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# Effect of probiotic, prebiotic, synbiotic and medicinal plants on Carcass Characteristics, Immune organs, blood immunological parameters and blood Hematological and biochemical of broiler Fed on Different Levels of Protein

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**Keywords:**
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**ABSTRACT**

The purpose of this study was to see how non-antibiotic growth boosters such as Probiotic, Prebiotic, Synbiotic, and medicinal herbs (Mixture of Origanum majorana, Foeniculum vulgare, and Carum carvi in a 1:1:1 ratio) affected carcass yield, immune response, blood hematological, and biochemical parameters in broilers fed test diets with two dietary protein levels (normal and low). The research was conducted at Alexandria University, Poultry Research Center, Faculty of Agriculture. The trial lasted 42 days in total. A total of 500 one-day-old Cobb broiler chicks with similar average live body weight were assigned to one of ten treatment groups. Each treatment has five replicates with ten chicks each. Ten experimental diets were developed to be nearly is caloric and to provide all nutrients required for broiler growth throughout two stages of development: starter diets (1 - 21 days) and grower diets (22 - 42 days). Ten experimental diets were made up of five feed-additive programs and two amounts of crude protein (recommended or low, 85 percent of recommended) (control, probiotic, prebiotic, synbiotic and medicinal plants). Among the additives, synbiotic had a positive effect on the WBCs, RBCs, in general, especially the Fabricius bursa Under the conditions of the current study, synbiotic had a significant impact on hematological parameters, carcass yield, and immunological organs of broiler. Furthermore, more research is required to confirm the existing findings.

# تأثير البروبيوتيك، البريبيوتيك، السنبيوتيك و الأعشاب الطبية على الذبيحة، الاستجابة المناعية، مُكونات الدم وقياسات الكيموحيوية لدم دجاج اللحم الذي تم تغذيته على نسب مُختلفة من البروتين

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**الكلمات المفتاحية:**
بروبيوتيك
بريبيوتيك
سنبيوتيك
الأعشاب الطبية
صفات الدم
الذبيحة
الاعضاء المناعية
دجاج اللحم
البروتين

**الملخص**

الهدف من هذه الدراسة كان معرفة مدي تأثير مُنشطات النمو الطبيعية، بروبيوتيك، بريبيوتيك، سانبيوتيك ومخلوط الأعشاب الطبية (مكونه من الشمر والبردقوش والكراوية بنسب خلط (1: 1: 1) مع مُستويين مُختلفين من البروتين (مثالي ومُنخفض) على صفات الذبيحة، والاستجابة المناعية، مُكونات الدم وقياسات الكيموحيوية . تمت هذه الدراسة في مركز بحوث الدواجن – كلية الزراعة- جامعة الاسكندرية –مصر. استمرت التجربة لمدة 42 يوم. تم توزيع عدد 500 كتكوت لحم عمر يوم من سلالة كوب ووزعت الطيور عشوائيًا على 10 مجاميع تجريبية بكل مجموعة خمس مُكررات وبكل مُكررة 10 كتاكيت. تم تجهيز عشرة تركيبات علفية (بادى و نامي) لتغطية جميع المُتطلبات الغذائية لكتاكيت اللحم خلال مرحلتي النمو البادي (1 – 21) يوم والنامي (22 – 42) يوم من العُمر. تتألف التركيبات العلفية من مُستويين من البروتين الخام الموصي به والمُنخفض (85% من الموصي به) وخمسة إضافات غذائية هي الشاهد، البروبايوتيك، البريبايوتيك،

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السنبيوتيك و مخلوط الأعشاب الطبية، تم إضافتها للعليقة الكونترول لتشكل العلائق المُختلفة ومدى تأثيرها على كل من الذبيحة، الاستجابة المناعية، مُكونات الدم والقياسات الكيموحيوية للدم . بشكل عام، كان لإضافة السنبيوتيك تأثيرات إيجابية على كرات الدم الحمراء والبيضاء وغدة فابيريسيوش، ويُمكن الاستنتاج أنه في ظل الدراسة الحالية فإن سنبيوتيك كان لها تأثيرات معنوية على صفات الدم والذبيحة والأجهزة المناعية لدجاج اللحم، ولكن هذا الأمر يحتاج إلى المزيد من البحوث للتحقق من النتائج الحالية.

## Introduction

Poultry farming is the most efficient animal farming technique now in use, and it is the foundation of world protein supply (USDA, 2019). With the use of antibiotics as a feed additive being prohibited, the poultry industry must develop new ways to improve productivity (Nang and Vidyarthi, 2019). Multiple additives are currently introduced to poultry nutrition to boost growth due to the widespread usage of various types of additives in nutrition and the enormous development in worldwide chicken outputs., reduce nutrition compound shortages, strengthen immunity systems, and prevent disease. It is simple to estimate the enormous amount of medicine and chemicals that endangers the environment and the health of consumers. Because of the important function that these chemicals play in poultry production efficiency, they are almost always used. Growth stimulants have piqued the interest of scientists and consumers in recent years, making the use of chemicals with desired properties that are not hazardous to health or the environment more important. The continuous sub-therapeutic use of antibiotics in chickens has raised worries about the possibility of antibiotic residue, the development of drug-resistant bacteria, Humans' ability to cure bacterial illnesses has deteriorated. The greater awareness of the potential problems associated with the use of antibiotics as feed additives has prompted researchers to look for alternatives. The use of probiotics, prebiotics, and synbiotics as feed additives in this study is one strategy for future research that would look into combining both natural growth enhancers (Huyghebaert et al., 2011).

(Patterson and Burkholder, 2003) Synbiotics are a mix of beneficial bacteria and substrates that have a synergetic impact on an animal's digestive tract. As a result, it has a lot of promise as a new antimicrobial growth promoter option in chicken production. Synbiotics were found to have a positive influence on In the gastrointestinal tract, there was an increase in the number of beneficial bacteria and a decrease in the growth of probable pathogens, as well as an increase in the number of helpful bacteria. due Synbiotics have been successfully employed as feed supplements for broiler chickens (Katarzyna et al., 2020). Among these options, probiotics are one of the most studied and fascinating groups (Daniel et al., 2019). Because of the multiple health and production benefits, the use of probiotics in animal agriculture has generated great attention. particularly in the context of more natural and antibiotic-free animals. "Live strains of precisely selected bacteria that, when administered in suitable proportions, provide the host with a health benefit, "according to the definition of probiotics (Tsuda and Miyamoto, 2010). Lactic acid bacteria (LAB) from the genera Lactobacillus, Pediococcus, Lactococcus, Enterococcus, Streptococcus, and Leuconostoc are the most prevalent microorganisms utilized as probiotics in animal production. Furthermore, probiotics are live bacteria, fungi, or yeasts that supplement the gut flora and aid in the maintenance of a healthy digestive tract, therefore enhancing poultry growth and general health.

Herbal extracts have been demonstrated to strengthen the immune system and lower blood cholesterol levels, as well as stimulate appetite and feed consumption (Mathivanan et al., 2007). These extracts, according to Sakine et al. (2006), have a mode of action based on changes in the gut microbiota through multiple pathways. This includes improved endogenous digestive enzyme production, immunological response activation and improvement, antibacterial, antiviral, antioxidant, and anthelminthic activities, morpho-histological gastrointestinal tract maintenance, and antioxidant activity improvement .In vitro effects against several pathogens, with antibacterial, antifungal, and/or anthelmintic activity, Several research studies have Anti-inflammatory and antioxidant activities have been proven (Petrolli et al., 2012). As a result, the current study was designed to see how non-antibiotic growth promoters (probiotic (BioPlus 2B), prebiotic (TechnoMos), symbiotic, and medicinal herbs (Mixture of Origanummajorana,Foeniculumvulgare, and Carumcarvi in a 1:1:1 ratio) affected carcass yield and immune response in broilers fed two different dietary protein levels (normal and low). Hematological and biochemical markers were also examined in the blood.

## MATERIALS AND METHODS:

The research was carried out at Alexandria University's Faculty of Agriculture's Broiler Production Unit, Poultry Research Center.

**Probiotic (BioPlus 2B) and prebiotic (TechnoMos):**

These products were obtained from the local market, and therapeutic herbs (a 1:1:1 mixture of Origanummajorana,Foeniculumvulgare, and Carumcarvi) were purchased from the local market and a sample was used for further chemical examination.

Probiotics, prebiotics, and herbs are examples of additives.

All of the additives were commercially available powders that were added to the meals at the levels indicated by the manufacturers. The following were the additives and their dosages:

**BioPlus 2B probiotic:**

At 1g/kg of the starter and finisher meals, a 1:1 mixture of Bacillus licheniformis spores and Bacillus subtitles spores (DSM5750) was used.

**(Prebiotic, TechnoMos):**

**Biological active materials derived from Saccharomyces cerevisiae cell wall fractions high in 1,3-glucans and mannans, 1000g, contains:** Total Glucans 24% -glucans (20%), -glucans (4%), and free glucans (4%).

Mannans make up 18% of the total.

Synbiotic: (A ratio of probiotics and prebiotics) (1:1)

Herbs: (A1:1:1mixtureofOriganummajorana,Foeniculumvulgare, and Carumcarvi).

**Experimental diets**:

The experiment was set up in a two-by-five factorial design. The recommended protein levels for starter and finisher diets were 230 and 200 g CP/kg, respectively (NRC, 1994), and low levels, 195 and 170 g CP/kg, for starter and finisher diets, respectively. The following were the feed additive programmes:

1. The control diet was a baseline diet without any feed additives.
2. A standard diet supplemented with probiotics (1g/Kg).
3. A standard diet supplemented with prebiotics (1g/Kg).
4. A standard diet supplemented with probiotic and prebiotic (Synbiotic) bacteria (1 g/kg).
5. A basic diet supplemented with therapeutic herbs (1.5g/Kg).

The compositions of the experimental diets are presented in Table 1. The two amounts of CP were either the NRC (1994) suggested level (23 percent CP for the starter and percent 21 grower diets, respectively) or the low level (23 percent CP for the starter and percent 21 grower diets, respectively) (19 percent CP for the starter and 17 percent finisher diets, respectively).

From 1 to 21 days and 22 to 42 days of age, the starter and grower meals in mash form were provided.

**Table (1): Composition and calculated analysis of experimental basal diets.**

| Ingredient, % | Experimental diets | | | |
|---|---|---|---|---|
| | Starter 1 Day to 21 Day | | Grower 21 Day to 42 day | |
| | Recommended of Protein | Protein of low | Recommended of Protein | Protein of low |
| Yellow maize | 555.00 | 660.00 | 603.00 | 705.00 |
| Soybean Meal 44% | 309.00 | 229.00 | 261.00 | 191.00 |
| Corn Gluten | 79.00 | 61.00 | 79.00 | 50.00 |
| Di-calcium phosphate | 15.00 | 15.00 | 15.00 | 15.00 |
| Limestone | 12.90 | 13.90 | 12.90 | 12.90 |
| Salt | 3.6 | 4.6 | 3.6 | 3.6 |
| Vegetable oil | 20.00 | 10.00 | 20.00 | 15.00 |
| L-lysine | 0.92 | 1.50 | 0.20 | 2.00 |
| DL-Methionine | 1.58 | 2.00 | 2.30 | 2.50 |
| Premix * | 3.00 | 3.00 | 3.00 | 3.00 |
| **Total** | **1000** | **1000** | **1000** | **1000** |
| Nutrient Composition: Crude Protein % | 23.45 | 19.1 | 21.2 | 17.3 |
| M.E. (kcal/ kg) | 3148 | 3155 | 3284 | 3296 |
| C/P | 133 | 163 | 153 | 188 |
| Fat | 5.7 | 7.19 | 6.3 | 7.7 |
| Crude Fiber, % | 2.43 | 2.89 | 2.62 | 3.00 |
| Calcium, % | 1.01 | 1.06 | 0.97 | 1.02 |
| Phosphorus | 0.50 | 0.50 | 0.50 | 0.50 |
| Methionine % | 0.45 | 0.46 | 0.43 | 0.42 |
| Lysine % | 1.18 | 1.17 | 1.06 | 1.04 |

** Premix each kg contain Vit A 8,25,000 IU, Vit D3 1,20,000 IU/, Vit K 100 mg, Riboflavin 500 mg, Thiamine 80 mg, Pyridoxine 160 mg, Vit E 800 mg, Cyanocobalamine 100 mcg, Niacin 1200 mg, Calcium pantothenate 80 mg, Manganese sulphate 25 g, Ferrous sulphate 10 g, Copper sulphate 500mg, Zinc oxide 8g Potassium Iodide 100 mg, Coccidiostat 60g.

**Slaughter Traits:**

Five birds from each treatment were chosen at random at the end of the trial (42 days of age) and slain by cutting the jugular veins of the neck according to Islamic faith instructions with a sharp knife. The slain weight was recorded when total bleeding was obtained. After that, the carcass was opened down and all entrails were removed, and the empty carcass, gizzard, liver, heart, and lymphoid organs (spleen and bursa) were all weighed separately and proportioned to the live pre-slaughter weight to arrive at a relative weight. The relative weights and lengths (cm.) of empty small intestine and caecum were also measured. Feathers and blood losses, as well as the weights and lengths of the digestive tract and its contents, were all documented. Dressing percentage was calculated according to Steven et al., (1981) as follows:

Dressing weight% = [dressing weight / per-slaughter live body weight] × 100

Equals dressing percent:

Where: Dressing weight = weight of empty carcass (Offal free) without head.

**Hematological and biochemical parameters:**

Blood samples were obtained from the same five birds from each treatment at 42 days of age. Following the slaughter (1 bird per replicate; the same broilers used for digestive tract measurements).

SRBC's reaction

Sheep red blood cells (SRBC) were washed three times in Phosphate buffer saline (PBS) before being diluted to 7% (vol/vol) in PBS. On the fifth week, 3 birds from each treatment (3 birds randomly picked from each cage) were injected intramuscularly with 1 mL of 7% SRBC into each thigh. The injected birds were then CO2 inhaled to euthanize them. Venipuncture was used to collect heparinized blood 7 days after SRBC immunisation. Half-maximal responses occur roughly 7 days following injection, according to preliminary investigations. Plasma was kept at a temperature of 20 °C until IgG assays were carried out.

**Blood samples:**

A sterile injector was used to draw blood samples from the wing vein. Following that, samples were placed in either a vacuum or a K3EDTA vacuum tube. An automatic blood analyzer was used to determine the concentrations of white blood cells (WBC), red blood cells (RBC), and lymphocytes in whole blood samples (BC 3200, Nanshan, Shenzhen 518057, P.R. China).

**Measurement of serum indices:**

The concentrations of total protein, albumin, globulin, IGg, glucose, triglyceride in serum samples were analyzed by an automatic biochemical analyzer (SPECTROPHOTOMETER. V 1.0. Revision for Alpha-1101, 1102, 1502. Laxco, Inc.) Using colorimetric methods, following the instructions of the manufacturer of the corresponding reagent kit (Diamond Diagnostic, Hanover, Germany).

**Statistical analysis:**

All response variables' data were subjected to a two-way ANOVA using the SAS programme (SAS, 2008) and the general liner model (GLM). Duncan's multiple range technique (Duncan, 1955) was used to distinguish significant differences between treatment means at 0.05, 0.01, and 0.001 probability.

## RESULTS AND DISCUSSION:

### Carcass yield and organs relative weight

The protein content, the various feed additives, and their combination had no significant effect on carcass yield (Table 2).

Previous research (Chumpawadee et al., 2009; Sharifi et al., 2011), which revealed that synbiotic, probiotic, and prebiotic supplements had no significant favourable effect on quail and broiler carcass yields, agree with the findings of this study. There were no differences in carcass and cut yields due to the use of protein levels, which is identical to Ghiyasi et alfindings. .'s (2008).

According to Salehimanesh et al., consumption of probiotic Primalac, prebiotic TechnoMos, and a combination of the two (synbiotic) in broiler feed had no significant effect on carcass characteristics (2015). Furthermore, prebiotic supplementation improves humoral immune responses better than symbiotic supplementation.

Our findings are in line with those of Midilli et al. (2008), who observed that probiotics and Mannan-oligosaccharides had no influence on carcass output.

The inclusion of additives or the restriction of their use had no effect on carcass yield.

Inclusion of different feed additives, on the other hand, resulted in a significant difference in liver relative weight (P≤0.05) (Table 2). The prebiotic and herb-treated groups were found to have the lowest liver relative weight when fed the prescribed protein quantity. When it came to the protein level effect, the low protein feeding group had the heaviest liver relative weight (P≤ 0.01). Meanwhile, giving prebiotic and herb diets resulted in the lowest liver relative weight. It's worth noting that the preceding groups and the treatment group had a significant difference (P≤0.01) The synbiotic increased carcass weight as a percentage of body weight. These findings, in addition to increasing gizzard weight, are similar with Vahid et al. (2018) findings.

According to Salehimanesh et al., consumption of probiotic Primalac, prebiotic TechnoMos, and a combination of the two (synbiotic) in broiler feed had no significant effect on carcass characteristics (2015). Furthermore, prebiotic supplementation improves humoral immune responses better than symbiotic supplementation.

Our findings are in line with those of Midilli et al. (2008), who observed that probiotics and Mannan-oligosaccharides had no influence on carcass output.

The inclusion of additives or the restriction of their use had no effect on carcass yield.

Probiotic, prebiotic, synbiotic, and herb treatments, on the other hand, reduced gizzard relative weight by 87, 91, 84, and 95 percent, respectively, when compared to control. With probiotic and synbiotic therapy, this reduction was considerable. Different feed additives had a significant (P≤0.05) effect on pancreas relative weight.

In previous research with probiotics (Yakhkeshi et al., 2012) and prebiotics (Midilli et al., 2008), non-significant alterations in carcass characteristics were identified.

Premavalli et al. (2018) and Abdel-Moneim et al. (2020) got similar results when growing Japanese quail,The beneficial effect of probiotics on avian survivability was attributed by the latter authors to the healthy digestive tract obtained by probiotic therapy.

With probiotic, prebiotic, synbiotic, and herb treatments, pancreas relative weight reached 106, 118, 135 and 135 percent of control, respectively. This could be due to greater activity in reaction to the study's higher blood glucose levels. A fennel-rich diet resulted in a rise in pancreatic weight, according to Mohammed and Abbas (2009).

Furthermore, intestine relative weight was affected by either protein level or alternative feed additives (Table 2), albeit a low protein diet increased intestinal relative weight by 16 percent (P0.05) when compared to the recommended level of protein. Additionally, probiotic, prebiotic, synbiotic, and herb treatments raised intestinal relative weight to 104, 105, 109, and 127 percent of control, respectively. Only the usage of herbs caused a significant increase.

For the relative weight of the gizzard, heart, and intestine, the interaction impact between examined protein levels and different sources of feed additive was not significant.

Table (2): Effect of experimental treatments on carcass yield of broilers chicken.

| Protein | Additive | Carcass (%) | Liver (%) | Gizzard (%) | Heart (%) | Pancreas (%) | Intestinal weight (%) |
|---|---|---|---|---|---|---|---|
| **Interaction Effect** | | | | | | | |
| Recommended | *Control* | 72.90 ± 2.08 | $2.40^{ab}$ ± 0.18 | 1.43 ± 0.14 | 0.42 ± 0.09 | 0.13 ± 0.01 | 4.75 ± 0.32 |
| | *Probiotic* | 75.50 ± 0.99 | $2.34^{b}$ ± 0.06 | 1.31 ± 0.10 | 0.38 ± 0.05 | 0.15 ± 0.01 | 4.39 ± 0.54 |
| | *Prebiotic* | 75.88 ± 0.44 | $1.98^{c}$ ± 0.06 | 1.41 ± 0.03 | 0.42 ± 0.01 | 0.25 ± 0.03 | 4.69 ± 0.18 |
| | *Synbiotic* | 76.14 ± 0.43 | $2.28^{b}$ ± 0.12 | 1.21 ± 0.04 | 0.48 ± 0.03 | 0.19 ± 0.01 | 4.43 ± 0.42 |
| | *Herb* | 73.60 ± 18.55 | $1.82^{c}$ ± 0.15 | 1.56 ± 0.05 | 0.48 ± 0.02 | 0.21 ± 0.04 | 4.92 ± 0.25 |
| Low | *Control* | 71.96 ± 0.64 | $2.67^{a}$ ± 0.09 | 1.95 ± 0.08 | 0.47 ± 0.01 | 0.22 ± 0.01 | 4.43 ± 0.16 |
| | *Probiotic* | 73.60 ± 0.70 | $2.27^{b}$ ± 0.07 | 1.63 ± 0.08 | 0.51 ± 0.03 | 0.21 ± 0.01 | 5.20 ± 0.27 |
| | *Prebiotic* | 73.18 ± 0.75 | $2.29^{b}$ ± 0.07 | 1.68 ± 0.11 | 0.49 ± 0.04 | 0.15 ± 0.02 | 4.92 ± 0.20 |
| | *Synbiotic* | 71.12 ± 1.27 | $2.48^{ab}$ ± 0.21 | 1.64 ± 0.06 | 0.49 ± 0.04 | 0.27 ± 0.01 | 5.55 ± 0.24 |
| | *Herb* | 70.34 ± 0.36 | $2.55^{ab}$ ± 0.19 | 1.67 ± 0.15 | 0.49 ± 0.01 | 0.24 ± 0.02 | 6.73 ± 1.20 |
| **Main Effects of Protein Level** | | | | | | | |
| Protein | *Recommended* | 74.80 ± 3.13 | $2.16^{b}$ ± 0.07 | $1.38^{b}$ ± 0.04 | $0.43^{b}$ ± 0.01 | 0.19 ± 0.01 | $4.64^{b}$ ± 0.15 |
| | *Low* | 72.04 ± 0.41 | $2.45^{a}$ ± 0.06 | $1.71^{a}$ ± 0.04 | $0.49^{a}$ ± 0.01 | 0.22 ± 0.01 | $5.37^{a}$ ± 0.28 |
| **Main Effects of Feed Additives** | | | | | | | |
| Additive | *Control* | 72.43 ± 1.04 | $2.53^{a}$ ± 0.10 | $1.69^{a}$ ± 0.11 | 0.45 ± 0.01 | $0.17^{b}$ ± 0.01 | $4.59^{b}$ ± 0.17 |
| | *Probiotic* | 74.55 ± 0.65 | $2.31^{ab}$ ± 0.04 | $1.47^{b}$ ± 0.08 | 0.45 ± 0.02 | $0.18^{b}$ ± 0.01 | $4.79^{ab}$ ± 0.31 |
| | *Prebiotic* | 74.53 ± 0.61 | $2.13^{b}$ ± 0.06 | $1.54^{ab}$ ± 0.07 | 0.45 ± 0.02 | $0.20^{ab}$ ± 0.02 | $4.81^{ab}$ ± 0.13 |
| | *Synbiotic* | 73.63 ± 1.04 | $2.38^{ab}$ ± 0.11 | $1.42^{b}$ ± 0.07 | 0.48 ± 0.02 | $0.23^{a}$ ± 0.01 | $4.99^{ab}$ ± 0.29 |
| | *Herb* | 71.79 ± 7.95 | $2.18^{b}$ ± 0.17 | $1.61^{ab}$ ± 0.08 | 0.48 ± 0.01 | $0.23^{a}$ ± 0.02 | $5.83^{a}$ ± 0.65 |
| **ANOVA** | | | | | | | |
| **Source of Variation** | | | | | | | |
| Pr× Add | | N.S | * | N.S | N.S | N.S | N.S |
| Protein | | N.S | ** | *** | * | N.S | * |
| Additives | | N.S | * | * | N.S | * | * |

*Values with different superscripts differ significantly (p≤0.05) within in certain column

NS= non-significant. (* P ≤0.05) (** P ≤0.01) (*** P ≤0.001)

**Immunity:**

**Lymphoid organs:**

Fabricius' spleen and bursa relative weights were unaffected by the interaction between different feed additives and the tested protein levels (Table 3). The amount of protein consumed had no effect on the spleen's relative weight, but low protein increased it. When compared to the appropriate amount, the bursa's relative weight increased by 40%. Although various feed additives had no effect on bursa relative weight, probiotic treatment reduced spleen relative weight to 67, 76, 81, and 86 percent of control, respectively.

Prebiotic TechnoMos had no effect on carcass efficiency, breast, thigh, thymus, or Bursa of Fabricius, according to Sojoudi et al. (2012), but had a significant effect on abdominal fat.

**Table (3):** Effect of experimental treatments and their interaction on lymphoid organs of broilers chicken..

| Protein | Additive | Spleen (%) | | Fabricius (%) | |
|---|---|---|---|---|---|
| **Interaction Effect** | | | | | |
| Recommended | *Control* | 0.22 | ± 0.04 | 0.07 | ± 0.01 |
| | *Probiotic* | 0.14 | ± 0.01 | 0.08 | ± 0.01 |
| | *Prebiotic* | 0.14 | ± 0.02 | 0.12 | ± 0.01 |
| | *Synbiotic* | 0.18 | ± 0.04 | 0.09 | ± 0.05 |
| | *Herb* | 0.23 | ± 0.04 | 0.14 | ± 0.01 |
| Low | *Control* | 0.20 | ± 0.02 | 0.14 | ± 0.02 |
| | *Probiotic* | 0.14 | ± 0.08 | 0.16 | ± 0.02 |
| | *Prebiotic* | 0.18 | ± 0.02 | 0.16 | ± 0.01 |
| | *Synbiotic* | 0.15 | ± 0.01 | 0.12 | ± 0.01 |
| | *Herb* | 0.14 | ± 0.01 | 0.11 | ± 0.01 |
| **Main Effects of Protein Level** | | | | | |
| Protein | *Recommended* | 0.18 | ± 0.01 | 0.10$^{b}$ | ± 0.08 |
| | *Low* | 0.16 | ± 0.02 | 0.14$^{a}$ | ± 0.09 |
| **Main Effects of Feed Additives** | | | | | |
| Additive | *Control* | 0.21$^{a}$ | ± 0.02 | 0.11 | ± 0.01 |
| | *Probiotic* | 0.14$^{b}$ | ± 0.09 | 0.12 | ± 0.02 |
| | *Prebiotic* | 0.16$^{ab}$ | ± 0.01 | 0.14 | ± 0.01 |
| | *Synbiotic* | 0.17$^{ab}$ | ± 0.02 | 0.11 | ± 0.08 |
| | *Herb* | 0.18$^{ab}$ | ± 0.02 | 0.13 | ± 0.01 |
| **ANOVA** | | | | | |
| Source of Variation | | | | | |
| Protein× Additives | | N.S | | N.S | |
| Protein | | N.S | | ** | |
| Additives | | * | | N.S | |

*Values with different superscripts differ significantly (p≤0.05) within in certain column
NS= non-significant. (* P ≤0.05) (** P ≤0.01) (*** P ≤0.001)

**Blood immunological parameters:**

Table 4 shows that the interaction of different feed additives and protein levels had a significant influence on white blood cell count (P≤0.05). The highest white blood cell (WBC) count was found in the synbiotic treatment with the recommended level of protein, reaching 114 percent of the recommended protein count, whereas the lowest WBC count was found in the low protein control, but different feed additives increased the count to 114, 104, 135, and 135 percent of the low protein control count in the probiotic, prebiotic, synbiotic, and herb treatments, respectively. Synbiotic and herbal therapies were able to raise WBC count from a low protein level to a recommended protein level equivalent. When compared to control, probiotic, prebiotic, synbiotic, and herb treatments increased WBC count by 7, 1, 23, and 17 percent, respectively. The findings back up those of Priya and Babu (2013), who discovered that using yeast as a probiotic increased the amount of WBC in chicks overall.

Low protein intake resulted in a 13 percent drop in WBC count when compared to the recommended protein intake (P≤0.01).

Total protein was unaffected by protein levels, feed additives,ortheir interactions.globulin, albumin/globulin ratio, or IgG. (Table 4). Protein level, on the other hand, had a considerable impact on albumin level. When compared to the required protein, inadequate protein reduced blood albumin by 29%.

Adding probiotics, prebiotics, or synbiotics had no effect on total protein, albumin, or globulin levels. The results backed up those of Alkhalf et al. (2010), who discovered that adding probiotics and prebiotics to broiler feed had no effect on total protein, albumin, or globulin levels. The three additions had no statistically significant effect on serum glucose levels, according to the findings. According to Mokhtari et al., synbiotics had no influence on broiler serum glucose levels (2010).

The findings are consistent with those of Alkhalf et al. (2010), who found that none of the three levels of probiotic supplementation utilised in their investigation changed serum total protein or albumin concentrations. Furthermore, those of Dimcho et al. (2005), who discovered that probiotic supplementation had no effect on chicken total protein concentrations. Furthermore, Mountzouris et al. (2010) found that using a 5-bacterial species probiotic in broiler feeding had no significant effect on IgG also analysed plasma immunoglobulin (IgA, IgM, and IgG) in probiotic-supplemented chickens and found that IgA, IgM, IgG, and total Ig concentrations did not alter across treatments. In addition, Midilli et al. (2008) found that dietary probiotic and/or prebiotic supplementation had no effect on immunoglobulin (IgG) concentrations in broiler serum.

Antibiotics, probiotics, oligosaccharides, enzymes, and organic acids are among the feed additives commonly utilised in chicken feed (Bin-Jumah et al., 2020; Hussein et al., 2020). They are introduced in the diets of poultry and animals to aid growth by enhancing feed intake ( Mahrose et al., 2019; Wang et al., 2019).

Low quantities of chemicals in poultry feed can also help enhance the production of poultry protein for human consumption, lowering the cost of animal and poultry production in some cases (El-Kholy et al., 2018; Johnson et al., 2019; Ismail, et al., 2020; Alagawany, et al., 2020; Reda, et al., 2020).

Table (4): Effect of experimental treatments and their interaction on blood immunological parameters of broilers chicken..

| Protein | Additive | White blood cell ($\cdot 10^6/mm^3$) | | Total protein (g/dl) | | Albumin (g/dl) | | Globulin (g/dl) | | A/G ratio (g/dl) | | IgG (ng/L) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Interaction Effect** | | | | | | | | | | | | | |
| Recommended | *Control* | 18.21$^{b}$ | ± 8.0 | 3.60 | ± 0.13 | 1.21 | ± 0.06 | 2.39 | ± 0.16 | 0.51 | ± 0.05 | 339.66 | ± 43.55 |
| | *Probiotic* | 18.54$^{b}$ | ± 6.4 | 4.11 | ± 0.54 | 1.45 | ± 0.09 | 2.66 | ± 0.44 | 0.56 | ± 0.05 | 428.05 | ± 30.31 |
| | *Prebiotic* | 18.03$^{b}$ | ± 2.8 | 4.93 | ± 0.36 | 1.41 | ± 0.11 | 3.52 | ± 0.26 | 0.40 | ± 0.01 | 260.36 | ± 12.74 |
| | *Synbiotic* | 20.79$^{a}$ | ± 2.2 | 5.09 | ± 0.60 | 1.22 | ± 0.20 | 3.87 | ± 0.55 | 0.32 | ± 0.06 | 196.33 | ± 27.50 |
| | *Herb* | 18.73$^{b}$ | ± 5.7 | 4.36 | ± 0.43 | 1.68 | ± 0.40 | 2.68 | ± 0.69 | 0.82 | ± 0.41 | 330.66 | ± 63.20 |
| Low | *Control* | 13.98$^{e}$ | ± 3.4 | 4.32 | ± 0.22 | 1.35 | ± 0.14 | 2.96 | ± 0.10 | 0.45 | ± 0.04 | 257.33 | ± 17.32 |
| | *Probiotic* | 15.91$^{c}$ | ± 2.3 | 3.76 | ± 0.31 | 0.81 | ± 0.09 | 2.95 | ± 0.22 | 0.27 | ± 0.01 | 391.00 | ± 10.26 |
| | *Prebiotic* | 14.53$^{d}$ | ± 0.6 | 4.36 | ± 0.03 | 0.98 | ± 0.06 | 3.39 | ± 0.08 | 0.29 | ± 0.02 | 332.00 | ± 34.07 |
| | *Synbiotic* | 18.88$^{b}$ | ± 8.7 | 4.14 | ± 0.30 | 0.98 | ± 0.26 | 3.16 | ± 0.57 | 0.38 | ± 0.19 | 365.66 | ± 23.90 |
| | *Herb* | 18.92$^{b}$ | ± 5.4 | 4.06 | ± 0.24 | 0.84 | ± 0.04 | 3.17 | ± 0.20 | 0.26 | ± 0.02 | 336.33 | ± 10.58 |
| **Main Effects of Protein Level** | | | | | | | | | | | | | |
| Protein | *Recommended* | 18.86$^{a}$ | ± 3.7 | 4.42 | ± 0.22 | 1.39$^{a}$ | ± 0.09 | 3.02 | ± 0.23 | 0.52 | ± 0.08 | 310.93 | ± 36.13 |
| | *Low* | 16.44$^{b}$ | ± 8.1 | 4.12 | ± 0.11 | 0.99$^{b}$ | ± 0.07 | 3.12 | ± 0.12 | 0.33 | ± 0.03 | 336.46 | ± 23.51 |
| **Main Effects of Feed Additives** | | | | | | | | | | | | | |
| Additive | *Control* | 16.09$^{b}$ | ± 8.1 | 3.96 | ± 0.19 | 1.28 | ± 0.07 | 2.67 | ± 0.15 | 0.48 | ± 0.03 | 298.50 | ± 27.89 |
| | *Probiotic* | 17.23$^{ab}$ | ± 7.8 | 3.93 | ± 0.29 | 1.13 | ± 0.15 | 2.80 | ± 0.23 | 0.41 | ± 0.07 | 409.50 | ± 51.80 |
| | *Prebiotic* | 16.28$^{b}$ | ± 5.9 | 4.65 | ± 0.20 | 1.19 | ± 0.11 | 3.45 | ± 0.12 | 0.34 | ± 0.02 | 296.05 | ± 22.88 |
| | *Synbiotic* | 19.83$^{a}$ | ± 7.7 | 4.62 | ± 0.37 | 1.10 | ± 0.16 | 3.52 | ± 0.39 | 0.35 | ± 0.09 | 281.10 | ± 41.22 |
| | *Herb* | 18.82$^{ab}$ | ± 3.7 | 4.18 | ± 0.23 | 1.26 | ± 0.26 | 2.92 | ± 0.34 | 0.54 | ± 0.22 | 333.50 | ± 73.15 |
| **ANOVA** | | | | | | | | | | | | | |
| Source of Variation | | | | | | | | | | | | | |
| Protein× Additives | | * | | N.S | | N.S | | N.S | | N.S | | N.S | |
| Protein | | ** | | N.S | | ** | | N.S | | N.S | | N.S | |
| Additives | | * | | N.S | | N.S | | N.S | | N.S | | N.S | |

*Values with different superscripts differ significantly (p≤0.05) within in certain column
NS= non-significant. (* P ≤0.05) (** P ≤0.01) (*** P ≤0.001)

**Blood hematological and biochemical characteristics**

**Red blood cells count :**

Red blood cells count of broilers as affected by different dietary additives under two levels of protein are presented in Table (5). Different feed additives had various effects under the two levels of protein. Probiotic treatment did not have significant effect on RBC count under the recommended protein level, whereas it reduced the count under the low protein to reach 84% of the count under the low protein control. Supplemented diet of the recommended protein with prebiotic significantly increased RBC count to reach 117% of the recommended protein control. The opposite was found with that of the low protein group, where feeding low protein diet supplemented with probiotic resulted to decrease RBC count to reach 82% of the protein control. On the other hand, synbiotic inclusion significantly increased RBC count of the birds fed the recommended protein diet to reach 152% of the recommended protein control. This additive was not critical when bird received the low protein. For both two experimental diet, Herbal treatment boosted RBC count in birds supplied recommended protein diets to 111 percent of control and was capable of increasing count by 38 percent in birds fed low protein diets to almost reach the count in birds fed recommended protein diets.

Table 5 shows the effects of various protein levels on RBC count regardless of feed additives. When compared to birds fed the recommended level of protein, feeding a low protein diet reduced red blood cell count by 53% ($P \leq 0.01$)

Table 5 shows the effects of various feed additives on RBC count regardless of protein content. Both probiotic and prebiotic therapies lowered RBC count by 18 and 14 percent, respectively, when compared to control ($P \leq 0.05$). Synbiotic and herb therapies, on the other hand, increased by 32 and 21 percent, respectively, when compared to the control.

**Hemoglobin concentration:**

Table (5) shows the effect of several dietary additives on broiler haemoglobin concentrations when fed two levels of protein-rich diet. Despite the fact that different feed additives had distinct impacts on RBC count under the two levels of protein, they all boosted haemoglobin concentration significantly ($P \leq 0.05$) in all circumstances. Probiotic, prebiotic, synbiotic, and herb treatments raised haemoglobin concentration by 3, 21, 53, and 23%, respectively, as compared to control at the recommended protein level. Under low protein conditions, probiotic, prebiotic, synbiotic, and herb treatments boosted haemoglobin concentration by 12, 16, 28, and 27%, respectively, compared to control, allowing it to meet and surpass the necessary protein level.

Table 5 shows the effects of various protein levels on haemoglobin regardless of feed additives. When compared to birds fed the required level of protein, feeding a low protein diet reduced red blood cell count by roughly 14% without having a noticeable impact.

Table 1 shows the effects of various feed additives on haemoglobin regardless of protein content (5). Probiotic, prebiotic, synbiotic, and herb treatments increased haemoglobin concentration to 107, 118, 141, and 125 percent of control, respectively ($P \leq 0.05$).

**Blood glucose concentration:**

Using two levels of protein, Table 5 shows the influence of several food additives on broiler glucose concentrations. Probiotics are beneficial at both Prebiotic, synbiotic, and herb treatments significantly improved glucose concentration ($P \leq 0.05$), raising it by 18, 4, 50, and 25%, respectively, compared to control at the recommended protein level and by 3, 15, 66, and 3%, respectively, compared to control under the low protein level. Table 5 depicts the impact of different protein amounts on glucose levels, regardless of dietary additives. When compared to birds fed the recommended amount of protein, serving a lower protein meal increased blood glucose by about 29% ($P \leq 0.01$). Table 5 shows how different feed additives affect glucose levels regardless of protein levels. Probiotics, prebiotics, synbiotics, and herbal treatments significantly ($P \leq 0.05$) increased glucose levels to 109, 105, 122, and 112 percent of control levels, respectively. This is in accordance with the findings of (Chichlowski et al., 2007, Awad et al., 2009), who discovered an increase in glucose and proline passive absorption in broiler chicks fed a probiotic.

Improved immune system, gut microbiota modification, reduced inflammatory reactions, decreased ammonia and urea excretion, lower serum cholesterol, and improved mineral absorption are all possible benefits of probiotics; on the other hand, probiotics may have an indirect positive impact on performance parameters and production profitability.

(Ashour et al., 2020) Feed additives are compounds that are added to feed to improve the efficacy of nutrients and their effects on poultry performance.

**Blood triglycerides concentration:**

Table 5 shows the concentrations of triglycerides in broilers as a function of several dietary additives at two levels of protein. The concentration of triglycerides was not affected by either the relationship between protein level and feed additives or the protein level alone. Meanwhile, the probiotic, prebiotic, synbiotic, and herb treatments significantly ($P \leq 0.01$) lowered triglycerides by 17, 21, 24, and 16 percent, respectively, when compared to the control.

These findings are consistent with those of Arun et al. (2006), who discovered that dietary treatment with probiotics substantially lowered blood total cholesterol and triglycerides. Additionally, adding pre- and probiotics to a wet wheat-based meal reduced blood triglyceride levels (Afsharmanesh et al., 2013). These findings are also in line with Ashayerizadeh et alresearch. .'s al. (2009), who reported that a reduction in the serum triglyceride level with use of probiotic and prebiotic can be due to an increase in the population of lactic acid bacterial in the gastrointestinal tract.

Furthermore, Sharifi et al. (2011) found that giving varying amounts of synbiotic to quails reduced blood triglycerides. Furthermore, Khajeali et al. (2012) found that increasing the amount of caraway in a broiler's diet can lower blood triglycerides significantly. Furthermore, this decrease in blood triglycerides could be attributed in some cases to a decrease in some hormones secreted by the adrenal cortex, which causes a decrease in fatty acid secretion from adipose tissues or a decrease in fat oxidation, resulting in a decrease in fatty acids, including cholesterol and triglycerides. Furthermore, according to Dina Bushuty (2012), blood cholesterol levels reduced considerably in groups treated with probiotics in Lactobacillus cholesterol assimilation compared to a control group given a baseline diet. Lactobacillus acidophilus and Lactobacillus casei in the food or water produce a reduction in gallbladder acids in digestive process, which results in a reduction in fat digestion capacity and, as a result, a drop in blood lipid levels (Getachew, 2016). The findings were consistent with those of Alkhalf et al., (2010) discovered no variations in (Hb) content as a result of adding probiotics to broiler diets. Probiotics and synbiotics, on the other hand, caused a considerable rise in Hb concentration, according to Beski and Sardary (2015). The greater Hb content in the chicks given probiotics and synbiotics might be owing to the acidic media of the gastrointestinal tract generated by probiotic fermentation, which allowed for improved iron salt absorption from the small intestine. This may result in improved vitamin B complex synthesis by beneficial bacteria, which might have a good impact on blood-forming processes. The findings of Hanamanta et al. (2009) on packed cell volume percent coincide with those of Hanamanta et al. (2009), who observed that adding probiotic and synbiotic to broiler diet had no significant influence on packed cell volume and Hb.

Recently, Ghasemi et al. (2016) also reported that a synbiotic decreased serum cholesterol and low-density lipoprotein cholesterol concentrations in broilers.

**Table (5):** Effect of experimental treatments and their interaction on blood Hematological and biochemical Parameters of broilers chicken.

| protein | Additive | Red blood cell ($\cdot 10^3/mm^3$) | Hemoglobin (g/dl) | Haematocrit (HCT) % | Glucose (mg/dl) | Triglycerides (mg/dl) |
|---|---|---|---|---|---|---|
| **Interaction Effect** | | | | | | |
| Recommended | *Control* | 1.98$^{c}$ ± 0.47 | 12.14$^{d}$ ± 2.40 | 37.76$^{b}$ ± 8.01 | 166.33$^{e}$ ± 16.55 | 68.66 ± 11.20 |
| | *Probiotic* | 1.98$^{c}$ ± 0.40 | 12.54$^{d}$ ± 2.65 | 34.60$^{c}$ ± 6.09 | 197.01$^{d}$ ± 7.23 | 43.66 ± 1.33 |
| | *Prebiotic* | 2.32$^{b}$ ± 0.03 | 14.66$^{b}$ ± 0.31 | 35.10$^{c}$ ± 4.02 | 172.33$^{e}$ ± 37.02 | 49.24 ± 3.05 |
| | *Synbiotic* | 3.02$^{a}$ ± 0.60 | 18.60$^{a}$ ± 2.60 | 49.60$^{a}$ ± 2.10 | 249.66$^{c}$ ± 12.44 | 43.33 ± 2.90 |
| | *Herb* | 2.20$^{b}$ ± 0.26 | 14.94$^{b}$ ± 0.99 | 34.14$^{c}$ ± 3.04 | 207.33$^{d}$ ± 22.57 | 47.41 ± 1.15 |
| Low | *Control* | 1.28$^{e}$ ± 0.30 | 10.76$^{e}$ ± 2.18 | 29.59$^{d}$ ± 3.06 | 247.16$^{c}$ ± 39.01 | 53.66 ± 9.52 |
| | *Probiotic* | 1.08$^{f}$ ± 0.04 | 12.00$^{d}$ ± 0.50 | 30.50$^{d}$ ± 3.11 | 254.23$^{b}$ ± 37.36 | 57.66 ± 7.88 |
| | *Prebiotic* | 1.05$^{f}$ ± 0.02 | 12.46$^{d}$ ± 0.32 | 27.96$^{e}$ ± 2.07 | 266.01$^{a}$ ± 43.06 | 47.20 ± 2.51 |
| | *Synbiotic* | 1.28$^{e}$ ± 0.31 | 13.72$^{c}$ ± 0.46 | 35.12$^{c}$ ± 1.09 | 257.24$^{b}$ ± 3.71 | 50.37 ± 4.16 |
| | *Herb* | 1.76$^{d}$ ± 0.33 | 13.70$^{c}$ ± 0.66 | 36.66$^{bc}$ ± 3.01 | 255.66$^{b}$ ± 54.54 | 55.33 ± 3.17 |
| **Main Effects of Protein Level** | | | | | | |
| Protein | *Recommended* | 2.30$^{a}$ ± 0.1 | 14.57 ± 0.95 | 38.24$^{a}$ ± 3.03 | 198.53$^{b}$ ± 11.42 | 50.33 ± 3.24 |
| | *Low* | 1.09$^{b}$ ± 0.1 | 12.52 ± 0.49 | 31.96$^{b}$ ± 2.07 | 256.06$^{a}$ ± 22.10 | 52.73 ± 2.52 |
| **Main Effects of Feed Additives** | | | | | | |
| Additive | *Control* | 1.63$^{ab}$ ± 0.33 | 11.45$^{b}$ ± 1.55 | 33.67$^{b}$ ± 6.35 | 206.66$^{b}$ ± 26.16 | 61.16$^{a}$ ± 7.38 |
| | *Probiotic* | 1.33$^{b}$ ± 0.30 | 12.27$^{b}$ ± 1.27 | 32.55$^{b}$ ± 3.05 | 225.50$^{ab}$ ± 21.26 | 50.66$^{ab}$ ± 4.75 |
| | *Prebiotic* | 1.40$^{ab}$ ± 0.37 | 13.56$^{ab}$ ± 0.42 | 31.53$^{b}$ ± 3.04 | 219.01$^{ab}$ ± 58.83 | 48.11$^{b}$ ± 1.82 |
| | *Synbiotic* | 2.15$^{a}$ ± 0.40 | 16.16$^{a}$ ± 1.49 | 42.36$^{a}$ ± 2.07 | 253.16$^{a}$ ± 9.74 | 46.66$^{b}$ ± 2.71 |
| | *Herb* | 1.98$^{ab}$ ± 0.21 | 14.32$^{ab}$ ± 0.60 | 35.40$^{ab}$ ± 304 | 231.50$^{ab}$ ± 28.52 | 51.16$^{ab}$ ± 2.40 |
| **ANOVA** | | | | | | |
| **Source of Variation** | | | | | | |
| Protein× Additives | | * | * | *** | * | N.S |
| Protein | | *** | N.S | *** | *** | N.S |
| Additives | | * | * | *** | * | *** |

*Values with different superscripts differ significantly ($p \leq 0.05$) within in certain column NS= non-significant. (* $P \leq 0.05$) (** $P \leq 0.01$) (*** $P \leq 0.001$)

**CONCLUSION:**

In conclusion, feeding broilers reduced crude protein diets (-10 percent NRC) was beneficial, which was slightly offset by non-antibiotic additions. Synbiotics were shown to be the most beneficial of the Effects on WBCs, RBCs, Intestinal length, and the Fabricius Bursa Furthermore, synbiotic exhibited substantial impacts on haematological, carcass, and immunological organs of broiler chickens under the settings of the current investigation. More study is needed to confirm the existing findings.

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