both human and animal health—is one of these techniques. It makes up the majority of the exterior skeleton of marine creatures like shrimp and crabs and is composed of many glucosamine units (25). Thus, it has a beneficial impact on the physiological and productive performance of broiler chickens as well as their ability to use energy and protein efficiently (17). It regulates the intestinal flora and creates suitable conditions for the spread of beneficial microorganisms and prevents the growth of harmful gram-negative and gram-positive microorganisms and fungi (12, 16, 20 and 21).

A manmade product that is added to poultry feed are probiotics, which are made up of helpful bacteria isolated from the intestinal flora of the chicken's digestive system (9). It aids in the transmission of these beneficial bacteria to freshly hatched chicks (8), and thus accelerates the occurrence of microbial balance after at least 14 days (18). This improves the body’s immune response (13) though it is necessary to correct the microbial imbalance, especially when adult birds are exposed to stressful elements such as high temperatures and disease infection, in addition to changes in the feed that

reduce productive performance (10 and 26). The role of probiotics in the digestive system of poultry is a process of competitive antagonism of microorganisms, modifying metabolism by intensifying the activity of digestive enzymes, reducing ammonia production, improving feed intake and digestion, absorption efficiency, improving the body's immune response, and reducing mortality rates (1 and 7).

The aim of this experiment was to examine the effects of supplementing chicken rations with different amounts of chitosan and probiotics, as well as their combinations, on the blood characteristics of 42-day-old broilers.

## Materials and Methods

This 42-day experiment was carried out at the University of Anbar/ college of Agriculture in chicken farm from 28 December 2023 until 8 February 2024. The experimental treatments were as follows: Treatment T1 (control without any addition), T2 and T3 had 1g/kg and 2g/kg of chitosan included in the diets, respectively, T4 and T5 included 1g/kg and 2g/kg of probiotic, T6 included a mixture of 0.5g/kg each of chitosan and probiotics and T7 a 1g/kg mixture of chitosan and probiotics each. A total of 210 one-day-old chicks from Rose 308 broilers weighing 40 g on average were used.

The chicks were divided into seven treatments at random, with three replicates for each treatment and ten chicks for each duplicate. As indicated in Table 1, three different diets were administered during the trial period: the starter diet, from 1 to 11 days, the grower diet (12 to 21 days), and the finisher diet (22 to 42 days). Probiotics and chitosan were acquired from the People's Republic of China through the Amazon website. At 42 days of age, three broiler chickens for each treatment had their jugular veins randomly sampled. Using K-EDTA tubes, tests were conducted for total erythrocyte and leukocyte counts (RBC and WBC), packed cell volume (PCV), hemoglobin concentration (Hb), heterophil, lymphocytes, and H/L.

After centrifuging the blood samples for 15 minutes at 3000 cycles, the blood plasma was separated, and subsequent tests carried out for total protein, albumin, globulin, glucose, AST, ALT, triglycerides, cholesterol, LDL, HDL, VLDL using a kit from AGAPPE-Switzerland. The experiment was a one-way study, and the trend contained a significant component as well as the impact of treatments using the general linear model and SAS (27) statistical program. Duncan's test (1955) was used to examine the difference between the mean values at the $P \leq 0.05$ significance level (11).

**Table 1: Components and nutritional makeup of diets used in experiments.**

| Ingredient (%) | Starter (1-11 d) | Grower (12-21 d) | Finisher (22-42 d) |
|---|---|---|---|
| Corn | 47 | 51 | **53** |
| Wheat | 10 | 10 | **10** |
| Soybean meal, (44% CP) | 33.8 | 29.3 | **25.39** |
| Animal protein (40% CP) * | 5 | 5 | **5** |
| Plant oil | 2 | 3 | **5** |
| DiCa ph. | 0.6 | 0.4 | **0.3** |
| CaCO3 | 1.5 | 1.2 | **1.2** |
| Sodium chloride | 0.1 | 0.1 | **0.1** |
| Total | 100% | 100% | **100%** |
| | **Calculated chemical composition**** | | |
| ME, kcal/kg diet | 2997 | 3111 | **3262** |
| Protein % | 23.4 | 21.5 | **19.8** |
| Lysine % | 1.35 | 1.22 | **1.11** |
| Cyst + meth % | 0.89 | 0.84 | **0.80** |
| Fiber % | 2.8 | 2.7 | **2.6** |
| Ca % | 1.07 | 0.90 | **0.86** |
| Available phosphorus % | 0.47 | 0.42 | **0.40** |
| Fat % | 4.6 | 5.7 | **7.8** |

*Concentrated protein for chicken feed, Alblasserdam, Holland, Wafi B.V. 40% crude protein, 5% crude fat, 2.20% crude fiber, 7.13% moisture, 28.32% ash, 4.50% calcium, 2.65% phosphorus, 4.68% available phosphorus, 3.85% lysine, 3.70% methionine, 4.12% methionine + cysteine, 0.42% tryptophan, 1.70% threonine, 2107 metabolizable energy, 2.30% selenium, and 4% copper.

** The chemical composition values were calculated using NRC (1994) (24).

## Results and Discussion

The PCV and hemoglobin values for treatment T5 were substantially better ($P \leq 0.05$) than for T1, T2, and T4, but not statistically different from the others (Table 2). Treatment T5 also continued to be superior in the number of red blood cells compared to T1, T2, T3, and T4 and did not differ significantly from the others. However, no discernible variations were found in the quantity of white blood cells, heterophil, lymphocytes, or the proportion of heterophil to lymphocytes throughout the treatments. By enhancing the microbial balance in the intestines, probiotics contribute to better digestive health by increasing the absorption of iron and vitamin B12, two components necessary for the synthesis of red blood cells and hemoglobin (20). Probiotics also play a role in reducing oxidative stress, which improves the efficiency of bone marrow in producing red blood cells (4). It has been proven that antioxidants play an important role in increasing the number of red blood cells and the level of hemoglobin in the blood (23 and 24).

**Table 2: Impact of dietary additions and mixtures of probiotics and chitosan on the cellular blood properties of broilers.**

| Treatment | Traits | | | | | | |
|---|---|---|---|---|---|---|---|
| | PCV (%) | Hb (g/dL) | RBC Cell/ml$^6$ | WBC Cell/ml$^3$ | Heterophil (%) | Lymphocytes (%) | H/L |
| T1 | 28.50 b | 8.50 b | 3.04 b | 9.05 | 39.50 | 51.50 | 0.76 |
| T2 | 29.00 b | 8.60 b | 3.07 b | 8.20 | 39.00 | 52.00 | 0.75 |
| T3 | 30.00 ab | 8.90 ab | 3.17 b | 6.30 | 31.00 | 59.50 | 0.52 |
| T4 | 29.00 b | 8.65 b | 3.10 b | 6.05 | 29.50 | 61.50 | 0.47 |
| T5 | 36.50 a | 11.05 a | 3.94 a | 8,25 | 32.50 | 59.00 | 0.55 |
| T6 | 30.50 ab | 9.15 ab | 3.26 ab | 8.90 | 36.00 | 54.50 | 0.66 |
| T7 | 32.00 ab | 9.65 ab | 3.44 ab | 7.60 | 33.00 | 58.00 | 0.56 |
| Sig. level | 0.05 | 0.05 | 0.05 | NS | NS | NS | NS |
| SEM | 0.88 | 0.29 | 0.103 | 0.761 | 2.12 | 1.897 | 0.066 |

The mean ± standard error

NS: not significant.

The letters in the same column (a, b, and c) show that notable variations between the treatments at level $p \leq 0.05$.

Treatments: T1 control, T2 and T3 (1g/kg and 2g/kg of chitosan, respectively), T4 and T5 (1g/kg and 2g/kg of probiotics, respectively), T6 (0.5g/kg each of chitosan + probiotics), T7 (1g/kg each of chitosan and probiotics).

Table 3 reveals that the concentration of low-density lipoproteins and blood plasma cholesterol in treatment T6 was considerably lower ($P \leq 0.05$) than in treatments T2 and T4, but was not statistically different from the other treatments. Triglyceride and high-density lipoprotein concentrations did not significantly differ across the treatments.

Lactobacillus and bifidobacterium strains can help break down bile acids are necessary for fat and cholesterol absorption so that they can be excreted instead of reabsorbed, which makes the liver use more cholesterol and lower its level (31). In addition, probiotics produce short-chain fatty acids that inhibit cholesterol production in the liver (14 and 15). Chitosan also has a positive charge that helps it bind to fatty acids and cholesterol, which have a negative charge. Chitosan also binds to bile acids that contain cholesterol, which encourages the liver to use more cholesterol to produce new bile acids (19).

**Table 3: Impact of dietary additions and mixtures of chitosan and probiotics on broiler blood profile lipid characteristics.**

| Treatment | Traits | | | |
|---|---|---|---|---|
| | mg/dl Cholesterol | TG mg/dl | HDL mg/dl | LDL mg/dl |
| T1 | 195.5 ab | 197.5 | 36.0 | 102.0 ab |
| T2 | 208.0 a | 173.0 | 39.5 | 108.5 a |
| T3 | 187.0 ab | 169.5 | 37.0 | 97.5 ab |
| T4 | 215.0 a | 151.0 | 39.0 | 112.0 a |
| T5 | 197.0 ab | 182.0 | 40.0 | 102.0 ab |
| T6 | 175.5 b | 160.0 | 37.5 | 91.0 b |
| T7 | 188.0 ab | 195.5 | 36.0 | 97.0 ab |
| Significance Level | 0.05 | NS | NS | 0.05 |
| SEM | 4.14 | 7.06 | 1.07 | 2.22 |

Treatments: T1 control, T2 and T3 (1g/kg and 2g/kg of chitosan, respectively), T4 and T5 (1g/kg and 2g/kg of probiotics, respectively), T6 (0.5g/kg each of chitosan + probiotics), T7 (1g/kg each of chitosan and probiotics).

Table 4 shows that the blood plasma glucose concentration for treatment T2 was significantly lower ($P \leq 0.05$) than for T6 and did not differ significantly from the others. In contrast, total protein concentration for T2 was significantly higher than for T3, T4, and T6 but not significantly different from the other treatments. Additionally, globulin concentrations in T2 were much higher than T3 and T4, but did not differ significantly from the other treatments. Also, there were no discernible variations in the albumin concentrations amongst the treatments. Chitosan acts as a soluble fibrous material that slows digestion and reduces the absorption of glucose from the intestines into the blood. It also improves the cells' response to insulin, which helps transport glucose from the blood to the cells more efficiently (30). As for the effect of chitosan in increasing the level of blood protein, it is due to improving liver function by reducing fat deposition and reducing inflammatory proteins in the blood (5).

**Table 4: Effect of adding chitosan and probiotics and their combinations to the diet on the biochemical blood characteristics of broilers.**

| Treatment | Traits | | | |
|---|---|---|---|---|
| | Glucose mg/dl | Total Protein g/l | Albumin g/l | Globulin g/l |
| T1 | 234.0 ab | 63.0 ab | 22.5 | 40.5 ab |
| T2 | 188.0 b | 68.0 a | 23.0 | 45.5 a |
| T3 | 250.5 ab | 55.5 c | 28.5 | 27.0 c |
| T4 | 239.5 ab | 55.5c | 25.0 | 30.5 bc |
| T5 | 218.5 ab | 66.5 ab | 31.5 | 35.0 abc |
| T6 | 260.0 ab | 60.0 bc | 25.0 | 35.0 abc |
| T7 | 284.0 a | 66.5 ab | 29.0 | 37.5 abc |
| Significance Level | 0.05 | 0.05 | NS | 0.05 |
| SEM | 7.97 | 1.49 | 1.24 | 1.79 |

Treatments: T1 control, T2 and T3 (1g/kg and 2g/kg of chitosan, respectively), T4 and T5 (1g/kg and 2g/kg of probiotics, respectively), T6 (0.5g/kg each of chitosan + probiotics), T7 (1g/kg each of chitosan and probiotics).

Table 5 shows that the ALT enzyme levels did not vary substantially across treatments, and were considerably lower in T2 (P≤0.05) than in T4 and T5. They also did not differ significantly from the other experimental treatments. To improve liver function, chitosan therapy lowers the amount of the AST enzyme because it reduces oxidative stress and inflammation, eliminates pollutants and heavy metals, and lessens body fat buildup (2).

**Table 5: Impact of dietary additions and mixtures of chitosan and probiotics on the liver enzymes of broiler.**

| Treatment | Traits | |
|---|---|---|
| | AST IU/ L | **ALT** IU/ L |
| T1 | 259 bc | 42.5 |
| T2 | 214 c | 23.5 |
| T3 | 259.5 bc | 35.5 |
| T4 | 342.5 ab | 30.0 |
| T5 | 374.5 a | 30.0 |
| T6 | 268.0 abc | 24.5 |
| T7 | 264.0 bc | 37.0 |
| Significance Level | 0.05 | NS |
| SEM | 16.58 | 2.64 |

Treatments: T1 control, T2 and T3 (1g/kg and 2g/kg of chitosan, respectively), T4 and T5 (1g/kg and 2g/kg of probiotics, respectively), T6 (0.5g/kg each of chitosan + probiotics), T7 (1g/kg each of chitosan and probiotics).

## Conclusions

The addition of probiotics improved the hemoglobin value and red blood cell count, while adding both probiotics and chitosan reduced the level of cholesterol and LDL. Chitosan had an effect in reducing the level of the AST enzyme. As such, it can be concluded that including the two additives has no negative effect on the health of broilers.

**Supplementary Materials:**

No Supplementary Materials.

**Author Contributions:**

Waleed Ismail Al-Jugifi methodology, writing—original draft preparation; Husam Hkmat Nafea, Noor Naji Alhayani: writing—review and editing. All authors have read and agreed to the published version of the manuscript.

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The authors declare no conflict of interest.

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