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Effect of *Euglena gracilis* algae derived dietary beta-glucan on serum protein fractions and liver enzyme activities of broiler chicken

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Abstract
This study investigated the potential impacts of graded levels of *Euglena gracilis* algae-derived beta-glucan supplementation on broiler serum proteins and liver enzyme activities. A total of 240 one-day-old Vencobb-430y broiler chicks were distributed among five dietary groups, with six replications and eight birds in each replication. Group I serving as the negative control receiving basal diet (T_1), Group II serving as the positive control receiving antibiotic growth promoter, avilamycin at 10 ppm with basal diet (T_2). In Group III, IV and V, the beta-glucan, procured from Kemin Industries, was incorporated into basal diets at 50, 100, and 150 g/ton respectively (T_3, T_4 and T_5). Serum protein fractions and liver enzyme activities were analyzed after a five-week feeding period. Supplementation of graded levels of algae-derived beta-glucan (T_3, T_4 and T_5) significantly ($p<0.05$) increased serum total protein (g/dl) levels and significantly increased ($p<0.01$) globulin (g/dl) levels and significantly ($p<0.01$) decreased albumin/globulin ratio compared to the negative control group (T_1) and 100 and 150g/ton supplementation of beta-glucan (T_4 and T_5) groups significantly ($p<0.01$) increased serum globulin (g/dl) levels and decreased albumin/globulin ratio compared to the positive control group (T_2) and no significant changes in the serum albumin (g/dl), ALT (U/L) and AST (U/L) levels were observed. The obtained results indicating that algae-derived beta-glucan had high immunity enhancement than antibiotic avilamycin supplementation in broiler diets which supports beta-glucan as an antibiotic alternative.

Keywords: Broiler, beta-glucan, serum protein fractions, albumin, globulin, serum liver enzymes, ALT, AST

Introduction
The poultry industry, crucial for global food demand, for decades used antibiotic growth promoters in broiler feed for enhanced growth and efficiency. However, concerns over disrupting gut microbiota, antibiotic resistance, and health risks led to European Union ban over antibiotic growth promoters (Ngangom *et al*., 2019) [8]. Without antibiotic growth promoters, poultry production and efficiency may decline. To address this, alternatives like herbs, prebiotics, probiotics, synbiotics, postbiotics, phytobiotics, enzymes, organic acids and others are explored for maintaining health and productivity in broilers. Among these, beta-glucans, classified under prebiotics, have shown promising effects. Prebiotics enhance gut health by promoting beneficial bacteria, producing short-chain fatty acids, and supporting intestinal tissue and immune function (Gibson *et al*., 2004) [5]. Glucans with beta-1,3 and beta-1,6 glycosidic linkages (beta-glucans) are major structural components of cell wall of many bacteria, fungi, yeast and algae (Soltanian *et al*., 2009) [10]. However, an alternative origin of beta-1,3-glucan lies in the unicellular algae *Euglena gracilis* which shares similarities in terms of molecular weight, structure, and size. This study aimed to assess the impact of graded levels of *Euglena gracilis* algae-derived beta-glucan on the serum protein fractions and liver enzyme activities in broiler chicks providing valuable insights into its role as a dietary supplement in poultry nutrition.

Materials and Methods
Euglena gracilis algae-derived beta-glucan powder (dry form) procured from Kemin Industries, SIPCOT Industrial Estate, Gummidipoondi, Chennai, Tamil Nadu, India. The dry form of powder containing 54% 1,3-beta-glucan. A total of 240 one-day-old Vencobb-430y broiler chicks were distributed among five dietary groups, with six replications and eight birds in each replication.

Group I was the negative control group (basal diet, antibiotics-free) (T_1); Group II, as the positive control, received a basal diet supplemented with a 10 ppm avilamycin, antibiotic growth promoter (Diet antibiotics, approved for broilers and commonly used) (T_2); Group III - algae derived beta-glucan 50 g/ton in the control diet (T_3), Group IV - algae derived beta-glucan 100 g/ton in the control diet (T_4) and Group V - algae derived beta-glucan 150 g/ton in the control diet (T_5). This study was approved by the Institutional Animal and Ethical care Committee at Veterinary College and Research Institute, Namakkal. (IAEC No.: 03/IAEC/VCRI-NKL/2023). Birds were slaughtered on 35th day of biological trial. For serological analysis, blood samples were obtained during slaughter in serum vial without anticoagulant, were allowed to clot, then centrifuged at 2000 RPM for 10 minutes to yield clear supernatant serum. Serum total protein quantified by the Lowry method (kit Code 83LS100-60), involving peptide-cupric ion reaction in alkaline pH, measured at 578nm (Johnson *et al*., 1999) [7]. Serum albumin measured with bromocresol green dye (pH 3.68), forming a green complex whose absorbance was measured at 630 nm (Johnson *et al*., 1999) [7] using kit Code 84LS100-60. Serum Globulin calculated as total protein minus albumin. Serum liver enzyme Alanine transferase and Aspartate transferase estimation utilized a modified IFCC method with a commercial kit (Coral Clinical Systems, India). ALT and AST catalyze amino group transfer, measured by NADH oxidation (340 nm) (Bergmeyer *et al*., 1986) [2].

Results and Discussion

Serum protein fractions

The serum protein fractions of broiler chicken supplemented with algae derived beta-glucan are presented in Table 1. Supplementation of graded levels of algae-derived beta-glucan significantly impacted serum protein fractions, except serum albumin (g/dl). Graded levels of algae derived beta-glucan supplementation resulted in significantly (p<0.05) increased serum total protein (g/dl) levels, significantly (p<0.01) increased serum globulin (g/dl) levels and significantly (p<0.01) decreased albumin/globulin ratio with compare to negative control group (T_1). However, Serum total protein levels were comparable between positive control group (T_2) and beta-glucan supplemented groups (T_3, T_4 and T_5) and serum globulin levels significantly increased in 100g/ton and 150 g/ton beta-glucan supplemented group (T_4 and T_5) compared to positive control group (T_2). Elevated globulin levels and a low (A/G) ratio indicate enhanced disease resistance and immune response (Griminger, 1986) [6]. Hence, the prebiotic exhibited a positive impact on broiler immunity, as indicated by Ferket, 2004 [4]. These findings are similar with Sirvydis *et al*. (2006) [9], who observed elevated serum total protein and globulin levels in broiler chickens fed prebiotic-supplemented diets. In contrast, in disagreement with El-Sawy *et al*. (2015) [3], no statistically significant differences were noted in serum total protein and globulin levels between beta-glucan-treated and control group.

Table 1: Effect of algae derived beta-glucan supplementation on serum protein fractions of broiler chicken at 35 days of age

Treatment groups	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	A/G ratio
T_1: Control diet	3.56^{a}±0.05	1.77±0.02	1.80^{a}±0.05	0.99^{c}±0.03
T_2: Control diet + AGP	3.77^{b}±0.06	1.79±0.06	1.97^{b}±0.07	0.93^{b}±0.05
T_3: 50 g/ton inclusion of algae derived beta-glucan	3.65^{ab}±0.10	1.75±0.03	1.89^{b}±0.08	0.95^{c}±0.04
T_4: 100 g/ton inclusion of algae derived beta-glucan	3.78^{b}±0.04	1.71±0.02	2.08^{bc}±0.04	0.83^{a}±0.02
T_5: 150 g/ton inclusion of algae derived beta-glucan	3.82^{b}±0.05	1.68±0.03	2.14^{c}±0.08	0.81^{a}±0.05
P Value	0.028*	0.146	0.005**	0.006**

Each value is a mean of twelve observations

Means having different superscripts in a column differ significantly *(p<0.05) ** (p<0.01)

Serum liver enzymes activity

The serum protein fractions of broiler chicken supplemented with algae derived beta-glucan are presented in Table 2. Supplementation of graded levels of algae-derived beta-glucan had no significant impact on liver enzymes such as ALT and AST. Consistent with this study, yeast-derived beta-glucan addition showed no significant difference in ALT and AST levels in broiler chicken (El-Sawy *et al*., 2015) [3]. Conversely, *Euglena gracilis* algae-derived beta-glucan inclusion in broiler chicken resulted in a significant difference in ALT and AST levels (Amer *et al*., 2023) [1]. In our study, beta-glucan supplementation showed no change in AST and ALT activities, affirming its safety as a biological modulator without adverse effects.

Table 2: Effect of algae derived beta-glucan supplementation on serum liver enzyme activity of broiler chicken at 35 days of age

Treatment groups	ALT (U/L)	AST (U/L)
T_1: Control diet	28.58±1.22	8.83±0.60
T_2: Control diet + AGP	28.17±1.04	10.17±0.83
T_3: 50 g/ton inclusion of algae derived beta-glucan	29.83±2.46	9.00±0.73
T_4: 100 g/ton inclusion of algae derived beta-glucan	30.67±3.93	9.75±0.72
T_5: 150 g/ton inclusion of algae derived beta-glucan	28.42±1.10	10.00±0.72
P Value	0.922	0.604

Conclusion

The results suggest that dietary algae-derived beta-glucan enhances serum total protein, globulin levels and decreases albumin/globulin ratios in broiler chickens, outperforming antibiotic supplementation. This indicates a positive impact on immunity, showcasing the potential of algae-derived beta-glucan as an antibiotic alternative.

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