RESEARCH ARTICLE
Open Access

Journal of Veterinary and Animal Sciences
ISSN (Print): 0971-0701, (Online): 2582-0605
https://doi.org/10.51966/jvas.2022.53.3.435-440

Effect of varied levels of dietary *Chlorella vulgaris* extract on blood biochemical profile and immune organ status in broilers[#]

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Citation: Merin, C. T., Bunglavan, S. J., Chako, B., Senthil Murugan, S. and Aswathi, P. B. 2022. Effect of varied levels of dietary *Chlorella vulgaris* extract on blood biochemical profile and immune organ status in broilers. *J. Vet. Anim. Sci.* **53** (3): 435-440
DOI: https://doi.org/10.51966/jvas.2022.53.3.435-440

Received:18.02.2022 *Accepted: 07.04.2022* *Published: 30.09.2022*

Abstract

A study was conducted to evaluate the effect of Chlorella vulgaris hydro-alcoholic extract on blood biochemistry and immune organs characteristics in broiler chicken. A total of 128 Vencobb 400, day-old broiler chicks were randomly assigned into four treatment groups having four replicates with eight birds per replicate. A basal diet was formulated in accordance with BIS recommendation and hydro-alcoholic extract of Chlorella vulgaris was added at an inclusion level of 0, 0.1, 0.2 and 0.4 per cent respectively to the basal diet to form 4 different treatment diets. After 42 days of experimental feeding trial, one bird from each replicate was sacrificed to study blood biochemical parameters and immune organs status. The results of the present study indicated that the birds fed with 0.4 percent C. vulgaris extract in their diet showed significantly higher WBC (P<0.001), RBC (P<0.01) and hemoglobin (P<0.05) count compared to other treatments while total protein, albumin, globulin and A:G ratios were found similar among the treatment groups. In the case of immune organ status, the bursal yield was significantly (P<0.05) higher for birds fed with 0.1 and 0.2 percent supplementation than other groups while the weight of thymus and spleen were similar between the treatment groups.

Keywords: *Chlorella vulgaris, blood parameters, immune organs*

[#]*Part of MVSc thesis submitted to Kerala Veterinary and Animal Sciences University, Pookode, Wayanad, Kerala.*

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Poultry industry is the most advancing and flourishing food industries in the world. Broiler meat being the cheapest source of protein without any religious taboo, is well accepted by the consumers in satisfying human nutrition especially the quality dietary protein demand. The recent increase in poultry meat demand requires proportional growth in poultry production. To achieve this, feed additives have immense role to play in animal nutrition. A large variety of natural feed additives are used in poultry industry to enhance production and thus safeguard consumer health.

Chlorella vulgaris is one among the fresh water green microalgae known to have high nutritional potential in human as well as animal nutrition due to the presence of high protein content, carbohydrates, lipids, various essential and non-essential amino acids, health promoting fatty acids and immune boosting bioactive molecules (Richmond, 2004). Earlier studies on various forms of this algae*viz*., dry powder (An *et al*., 2016), chlorella growth factor (Kang *et al*., 2013), fermented (Oh *et al*., 2015) and lyophilized (Kholif*et al*., 2017) forms had indicated enhanced growth and immune potential in humans and animals. However, the studies on the potential of extracted form of *C. vulgaris* on blood biochemical profile and immune organ status in poultry is limited. Hence the present study was designed to evaluate the blood biochemical parameters and immune organ status in birds supplemented with graded levels of hydro-alcoholic extract of *C. vulgaris* as feed additive.

Materials and methods

The experimental work was conducted at Poultry farm, Instructional Livestock Farm Complex (ILFC), College of Veterinary and Animal Sciences, Pookode, Wayanad. A total of 128 Vencobb 400, day-old broiler chicks were randomly distributed into four treatment groups (G1, G2, G3 and G4) with four replicate and eight birds per replicate. A corn-soyabean meal based basal diet (pre-starter, starter and finisher) was formulated according to BIS (IS 1374: 2007) recommendation and hydro-alcoholic extract of *C. vulgaris* was added to the diet at inclusion levels of 0, 0.1, 0.2 and 0.4 percent respectively for G1, G2, G3 and G4. The birds were reared for 42 days with standard brooding, housing and management practices. Birds were maintained on pre-starter (0-7 days), starter (8-21 days) and finisher (22-42 days) feed. Feed and water were provided *adlibitum*.

On the 42nd day of feeding trial, one bird from each replicate was randomly selected and humanely sacrificed to study the blood biochemical parameters and immune organ status. Immediately after bleeding, blood samples (5 ml) were collected from jugular vein of each bird in separate vials with and without anticoagulant respectively. The WBC, RBC and haemoglobin counts were analysed from blood collected with anticoagulant (EDTA) using automatic blood analyzer whereas serum was separated from whole blood collected without anticoagulant and analysed for total protein, albumin, globulin and A:G ratio. The immune organs like thymus, spleen and bursa were collected and weighed. Percentage yield of immune organs were calculated by dividing the individual weight of organs by weight of carcass in each treatment groups. Data collected on various parameters were statistically analysed by one-way ANOVA (Snedecor and Cochran, 1994) using SPSS version 24.0.

Results and discussion

Blood biochemical parameters

The blood biochemical parameters of broiler birds supplemented with graded levels of *C. vulgaris* extract is given in Table 1. The results indicated that G3 and G4 had significantly higher values for WBC ($P< 0.01$) and G4 had significantly higher values for RBC($P<0.01$) as well as haemoglobin ($P< 0.05$) concentration compared to G1 and G2. Total protein, albumin, globulin and A:G ratio were not affected by the treatments. Our result was in agreement with Kang *et al*. (2013) who reported that free liquid Chlorella supplementation in broiler significantly increased ($P<0.05$) the total leukocyte count whereas the feeding of Chlorellain other forms *viz*. Chlorella growth factor and dried Chlorella powder showed similar values with control which could be due to difference in processing technique or forms of supplementation. Similarly, our result

Table 1. Blood biochemical parameters[#] of birds supplemented with graded levels of *C. vulgaris* extract

Blood Parameter	Group				F Value	P Value
	G1	G2	G3	G4		
WBC (10^3/µL)	23.37 ± 0.21[c]	24.63 ± 0.36[b]	25.48 ± 0.25[a]	24.47 ± 0.17[a]	14.795	0.000**
RBC (10^6/µL)	3.06 ± 0.26[b]	3.02 ± 0.16[b]	3.56 ± 0.26[b]	4.30 ± 0.24[a]	6.518	0.007**
Hb (g/dL)	16.02 ± 1.34[b]	15.85 ± 0.91[b]	18.72 ± 1.69[ab]	22.32 ± 1.48[a]	4.791	0.020*
TP (g/dL)	3.67 ± 0.36	3.52 ± 0.18	3.87 ± 0.35	4.04 ± 0.31	0.533	0.669[ns]
Albumin (g/dL)	1.68 ± 0.11	1.76 ± 0.01	1.92 ± 0.12	1.86 ± 0.12	1.168	0.362[ns]
Globulin (g/dL)	1.99 ± 0.29	1.76 ± 0.18	1.95 ± 0.30	2.18 ± 0.22	0.468	0.711[ns]
A:G Ratio	0.88 ± 0.13	1.05 ± 0.14	1.07 ± 0.20	0.87 ± 0.07	0.529	0.671[ns]

[#]Average of four values with SE

[a, b, c] Mean values with different superscripts within a row different significantly

**Significance at P<0.01 *Significance at P < 0.05; [ns] Non significant

Table 2. Yield of Immune organs[#] (%) in birds supplemented with graded levels of *C. vulgaris* extract

Immune Organ	Groups				F Value	P Value
	G1	G2	G3	G4		
Thymus	0.73 ± 0.33	0.44 ± 0.04	0.55 ± 0.11	0.56 ± 0.17	1.294	0.321[ns]
Spleen	0.31 ± 0.02	0.28 ± 0.03	0.32 ± 0.04	0.34 ± 0.07	0.240	0.867[ns]
Bursa	0.35 ± 0.01[c]	0.64 ± 0.04[a]	0.61 ± 0.04[ab]	0.40 ± 0.12[bc]	4.428	0.026*

[#]Average of four values with SE

[a, b, c] Mean values with different superscript within a row different significantly

*Significance at p<0.05; [ns] Non significant

was confirmed by Kotrbacek *et al.* (1994) and Fathi *et al*. (2018) who supplemented *C. vulgaris* and *Spirulina platensis* in broiler and observed improved (P<0.05) phagocytic activity of leukocyte and total leukocyte count respectively. In addition, an improved (P<0.05) leukocyte count in turn promoting immunity with supplementation of fresh *S. platensis* through water in Avian influenza challenged broiler birds observed by Lokapirnasari *et al*. (2016) confirmed our findings. On contrary to our results, Choi *et al*. (2017) concluded that recombinant Chlorella supplementation in broiler had no effect in total leukocyte and RBC count of experimental birds. The enhanced blood values in our study could be due to the rich content of minerals in *C. vulgaris* extract especially zinc, copper and iron (Tokusoglu and Uunal, 2003) which contribute to RBC and haemoglobin biosynthesis. The Chlorella has potential to stimulate the activity of leukocyte especially T-lymphocyte and macrophages through the production of gamma-interferons and interleukins whose synthesis is triggered by various immune modulating polymers and biomolecules present in it (An *et al*., 2008; Lee and Lim, 2012)

Total protein, albumin, globulin and A:G ratio, were not significantly increased by treatments in our study as reported by Fathi *et al.* (2018) and El-Abd and Hamouda (2017) who observed similar values of total protein, albumin and A:G ratio in birds fed on *S. platensis* and *C. vulgaris* respectively as additive but reported numerical improvement between treatments. Similarly, an improved concentration of total protein and albumin reported by An *et al*. (2010) in experimental rats fed on hot water extract of *C. vulgaris* which was in partial accordance to our study due to numerical improvement in our respective serum values of birds fed on the additive. The dietary protein and amino acid content from the Chlorella which is highly available through the process of extraction could be a contributing factor for improved albumin concentration whereas enhanced globulin count can be correlated to increased immunoglobulin thus immunity in birds (Busher, 1990). Along with, the presence of various

bioactive molecules possessed by Chlorella like β-carotene and immune modulating polysaccharides like β-glucan or immurella (Kang *et al*., 2013) might be the responsible for their improved immune response. Moreover, the extraction process done in our experimental material *C. vulgaris* resulted in better disruption of cell which could have promoted the release and availability of more cell contents (Janczyk *et al*., 2007) which beneficially affect the immunity of birds.

Immune organ status

The immune organ status of experimental birds is presented in Table 2. The results indicated that G2 had significantly (P< 0.05) higher bursal yield (%) compared to other groups. However, the percentage yield of spleen and thymus were found similar between groups. The results are in accordance with Fathi *et al.* (2018) who observed that the supplementation of *S. platensis* meal at an inclusion level of 0.7 and 0.9 g /kg feed resulted in significant (P<0.05) increase in percentage of immune organs *viz*. thymus, spleen and bursa in broiler chicken. In addition, earlier studies reported by Kaoud (2015) and El-Abd and Hamouda (2017) on significant (P< 0.05) improvement in absolute as well as per cent yield of thymus and bursa in birds fed on *S. platensis* in feed and *C. vulgaris* in water respectively confirms our result. Mirzaie *et al*. (2020) also reported that the supplementation of *C. vulgaris* byproduct produced through lipid extraction significantly (P<0.05) increased the weight of spleen in broilers whereas the weight of bursa was found similar between groups. Similarly, Qureshi *et al*. (1994) also observed a significantly (P< 0.05) higher relative weight of thymus in *S. platensis* supplemented birds compared to control group.

The increased bursal yield in birds fed on additive from our study is an indicative of the potential of *C. vulgaris* extract in improving the immune status and health of birds. This is confirmed by Bennet and Stephens (2006) who stated the linear relationship of size of bursa with health of birds in flock. Moreover, improved immune response through increased bursal size from microalgae supplementation is proved by Qiu *et al*. (2021) who stated the ability of microalgal polysaccharide in regulating immune related signaling pathway thus improving immune function.

Conclusion

The supplementation of hydro-alcoholic extract of *C. vulgaris* in broiler diet could improve immunity of broiler chicken especially by enhancing the haemato-biochemical parameters and immune organ status through improving the availability of immune modulating biomolecules. Hence the hydro-alcoholic extract of *C. vulgaris* is safe to be used as a herbal feed additive up to a level of 0.4 per cent for healthy broiler production.

Acknowledgement

We are grateful to Kerala Veterinary and Animal Sciences University for providing facilities for carrying out our research.

Conflict of interest

The authors declare that they have no conflict of interest

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