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Harnessing the Nutritional Potential of Duckweed as a Protein Source in Ruminant Diet

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ABSTRACT

Duckweed is a promising protein source as a ruminant diet. Therefore, the duckweed samples were evaluated at varying levels to study the effect of its supplementation as a key protein source by *in-vitro* gas production technique. The extent of their dry matter (DM), crude protein (CP), ether extract (EE), and ash content were analyzed and there was 7.55 % DM, 24.5% CP, 3.5% EE and 20.07% ash. *In-vitro* studies revealed that the duckweed supplementation affected total gas production linearly and quadratically with concentrate ration ($P<0.001$). IVDMD and IVOMD were significantly higher in TMR-10. The partition factor (PF) was 4.25mg/dl in the control which was significantly lower as compared to 4.82 mg/dl in TMR-30 (30% duckweed). The ME value decreased significantly with an increase in duckweed inclusion and was minimal in TMR-30 (5.15 MJ/kg). It can be concluded that 10% replacement of the concentrate mixture can be done by duckweed in a TMR having 70:30 roughage to concentrate mixture ratio without any adverse effect.

KEYWORDS: Degradability, Duckweed, Gas Production, *In-vitro* studies

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INTRODUCTION

Feed shortage is the major constraint for the optimum production performance of ruminants and these animals are generally fed on high roughage diet to meet their requirement, particularly in tropical countries. Therefore, the addition of a concentrate mixture is necessary to meet the nutrient requirement, particularly the protein requirement. Globally, the demand for alternative protein ingredients has risen sharply due to hike in the prices of traditional protein sources like soybean meal in the past few years due to increasing human population and urbanisation. In this context, duckweed, a fastest-growing aquatic ûowering plant has gained the interest of farmers as well as animal nutritionist as it can produce about 10-30 tons of DM/ha/year. Apart from its exponential growth, it contains about 15-35 % crude protein (CP) with a superior amino acid profile (especially lysine 6.9 %, methionine 1.4%, and histidine 2.7%) than most plant proteins, 10-30 % total ash and rich in minerals, vitamin A other secondary metabolites (Appenroth et al., 2017, Kaplan et al., 2019; Demann et al., 2023). Further, the protein production of soybean, rice, and corn per harvested area is lower than that of duckweed (Ibrahim et al., 2017). Owing to their economic advantage, yield potential, nutritional quality, and positive perceptivity of people, duckweeds have been accepted and employed as plant-based food and feed resources without public aversion (de Beukelaar et al., 2019). It has been reported that duckweed derived food lowers the cardiometabolic risks and diabetes as compared to red meat (Chalvon-Demersay et al., 2017). Further, clinical nutrition studies have validated that the duckweeds consists of essential amino acids and vitamin B_{12} contents that are equipollent to peas and cheese (Kaplan et al., 2019). Iron and zinc contents in duckweeds are adequate for the recommended allowance alike the sodium/potassium ratio and fiber content (Ifie et al., 2021). The coupling of fresh duckweeds and agricultural residues in a diet for ruminants appears to offer a nutrient balance that is proficient in optimizing rumen microbial fermentative capacity (Leng et al., 1995). Duckweeds are claimed as novel ingredient to substitute soybeans, thus lowering cost of feed in dairy farming and ameliorating the shortcomings of feed production (Tallentire et al., 2018). Therefore, this research aims to explore the nutritional potential of duckweed to enable its adoption as a promising protein source in the diet of ruminants.

MATERIALS AND METHODS

The duckweed sample used in the in-*vitro* study was obtained from the Department of Fisheries, GADVASU, Ludhiana. The samples were air-dried and then ground in a Wiley mill through a 2mm screen and were analyzed for dry matter (DM), crude protein (CP), ether extract (EE), ash content, acid detergent fibre (ADF) and neutral detergent fibre (NDF). In the present study, duckweed was used as a protein source replacing concentrate in a total mixed ration (TMR) having concentrate mixture and roughage in 70:30 ratio (Table 1). Six different experimental substrates of TMR were prepared with graded duckweed levels i.e., Control (0 duckweed), TMR-5 (5% duckweed), TMR-10 (10% duckweed), TMR-15 (15% duckweed), TMR-20 (20 % duckweed) and TMR-30 (30% duckweed).

Table 1. Ingredient composition of total mixed ration (roughage: concentrate ratio, 70:30) having graded level of duckweed

Attributes	Duckweed	Concentrate mixture	Maize silage	Wheat straw
Control	0	30	50	20
TMR-5	5	25	51	19
TMR-10	10	20	53	17
TMR-15	15	15	54	16
TMR-20	20	10	55	15
TMR-30	30	0	57	13

For *in-vitro* gas production (Menke et al., 1979) study, rumen liquor was collected from fistulated buffalo bulls fed on a standard diet (roughage: concentrate in 70: 30 proportion) in the morning (6 AM) before feeding and watering into a pre-warmed thermo-flask and brought to the laboratory. Each substrate was incubated (triplicate) for 24 h in Menke glass syringe and after 24 h of incubation total gas production was recorded in each syringe. For determining the true dry matter (IVDMD) and organic matter (IVOMD) digestibility, the contents of syringes were transferred to a spout-less beaker and boiled with a neutral detergent. The partitioning factor (PF) and microbial biomass production (MBP) were calculated on the basis of IVTDOM (Blummel et al., 1999). Volatile fatty acids (VFAs) were estimated by (Cottoyn and Boucque, 1968) using gas liquid chromatography (GLC) technique using Net Chrom-9100 model. VFA was estimated using a gas column (6 ft length and 1/8 inch diameter) packed with chromosorb 101. The gas flow rates were 30, 30, and 320 µl/ min for nitrogen, hydrogen and zero air, respectively. Temperature of injector oven, column oven and detector was set at 270°C, 172°C respectively.

Data from *in-vitro* study was analyzed 1x3 factorial design (Snedecor and Cochran, 1994), by using SPSS Version 19. The differences in means were tested by Tukey B. The statistical model used was

$$Y_{ij} = \mu + T_i + \epsilon_{ij}$$

where,

Y_{ij} is each observation

μ is overall mean

T_i is effect of i[th] treatment

ϵ_{ij} is residual error

RESULTS AND DISCUSSION

Duckweed plays a multifaceted role in animal feed, human nutrition, bioenergy resource, wastewater purification, and as plant-derived cell factories (Ziegler et al., 2015). Duckweed used in the present study contained about 7.55% DM, 24.5% CP, 3.5% EE and 20.07% ash (Table 2). These results are in agreement with the results of previous studies (Negesse et al., 2009 and Hlophe and Moyo, 2011) who reported that duckweed contain about 6.7% DM, 25.8-27.4% CP, 2.8-4.2% EE, and 12.3-23.3 % ash in duckweeds. Further, the fiber content i.e., NDF and ADF content of duckweed in present study is 39.05% and 20.25% respectively and is concordance with reports of Huque et al. (1996) and Negesse et al. (2009) who reported that in duckweed NDF and ADF content varies from 47.1-57.4 % and 20.3-23.4 % respectively.

Table 2. Chemical composition of concentrates of duckweed, % DM basis

Parameter	Duckweed	Concentrate	Maize silage	Wheat straw
Total ash	20.1	4.80	6.5	8.15
Organic matter	79.9	95.2	93.5	91.8
Crude protein	24.5	25.6	8.86	2.94
Ether extract	3.50	5.70	3.10	0.45
Neutral detergent fiber	39.0	22.4	52.1	78.6
Acid detergent fiber	20.2	13.1	26.8	50.8
Acid detergent lignin	7.80	4.83	2.27	7.80
Cellulose	12.4	8.27	24.5	43.0
Hemicellulose	18.8	9.30	25.3	27.8

The results of *in-vitro* study showed that total gas production (TGP) was affected linearly ($P<0.001$) and quadratically ($P<0.001$) with the inclusion of duckweed. It was observed that the TGP of TMR-5 and TMR-10 remained statistically similar to the control diet and decreased significantly thereafter with the lowest value at TMR-30 (Table 3). The results are in accordance with the studies conducted by Subhaiah et al. (2018) who observed higher ($P<0.05$) net gas production at 15.5 % inclusion of duckweed in the concentrate mixture and thereafter there was decrease in NGP at 31 and 46 % inclusion level in the concentrate mixture as compared to control group. IVDMD and IVOMD were statistically higher in TMR-10 (75.0 and 75.8% respectively) as compared to TMR-30 and remained statistically comparable to all other groups. *In vitro* NDF digestibility provides us with the precise estimates of total digestible nutrients, net energy, and feed intake potential while ADF is vital for the organic matter degradation and gas production in the rumen of ruminant (Videv et al., 2017). Khan et al. (20002) reported that in case of duckweed *in-vitro* organic matter digestibility of duckweed at 24 hour was about 529-539 g/kg DM. They reported that rumen degradable N and microbial protein synthesis in duckweed was affected by the level of ADF and ADL content of the plant which influences the microbial protein synthesis and rumen degradability of nitrogen ultimately total gas production and nutrient digestibility. PF is a measure of the efficiency of MBP *in-vitro* and the theoretical range of PF in ruminant diets should be 2.74-4.41 (Blümmel et al., 1999, Sarkar et al., 2018). A higher partitioning factor means that proportionally more of the degraded matter is incorporated into microbial mass i.e. the efficiency of microbial protein synthesis is higher. In present study, PF value was observed to be statistically higher in TMR-30 (4.82mg/dl) as compared to all other treatments except TMR-20, whereas MBP production remained statistically similar among all treatment groups. Moreover, the mean values of PF ranged within the theoretical range of up to 15 % replacement of the concentrate mixture. The ME value of various TMR substrates was found to be decreased significantly both linearly ($P<0.001$) and quadratically ($P<0.001$) after 10 % replacement of concentrate mixture with duckweed with lowest value at TMR-30 (5.15 MJ/kg).

Table 3. *In vitro* evaluation of the different levels of duckweed as a protein source in the total mixed ration (70:30 roughage to concentrate ratio)

Parameters	Control	TMR-5	TMR-10	TMR-15	TMR-20	TMR-30	SEM	P-Value	
								Linear	Quadratic
Total gas (ml)	161.5^{a}	158.2^{a}	156.5^{ab}	151.5^{b}	143.2^{c}	128.2^{d}	2.20	<0.001	<0.001
IVDMD%	74.2^{ab}	73.3^{ab}	75.0^{a}	73.3^{ab}	72.5^{ab}	70.5^{b}	1.13	0.028	0.052
IVOMD%	74.8^{a}	73.8^{ab}	75.8^{a}	74.1^{ab}	72.9^{ab}	70.9^{b}	1.07	0.003	0.028
PF, mg/dl	4.25^{b}	4.25^{b}	4.37^{b}	4.37^{b}	4.51^{ab}	4.82^{a}	0.11	<0.001	0.048

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

The effect of different concentrate mixtures containing different levels of duckweed on total and individual volatile fatty acids *in vitro* is presented in Table 4. The TVFA was significantly lower in TMR-5 (4.05 mM/dl) and was higher in TMR-20 (4.66 mM/dl). The relative percent of acetate, a major substrate for lipogenesis, showed a significant decrease in control (57.4%) and increase in TMR-20 (62.6%). The propionate content, reserved for gluconeogenesis was statistically higher in control (1.30 mM/dl) and lower (1.01 mM/dl) in concentrate containing higher levels of duckweed (TMR-30). The butyrate percent was observed to be highest in control (13.5%) followed by TMR-5 (13.58%) and TMR-10 (12.74%) and lowest in TMR-30 (11.5%). The acetate to propionate ratio was significantly lower (P<0.05) in control (1.99) and higher in TMR-20 duckweed based concentrate mixture. Earlier, *in-vivo* study of Zetina-Córdoba et al. (2013) in lambs reported that Molar concentrations of acetate, propionate and butyrate showed no differences for diets having duckweed upto 30 % as partial replacement of Taiwan grass. The discrepancy among the results could be due to the fact that experimental designs as in above study Taiwan grass is substituted while in present study concentrate.

Chemical composition of duckweed showed that it is a good source of protein, fiber and minerals and it have the potentiality as feed ingredient for dairy animals. Further, substitutions of 10 % concentrate mixture of TMR having 70:30 roughage to concentrate mixture ratio with duckweed resulted in higher total gas, nutrient digestibility and improved rumen fermentation characteristics. So, it can be concluded 10 % inclusion of duckweed in TMR of dairy animals has a potential to improve sustainability of ruminant feed without any adverse effect.

Table 4. *In vitro* volatile fatty acids production (mM/dl) of different concentrates containing different levels of duckweed

Parameter	Control	TMR-5	TMR-10	TMR-15	TMR-20	TMR-30	SEM	P value
Acetate	2.58^{c}	2.38^{b}	2.82^{d}	2.83^{d}	2.92^{e}	2.30^{a}	0.016	<0.001
Propionate	1.30^{d}	1.12^{b}	1.22^{c}	1.21^{c}	1.18^{c}	1.01^{a}	0.02	<0.001
Butyrate	0.61^{c}	0.55^{b}	0.59^{bc}	0.59^{bc}	0.56^{b}	0.43^{a}	0.02	<0.001
A/P Ratio	1.99^{a}	2.12^{b}	2.31^{c}	2.34^{c}	2.48^{d}	2.28^{c}	0.03	<0.001

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