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The effect of biofloc produced from a fish farming system on nutrients digestibility, rumen fermentation, feeding behavior, and blood parameters of Najdi goats

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Abstract

The aim of this study was to evaluate the effect of biofloc produced from a fish pond on digestibility, rumen fermentation, rumination activity, and blood parameters of Najdi goats. At the first, 200 common carp fish (1.00 g) in two water tanks were used for producing biofloc. Then, 12 goats (average body weight of 32.00 ± 1.50 kg aging eight months) were assigned through a completely randomized design to two treatments (six replicates) and fed for one month. The treatments were comprised of a control diet and a trial diet containing 1.50% biofloc. At the end of the experiment, digestibility of nutrients, rumen fermentation, blood metabolites, and rumination activity were measured. The results showed that the digestibility of dry matter (DM), crude protein, neutral detergent fiber (NDF), and acid detergent fiber (ADF) significantly increased in goats fed with 1.50% biofloc. Also, the rumen protozoa population, plasma glucose, average time of eating, rumination, and chewing per NDF and ADF intakes, total rumination time, and chewing activity were significantly increased. There was no significant difference between treatments for DM intake, ruminal ammonia nitrogen, blood urea nitrogen, cholesterol, and triglyceride. The pH of rumen fluid significantly decreased in treatment containing biofloc. The results of the current study confessed that the use of biofloc (1.50% DM) as a supplement in Najdi goat's diet can improve the digestibility of the diet. It is recommended further and detailed trials with different levels of biofloc in sheep or different livestock species to know more about this untapped supplement source.

Introduction

Typically, the fish utilize only 30.00% of the ingested nitrogen (N) and phosphorus (P), the remaining is wasted. Most of the wasted N is dissolved, whereas the majority of P is associated with the solid material.[1]

An increase in the density of fish causes an increase in nitrogen uptake. By adding a carbon source or carbohydrate, new biomass of micro-algae and heterotrophic bacteria called biofloc grows on nutrients wastes excreted by aquatic organisms.[1,2] This process also results in water purification from discharged nitrogen and creates a cheap food source in the pool.[1,3]

The biofloc production system was originally developed in 1970 for the production of shrimp.[4] Following a lot of studies on aquatic nutrition in 2009, a book called biofloc technology was published.[5] Also, some researchers produced biofloc powder in bio-reactors.[6] Besides, the rapid development and spread of biofloc farms around the world attracted the attention of researchers.[3]

Biofloc is a collection of algae, bacteria, nematodes, protozoa, and compounds such as fatty acids, amino acids, isoacids, and vitamins.[1] The researchers have reported that biofloc contains more than 50.00% protein, 4.00% fiber, 7.00% ash, and 22.00 kJ g^{-1} dry matter (DM)-energy.[7] Saturated fatty acids, unsaturated fatty acids[8]

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and volatile fatty acids also exist in biofloc.[9] Also, biofloc contains the amino acids (glutamic acid, aspartic acid, leucine, lysine, isoleucine, and methionine) and vitamins (niacin, riboflavin, thiamine, cobalamin, and E).[10]

Considering the compounds of biofloc, it can be used in different ways in animal nutrition, so it can be concluded that biofloc may have effective and similar effects to the effects of algae and bacteria used as a supplement in livestock feeding.[1] Red algae found in tropical waters contain high protein content (10.00 - 30.00%).[2,11] Also Azolla, Spirulina algae, and Tasco (seaweed *Ascophyllum nodosum*) have similar compounds with biofloc that can be used in livestock feeding. The components of biofloc are comparable with the compounds of some dietary supplements (such as algae) used in livestock feeding.[12] Biofloc has lower alanine, arginine, glutamic acid, and glycine, higher leucine, and isoleucine, and the same value of aspartic acid, histidine, and lysine compared to Tasco algae.[12] Biofloc also has a much higher protein content, lower carbohydrates, and ash and crude fiber similar to Tasco.[12] There is rare information on using biofloc in the animal or ruminant diet. Thus, considering the possible effect of biofloc ingredients on digestibility and rumen fermentation parameters, this experiment was designed to evaluate the effect of biofloc produced from fish farming as a supplement on digestibility, rumen fermentation, and rumination and blood parameters of Najdi goats.

Materials and Methods

This experiment was conducted with 12 male Najdi goats (32.00 ± 1.50 kg, aging eight months) for one month. The diets of goats were determined based on their weight and according to the dietary requirements of goats (Table 1).[13] The treatments consisted of biofloc (1.50% DM) and control (without biofloc) that were randomly assigned to each group. The basal diet ratio was 50:50 concentrate to forage. All animal management and sampling procedures were conducted according to the Care and Use of Agricultural Animals in Research and Teaching guidelines.

Table 1. Components and chemical composition of the basal diet.

Item	Content (%)
Alfalfa	30.00
Wheat straw	20.00
Wheat bran	10.00
Soybean meal	2.00
Barley	36.00
Lime	0.70
Salt	0.30
Mineral and vitamin	1.00
Chemical analysis	
Metabolizable energy (Mcal kg^{-1})	2.30
Neutral detergent fiber (%)	39.19
Acid detergent fiber (%)	19.25
Crude protein (%)	13.90
Organic matter (%)	97.97

The biofloc production was conducted using 200 carps (1.00 g) farmed in tanks having 250 L of water as described by Wang *et al.*[14] Diet of carps contained 25.00% crude protein (CP), 21.00% carbohydrate, 15.00% moisture content, 12.00% fat, 12.00% fiber and 15.00% ash for 56 days and 14 days were considered for adaptation of fish. The feeding amount was 5.00% of fish body weight per day in the first two weeks and then reduced to 4.00% of fish body weight per day. In the second and the last two weeks, the feeding amount was 3.00% per day. Each meal was fed three times per day. Daily, 30.00 g kg^{-1} of live weight sugar was added in a farmed tank and two hr after feeding, the balance between carbon and nitrogen was about 10, without any change in the water of the system. Physical-chemical parameters of water including temperature: 28.00 ˚C, pH: 7.30, TSS: 330 mg L^{-1}, NH_3: 0.63-0.75 mg L^{-1}, NO_2: 0.36 mg L^{-1}, alkalinity: 25.00-250 mg L^{-1}, and N/P ratio 5.80:1.00 were also monitored daily. During 56 days, every three days, one-third of water was poured out of the tank into another vessel and after biofloc suspension precipitating, the water was evacuated. The suspension remained in the bottom of the dish was filtered with a 30.00 µm-pore-sized paper and dried by oven at 60.00 ˚C. The biofloc meal (1.00 mm size) was collected for two months.[5,14,15] The chemical composition of the produced biofloc was analyzed. Dry matter, ether extract, ash, and N content of produced biofloc were analyzed following Association of Official Analytical Chemists (AOAC)[16] as follows: CP: 36.00%, crude fiber: 4.20%, ether extract: 2.50%, ash: 10.20% and metabolizable energy: 18.60 kJ g^{-1}. Normally, the content of alanine of bioflocs was 3.22%, glutamic acid was 7.80%, histidine was 1.72%, lysine was 3.20%, isoleucine was 2.78%, leucine was 4.80%, valine was 3.42% and niacin was 7.90%.[10]

Daily, feed intake was recorded (30 days) for the experimental animals kept in individual metabolic cages. The feed intake was calculated by extracting the residual feed from the total feed offered. For the determination of nutrient digestibility, sampling was done for five days at the end of the experimental period and feed intake and feces excretion were recorded daily. Samples of feed, orts, and feces (about 10.00%) were collected every morning, then, oven-dried, grounded, and analyzed for DM, ash, acid detergent fiber (ADF), and N based on AOAC.[16] Neutral detergent fiber (NDF) was determined without the use of sodium sulfite or a-amylase according to Van Soest *et al.*[17]

At the end of the experiment, rumen fluid samples were taken from each animal 3 hr after morning feeding by stomach tube. Rumen liquor pH was recorded immediately after collection using a pH meter (Metrohm, Buchs, Switzerland). Later on, the rumen fluid was strained through four layers of muslin cloth. The NH_3-N concentration was determined using phenol-hypochlorite as the main reagent.[18] The rumen fluid was used to count protozoa using the method described by Veira *et al.*[19]

The chewing activities of each animal were recorded through a visual observation method for a period of 24 hr, continuously at 5-min intervals, at the end of the experiment. The total number of min of eating, ruminating, and resting activity was estimated.[20] The chewing activities were adjusted for DM, NDF, ADF, and CP intakes.[21]

On the final days of the experiment, blood samples were collected from the jugular vein in the serum tubes containing anticoagulant agent (10.00% EDTA), approximately 4 hr after the morning feeding.[22] Collected samples were centrifuged for 3000 *g* at 4.00 ˚C for 15 min (Hermle AG, Gosheim, Germany) and immediately analyzed. Measurement of glucose, urea nitrogen, cholesterol, and triglyceride was performed using a quantitative diagnostic kit (Pars Azmon, Tehran, Iran) and an auto-analyzer (BS200; Mindry, Guangdong, China).

Data were analyzed as a completely randomized design using the GLM procedure of SAS (9.1) based on the following statistical model:

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

where, Y_{ij} is the observation, μ is the general mean, T_i is the effect of treatment on observed parameters and e_{ij} is the standard error of the mean. Means were compared by the Tukey multiple comparison tests at $p < 0.05$.

Results

The addition of biofloc to goat's diet had no significant effect on DM intake ($p > 0.05$). However, the NDF and ADF intakes decreased in the diet containing biofloc ($p < 0.05$; Table 2). The data in Table 2 show that the digestibility of DM, NDF, ADF, and CP in the diet containing biofloc was higher than the control diet ($p < 0.05$).

Table 2. Effect of a diet containing biofloc on nutrient intake and apparent digestibility in Najdi goats.

Item	Control	Biofloc	SEM	*p*-Value
Intake (kg dry matter per day)				
Dry matter	1.22	1.19	0.09	0.11
Neutral detergent fiber	0.34[a]	0.39[b]	0.01	0.03
Acid detergent fiber	0.29[a]	0.34[b]	0.01	0.01
Digestibility (%)				
Dry matter	75.78[a]	84.44[b]	1.26	0.0028
Neutral detergent fiber	26.35[a]	34.97[b]	1.58	0.0083
Acid detergent fiber	22.83[a]	31.19[b]	1.49	0.0017
Crude protein	76.45[a]	84.75[b]	0.93	0.0007

[ab] Means with common superscripts do not differ ($p > 0.05$). SEM: Standard error of mean.

The results showed that using of biofloc in diet did not have any significant effect on ammonia nitrogen ($p > 0.05$). However, the pH of rumen fluid was 5.60 and 6.22 in control and biofloc diets, respectively ($p < 0.05$). The addition of biofloc increased the protozoa population of the goat's rumen ($p < 0.05$). The results indicated that the addition of biofloc did not influence blood urea nitrogen, triglyceride, and cholesterol ($p > 0.05$). But, blood glucose in the goats fed with the diet containing biofloc was higher than control ($p < 0.05$), (Table 3).

The average eating time, eating time for DM, rumination time, and total chewing activity per DM intake in goats fed with control diet and diet containing biofloc had no significant difference ($p < 0.05$). The average eating and rumination times per NDF and ADF intakes, total chewing activity, and total chewing activity per NDF and ADF intakes in the diet containing biofloc were higher compared to the control diet ($p < 0.05$; Table 4).

Discussion

The use of biofloc in the goats diet had no significant effect on DM intake, but the NDF and ADF intakes decreased in the diet containing biofloc. Dry matter intake in this study was similar to the results of the others,[23] used Azolla algae containing some similar compounds with biofloc. The goats received biofloc supplement had a numerically higher DM intake than a diet without biofloc. Accordingly, the increase in the NDF and ADF intakes in the biofloc diet can be justified. The presence of niacin in biofloc can increase the number of Entodinia protozoa in rumen fluid[24] swallowing and digesting a large number of rumen bacteria.[25] Thus, changes in the bacteria population might cause changes in the rumen fermentation pattern and reduce the intake of NDF and ADF.

The use of 1.50 % biofloc in the diet significantly increased the digestibility of nutrients (DM, CP, NDF, and ADF). Considering that no studies have been done on biofloc produced from the fish farming system in animal nutrition; therefore, studies on similar supplements such as Tasco, Azolla, Spirulina, and *Chlorella vulgaris* algae (some similar compounds with biofloc) were investigated. In agreement with the current results, Thompson has reported that Tasco supplementation in cows exposed to heat stress increases digestibility due to reducing the effect of heat stress on rumen function and microorganisms.[26] It has been reported that the addition of 20.00 to 40.00 mg kg^{-1} *Chlorella vulgaris*

Table 3. Effect of a diet containing biofloc on rumen pH, ammonia nitrogen, protozoa population, and blood metabolites compared to control diet in Najdi goats.

Treatment	pH	Ammonia (mg dL^{-1})	Protozoa	Triglyceride (mg dL^{-1})	Glucose (mg dL^{-1})	Cholesterol (mg dL^{-1})	Urea (mg dL^{-1})
Control	5.60[a]	191.15	17.50×10^{4}[a]	34.58	52.98[a]	53.48	36.99
Biofloc	6.80[b]	127.20	35.00×10^{4}[b]	34.38	57.85[b]	50.53	37.36
SEM	0.12	33.07	5.68	1.20	0.918	2.76	1.05
***p*-Value**	0.0004	0.097	0.07	0.9103	0.0094	0.4781	0.8182

[ab] Means in columns with common superscripts do not differ ($p > 0.05$). SEM: Standard error of mean.

Table 4. Effect of a diet containing biofloc on feeding behavior in Najdi goats.

Parameters	Control	Biofloc	SEM	*p*-Value
Eating time (min per day)	381.25	382.50	1.98	0.6704
Dry matter (min kg^{-1})	257.46	237.59	10.78	0.1608
Neutral detergent fiber	968.87^{a}	1134.32^{b}	44.72	0.0398
Acid detergent fiber	2038.00^{a}	2479.1^{b}	78.99	0.0076
Rumination time (min per day)	301.25^{a}	326.25^{b}	3.15	0.0014
DM (min kg^{-1})	203.18	202.54	5.61	0.9379
Neutral detergent fiber	765.49^{a}	966.77^{b}	34.53	0.0062
Acid detergent fiber	899.21^{a}	1140.76^{b}	33.63	0.0023
Chewing activity (min per day)	682.50^{a}	708.75^{b}	1.98	0.0001
DM (min kg^{-1})	460.64	440.12	14.11	0.3436
Neutral detergent fiber	1734.4^{a}	2101.10^{b}	78.33	0.0162
Acid detergent fiber	1138.82^{a}	1338.32^{b}	46.64	0.0233
Resting time (min per day)	757.50^{a}	731.25^{b}	1.98	0.0001

^{ab} Means in rows with common superscripts do not differ ($p > 0.05$). SEM: Standard error of mean.

algae in the diet with 25.00% concentrate and 75.00% maize silage has the highest *in vitro* DM disappearance, but NDF digestibility has not been affected.[27] But, the others[28] have reported that adding extract of *Ascophyllum nodosum* algae results in no significant decrease of fiber digestion. Also, Gardiner *et al*. did not observe any difference in the DM digestibility in the pigs fed with the 2.50 g kg^{-1} DM of *Ascophyllum nodosum* extract.[29] there was no significant difference in DM intake and nutrients digestibility in the animals fed a diet supplemented with 1.00 kg *Azolla microphylla*.[23]

Several studies have shown a negative relationship between feed intake and digestion in the rumen. This relation is due to an increase in passage rate and a reduction in the retention time of feed in the rumen.[30] This could justify the increase in the digestion of NDF and ADF due to the reduction of their intake in the biofloc treatment. The most abundant amino acid in the biofloc is glutamic acid that is involved in the synthesis of alanine and glutamine; which are effective in gluconeogenesis. This can be a reason for amylolytic bacteria functions improvement and consequently digestibility increase.[31]

Biofloc contains isoacids such as isobutyric acid and isovaleric acid and amino acids including valine, leucine, and isoleucine; that isoacids will be effective in providing a carbon skeleton for cellulolytic bacteria and NDF and ADF digestions increase.[17] Also, riboflavin is the second highest vitamin in the biofloc synthesizing flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN) and influencing metabolic energy production from carbohydrates, fat, and protein. Therefore, improving the metabolism of anaerobic bacteria in the rumen will enhance the digestibility of nutrients.[32,33]

There was no significant difference in ammonia nitrogen level among treatments. In agreement with the results, Gardiner *et al.* did not observe any difference in the ammonia nitrogen level using the extract of *Ascophyllum nodosum* (2.50 g kg^{-1} DM) in pigs.[29] Also, it has been reported that there is no significant difference in ammonia nitrogen level in lambs fed with Tasco, which is consistent with the results of this trial.[34] Thompson has reported that supplementation of Tasco algae in cows does not have any negative effect on rumen pH and ammonia concentration, which is consistent with the results of the current experiment.[26] Moreover, the others [34,35] have stated that using Tasco algae in the diet of sheep and lambs significantly reduces rumen pH. The researchers have observed that the addition of Azolla algae does not affect rumen pH.[36] One of the reasons for the beneficial effects of biofloc on appropriate pH in the rumen can be the improvement of cellulolytic bacteria activity and thus lower propionic acid and butyric acid production.[28] On the other hand, biofloc contains various amino acids, which are weak acids and amides (histidine) with buffering property. This can be another reason for rumen pH adjustment. The presence of amino acids such as lysine and histidine can prevent the loss of rumen pH in a high-concentrate diet. Also, the presence of niacin in biofloc due to transferring the amides hydrogen can be effective in increasing the rumen pH of the goats fed with a high level of concentration.[37]

The addition of biofloc did not affect blood urea nitrogen. As previously mentioned the addition of biofloc increases the rumen pH and elevates the ratio of ammonia to its ions in the rumen consequently. In this experiment, it was expected that blood urea nitrogen would be higher in biofloc treatment. But the increase in the pH of rumen fluid is not enough to affect the absorption of ammonia from the rumen into the blood.[28,38]

Plasma glucose in goats fed with biofloc was higher than the control group. According to former researches, *the Ascophyllum nodosum* did not affect blood glucose.[39] But, it has been reported that the addition of Spirulina algae to sheep diet reduces blood glucose levels.[40] As previously explained, the presence of different amino acids in biofloc can improve the function of amylolytic bacteria in starch digestion. Due to the high content of starch in the experimental diet, the ratio of NADH/NAD increased and the ratio of acetate/propionate reduced. Then, the production of propionic acid rose as a result of acrylate

pathway stimulation, and consequently, a small increase in blood glucose can be expected. Further, biofloc with an effect on fiber digestion can also lead to a slight reduction in the production of propionic acid. But this effect has not been able to overcome the increased production of propionic acid under the effect of starch fermentation. Meanwhile, according to the results, it can be concluded that the absorption rate of propionic acid in the rumen of goats was high in the biofloc treatment, which did not decrease rumen pH.[28,41]

No significant differences in the content of cholesterol and triglyceride were observed among treatments, but in contrast to our results, El-Sabagh *et al*. reported that adding Spirulina algae to sheep diet reduces blood cholesterol.[40] Regarding the uniformity of all conditions in the experiment, no significant reduction in the amount of blood triglyceride and cholesterol in the goats fed with biofloc can be expected. Increasing the propionic acid content will reduce the production of acetic acid and butyric acid and the pathways for the metabolism of acetic acid and butyric acid will eventually result in production of fatty acids, triglyceride, and cholesterol. Therefore, a reduction in the production of these fatty acids along with triglyceride and cholesterol is not expected. But, the presence of high niacin in biofloc can reduce triglyceride and cholesterol by reducing the body fat decomposition.[33]

Protozoa population in biofloc treatment significantly increased compared to control. The researchers have stated that *Ascophyllum nodosum* alga hurts fiber degrader bacteria, but there is no report regarding its negative effect on rumen protozoa.[42] As previously stated, rumen protozoa need niacin and due to the presence of niacin in the treatment containing biofloc, the increase in rumen protozoa population was not expected.[14,23]

The average eating time, rumination, and chewing for NDF and ADF, total rumination time, and chewing activity were also significantly increased. Increasing the size of forage increases the amount of chewing activity in ruminants.[43] In this study, the basal diet was not different, therefore, there was no significant difference between the average eating time, eating time, and rumination for DM and total chewing activity for DM.

In this experiment, fibers contents and particle size were not significantly different in experimental diets. Considering the diet containing biofloc increased digestive function in the rumen, it can be concluded that this increase in digestibility resulted in an increase in the reduction of particle size and consequently, in the speed of food transfer from the rumen to the abomasum. Increases in the passage of small particles and the accumulation of large particles in the rumen induce mechanical stimulation in mucus receptors and ultimately cause ruminal reflux. Therefore, nutrients digestibility increase in the treatment containing biofloc can be a reason for total rumination time elevation.

In this experiment, no differences in eating time and rumination time increase will lead to a reduction in resting time of goats fed with biofloc. By increasing the effective physical fiber of the diet; rumination and chewing activity will increase.[44] There is a positive correlation between the size of forage components and the amount of daily activity of chewing and rumination.[45] But, because of the same diet, it is unlikely that there are differences between treatments in case of these factors. Therefore, the lack of change in eating time and an increase in rumination time due to biofloc will increase the total chewing time.

The results indicated that additional biofloc improved the digestibility of nutrients and rumen fermentation parameters. As previously mentioned, this can be explained by the effect of components of biofloc on the improvement of amylolytic and cellulolytic bacteria function, adjustment of rumen fluid pH, and balance of ruminal microorganism's population. It seems that 1.50% biofloc produced from a fish farming system can be used as a supplement in Najdi goats and other ruminant's diet. Our team agreed that furthers studies with more parameters and other ruminant species and breeds can be carried out to clarify the picture even more.

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Conflict of interest

The authors declare that they have no financial or personal relationships which may have inappropriately influenced them in writing this article.

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