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Effect of dietary supplementation of microalgae Spirulina and Chlorella on growth performance and blood profile of broiler chicken at high altitude

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ABSTRACT

The objective of this study was to explore the effect of Spirulina and Chlorella as a dietary supplement on the growth performance of broiler chicken at high altitude. In this experiment, birds were divided into groups of 30 and their basal diet was supplemented with Spirulina and Chlorella in varying composition up to 5%. The study showed that supplementation of the basal diet with Spirulina and Chlorella significantly influenced the growth performance of broiler chicken. The body weight and FCR improved during the study. The diet supplemented with 2.5% of Spirulina was the most suitable and economically feasible for broiler chicken at high altitudes

Keywords: Chlorella, High-Altitude, Hypoxia, Microalgae, Poultry, Spirulina

The establishment of successful poultry farming in high altitude (Himalayan region) areas remains a challenge till date. Ladakh lies in the north-western part of the Himalayas at 3500 m above sea level with extremely harsh weather conditions like very low temperatures in winters (-35°C), hypobaric hypoxic condition, and increased UV radiation with a shortage of poultry feed and proper medication which drastically affects the growth performance and production of poultry leading to unsuccessful poultry farming in this region (Kalia *et al.* 2018). Hyperpulmonary-hypertension at high altitude results in the overburdening of the heart's right ventricle contributing to the pathophysiological conditions such as ascites in broilers (Monge and León-Velarde 1991, León-Velarde *et al.* 2010, Druyan 2012).

In the early 80s, the use of microalgae as a protein source was introduced in poultry feed (Lipstein *et al.* 1980). Microalgae such as Spirulina and Chlorella have recently been studied for their role in the poultry feeding due to high nutritional and functional properties (Toyomizu *et al.* 2001). It was observed that slow growth and impaired production performance with environmental stress are commonly observed at high altitudes (Swati 2020). Therefore, the present study was carried out to explore the potential of Spirulina and Chlorella as a dietary supplement in broiler chicken at high altitude.

MATERIALS AND METHODS

The experiment was carried out at the Poultry Research Unit of Defence Institute of High-Altitude Research at 3500 m above sea level in Leh-Ladakh, India during August 2021 to October 2021. The temperature was maintained around 21-24°C and relative humidity was 29-34%.

Experimental design: Unsexed day-old broiler chickens (180) were acquired from in-housing facility. The birds were randomly distributed into 6 groups of 3 replicates with 10 chicks in each replicate. All the experimental birds were vaccinated against the Newcastle Disease virus. Birds in the Control group (C) were fed with the basal diet without any addition, while treatment groups were fed diet supplemented with 2.5% and 5% of dried *Spirulina platensis*, 2.5% and 5% of dried *Chlorella vulgaris* and a combination of both *Spirulina platensis* and *Chlorella vulgaris* (1.25% each). The dried powder of both *Spirulina platensis* and *Chlorella vulgaris* were purchased from commercial source. All the birds were given the same basal diet, a standard in-house feed formulation developed by our laboratory specifically for high altitude broiler chickens.

Growth performance: During the trial, body weight gain (BWG), feed consumption (FC) and feed conversion ratio (FCR) were recorded on weekly basis. The FCR was calculated as feed consumed / BWG.

Blood sample collection: At the end of experiment (8 weeks), 6 birds were randomly selected from each treatment group at fasting and about 2 ml of blood was drawn out from the wing vein and stored in EDTA vacutainer tubes. Shortly after the collection, blood was used for hematology analysis which was performed using Spin Compact 5 hematology analyzer.

Statistical analysis: The statistical analysis was

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Table 1. Effect of Spirulina and Chlorella supplementation on body weight gain and FCR

Group	BWG (g) Week 1	BWG (g) Week 4	BWG (g) Week 8	FCR
Control	56.46±1.0	261.6±7.4[b]	854.06±6.6[c]	2.18
Spirulina (2.5%)	59.26±1.3	306.93±6.7[a]	997.73±10.67[a,b]	1.85
Spirulina (5%)	58.73±1.4	294.8±4.1[a,b]	894.13±14.6[c]	2.08
Chlorella (2.5%)	59.4±1.2	325.4±9.06[a]	1019.13±10.04[a]	1.81
Chlorella (5%)	59.93±1.2	321.86±8.00[a]	971.4±7.5[a,b]	1.9
Spirulina (1.25%) + Chlorella (1.25%)	60.93±1.5	291.86±8.44[a,b]	954.66±5.44[b]	1.94
P value	0.264	<0.001	<0.001	

Means bearing different superscript (a,b and c) in a column differ significantly (P<0.05).

performed using SPSS (version 26; IBM, Chicago, IL, USA) using one way-ANOVA. The differences among groups were considered significant at P value < 0.05.

RESULTS AND DISCUSSION

Both *Spirulina platensis* and *Chlorella vulgaris* supplementation showed a significant difference in broiler performance (P<0.001). At the age of 8th week, it was observed that the group fed with 2.5% *Chlorella vulgaris* had improved body weight growth followed by the group fed 2.5% *Spirulina platensis* as compared to other treatment groups and control. In terms of feed consumption, no significant difference was observed. The results are in agreement with previously published studies (Rezvani *et al.* 2012, Kang *et al.* 2013, Abou-Zeid *et al.* 2015, An *et al.* 2016, Alwaleed *et al.* 2021).

In terms of FCR, improved values were observed in treatment groups compared to control. Higher FCR was observed in broiler chicken at high altitude (Chuskit *et al.* 2021) The growth performance of broiler chicken was lower at high altitude than the plain region which may be due to high altitude physiology. Earlier, it was reported that chicken reared at high altitude showed slower growth rate as compared to chicken grown at lowland (Hassanzadeh *et al.* 2004, Swati 2020).

It was found that different levels of Spirulina in the broiler diet improved BWG, EPEI, and FCR (Park *et al.* 2018). Our study is also in agreement with previous reports suggesting that higher inclusion of *Spirulina platensis* may decrease the growth performance as evident in the group treated with 5% *Spirulina platensis* which showed only 4.88% and 4.58% improvement in BWG and FCR in comparison to group treated with 2.5% *Spirulina platensis* which showed 17.5% and 15.13% improvement in BWG and FCR respectively when compared to control group (Ross and Dominy 1990, Venkataraman *et al.* 1994, Evans *et al.* 2015). Similar trend was observed in 5% *Chlorella vulgaris* and combined supplementation of Chlorella and Spirulina (1.25% each) groups which showed decreased performance of BWG and FCR (14.29% and 12.84%), in contrast to 2.5% *Chlorella vulgaris* which showed 20.11% and 16.97% improvement in BWG and FCR when compared to control (Table 1). The improved BWG may be due to the improved feed utilization efficiency and absorption of minerals and vitamins.

The RBC count, hemoglobin concentration and HCT levels were similar among the groups. In terms of MCV, MCH, and MCHC, minor difference was observed, except the increased level of supplementation of *Spirulina platensis* (5%) and *Chlorella vulgaris* (5%) resulted in decreased MCV values. Also, the group with 1.25% supplementation of both *Spirulina platensis* and *Chlorella vulgaris* showed an increased MCV value when compared to the control group (Table 2).

The Heterophil: Lymphocyte (H:L) ratio is a sensitive indicator of long term stress in the chicken population. In the present study, H:L ratio in control and treatment groups (*Spirulina platensis* 2.5%, *Chlorella vulgaris* 2.5%, *Spirulina platensis* 1.25% + *Chlorella vulgaris* 1.25%) showed no stress induced due to microalgae supplementation (Table 3). However, an increase in the level of supplementation to 5% increased the H:L ratio suggesting that an increase in the level of microalgae supplementation induces stress in the chicken population. This may be due to the release of stress hormone corticosterone or stimulation of the adrenal cortical cell

Table 2. Effect of Spirulina and Chlorella supplementation on blood profile

Group	RBC (10^12/L)	Hemoglobin (g/dL)	HCT (%)	MCV (fL)	MCH (pg)	MCHC (g/dL)
Control	3.32±0.27	18.98±1.62	43.35±3.76	130.28±0.55[a]	56.8±0.53[a,b]	43.58±0.29[b]
Spirulina (2.5%)	3.80±0.48	21.3±2.64	48.88±5.86	129.25±1.66[a,b]	56.083±0.65[a,b]	43.41±0.38[b]
Spirulina (5%)	2.92±0.17	15.96±0.75	36.41±2.02	123.83±3.24[b]	54.21±1.24[b]	43.86±0.52[b]
Chlorella (2.5%)	3.30±0.22	19.41±1.35	43.56±2.67	132.26±1.52[a]	58.7±0.57[a]	44.41±0.61[b]
Chlorella (5%)	3.16±0.24	18.65±1.38	39.78±3.59	128.11±2.32[a,b]	57.7±1.51[a]	46.18±.78[a]
Spirulina (1.25%) + chlorella (1.25%)	2.64±0.26	15.33±1.47	35.36±3.48	133.98±1.63[a]	58.06±0.62[a]	43.35±0.20[b]
P value	0.143	0.129	0.141	0.022	0.027	0.003

Means bearing different superscript (a,b and c) in a column differ significantly (P<0.05).

Table 3. Effect of Spirulina and Chlorella supplementation on differential WBC count

Group	WBC (10^9/L)	Lymphocytes (%)	Monocytes (%)	Basophils (%)	Eosinophils (%)	Heterophills (%)	H:L Ratio
Control	38.55±1.4[a]	54.04±13.17	27.18±10.75	0.1±0.06	0.81±0.37	17.86±13.33	0.33
Spirulina (2.5%)	40.31±2.22[a]	30.86±5.75	58.34±7.61	0.026±0.01	0.40±0.17	10.38±2.73	0.33
Spirulina (5%)	35.64±1.21[a,b]	35.08±10.00	30.37±9.33	0.08±0.07	0.76±0.33	37.31±15.07	1.06
Chlorella (2.5%)	39.31±1.34[a]	59.46±14.75	20.75±11.73	0.10±0.06	0.50±0.48	19.04±13.58	0.32
Chlorella (5%)	37.39±1.17[b]	35.56±12.20	30.86±15.47	0.13±0.07	0.7±0.44	26.73±15.42	0.75
Spirulina (1.25%) + Chlorella (1.25%)	32.61±2.09[b]	55.48±9.45	34.78±11.31	0.13±0.01	0.12±0.03	9.59±3.06	0.17
P value	0.029	0.319	0.286	0.707	0.722	0.570	

Means bearing different superscript (a,b and c) in a column differ significantly ($p<0.05$).

Table 4. Economic feasibility of using Spirulina and Chlorella as broiler chicken dietary supplement at high altitude

Particular	Control	Spirulina (2.5%)	Spirulina (5%)	Chlorella (2.5%)	Chlorella (5%)	Spirulina + Chlorella (1.25% each)
Cost of supplement/chicken (₹)	NiL	32.71	65.42	150.99	905.4	91.75
Cost of feed/chicken (@ 39/kg) (₹)	71.66	71.66	71.66	71.66	71.66	71.66
Total cost (₹)	71.66	104.37	137.08	222.65	977.06	163.41
Sale of chicken at end of 8th week (@ ₹ 350/kg live weight) *	298.9	349.2	312.94	356.69	339.99	334.13
Total profit from each bird (₹)	227.24	244.83	175.86	134.04	-637.07	170.72
Total profit from each group (₹)	6817.2	7344.9	5275.8	4021.2	-28971.81	5121.6
Profit/Loss percentage compared to control	-	(+)7.7%	(-)22.0%	(-)41.01%	(-)324%	(-)24.8%

*Limited availability of fresh chicken results in increased demand and price. Market rate of frozen chicken is approx. ₹ 380/kg.

due to adrenocorticotropic hormone (Davison and Flack 1981, Gross and Siegel 1983).

The economic efficiency of adding the supplement to the basal diet remains a major concern for small-scale and large-scale commercial poultry farming; opting for feed supplement which provides desired growth in poultry performance and profit as well are the ideal characteristic for such supplements. Though supplementation of *Spirulina platensis* and *Chlorella vulgaris* resulted in improved growth performance, it was found that supplementation at higher concentration (5%), and combinatorial approach of supplementation is not economically feasible and can incur a higher percentage of economic loss when compared to the control group (Table 4). The profitability is only feasible using the supplementation of *Spirulina platensis* at 2.5% which showed desired growth in terms of growth performance also.

In conclusion, the supplementation of up to 2.5% of dietary *Spirulina platensis* showed improved broiler chicken performance and economic feasibility at high altitude.

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