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Influence of spirulina supplementation on nutrient digestibility, serum biochemical factors in Nellore ram lambs

B Murali Mohan, Suryanarayana Mvan, Devasena B and Gangaraju G

Sri Venkateswara Veterinary University, College of Veterinary Science, Tirupati, Andhra Pradesh, India

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Corresponding Author: B Murali Mohan

Abstract

A growth trial was conducted for 90 days with 18 Nellore brown ram lambs with average body weight of 15±0.37 kg which were randomly divided into three groups containing six animals in each group and were evaluated with / without supplementation of *Spirulina platensis* on nutrient digestibility and serum biochemical factors in a CRD model. To a basal diet acting as control (T_1) the *Spirulina* was supplemented at 1 g and 2 g / 10 kg body weight to 2nd and 3rd groups to make them as T_2 and T_3, respectively. The animals were stall fed with the concentrate mixture and super napier green fodder. Significantly higher (p<0.05) protein digestibility was recorded for T_2 followed by T_3 and T_1 respectively. Crude protein digestibility was in the order of $T_2 > T_3 > T_1$. The digestibility of ether extract, crude fibre, NDF, ADF were found to be non-significant and comparable among the treatments. Significant difference (p<0.05) was noticed in DCP (%DM) content, with lowest value in T1 fed group. However, no significant difference was noticed in total DMI (% BW basis) and TDN (% DM) among the treatments. The results of the serum biochemical profile of ram lambs at the beginning of the experiment indicated no significant (p>0.05) difference for serum total protein, albumin**,** globulin, ALT, AST, cholesterol, triglycerides, urea, creatinine, HDL, LDL, VLDL, calcium and glucose. Except for total protein none of the serum biochemical parameters were found to be significant at 90th day of trial. The serum total protein in T_2 was 9.8% and 3.9% more as compared to T_1 and T_3 respectively. The remaining parameters albumin**,** globulin, ALT, AST, cholesterol, triglycerides, urea, creatinine, HDL, LDL, VLDL, calcium and glucose were non-significant among the treatments.

Keywords: Growth trial, *Spirulina*, nutrient digestibility, serum biochemical parameters, feed efficiency

Introduction

The demand for the consumption of livestock products is increasing day by day because of the consumer preferences for tastes, awareness about consumption of quality protein through livestock products and also urbanization is also one of the major factors for this demand. But 2 reasons strongly oppose the working conditions for meeting this animal product demand: increased competition for the land and the climate change which negatively affects water and animal feed availability (Poppi and McLennan, 2010) [31]. The identification of other alternate resources is therefore important for sustainable animal production which has high nutritive value and conversion efficiency, be able to optimise animal product quality. Consequently, *Spirulina* is emerging as a potential alternate to meet these criteria. It contains all essential amino acids, vitamins and minerals. It also is a rich source of carotenoids and fatty acids (Holman and Malau -Aduli, 2013) [16]. Intensive livestock production systems may be associated with multiple stressful incidents that negatively impact immune response and animal performance. The high metabolic rate during intensive feeding is accompanied by an increased production of free radicals, and any imbalance between production of these molecules and their safe disposal may end in oxidative stress, which can damage cells and tissues (Lykkesfeldt & Svendsen, 2007) [22]. Therefore, under oxidative stress conditions, there is an increased demand for antioxidants to reduce the deleterious effects of free radicals on the immune system (Carroll & Forsberg, 2007) [7]. Interestingly, feeding natural, rather than synthetic, antioxidant could be advantageous to animal welfare and consumer safety. The blue-green algae, *Spirulina platensis*, have been considered as a suitable natural antioxidant and immune-stimulant to humans and animals with fewer side effects and more cost effectiveness than synthetic products (Abdel-Daim *et al*., 2013) [2]. The present trial was conducted to study the effect of optimum level of supplementation of *Spirulina* in the diet of growing ram lambs and also, it's effect on nutrient digestibility and serum biochemical parameters.

Materials and Methods

The present study was conducted at the Department of Animal Nutrition, College of Veterinary Science, Tirupati, to study the effect of dietary supplementation of *Spirulina* in Nellore ram lambs on their growth performance, nutrient digestibility, serum biochemical and carcass characteristics. The laboratory analysis was carried out at the Department of Animal Nutrition and Department of Veterinary Biochemistry, College of Veterinary Science, Tirupati and the animal experimentation was carried out at the Department of Livestock Farm Complex, College of Veterinary Science, Tirupati. Feed ingredients were procured from the local market, *Spirulina* was procured from Inway *Spirulina* private limited, fresh super napier

green fodder was harvested at optimum stage from the fodder plots, Department of Livestock Farm Complex, Tirupati was used for feeding experimental animals after cutting and chopping each day. Eighteen Nellore brown ram lambs (age-3-5 months old, Avg body wt. 15±0.37 kg) were purchased from a local shandy. The ram lambs were adapted for stall feeding conditions, dewormed and vaccinated against F&M disease before the commencement of the study. The animals were randomly allotted to three treatment groups with six animals per treatment. A basal diet with *ad libitum* feeding of super napier and concentrate mixture @ 1.5% of body weight was fed for all the three groups and were named as T_1, T_2 and T_3. The basal diet with no supplementation of *Spirulina* was the control (T_1), T_1 with *Spirulina* supplementation in concentrate mixture @ 1 g/10 kg bodyweight is T_2 and @ 2 g /10 kg bodyweight is T_3. A growth trial was conducted for a period of 90 days under intensive system of rearing and the ram lambs were housed in a pucca shed in individual pens of 2.1 x 1.2 x 1m dimensions having facilities for feeding and watering. The ram lambs were fed with weighed quantities of concentrate mixture according to the experimental diets at 9:00AM daily and *ad libitum* feeding of super napier was done twice daily at 12:00 PM and 4:00 PM and the leftover super napier if any was recorded next day morning to calculate dry matter intake (DMI). The animals had free access to fresh and clean drinking water throughout the day. Deworming was done once in a month during the experimental period. Periodical weighing of the animals was practiced before feeding at fortnightly intervals to know the effect of experimental diets on growth performance. At the end of experiment, 5 lambs per treatment were slaughtered to study effect of treatments on carcass traits. Animals were slaughtered by "Halal" method and sticking, legging, dressing and evisceration were performed as per the procedures described by Gerrard (1964) [12].

Results

Nutrient digestibility

The apparent nutrient digestibility coefficients of ram lambs fed with experimental rations are presented in Table 1. The digestibility coefficient of CP was significantly higher ($p<0.05$) in T_2 and the values were 71.58, 76.16 and 73.73 in T_1, T_2 and T_3 groups, respectively. No significant difference ($p>0.05$) was noticed among the treatment groups in digestibility coefficients of DM, OM, CF, EE, NFE, NDF and ADF and the values were 69.61, 70.08 and 69.4; 72.29, 73.41 and 72.5; 56.51, 57.30 and 56.59; 63.58, 62.61 and 63.57; 72.71, 72.67 and 72.74; and 54.33, 55.31 and 55.65; 44.53, 45.40 and 44.54 in T_1, T_2 and T_3 groups, respectively.

Table 1: Effect of dietary supplementation of *Spirulina* on nutrient digestibility of ram lambs

Parameter	T_1	T_2	T_3	P value
Dry matter	69.61±1.14	70.08±1.12	69.4±1.19	0.913
Organic matter	72.29±1.21	73.41±1.33	72.5±1.32	0.811
Crude protein*	71.58^{a}±1.31	76.16^{b}±1.41	73.73^{ab}±1.25	0.045
Ether extract	63.58±1.04	62.61±1.03	63.57±1.39	0.798
Crude fibre	56.51±1.24	57.30±1.37	56.59±1.42	0.901
NFE	72.71±0.43	72.67±0.33	72.74±0.27	0.989
NDF	54.33±1.63	55.31±1.55	55.65±1.27	0.896
ADF	44.53±1.68	45.40±1.5	44.54±1.21	0.894

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

The plane of nutrition of the ram lambs supplemented with or without *Spirulina* is presented in Table 2. The% DCP content was 10.36, 11.20 and 10.90 in T_1, T_2 and T_3 groups respectively and was significantly lower ($p<0.05$) in T_1 among the treatments. There was no significant difference ($p>0.05$) observed in the total DMI (% BW) and TDN (%DM) and the values were 3.32, 3.28 and 3.24 and 63.07, 64.09 and 63.79 in T_1, T_2 and T_3 groups, respectively.

The DCP% was significantly ($p<0.05$) higher in T_2 and lower in T_1. However, no significant difference ($p>0.05$) was observed between T_1 & T_3 and T_2 & T_3.

Table 2: Effect of dietary supplementation of *Spirulina* on Plane of nutrition of ram lambs during growth trial

Treatment	BW (kg)	DMI (g /day)	DMI (% BW)	TDN (% DM)	DCP (% DM)*
T_1	23.58±1.07	779.94±18.79	3.32±0.06	63.07±0.45	10.36^{a}±0.18
T_2	25.48±0.97	834.52±23.74	3.28±0.03	64.09±0.28	11.20^{b}±0.21
T_3	24.37±0.44	790.28±8.57	3.24±0.04	63.79±0.45	10.90^{ab}±0.19
P value	0.329	0.112	0.541	0.216	0.022

^{ab} values bearing different superscripts in a same column differ significantly *($p<0.05$).

Serum Biochemical Profile

Serum biochemical profile of experimental animals at the beginning and at the end of the experiment is presented in Table 3 and Table 4, respectively.

The results of serum biochemical profile of ram lambs at the beginning of the experiment indicated no significant ($p>0.05$) difference for serum total protein, albumin, globulin, ALT, AST, cholesterol, triglycerides, urea, creatinine, HDL, LDL, VLDL, calcium and glucose.

Serum biochemical profile of ram lambs at the end of the experiment revealed significant ($p<0.05$) increase in total protein (g/dL) in T_2 (7.45) as compared to T_1 (6.78) and T_3 (7.05). However, the other parameters like ALT, AST, albumin, globulin, urea, triglycerides, cholesterol, creatinine were found to be non-significant ($p>0.05$) among treatments. ALT (IU/L) and AST (IU/L) was lower in T_2 (23.95 and139.8) and T_3 (21.13 and 136.62) as compared to T_1 (25.45 and 23.95).

Cholesterol (mg/dL) and triglyceride (mg/dL) content were non significantly ($p>0.05$) lower in T_2 (78.75 and 93.75) and T_3 (76.5 and 91.75) as compared to T_1 (79.75 and 94.25). HDL (mg/dL) was higher in T_3 (32.8) as compared to T_1 (31.45) and T_2 (31.38); LDL (mg/dL) and VLDL (mg/dL) were higher in T_1 (29.45 and 18.85) as compared to T_2 (28.63 and 18.75) and T_3 (28.85 and 18.35). Calcium and glucose were not influenced among the treatments with or without *Spirulina* supplementation at the start of trial. However, at the end of the trial, the values for calcium

(mg/dL) were higher in T_3 (11.25) as compared to T_1 (10.99) and lower in T_2 (10.69) and glucose (mg/dL) levels were higher in T1 (97.25) as compared to *Spirulina* supplemented groups T_2 (95.75) and lower in T_3 (94.75).
Serum globulin (g/dL) was higher in T_2 (3.73) as compared to T_1 (3.02) and T_3 (3.56). The values for urea (mg/dL) and creatinine (mg/dL) were higher in T_2 (8.25 and 2.97) as compared to T_3 (7.85 and 1.45) and lower in T_1 (7.1 and 1.25), respectively.

Table 3: Effect of dietary supplementation of *Spirulina* on serum biochemical parameters of ram lambs (0^{th} day)

Parameter	T_1	T_2	T_3	P value
Total protein (g/dL)	6.65±0.17	6.3±0.18	6.18±0.48	0.103
Albumin (g/dL)	3.5±0.11	3.45±0.09	3.6±0.12	0.616
Globulin (g/dL)	2.97±0.05	2.85±0.21	2.57±0.22	0.174
ALT (IU/L)	22.13±2.31	19.35±1.85	21.15±2.03	0.644
AST (IU/L)	130.88±6.96	132.75±5.41	138.38±8.76	0.751
Triglycerides (mg/dL)	81.5±2.4	84.00±3.43	80.75±2.78	0718
Urea (mg/dL)	9.58±1.1	8.62±0.45	10.52±0.4	0.238
Cholesterol (mg/dL)	77.5±1.94	77.75±1.75	77.25±1.89	0.777
Creatinine (mg/dL)	1.16±0.09	1.14±0.04	1.24±0.03	0.476
HDL (mg/dL)	30.85±0.74	30.6±0.61	31.95±0.65	0.360
LDL (mg/dL)	30.35±1.04	30.6±0.63	31.15±0.99	0.819
VLDL (mg/dL)	16.3±0.48	16.8±0.69	16.15±0.56	0.718
Calcium (mg/dL)	6.15±0.29	5.81±0.22	5.55±0.08	0.194
Glucose (mg/dL)	103.5±3.88	95.5±3.01	102.5±1.26	0.165

Table 4: Effect of dietary supplementation of *Spirulina* on serum biochemical parameters of ram lambs (90^{th} day)

Parameter	T_1	T_2	T_3	P value
Total protein (g/dL)*	$6.78±0.11^a$	$7.45±0.06^b$	$7.05±0.25^{ab}$	0.049
Albumin (g/dL)	3.47±0.13	3.73±0.08	3.75±0.06	0.132
Globulin (g/dL)	3.02±0.8	3.73±0.09	3.56±0.32	0.074
ALT (IU/L)	25.45±3.87	23.95±1.56	21.13±3.29	0.616
AST (IU/L)	149.35±7.69	139.8±19.25	136.62±6.19	0.779
Triglycerides (mg/dL)	94.25±0.75	93.75±0.85	91.75±2.21	0.46
Urea (mg/dL)	7.1±0.6	8.25±0.06	7.85±0.17	0.129
Cholesterol (mg/dL)	79.75±2.78	78.75±2.56	76.5±0.65	0.587
Creatinine (mg/dL)	1.25±0.06	1.97±1.71	1.45±0.18	0.44
HDL (mg/dL)	31.45±1.56	31.38±1.42	32.8±1.36	0.744
LDL (mg/dL)	29.45±1.26	28.63±1.02	25.85±1.03	0.108
VLDL (mg/dL)	18.85±0.15	18.75±0.17	18.35±0.44	0.460
Calcium (mg/dL)	10.99±0.53	10.69±0.36	11.25±0.69	0.771
Glucose (mg/dL)	97.25±1.03	95.75±5.59	94.75±3.12	0.181

[ab] values in a column bearing different superscripts differ significantly *(p<0.05).

Discussion

Nutrient Digestibility

The apparent digestibility of nutrients indicated that, except for crude protein, none of the parameters were statistically significant (p>0.05). The digestibility of crude protein was significantly higher in lambs supplemented with *Spirulina* at 1 g /10 kg body weight compared to those supplemented with 2 g /10kg body weight. However, the digestibility of other nutrients did not show significant differences among treatments.
Contrary to reports by El-Ashry *et al*. (2003) [9] suggesting that *Spirulina* increases fibre digestibility in the rumen due to enhanced cellulolytic activity, our findings indicated no significant change in fiber digestibility across treatments. However, based on El-Ashry *et al*. (2003) [9], it can be inferred that *Spirulina* supplementation increases fibrolytic activity in the rumen, possibly due to an increased number of cellulolytic bacteria, subsequently enhancing the fermentation capacity of the rumen. Accelerated cellulolytic bacteria activity can break down fibre particles, releasing cell-bound nutrients, including cell-bound proteins, potentially contributing to the observed enhancement in protein digestibility in the 1 g /10kg body weight *Spirulina*-supplemented group. Thus, it is assumed that cell bound protein could have contributed to enhanced protein digestibility in *Spirulina* supplemented groups.
Spirulina has been reported to increase microbial crude protein synthesis and reduce retention time within the rumen (Quingley and Poppi, 2009) [33]. Approximately 20% of dietary *Spirulina* was reported to bypass rumen degradation, reaching the abomasum for increased absorption (Quingley and Poppi, 2009; Panjaitan *et al.*, 2010 and Zhang *et al*., 2010) [33, 31, 45]. Rumen development in lambs occurs in three stages, with the diversity of rumen microbial populations playing a crucial role in understanding rumen function (Matthews *et al*., 2019) [26]. Rumen microbes are vital for animal productivity and nutrient digestibility as they degrade carbohydrates, proteins, and other components, influencing rumen fermentation and volatile fatty acid production, which is essential for metabolic functions (Wang *et al.*, 2016) [43].
Earlier reports suggest that nutrient levels and feed composition significantly influence rumen histological morphology, affecting parameters such as rumen papilla length and thickness, as well as rumen wall-related characteristics, microbial populations, and pH (Wang *et al*. 2022; Sun *et al*. 2021) [42, 40]. The addition of *Spirulina* may mitigate dietary nutrient composition discrepancies in the rumen. In our study, the improvement in the digestibility of certain nutrients may be attributed to enhanced rumen function. However, it's important to note that dietary protein digestibility decreases with increasing levels of Spirulina, potentially due to its complex cell wall structure that withstands gut enzymes secreted by the mucosa.

Serum Biochemical Profile

The serum biochemical profile remained unaffected by *Spirulina* at both the commencement and conclusion of the 90-day trial. Initial serum biochemical profile results for ram lambs showed no significant (p>0.05) differences in serum total protein, albumin, globulin, ALT, AST, cholesterol, triglycerides, urea, creatinine, HDL, LDL, VLDL, calcium, and glucose. However, at the trial's end, there was a significant increase (p<0.05) in total protein for the group supplemented with 1 g /10kg body weight of *Spirulina* compared to the control and the group supplemented with 2 g /10kg body weight of *Spirulina*. Other parameters, including albumin, globulin, ALT, AST, cholesterol, triglycerides, urea, creatinine, HDL, LDL, VLDL, calcium, and glucose, showed non-significant differences (p>0.05) among treatments.
In the present findings, it was documented with an increase serum total protein content in *Spirulina* supplemented groups which is attributed to an elevated protein content.

These results are in agreement with Parimi *et al*. (2015) [32]; Hanafy (2023) [15]; Mokhtar *et al.* (2023) [28] Assar *et al.* (2023) [4]; Hafez *et al*. (2013) [14]. While the concentration of globulin increased non-significantly in *Spirulina*-supplemented groups, these results corroborate with the findings of Hanafy (2023) [15], El-sabagh *et al*. (2014) [10], and Hafez *et al*. (2013) [14]. The higher plasma globulin and albumin concentrations may be attributed to *Spirulina's* high protein content (Gershwin and Belay, 2008) [13], and increased plasma globulin levels could signify a stronger innate response and higher resistance in lambs.

ALT and AST exhibited a decreasing trend in the *Spirulina*-supplemented group, with a non-significant effect (p>0.05). This trend suggests that *Spirulina* may possess a protective action against liver dysfunction which are in agreement as suggested by Bhattacharya and Mehta (2012); Hanafy A (2023) [15]; Assar *et al.* (2023) [4]; Mansour *et al*. (2023) [24]; Mokhtar *et al.* (2023) [28]; Liang *et al*. (2020) [21]; El-sabagh *et al*. (2014) [10] and Hafez *et al*. (2013) [14]. Abdel-Daim *et al*. (2013) [2] also reported a significant decrease (p<0.05) in ALT in the serum of ewes supplemented with *Spirulina*.

Cholesterol and triglycerides concentrations showed a non-significant decrease (p>0.05) in *Spirulina*-supplemented groups T_2 and T_3 compared to the control which are in consistent with the findings of Hanafy A (2023) [15]; Assar *et al.* (2023) [4]; Mansour *et al.* (2023) [24]; El-sayed *et al.* (2018); El-sabagh *et al*. (2014) [10]; and Hafez *et al*. (2013) [14]. The hypolipidemic activity of *Spirulina* may be attributed to β-carotene (Seo *et al*., 2004) [39] and linolenic acid (Morsie *et al*., 2004) [29]. *Spirulina's* hypercholesterolemic action involves reducing plasma and liver cholesterol levels through increased lipoprotein lipase and hepatic triglyceride lipase activity (Karkos *et al.*, 2011) [19]. *Spirulina's* hypercholesterolemic activity is related to the large amount of cysteine found in C-phycocyanin content (Nagaoka *et al*., 2005) [30] and *Spirulina* was reported to inhibit pancreatic lipase activity also in a dose-dependent manner (Torres-Duran *et al.*, 2007). Another finding for the decreased cholesterol concentration with *Spirulina platensis* may be due to the ileal bile acid absorption and inhibition of both jejunal cholesterol absorption (Nagaoka *et al*., 2005) [30]. *Spirulina platensis* reduced triglyceride and cholesterol (Hormat al-Sadat *et al*., 2022) [17]. The same finding was seen in rabbits by (Cheong *et al.*, 2010) [8], in rats (Kato *et al*., 1984) [20], in hamsters (Riss *et al.*, 2007) [35], in humans (Ruitang *and* chow., 2010) [36]. Decreased cholesterol is also due to *Spirulina's* anti-oxidant characteristics (Liang *et al*., 2020) [21].

Serum glucose levels, were lower in the *Spirulina*-supplemented groups T_2 and T_3 and were non-significant (p>0.05). These finding aligns with Hanafy (2023) [15] and El-sabagh *et al*. (2014) [10]. The decrease in serum glucose may result from *Spirulina*'s iron-chelating effect, as phycocyanin isolated from *Spirulina* can bind ferrous ions (Bermejo *et al.*, 2008) [5]. Iron depletion is known to upregulate glucose uptake and increase insulin activity, potentially explaining the decreased glucose levels in *Spirulina* supplementation. *Spirulina platensis* has been recognized for its anti-diabetic properties (Metin Guldas *et al*., 2021) [27].

Creatinine concentration increased non-significantly (p>0.05) in *Spirulina*-supplemented groups T_2 and T_3 compared to the control which are consistent with Abdel-Daim *et al*. (2013) [2] and Malau Adauli and Holman (2015) [23]. This increase is linked to the positive linear relationship between creatinine concentrations and muscle tissue mass, suggesting that lambs with higher serum creatinine concentrations have greater muscularity. The protective effects of *Spirulina* may be attributed to its antiapoptotic, anti-inflammatory, and antioxidant properties (Zakaria *et al*., 2019) [44].

Urea concentration increased non-significantly (p>0.05) in *Spirulina*-supplemented groups (T_2 and T_3) compared to the control which are consistent with the results reported by Mokhtar *et al.* (2023) [28], Malau-Adauli and Holman (2015) [23], El-sabagh *et al*. (2014) [10], and Hafez *et al*. (2013) [14]. Urea, the principal byproduct of protein metabolism synthesized in the liver, is transported for excretion by the kidneys (Russel and *Roussel.* 2007) [37]. The observed increase in urea concentration may be linked to the higher provision of protein through *Spirulina* supplementation, as it relates to dietary protein intake (Huntington *et al.*, 2001) [18]. The serum urea concentration is a reflection of dietary protein intake. In the present findings, higher urea concentrations were recorded to the *Spirulina* supplemented groups owing to the higher protein content in *Spirulina* and also due to enhanced protein digestibility which is in agreement with the findings reported by Sargison and scott, (2010) [38].

Conclusion

General addition of algae extract powder as feed additive to the ration of sheep will show the positive return on the health and production. From the present trial, it can be concluded that supplementation of *Spirulina* at 1 g /10 kg could improve the performance of animal with positive growth rate, however supplementation of *Spirulina* at higher level as discussed in the present study could not be proved economical. Owing to its high protein content and quality *Spirulina* can be added as a feed additive @ 1 g /10 kg body weight in the rations to improve the productive performance of ram lambs.

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