STUDY OF BIOFLOC PRODUCTION FROM FISH BREEDING SYSTEM AND USING AS SUPPLEMENT IN RUMINANT DIET

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ABSTRACT

The aim of this work was to produce biofloc from the fish breeding system and study of its effect on *in vitro* ruminal digestibility and gas production in the ruminants. In the first phase of the experiment, two hundred common carp fishes (1gr) in two tanks with capacity 250 liters' water were used for producing biofloc. In the second stage, the effect of 0, 0.5, 1, 1.5 and 2% of produced biofloc with ration 70 to 30 concentrate to forage on *in vitro* gas production and digestibility was determined. The data were analyzed in a completely randomized design (5 treatments and 4 replicates). The results showed that there was no significant difference in potential and rate of gas production and dry matter digestibility between treatments. The highest amount of truly digested organic matter, cell wall degradability, and NDF digestibility was related to the treatment containing 2% compared with the other treatments (108.4, 67.8, and 32.5, respectively). The

microbial biomass efficiency, microbial biomass and PF of treatments containing biofloc were more than control treatment ($P < 0.05$). On the base of this trial, treatments containing biofloc especially the high levels of biofloc had a more positive effect on digestibility and fermentation of experimental diets, therefore biofloc produced from the fish breeding system can be used as a useful supplement in ruminants.

INTRODUCTION

The fish feed has main nutrients, such as nitrogen, phosphorus, as well as iron, zinc, copper, and manganese. Approximately 70% of phosphorus and 15% of nitrogen fed in fish may be excreted through in feces (4). Therefore, an increase in density of fish, followed by an increase in nitrogen uptake. By adding a carbon source such as carbohydrate, a new biomass including algae and heterotrophic bacteria that called biofloc grows on nutrients excreted by aquatic organisms (1,9). This also results in water purification from discharged nitrogen and create a cheap food source in the pool. This system can be compared to an artificial stomach suitable for aquaculture (2). Biofloc contains more than 50% protein, 4% fiber, 7% ash and 22 KJ/g dry matter-energy (21). Saturated fatty acids (palmitic acid, stearic acid and arachidonic acid), unsaturated fatty acids (oleic acid, linolenic acid and linoleic acid) (11), and volatile fatty acids (acetic acid, propionic acid, butyric acid, isobutyric acid, isovaleric acid and valeric acid), also exist in biofloc (14). In addition, biofloc contains the amino acids (glutamic acid, aspartic acid, leucine, lysine, isoleucine, and methionine and vitamins (niacin, riboflavin, thiamine, B12, and E) (12). Due to the compounds of biofloc, this substance can be effective in animal nutrition. The information on using of biofloc in the ruminant diet is little. Therefore, the current study evaluates

producing of biofloc and its effect as a supplement on rumen digestibility and gas production in the ruminants.

MATERIALS AND METHODS

In the first stage of the experiment, for the production of biofloc, 200 pieces of 1 g of carp were farmed in two tanks 250 liters of water. During a 56-day period, a diet containing 25% crude protein, 21% carbohydrate, 15% moisture content, 12% fat, 12% fiber and 15% ash fed to fishes and one 14-day period was considered for adaptation of fish. Feeding amount was 5% of fish body weight per day in the first 2 weeks and then reduced to 4% of fish body weight per day. In the second 2 weeks and at the last 2 weeks, the feeding amount was 3% per day. Each meal was fed three times per day at 8:00–9:00 am, 12:00–13:00, pm and 17:00–18:00 pm, respectively. By adding sugar by 30 g per kg of live weight in a farmed tank, two hours after feeding, the balance between carbon and nitrogen was about 10, and there was no change in water of system. Parameters such as temperature and pH of water were measured daily. After 56 days, every third day, one-third of water was poured out of the tank into another vessel and after precipitate of the biofloc suspension, the water was evacuated. The suspension remained in the bottom of the dish was filtered with a 30µ pore tissue and dried by the oven at 60° C. The biofloc collected during two months (19).

Fig. 1: Process of biofloc production from fish farming system

In the second stage, the effect of produced biofloc on *in vitro* gas production and digestibility was determined by *in vitro* techniques. The base diet formulated by using NRC (16), was containing 30% forage and 70% concentrate with 0, 0.5, 1, 1.5 and 2% of produced biofloc (Table 1)

Table 1: Components and chemical composition of base diet

Feeds	%
Alfalfa	30
Corn Seed	21
Soybean meal	12.35
Barely	35.50
Lime	0.40
Salt	0.25
Mineral and vitamin supplement	0.50
Chemical composition	
ME(Mcal/kg)	2.57
NDF(%)	20.75
ADF(%)	15.83
CP(%)	16.5
OM	97.97

For determining of gas production of experimental diets containing different amounts of produced biofloc (4 replicates per treatment), the rumen fluid was taken from two goats that had been fed with a forage based diet before morning feeding. The rumen fluid was then strained through four layers of cheesecloth. Gas production of experimental diets was measured in 100 ml glass vials containing 300 mg of diets (milled with 1 mm sieve) and 35 ml buffered rumen fluid. Vials then were incubated in a water bath at 39°C for 2, 4, 6, 8, 10, 12, 16, 24, 48, 72 and 96 h (14). After 96 h of incubation, cumulative gas production data were fitted to the exponential equation $Y=B\ (1-e^{-Ct})$, where B is the gas production (ml) from the fermentable fraction, C is the rate constant of gas production (ml/h), t is the incubation time (h), and Y is the volume of gas produced at time (3).

For determination of the partitioning factor (Pf) at end of each incubation period, the content of syringes was transferred into an Erlenmeyer flask, mixed with 20 ml neutral detergent fiber solution, boiled for 1 hour, filtered, dried (in oven at 60 °C for 48 h) and ashed (in furnace, at 550 °C for 3 h). Partitioning factor, microbial biomass, and actual digested organic matter were then calculated. Digestibility of dry matter and NDF of experimental diets were determined using Tilly and Terry method (18). Rumen fluid was collected and mixed with McDougall buffer in a ratio 1:4. After gasifying with CO_2, tubes were incubated at 39 ˚C for 48 h incubation, 6 ml of 20% HCl solution and 5 ml pepsin solution were added and then incubated for 48 h simulating post-ruminal degradation. After that, the residual substrates of each tube were filtered and used to determine the digestibility of dry matter (DM) and neutral detergent fiber (NDF).

The data were then subjected to analysis of variance as a completely randomized design using the General Linear Model (GLM) procedure of the SAS (17). The Duncan's multiple range test was used to compare the mean difference at $P < 0.05$.

RESULT AND DISCUSSION

The production of the required amount of biofloc for use in experimental diets are shown in Figure 2. Addition biofloc to 2% in experimental diets showed no significant difference in potential and gas production rates during 96 h incubation ($P > 0.05$) (Table 2).

Fig. 2: Produced biofloc

Table 2: Potential and gas production rate of diets containing various levels of biofloc

Biofloc (%)	Potential of gas production (ml)	Gas production rate (ml/h)
0	57.2	0.06
0.5	58.3	0.07
1	59.1	0.06
1.5	59.9	0.07
2	60.6	0.08
SEM	2.64	0.01
P value	0.91	0.57

SEM = standard error of mean

The highest amount of truly digested organic matter, cell wall degradability, and NDF digestibility was related to treatment containing 2% biofloc in comparison to the other treatments (108.4, 67.8 and 32.5 %, respectively) (Table 3). The microbial biomass efficiency, microbial biomass and PF of treatments containing biofloc were more than control treatment ($P < 0.05$).

There are rare studies on effects of biofloc on digestibility and fermentation in the ruminant. As the results showed, truly digested organic matter and cell wall degradability was the most in high levels of biofloc in diet. This finding is in agreement with previous study (8). It has been found that Tesco algae (contains some compounds similar to biofioc) might have beneficial effects on digestibility due to effect of this supplement on rumen microorganisms and fatty acid metabolism. However, the addition of Tesco algae reduces food digestibility due to its effect on fibrolytic bacteria (10).

Table 3: Fermentation parameters of gas production of diets containing different levels of biofloc

Biofloc (%)	Truly digested organic matter(g/kg)	Microbial biomass efficiency (mg)	Microbial biomass(%)	PF(mg/ml)	Cell wall degradability(%)
Control	102.3^{c}	36.1^{c}	36.9^{b}	3.44^{c}	65.6^{c}
0.5	105.9^{b}	38.2^{a}	40.5^{a}	3.56^{a}	66.5^{bc}
1	105.7^{b}	37.5^{ab}	39.7^{a}	3.52^{ab}	66.6^{b}
1.5	107.5^{a}	37.8^{a}	40.7^{a}	3.54^{a}	67.1^{ab}
2	108.4^{a}	37.0^{b}	40.2^{a}	3.5^{b}	67.8^{a}
SEM	0.28	0.17	0.28	0.01	0.26
P value	0.0002**	0.0023**	0.0012**	0.0027**	0.0172*

$^{a\,b\,c}$ Column means with common superscripts do not differ (P > 0.05); SEM = standard error of mean; ** = P < 0.01; * = P < 0.05;

PF= Partitioning Factor

Increasing microbial biomass and biomass efficiency in diets containing biofloc might be present of niacin as one of the components of biofloc. Glutamate dehydrogenase enzyme is one of the important enzymes in the cell that plays a key role in protein synthesis and energy production (6). Niacin is a coenzyme which ultimately causes increase the synthesis of microbial protein (6). This could also justify an increase in the amount of PF in treatments containing biofloc, because increasing the PF that actually means more microbial material had released into the biomass, in other words, it increases the microbial protein synthesis efficiency (3).

In vitro digestibility of diets containing various levels of biofloc are shown in Table 4. Digestibility of dry matter was not significantly different between experimental diets

containing different levels of biofloc (P > 0.05). However, the amount of NDF digestibility was highest in treatments containing 1.5 and 2 % biofloc (P < 0.05)

Table 4: *In vitro* digestibility of diets containing various levels of biofloc (%)

Biofloc (%)	Digestibility of DM	Digestibility of NDF
Control	39.2	30.7^{c}
0.5	39.2	31.3^{bc}
1	37.8	31.7^{b}
1.5	37.7	31.9^{ab}
2	39.2	32.5^{a}
SEM	0.879	0.198
P value	0.3672	0.0084**

a b c Column means with common superscripts do not differ (P > 0.05); SEM = standard error of mean; ** = P < 0.01

It has been found that the addition of one kg of Azulla algae (containing some compounds same with biofloc) in dairy cattle diet did not affect digestibility of nutrients (5). The addition of higher amounts of biofloc in this experiment, significantly increased NDF digestibility that could due to the presence of amino acids and maintaining the appropriate pH of the rumen and improving the activity of cellulolytic bacteria, resulting lower propionic and butyric acid production (20). Biofloc contains various amino acids, such as histidine, which are weak acids and amides and has a buffering effect. Amino acids such as lysine and histidine in biofloc also reduce the pH of rumen contents. Also, niacin is effective in reducing the rumen pH. On the other hand, the presence of isoacids such as isobutyric acid, isovaleric acid and 2 methyl valeric acid in biofloc and appropriate pH resulted in the better activity of cellulolytic bacteria (13).

It has been found that rumen protozoa need to niacin. The presence of niacin in the treatments containing biofloc, caused to increase in the protozoa population of the rumen and consequently the improvement of NDF digestibility (7). Riboflavin, the second vitamin in biofloc, is in form of FAD and FMN, which has a coenzyme role in butyric acid metabolism and oxidative reactions. The result of these reactions is getting energy from carbohydrates, fat, and protein. Therefore, the improvement of the metabolism of the anaerobic bacteria in the rumen and helping to create anaerobic conditions for better performance of other microorganisms observed, which would result in improved fermentation and digestibility parameters (6).

CONCLUSION

Biofloc as one useful supplement produced from the fish breeding system in the first stage of the experiment. So according to the current results, treatments containing biofloc especially the high levels of biofloc had a more positive effect on digestibility and fermentation of ruminant diets, therefore biofloc produced from the fish breeding system could use as a useful supplement in ruminants.

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