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Effect of supplementation of spirulina (*Arthrospira*) and exogenous protease enzyme on growth performance and nutrient retention of broilers

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ABSTRACT

The present study was carried out to assess the effect of dietary supplementation of different levels of spirulina (*Arthrospira*), a blue-green microalgae with or without exogenous protease enzyme on performance and nutrient retention of broiler chicken. Two hundred and forty commercial broiler chicks were randomly distributed into five dietary treatments with four replicates containing 12 birds each. The dietary treatments were: basal diet prepared as per ICAR (2013) specifications (control: T_1), basal diet containing 1% spirulina (T_2), basal diet containing 1% spirulina + 0.05% exogenous protease (T_3), basal diet containing 2% spirulina (T_4) and basal diet containing 2% spirulina+0.05% exogenous protease (T_5). Birds were reared under deep litter system for 35 days and fed with pre-starter, starter and finisher diets. Results revealed that the body weight gain, performance index, protein efficiency ratio and feed conversion ratio were increased significantly in T_3 groups compared to T_1, T_2, T_4 and T_5 groups. Nutrient retention was not affected by supplementing spirulina and exogenous protease in broiler rations. Therefore, it could be concluded that although the supplementation of 1% spirulina and 0.05% exogenous protease improve the growth performance of broiler birds, it should be used with caution under the prescribed limit.

Keywords: Broilers, Exogenous protease, Nutrient retention, Performance, Spirulina

Spirulina, a biomass of cyanobacterium, containing high protein, vitamins, minerals, essential amino and fatty acids, phytopigments, bioactive compounds, reported to have growth promoting, immuno- modulating and anti-inflammatory effects (Farag *et al*. 2016, Mariey *et al*. 2012). It may act as an alternative to antibiotics for enhancing growth performance of poultry birds. Dietary inclusion of spirulina in broiler nutrition has favourable effects on health and performance (Kharde *et al*. 2012, Shanmugapriya and Saravana babu 2014). Spirulina has also been associated with increased growth rate (Evans *et al*. 2015, Park *et al*. 2018) and nutrient digestibility (Park *et al*. 2018). The efficiency of its utilization may enhance by supplementation of exogenous enzymes. Protease enzyme has several benefits including decreasing undigested proteins in the diet, increasing amino acid availability and reducing protein needs in the diet (Buttin *et al*. 2016). However present knowledge of broiler chicken response to dietary supplementation of spirulina and exogenous protease enzyme is unknown. Therefore, the present study was planned to observe the effect of supplementation of different levels of spirulina with or without exogenous protease enzyme on growth performance and nutrient utilization of broilers.

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MATERIALS AND METHODS

The experiment was carried out at Department of Animal Nutrition, College of Veterinary Science, Sri Venkateswara Veterinary University (SVVU), Tirupati, after taking approval by the Institutional Animal Ethics Committee vide reference number 281/go/ReBi/S/2000/CPCSEA/CVSc/TPTY/028/Animal Nutrition/2021 dated 24-08-2021.

Two hundred and forty commercial broiler chicks (Cobb - 400 strain) were randomly distributed into five dietary treatments with four replicates containing 12 birds each. The dietary treatments were: basal diet prepared as per ICAR (2013) specification (control: T_1), basal diet containing 1% spirulina (T_2), basal diet containing 1% spirulina+0.05% exogenous protease (T_3), basal diet containing 2% spirulina (T_4), basal diet containing 2% spirulina+0.05% exogenous protease (T_5). Birds were reared under deep litter system for 35 days and fed with pre-starter, starter and finisher diets. Clean and fresh drinking water was made available at all the times. All the birds were vaccinated as per standard vaccination schedule. Nutrient composition of spirulina used in experimental diets is summarized in Table 1.

The individual body weight of the birds was recorded at weekly interval up to 5 weeks of age. Average weekly body weight gain was calculated in all the replicates of the five treatments. Total feed offered and left-over feed for each replicate was measured daily to calculate daily feed consumption. Feed conversion ratio (FCR) was calculated

Table 1. Nutrient composition (% DM) of spirulina used in experimental diets

Nutrient	Spirulina
Dry matter	89.26
Organic matter	88.53
Crude protein	62.3
Ether extract	0.83
Crude fibre	0.76
Total ash	11.47
Acid insoluble ash	1.8
GE (Kcal/kg)	4043.13
ME (Kcal/kg)*	2850
Calcium	0.59
Phosphorous	1.31

*Calculated values

based on total feed consumed and total weight gain of each replicate. Performance index was calculated for each treatment by dividing the average weight gain by the feed conversion ratio and the protein efficiency ratio was calculated by dividing the weight gain to protein consumed.

To determine the nutrient retention (%), a metabolism trial was conducted at the end of 5 weeks of age and proper records of total feed offered, left over feed and faeces voided were recorded daily. Moisture content of excreta samples was analysed to know the total faecal dry matter output per day. At the end of collection period, excreta sample collected from each replicate was thawed, mixed thoroughly, dried in oven and ground in wielly mill for further analysis. The representative samples of experimental diets offered and excreta samples from each replicate were analysed for various nutrients as per AOAC (2007) and the nutrient retention (%) of dry matter, crude protein, ether extract and crude fibre was calculated. Data were analysed by the method of Snedecor and Cochran (1994) and using SPSS version 22.0. Statistical comparisons of the results were made using one way analysis of variance (ANOVA) and significant differences ($p<0.05$) between different experimental groups were analysed by Duncan's test.

RESULTS AND DISCUSSION

Growth performance: Body weight gain was significantly ($p<0.05$) higher in 1% spirulina along with protease enzyme supplementation compared to control and 2% spirulina supplemented groups (Table 2). The obtained results approved the findings of Park *et al.* (2018) where spirulina at 0, 0.25, 0.5, 0.75 and 1 % in the broiler diet showed linear increase ($p<0.01$) in body weight gain as the level of spirulina increased from 0 to 1 %. Similarly, Shanmugapriya *et al.* (2015) in normal broiler birds and Moustafa *et al.* (2021) in heat stress broiler birds fed diets containing 0.5, 1 and 1.5 % spirulina and reported significant increase ($p<0.05$) in body weight gain up to 1 % level and was decreased in 1.5 % level compared to control in both type of birds. On the other hand, Pestana *et al.* (2020) observed very high levels of spirulina (15%) had significant reduction ($p<0.01$) in the body weight gain compared to control even after supplemented with various enzymes *i.e.,* lysozyme and cocktail enzymes. Possible reasons for increased body weight gain in 1 % spirulina supplemented groups might be due to availability of quality amino acids from spirulina microalgae compared to other vegetable proteins (Park *et al.* 2018, Evans *et al.* 2015, Tavernari *et al.* 2018), antimicrobial, antioxidant and anti-inflammatory properties due to presence of physiologically active compounds like carotenoid pigments, phycocyanin, poly unsaturated fatty acids, vitamins, macro and micro minerals (Becker 2007, Eriksen 2008, Maoka 2011) and beneficially alters the intestinal microbial population (Alwaleed *et al.* 2020). On the other hand, decrease in the body weight gain in higher spirulina incorporation (2%) was due to lack of proper endogenous enzymes to completely break the cell wall of spirulina (Pestana *et al.* 2020), higher viscosity of digesta due to gelation of proteins and faster passage rate may limit the access of the endogenous enzymes to their target substrates which resulted in decreased digestibility and absorption of nutrients leading to poor performance (Evans *et al.* 2015). The improved body weight gain in protease supplemented groups compared to non-supplemented groups might be attributed to augmenting the endogenous peptidases secretion resulted in increased protein digestibility, stimulation of digestive enzymes secretion, hydrolyzing the proteinaceous anti nutritional factors (Ghazi *et al.* 2002, Law *et al.* 2018) and modification of intestinal microflora.

Feed intake was significantly higher ($p<0.05$) in 1% spirulina supplemented groups and lower ($p<0.05$) in 2% spirulina supplemented groups when compared to control group (Table 3). The feed intake observed in the present

Table 2. Effect of supplementation of Spirulina and exogenous Protease enzyme on weekly body weight gain (g) in broilers

Weekly body weight gain (g)	Treatment				
	T_1	T_2	T_3	T_4	T_5
1st week	103.56 ± 1.25	107.41 ± 2.31	108.56 ± 1.02	108.79 ± 1.76	108.48 ± 2.15
2nd week	270.66 ± 4.97	278.47 ± 5.52	283.00 ± 2.83	281.54 ± 4.61	283.33 ± 4.65
3rd week *	421.83^{ab} ± 2.67	424.85^{ab} ± 2.40	427.54^{a} ± 4.71	408.27^{c} ± 5.20	412.08^{bc} ± 4.96
4th week *	448.08^{bc} ± 3.91	467.21^{ab} ± 7.27	474.25^{a} ± 4.63	443.27^{c} ± 9.85	449.83^{bc} ± 3.72
5th week *	488.85^{bc} ± 6.98	502.06^{ab} ± 8.68	520.95^{a} ± 7.29	469.77^{cd} ± 3.96	464.16^{d} ± 4.39
Cumulative (0-5 weeks) *	1732.99^{c} ± 12.58	1780.02^{b} ± 8.88	1814.31^{a} ± 9.60	1711.65^{c} ± 7.49	1717.89^{c} ± 10.02

^{abcd} Values bearing different superscripts in a row differ significantly * ($p<0.05$)

Table 3. Effect of supplementation of Spirulina and exogenous Protease enzyme on weekly feed intake (g) in broilers

Weekly feed intake (g)	Treatment				
	T_1	T_2	T_3	T_4	T_5
1st week	123.21 ± 0.44	122.68 ± 1.02	121.27 ± 1.80	120.87 ± 1.32	120.91 ± 1.31
2nd week	375.41 ± 2.19	377.70 ± 1.78	376.04 ± 2.27	376.49 ± 1.47	375.95 ± 1.55
3rd week *	646.29 ^{a} ± 6.06	644.66 ^{a} ± 5.55	641.54 ^{ab} ± 4.18	623.54 ^{c} ± 4.59	626.87 ^{bc} ± 4.58
4th week *	866.20 ^{abc} ± 11.45	872.21 ^{ab} ± 5.79	878.24 ^{a} ± 1.82	841.50 ^{c} ± 10.80	848.41 ^{bc} ± 6.78
5th week *	1152.71 ^{ab} ± 5.19	1173.51 ^{a} ± 12.30	1170.37 ^{a} ± 8.45	1124.33 ^{bc} ± 10.70	1109.33 ^{c} ± 13.10
Cumulative (0-5 weeks) *	3163.83 ^{a} ± 20.83	3190.78 ^{a} ± 12.79	3187.48 ^{a} ± 9.41	3086.75 ^{b} ± 13.74	3081.50 ^{b} ± 18.04

^{abc} Values bearing different superscripts in a row differ significantly * ($p<0.05$)

study are corroborated with the findings of Park *et al.* (2018) who reported non-significant increase ($p>0.05$) in feed intake as spirulina level increased from 0 to 1%. Similarly, Abbass *et al.* (2020) observed non-significant increase ($p>0.05$) in feed intake up to 1% level of spirulina, thereafter decrease in feed intake. In contrast to results reported in the present study, Raach-Moujahed *et al.* (2011) and Shanmugapriya *et al.* (2015) observed decreased feed intake as the level of spirulina increased in diet.

Significantly better ($p<0.05$) feed conversion ratio was observed in 1% spirulina along with protease enzyme supplementation compared to other treatment groups and control group (Table 4). The obtained results of FCR were in agreement with Shanmugapriya *et al.* (2015) who reported significantly ($p<0.01$) better FCR in broilers supplemented with spirulina up to 1% level and poor FCR was observed as the levels further increased to 1.5 %. Similarly, Park *et al.* (2018) observed linear decrease in FCR as the level of spirulina increased from 0 to 1% level. Similar findings were also reported by Jamil *et al.* (2015), Fathi *et al*. (2018), Shinde *et al*. (2018) and Khan *et al*. (2020) who observed better FCR in broilers supplemented with low levels of spirulina. In contrast to present study, Abbass *et al.* (2020) reported linear decrease ($P<0.05$) in FCR from 1.63 to 1.55 with increasing the spirulina from 0 to 4%. The increased FCR in protease enzyme supplemented group might be attributed to the increased digestibility of nutrients especially proteins, stimulation of digestive enzymes and partially degrading the cell wall of feed resulted in better availability of nutrients present in feeds. Non significantly better FCR in spirulina supplemented groups compared to control might be due to increased antioxidant activity of spirulina which might have diverted more nutrients towards growth by minimizing the nutrient expenditure for the wear and tear of body tissues due to oxidative stress.

The performance index and protein efficiency ratio were increased from control to 1% spirulina supplemented group and decreased in 2% spirulina supplemented group (Table 5). Better performance index and protein efficiency ratio in spirulina supplemented groups compared to control might be attributed to the improved feed utilization efficiency, enhanced absorption of vitamins and minerals (Gruzauskas *et al.* 2004) and beneficially alter the intestinal microbial population with increase in *Lactobacillus* and decrease in *E. Coli* population (Alwaleed *et al.* 2020). Further better performance in exogenous protease supplemented groups might be due to better peptide digestion of spirulina proteins along with other ingredients in ration and increased absorption of amino acids.

Nutrient retention (%): The spirulina supplementation at 1% and 2% levels with and without exogenous protease enzyme supplementation had no effect ($p>0.05$) on retention of Dry Matter (DM), Organic Matter (OM), Crude Protein (CP), Ether Extract (EE) and Crude Fiber (CF) as compared to control (Table 6). However, there was numeric increase in CP retention and decrease in EE retention in spirulina supplemented groups compared to control. CP retention reported in the present study is similar to that of Becker (2007), who reported that spirulina addition had no beneficial effect on protein digestibility as it contains approximately 11.5% of non-protein nitrogen.

Although current results show non significant improvement in supplementation of spirulina at 1% level along with exogenous protease enzyme to broilers diet on the body weight gain, performance index, protein efficiency ratio and feed efficiency, further research needs to be

Table 4. Effect of supplementation of Spirulina and exogenous Protease enzyme on feed conversion ratio in broilers

Feed conversion ratio	Treatment				
	T_1	T_2	T_3	T_4	T_5
1st week *	1.19 ^{a} ± 0.017	1.14 ^{ab} ± 0.017	1.11 ^{b} ± 0.023	1.11 ^{b} ± 0.007	1.11 ^{b} ± 0.013
2nd week	1.38 ± 0.020	1.35 ± 0.023	1.32 ± 0.017	1.34 ± 0.020	1.32 ± 0.017
3rd week	1.53 ± 0.014	1.51 ± 0.020	1.50 ± 0.020	1.52 ± 0.007	1.52 ± 0.016
4th week	1.93 ± 0.013	1.86 ± 0.023	1.85 ± 0.018	1.90 ± 0.050	1.88 ± 0.015
5th week *	2.36 ^{a} ± 0.029	2.33 ^{a} ± 0.029	2.24 ^{b} ± 0.026	2.39 ^{a} ± 0.011	2.38 ^{a} ± 0.030
Cumulative (0-5 weeks) *	1.82 ^{a} ± 0.005	1.79 ^{a} ± 0.013	1.75 ^{b} ± 0.006	1.80 ^{a} ± 0.013	1.79 ^{a} ± 0.006

^{ab} Values bearing different superscripts in a row differ significantly * ($P<0.05$)

Table 5. Effect of supplementation of Spirulina and exogenous Protease enzyme on overall performance in broilers

Parameter	Treatment				
	T_1	T_2	T_3	T_4	T_5
Initial body weight	43.36 ± 0.43	43.42 ± 0.35	43.30 ± 0.65	43.20 ± 0.16	43.60 ± 0.25
Final body weight*	1776.35 [c] ± 2.83	1823.44 [b] ± 2.45	1857.61 [a] ± 3.30	1754.85 [d] ± 1.73	1761.50 [d] ± 0.99
Body weight gain*	1732.99 [c] ± 12.58	1780.02 [b] ± 8.88	1814.31 [a] ± 9.60	1711.65 [c] ± 7.49	1717.89 [c] ± 10.02
Feed intake*	3163.83 [a] ± 20.83	3190.78 [a] ± 12.79	3187.48 [a] ± 9.41	3086.75 [b] ± 13.74	3081.50 [b] ± 18.04
Feed conversion ratio*	1.82 [a] ± 0.005	1.79 [a] ± 0.013	1.75 [b] ± 0.006	1.80 [a] ± 0.013	1.79 [a] ± 0.006
Performance index (g)*	949.27 [c] ± 8.37	993.18 [b] ± 12.10	1032.75 [a] ± 8.90	949.30 [c] ± 11.12	957.73 [c] ± 7.17
Protein efficiency ratio*	2.74 [d] ± 0.004	2.78 [b] ± 0.002	2.84 [a] ± 0.006	2.77 [c] ± 0.002	2.79 [b] ± 0.001

[abcd] Values bearing different superscripts in a row differ significantly * ($p<0.05$)

Table 6. Effect of supplementation of Spirulina and exogenous Protease enzyme on nutrient retention (%) in broilers

Parameter	Treatment				
	T_1	T_2	T_3	T_4	T_5
Dry Matter	74.03 ± 0.29	73.57 ± 1.87	74.59 ± 1.16	72.69 ± 0.62	71.55 ± 0.32
Organic Matter	75.81 ± 0.96	74.72 ± 1.92	74.99 ± 1.67	74.36 ± 1.48	73.85 ± 0.92
Crude Protein	67.49 ± 1.42	69.14 ± 3.57	70.35 ± 0.89	67.03 ± 0.38	69.82 ± 0.87
Ether Extract	75.21 ± 1.14	70.67 ± 4.13	71.48 ± 3.55	69.13 ± 1.45	71.06 ± 0.53
Crude Fiber	29.11 ± 0.52	30.02 ± 0.58	30.06 ± 0.54	29.35 ± 0.42	29.33 ± 0.43

All the parameters were non- significant (NS) among the treatments ($p>0.05$)

carried out for efficient utilization of spirulina at different levels with different combination of exogenous enzymes to determine optimum levels in broiler and layer diets for profitable poultry farming.

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