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Effect of Duckweed as Alternate Protein Source on Rumen Fermentation and Health Status of Beetal Goats

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***Abstract*:** "Duckweed" to refer to members of the aquatic plant family *Lemnaceae*. A duckweed feeding trial was carried out at the Goat Farm of the Department of Livestock Production and Management, GADVASU, Ludhiana with 20 male goats fed four different diets. The objective of the trial was to evaluate the use of duckweed (*Spirodela polyrhiza*) and duckweed-based TMRs and use as a protein supplement for ruminants. The hypothesis was that duckweed is a suitable protein source for goats and will behave in a similar fashion to soybean meal. The diets included a control group (all supplemental protein from soybean meal), 1/3 duckweed, 2/3 duckweed, and 100% duckweed (corresponding to 1/3, 2/3, and 100% of the supplemental protein from duckweed, respectively). The goats were fed equal amounts of fodder and concentrate ration in 50: 50 R:C at 4% of body weight (as fed). Duckweed supplementation in male goats significantly increased blood cholesterol and creatinine levels as compared to the control group, but the values were within the physiological range. Duckweed supplementation did not have any adverse effects on rumen fermentation parameters (pH, TN, NPN, NH_3, TVFA and TCA-pptN). Based on the above results concluded that duckweed can be used as alternate protein source in goats' feeding without any adverse effects.

***Keywords*:** Duckweed (*Spirodela polyrhiza*), Goats, Rumen fermentation, Retention, Blood parameters

Feed cost is most important in animal production systems and chiefly determines the profit margins for the farmer. Dairy producers strive to acquire the most economically efficient sources of nutrients to meet the precise nutrient requirements of the dairy cows. By-products present a cost-effective alternative to traditional feed ingredients. However, before substituting traditional feed ingredients with by-products, several factors such as acceptability, consistency, availability, and quality must be carefully considered. One noteworthy example of such a feedstuff is duckweed, which is a tiny, free-floating, vascular aquatic plant. It has similar crude protein levels and also contains the essential amino acids needed in ruminant rations. Studies have shown that duckweed can have crude protein levels as high as 45%. Moreover, duckweed offers high-quality protein with an amino acid profile similar to most plant proteins, rivalling animal protein sources. When comparing the annual dry matter yield, duckweed excels, producing between 10 to 30 tons per hour (Leng et al 1995), while alfalfa only yields about 11 metric tons per hectare per year. Another advantageous aspect of duckweed is the low lignin content in its cell walls, which contributes to increased fiber digestibility . The entire body of duckweed consists of non-structural, metabolically active tissue, making it remarkably high in nutritional value (FAO 2009).

Despite the potentially beneficial attributes of duckweed on ruminant animal performance, the practice of feeding aquatic plants has received limited attention. This plant may offer a viable alternative to crop production in areas where land is scarce, of poor quality or experiencing inadequate and variable precipitation. Given the future challenge of meeting the global protein demand, the importance of duckweed as an alternative protein source in animal diets is growing significantly. Hence, this research aims to explore the nutritional quality of duckweed, particularly as a protein source, and its effects on rumen fermentation and blood profiles in ruminants.

MATERIAL AND METHODS

The duckweed sample for this study was obtained from the College of Fisheries, GADVASU, Ludhiana. The sun/ air-dried duckweed was ground in a Wiley mill through a 2mm screen. The total mixed rations (TMR) were prepared by incorporating different levels of duckweed, namely control, 1/3 duckweed, 2/3 duckweed, and 100% duckweed, which replaced the total crude protein (CP) derived from soybean in a 50:50 ratio (R:C), as indicated in Table 1. All the TMR prepared were iso-nitrogenous, having approximately 15% CP.

Animal Feeding: Male goats (20; 15kg body weight) were divided into 4 equal groups and were offered with four different total mixed rations i.e. TMR1 (control), TMR2 (1/3

duckweed), TMR3 (2/3 duckweed) and TMR4 (100% duckweed) for a duration of 120 days.

Housing: The male goats were accommodated in a concrete shed and provided with group stall feeding at 9:00 am each day. They were given unrestricted access to water twice a day and were allowed for 1-hour at the yard on a daily basis.

Chemical analysis: Samples of feed, faeces, and orts were subjected to analysis for proximate constituents following the AOAC (2000) guidelines. Cellulose analysis was performed according to Crompton and Maynard (1938), while cell wall constituents were determined following the methodology outlined by Robertson, Van Soest et al (1981).

Rumen liquor analysis: All the male goats were given the respective experimental diets as per the experimental design. Rumen liquor was collected four hours after the experimental feeding. Specialized stomach tubes were used to collect rumen liquor from various sites of the rumen. Subsequently, all the collected rumen liquor samples were pooled based on their respective sampling hours and stored in a refrigerator until analysis. The strained rumen liquor was then analyzed for various parameters, including pH, total volatile fatty acid (using the method described by Cottyn and Boucque in 1968), total nitrogen, TCA-precipitable nitrogen, and ammonia nitrogen, following standard procedures.

Estimation of blood biochemical profile: Blood samples were collected from male goats through the jugular vein both on day 0 and after the experimental feeding. The serum was then stored at -20°C for further analysis of various parameters, including glucose, BUN, cholesterol, GGT, AST, ALT, triglycerides, total protein, and creatinine. Diagnostic kits from Siemens Autopack were used to estimate these biochemical parameters, which were subsequently analysed using the RA-50 blood analyser.

Statistical analysis: The data was analyzed using SPSS Version 19. To assess the differences in means, the Tukey B test was employed.

RESULTS AND DISCUSSION

Rumen fermentation parameters *in vivo*: The pH of the rumen liquor remained unchanged with duckweed supplementation, indicating that it has no adverse effects on rumen microflora. Typically, the normal rumen pH values for ruminants offered mixed rations range from 5.8 to 7.0, depending on factors such as protein content, degradability, carbohydrate quantity and type, roughage characteristics, and roughage to concentrate ratio. In general, the pH level in the rumen liquor shows an inverse correlation with total volatile fatty acid) concentration. The rumen pH did not show a significant difference among the groups supplemented with varying levels of duckweed and the control group (Table 2). Moore et al (2002) observed that in soybean meal (pH 6.52) and soybean hull (pH 6.41) diet, the rumen pH values for in duckweed diet in present study were lower. This discrepancy may be attributed to the fact that our diets consisted of 50% concentrate and 50% fodder, whereas they fed a lower level of concentrate.

The mean ammonia-N concentration (mg/dl SRL) did not show a significant effect (values ranged from 49.52- 59.67, groups I, II respectively). The ammonia-N concentration in this study surpassed the minimum threshold of 5-8 mg/100 ml SRL, as proposed for optimum microbial growth in all four groups. The values were consistently higher than the proposed threshold. During the conversion of dietary nitrogen (N) into microbial protein, NH3 plays a crucial role as an intermediate in the rumen. Consumption of a substantial amount of protein can lead to an excessive production of NH3 in the rumen. If the rate of NH3 production surpasses its utilization by rumen microbes, the concentration of NH3 in

Table 1. Ingredient composition of different concentrate mixtures containing duckweed

Ingredient	Control	1/3 duckweed	2/3 duckweed	100% duckweed
Maize	35	35	35	35
Soybean	28	18.6	9.3	0
Duckweed	0	15.5	31	46
Wheat bran	17	13	8	4
Rice bran	14.75	12.6	11.5	9.5
Mineral Mixture	2	2	2	2
Salt	1	1	1	1
Urea	0	0.3	0.7	1
Bypass fat	2.25	2	1.5	1.5
NFC	26.63	21.43	14.25	15.10

R:C 50:50 (Roughage: concentrate)

the rumen increases. This increase is particularly noticeable when the diet lacks readily available carbohydrates.

The total nitrogen content in strained rumen liquor (SRL) primarily reflects the solubility of ingested protein in the rumen and may also vary depending on the amount of protein intake. In the duckweed supplemented groups (group II, III, and IV), the total nitrogen levels were 239.49, 176.57, and 189.70 mg/dl, respectively. These values did not show a statistically significant difference compared to the control group, where it was 250.07 mg/dl (Table 2). The TCA-ppt N mainly represents microbial nitrogen. The supplementation of 1/3 duckweed resulted in an increase in the concentration of TCA-ppt N (mg/100 ml) in group II, followed by group I, and the lowest increase was observed in the 2/3 supplemented duckweed group.-The TCA-ppt N concentrations (mg/dl) in the rumen liquor were 121.34, 135.38, 79.62, and 99.89 in groups I, II, III, and IV, respectively. The observed increase in TCA-ppt N can be partly attributed to the enhanced utilization of ammonia and feed nitrogen by rumen microorganisms for their body protein synthesis, and partly to an increase in protozoal numbers. The non-protein nitrogen fraction primarily comprises ammonia nitrogen, along with small quantities of amides, amino acids, etc. Consequently, the concentration of non-protein nitrogen in the rumen fluid primarily relies on the production of ammonia, its uptake by microbes, and absorption through the rumen wall. Additionally, the non-protein nitrogen concentration (mg/100ml SRL) was lower in group IV (89.81) and group III (96.95) when compared to both the control group (128.73) and the group supplemented with 1/3 duckweed (104.02) (Fig. 1).

The concentration of total volatile fatty acids (TVFA) in the SRL (rumen liquid) depends on the amount of easily digestible carbohydrates, fermentable sugars, and the quantity and quality of CF. In this study, the concentration of TVFA ranged from 8.35 to 9.68 mM/dl SRL (Table 3). This variation can be attributed to the succession of events that occurred during carbohydrate fermentation in the rumen. The shift in substrate utilization during carbohydrate fermentation involves the initial utilization of easily fermentable sugars followed by structural carbohydrates (cellulose), which are fermented at a slower rate, with the maximum rate of

Table 2. *In vivo* Rumen fermentation parameters

Parameters	Group 1	Group 2	Group 3	Group 4	SEM
Total nitrogen mg	250.07^{c}	239.49^{bc}	176.57^{a}	189.70^{ab}	11.01
NPN mg	128.73	104.02	96.95	89.81	6.97
TCA-N mg	121.34	135.38	79.62	99.89	10.79
NH3 mg	49.52	59.67	51.80	50.40	3.64
pH	5.82	5.76	6.14	6.11	0.059

Table 3. *In vivo* volatile fatty acids production (mM/dl) of different total mixed rations containing different levels of duckweed

Parameters	Group 1	Group 2	Group 3	Group 4	SEM
Acetic acid	5.62	6.64	5.91	5.84	0.17
Propionic acid	1.60	1.85	1.46	1.49	0.060
Iso butyric acid	0.037	0.048	0.036	0.051	0.003
Butyric acid	0.97	1.01	0.87	1.05	0.038
Iso valeric acid	0.055	0.068	0.051	0.061	0.005
Valeric acid	0.063^{b}	0.067^{b}	0.051^{a}	0.064^{b}	0.002
TVFA	8.35	9.68	8.38	8.57	0.24
Relative proportion (%)					
Acetate	67.24	68.60	70.62	68.02	0.54
Propionate	19.22	19.08	17.37	17.56	0.38
Iso butyrate	0.45	0.50	0.43	0.59	0.027
Butyrate	11.67	10.42	10.34	12.34	0.35
Isovalerate	0.66	0.69	0.61	0.71	0.041
Valerate	0.75^{b}	0.69^{ab}	0.61^{a}	0.76^{b}	0.021
A:P ratio`	3.50	3.59	4.11	3.91	0.11

Means bearing different superscripts in a row differ significantly (P<0.05)

breakdown occurring in the later stages of the digestion process. The results showed a non-significant difference among all four groups regarding TVFA concentrations. Specifically, the TVFA concentrations in the control and duckweed-supplemented groups varied from 8.35 -9.68 mM/dl for group and I and II (1/3 duckweed). However, a statistically significant difference was observed in valeric acid production, with the lowest value in the 2/3 duckweed supplemented group (III) and the highest value in the 1/3 duckweed supplemented group (II). The mean percentage of acetate was similar in all four groups (67.24- 70.62%, in groups I and III) .The percentage of propionate showed no significant variation among the groups. The butyrate percentage was also comparable across all four groups, ranging from 10.34% to 12.34, However, the percentage of valerate in rumen liquor was statistically higher in the 100% duckweed supplemented group (0.76) and the control group (0.75), and statistically lower in the 2/3 duckweed supplemented group (0.61). No significant effect on percent isovalerate was observed in both control and duckweed supplemented groups. The A: P ratio was lowest in the control group (3.50) and numerically higher in the 2/3 duckweed supplemented group (4.11) (Fig. 2).

The duckweed exhibits a VFA profile similar to that of soybean meal. The VFAs showed no significant differences between the control and duckweed supplemented diets. Comparing with study by Moore et al. (2002) on soybean meal and soybean hull diets, the present diet showed higher total VFA amounts than those reported for soybean meal and soybean hull. Additionally, the acetate to propionate ratio in diets was higher (3.50 mM for control to 4.11 for 2/3 duckweed) than in the soybean meal (3.06) or soybean hull (3.26) diets of Moore et al (2002). Damry et al (2001) and Huque et al (1996) concluded that incorporation of duckweed into a ruminant's diet had no detrimental effects. Damry et al (2001) reported that duckweed served as a good source of undegradable protein for ruminants, contrasting with the earlier findings of Huque et al. (1996), which demonstrated high degradability of duckweed (87% for *Lemna*) when exposed to rumen conditions in bulls for 72 hours.

Blood biochemical aspects: Prior to the commencement of the experiment, blood samples were collected from the goats to evaluate their baseline values (Table 4, 5). All blood parameters were within the physiological range, indicating that the goats were in good health at the beginning of the study. Furthermore, no statistically significant effects on any

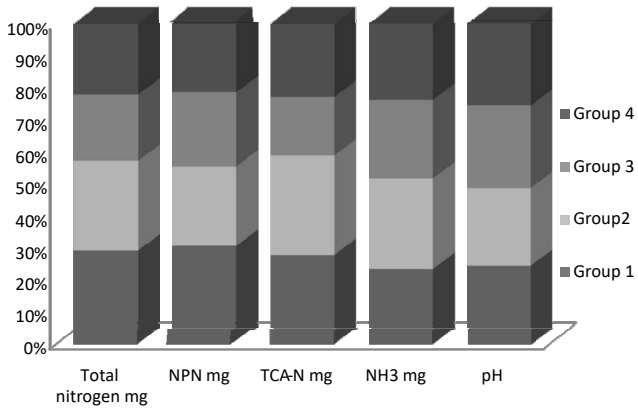

Fig. 1. *In vivo* Rumen fermentation parameters

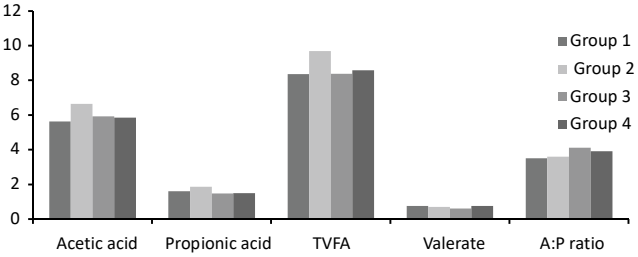

Fig. 2. *In vivo* volatile fatty acids production (mM/dl) of different total mixed rations containing different levels of duckweed

Table 4. Blood constituents in male beetal goats at the start of experiment

Parameters	Group 1	Group 2	Group 3	Group 4	SEM
Triglycerides, mg/dl	12.40	13.17	16.88	12.71	2.37
GGT, (U/L)	47.63	45.00	46.66	54.95	3.17
Glucose, mg/dl	43.0	35.33	35.33	34.00	2.02
BUN, mg/dl	24.20	25.04	25.49	24.93	0.68
Creatinine, mg/dl	0.90	0.96	1.02	0.91	0.022
Cholesterol, mg/dl	56.42	75.36	71.27	57.0	3.74
ALT, (U/L)	16.38	17.40	20.19	20.11	0.93
AST, (U/L)	100.56	109.10	97.22	89.38	7.54
Total protein	6.58	6.47	6.74	6.58	0.15

Means bearing different superscripts in a row differ significantly (P<0.05)

Table 5. Blood parameters after feeding

Parameters	Group 1	Group 2	Group 3	Group 4	SEM
Triglycerides, mg/dl	15.68	15.27	16.28	15.86	2.03
GGT, (U/L)	42.61^{ab}	43.26^{ab}	38.44^{a}	57.65^{b}	2.86
Glucose, mg/dl	39.39	34.13	36.37	37.07	2.40
BUN, mg/dl	24.30	24.65	27.07	26.53	1.04
Creatinine, mg/dl	0.77^{a}	0.85^{ab}	0.93^{b}	0.84^{ab}	0.018
Cholesterol, mg/dl	40.50^{a}	58.16^{ab}	63.42^{b}	49.54^{ab}	3.69
ALT, (U/L)	20.22	19.60	20.37	20.39	0.57
AST, (U/L)	101.24	90.72	101.42	101.57	4.38
Total protein (g/dl)	6.29	6.37	6.73	6.74	0.13

Means bearing different superscripts in a row differ significantly (P<0.05)

of the blood parameters were observed throughout the course of the experiment. Effect of duckweed supplementation on blood glucose, triglycerides, GGT, creatinine, urea-nitrogen, cholesterol, ALT, AST and total indicate no significant effect of duckweed supplementation on triglycerides, GGT, Creatinine, ALT and AST in all groups. The serum triglycerides (mg/dl) were 15.68, 15.27, 16.28 and 15.86 respectively in all the four groups. The GGT (U/L) values were 42.61, 43.26, 38.44 and 68.11 whereas AST value (U/L) were 101.24, 90.72, 101.42 and 101.57 in groups I. II, III and IV respectively. The serum urea nitrogen concentration is closely associated with the breakdown of protein to amino acids and their deamination in rumen and the rate of utilization of NH_3 for bacterial protein synthesis. The increase in serum urea level may reflect an accelerated rate of protein catabolism rather than decrease in urinary excretion. The serum urea level also increases in renal tubular necrosis and decreases in hepatic insufficiency and low protein intake. Concentration of urea-N in blood serum are indicator of the adequacy or inadequacy of the nitrogen in the diet of animals and results revealed no statistically significant difference in 4 groups. The blood urea concentration (mg/dl) were 24.30, 24.65, 27.07 and 26.53 in all four groups respectively. Increased serum urea nitrogen is a sign of inefficient nitrogen utilization, but also indicates the diets were providing adequate N for the goat's requirements. With more N in the blood, more N is passed to the liver and consequently excreted as waste. Moore et al (2002) reports a serum urea nitrogen level of 15.39 mM for a hay and soybean meal diet and 13.75 mM for a soyhull diet. Their serum urea nitrogen was taken at 2.4 hours after feeding. Similar findings of our results were reported by Jhonson (1990) in sheep and observed no differences in plasma urea nitrogen (PUN) concentrations across treatments at the beginning of the trial. Plasma urea nitrogen concentrations were depressed for the steers on the 100% DW diet relative to the steers on the 100% SBM diet (0% DW) at day 14 (3.64 vs. 6.05 mg/dl) and again on day 21 (4.66 vs. 7.15 mg/dl; P < 0.03). Additionally, PUN concentrations were depressed for steers on the 25% DW diet compared to the steers on the 0% DW treatment (5.13 vs. 7.15 mg/dl). This would allow one to hypothesize that the ruminant animal more efficiently utilizes the protein found in duckweed. The serum creatinine concentration (mg/dl) varied from 0.77 to 1.03 in all four groups. The cholesterol is synthesized from fatty acids inside the body of animal. Its concentration in the serum is the reflection of the body fat metabolism. There no significant difference in serum cholesterol concentration in control and duckweed supplemented groups. The serum cholesterol concentration (mg/dl) was 40.50, 58.16, 63.42 and 49.54 in groups I, II, III and IV, respectively. The results were statistically non-significant. After the duckweed supplementation, blood glucose levels (mg/dl) were 39.39, 34.13, 36.37, and 27.07 for groups I, II, III, and IV, respectively. Similarly, the AST (aspartate aminotransferase -U/L) were 20.22, 19.60, 20.37, and 20.39 for the corresponding groups. However, statistical analysis revealed no significant differences in blood glucose levels or AST values among the groups. The total protein levels (did not differ significantly across all four groups.

CONCLUSION

The inclusion of duckweed in the diet of male goats led to a significant increase in blood cholesterol and creatinine levels compared to the control group, but these values remained within the physiological range. However, it is important to note that duckweed supplementation did not adversely affect rumen fermentation parameters, including pH, total nitrogen, non-protein nitrogen, ammonia nitrogen, total volatile fatty acids and trichloroacetic acid-precipitable nitrogen. Based on the results obtained, it can be concluded that the addition of duckweed meal to the diet did not have any adverse effects on the health status of the goats.

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