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Bernadete Barek Koten, Emanuel Natal, Redempta Wea, Melkianus D. Randu, Aholiab Aoetpah, Cytske Sabuna

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Feed Digestibility and Feeding Behaviour of Sheep Fed *Gracilaria Sp.* and Chromium Organic Supplementation

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ABSTRACT

Bioactive compound in *Gracilaria sp* and organic chromium supplementation could improve feed digestibility through methanogen inhibition, increase carbohydrate metabolism effectiveness and feeding behaviour of sheep. The research conducted for 90 days using 18 heads of male local sheep and 3 heads as replacements stock, 7 months. The research was arranged using CRD (Completely Randomized Design), treatment consisted of: R0: 45% forage (42% native forage and 3% indigofera leaf meal) + 55% concentrate, R1: R0 + 3.6% *Gracilaria sp.* meal, R2: R1 + 0.3 ppm Organic Cr. Data were analyzed by ANOVA and continued with Orthogonal Polynomial test. The result showed that combination of 3.6% *Gracilaria sp.* meal and 0.3 ppm organic Cr (R2) were increasing crude fiber consumption ($P < 0.05$) and decreasing duration of rumination ($P < 0.05$) but no significant effect on dry matter consumption, organic matter consumption, crude fiber digestibility, crude protein digestibility and rumination frequency. *Gracilaria sp.* supplementation tends to increase crude fiber consumption but it does not decrease rumination duration compared with the control. The research could be concluded that supplementation of *Gracilaria sp.* meal increasing crude fiber consumption without disturbing duration of rumination.

Keywords: Consumption, Digestibility, *Gracilaria sp.*, Local male sheep, Organic chrom, Rumination duration, Rumination frequency

1. INTRODUCTION

Sheep is a small ruminant that potentially used as a meat source and is majorly bred in Indonesia. In general, sheep are traditionally maintained which often caused low productivity due to the poor feed quality. Furthermore, some specific microbe metabolism like rumen methanogen could possibly cause the lack of feed nutrient optimization by the sheep. Methanogen would transform rumen fermentation into methane gas which depicts the loss of feed energy. Antibiotic and antimethanogen have been used in a considerable number for ruminants in order to increase the growth [1], however the usage has been restricted these days since it might generate resistance towards particular pathogen bacteria and antibiotic residue within the meat and milk. For the past years, there are many researches regarding the utilization of plant bioactive compounds and certain minerals as methanogen inhibitor agent subsidiary.

Gracilaria sp. is a type of a red seaweed that could be used as a feed supplement because of its antimethanogen compound content. *Gracilaria sp.* and other types of red seaweed could obstruct the transfer of metal during methanogenesis [2]. Halogenated compounds could be found inside sea algae, one of them is in the form of terpenes, where chlorine and bromine contents are predominant halogens that used to increase other metabolite biological compound activity [3]. *Asparagopsis taxiformis* type of red seaweed is recognized to consist of antimethanogen compounds which are bromoform, dibromochloromethane, bromochloroacetic acid, dibromoacetic acid, and dichloromethane. Bromoform could prevent the activity of enzyme by binding the vitamin B12 that resembles methanogenesis co-factor [4]. The result of the previous research shows that *Gracilaria verrucosa* which was observed from Pasuruan, Central Java, contained alkaloid compounds, flavonoids, tannin and phenolic, and did not contain steroid, saponin and terpenoid [5]. This

indicates that bioactive compound content of *Gracilaria sp.* Varies and influenced by environment factors, such as salt rate, light, temperature and pH [6].

Gracilaria sp. contains high nutritional compounds mainly for the availability of carbohydrate source and quite high macro mineral consisting of Mg 0.74%, K 11.26%, Na 1.16%, Ca 0.03% and P 0.06% [7]. Previous study [6] reported that *Gracilaria edule* which observed from the seaweed cultivation in Jepara's shore each contains 72.95% water and ash level 3.19% and crude fat 27.12%. Carbohydrate content inside *Gracilaria sp.* is high and could supplement microbe population in spite of the benefits of antimetagen compound. Microbe population affected the feed digestivity is one of the factor that influenced the length of rumination [8]. Meanwhile, Cr mineral take part to rise the glucose absorption and protein tissue synthesis by rising the insulin activity inside the body that play a role to provide cell glucose. The addition of Cr increase glucose supply into the body cells. Glucose Tolerance Factor (GTF) rise the insulin binding by receptor on cell membrane that used transport glucose and amino acid as the protein synthesis raw material to increase the supply. The combination of *Gracilaria sp.* And organic Cr hopefully could increase the feed digestivity which affected the increasement of sheep's daily weight.

2. MATERIALS AND METHOD

2.1. Livestock and Treatment Design

The research conducted for 90 days using 18 heads of male local sheep and 3 heads as replacements stock (7 months, 16 kg body weight average). The research was arrange using Completely Randomized Design (CRD), treatment consisted of : R0: 45% forage (42% native forage and 3% indigofera leaf meal) + 55% concentrate (13% Crude Protein (CP); 65% Total Digestible Nutrient (TDN)), R1 : R0 + 3.6% *Gracilaria sp.* meal, R2 : R1 + 0.3 ppm Organic Cr, each treatments were six time repetition. Data were analyzed by analysis of variance (ANOVA) and continued with Orthogonal Polynomial test procedure of Steel et al. [8]. Feed was given as much as 4% of body weight and drinking water was given ad libitum. Feeding was done twice, in the morning and afternoon. Feeding treatment was given after 14 preliminary days for 56 days. Making flour *Gracilaria sp.* based on previous study [9] and manufacture of organic Cr minerals based on previous study [10].

2.2. Measurement of Nutrient Consumption and Digestibility

Recording the amount of consumption and leftover feed was carried out for 4 weeks, in the last 7 days sampling and collection were carried out for fresh diet and orts. The administration and the remaining forage

were taken as much as 100 g and the administration and the remaining concentrate were taken as much as 10 g from the daily amount and then composited for 3 days of sampling to determine the dry matter and organic matter consumption (DMC and OMC). The samples were dried in an oven at 70°C for 2 days, and then again in an oven at 105°C for 1 day. The dried samples were then ground and used for analysis of feed ingredients according to AOAC [11]

3. RESULT AND DISCUSSION

Gracilaria sp. meal supplementation 3.6% and 0.3 ppm organic Cr affected ($P < 0.05$) crude fiber consumption but did not affect ($P > 0.05$) dry matter consumption (DMC), organic matter consumption (OMC), crude protein consumption (CPC), crude fiber consumption (CFC), dry matter digestibility (DMD), and organic matter digestibility (OMD), crude protein digestibility (CPD), crude fiber digestibility (CFD). The average value of consumption and nutrient digestibility of male local sheep supplemented with *Gracilaria sp.* meal and organic Cr are presented in Table 2.

Gracilaria sp. flour supplementation at 3.6% and 0.3 ppm organic Cr affected ($P < 0.05$) the duration of rumination but did not affect ($P > 0.05$) the rumination frequency of male local sheep. The average duration of rumination and the frequency of rumination of male local sheep supplemented with *Gracilaria sp.* flour. and organic Cr are presented in Table 3

Consumption and digestibility of dry matter (DMD) and organic matter (OMD) were not affected ($P > 0.05$) by *Gracilaria sp.* flour supplementation and organic Cr. This indicates that the supplementation of *Gracilaria sp.* and organic Cr did not significantly increase the development of feed digesting microbes in the rumen. Although the supplementation of *Gracilaria sp.* increase in total carbohydrates and easily digestible fiber in the rumen, but there is no addition of easily available sources of ammonia at the same time so that rumen microbes are not optimally utilized for microbial protein synthesis. The use of indigofera legumes increases the fiber and protein content of feed and it was assumed that synchronization occurs in basal feed (R0) because indigofera was easily fermented and degraded in the rumen. The results of the research by Jayanegara et al. [12] showed that the DMD and OMD indigofera was quite high, namely 70.1% and 69.5%, followed by high gas production in the first two hours and relatively high Volatily Fatty Acid (VFA) and Ammonia (NH₃). *Gracilaria sp.* flour supplementation increase the total amount of carbohydrates and dietary fiber that are easily fermentable but contain low protein so that it does not support an easily available source of ammonia in the rumen for microbial protein synthesis related to the digestive process of food [13]. Different results were shown in the study of Nugroho et al. [14] showed that

Gracilaria sp. taken from around Nusakambangan Island increased DMD and OMD at levels above 1.8 % and very

high at 8% levels *in vitro*. In addition to the difference in the type of basal feed, this difference the

Table 2. Consumption and nutrient digestibility of male local sheep supplemented with *Gracilaria sp.* flour and organic Cr

Variables	R0	R1	R2
Dry Matter Consumption			
g/head/day	838.00±83.28	870.7±98.64	935.00±61.9
%BW	3.63± 0.23	3.70± 0.14	3.70± 0.14
Organic Matter Consumption			
g/head/day	357.14±53.12	362.5±34.03	395.7±69.9
%BW	1.50± 0.23	1.50± 0.24	1.62± 0.28
Crude Protein Consumption			
g/head/day	107.47± 9.37	108.5±10.83	107.56±7.9
Crude Fiber Consumption			
g/head/day	149.17±15.1 ^a	157.28±17.6 ^a	177.7±11. ^b
Digestibility			
DMD (%)	71.0± 4.46	69.08±2.62	70.84± 4.8
OMD (%)	41.23± 6.10	36.59±11.85	43.36±13.9
CPD (%)	71.15± 6.04	71.52±3.64	66.32± 6.6
CFD (%)	49.75± 9.63	50.73±6.88	57.75±16.7

Note: R0 = 45% forage (42% field grass and 3% Indigofera flour) + 55% concentrate (PK 13%; TDN 65%), R1 = R0 + 3.6% *Gracilaria sp.* flour, R2 = R1 + 0.3 ppm Chromium (Cr) organic. Different superscripts in the same line showed significantly different

Table 3. The duration of rumination and the frequency of rumination of local rams supplemented with *Gracilaria sp.* flour. and organic Cr

Variables	R0	R1	R2
Duration of Rumination (minute/day)			
Days	115.0±10.10	114.86±12.20	110.14±9.34
Night	213.14±14.59	214.86±11.75	201.29±10.75
Total	328.14 ^b	329.72 ^b	311.43 ^a
Frequency of Rumination (time/days)			
Days	106.29±11.54	109.00±9.59	105.57±12.53
Night	204.86±13.57	206.14±9.06	200.57±7.28
Total	311.15	315.14	306.14

Note: R0 = 45% forage (42% field grass and 3% Indofera flour) + 55% concentrate (PK 13%; TDN 65%), R1 = R0 + 3.6% *Gracilaria sp.* flour, R2 = R1 + 0.3 ppm Chromium (Cr) organic. Different superscripts in the same line showed significantly different.

source of *Gracilaria sp.* used. Environmental factors such as salt content, temperature, light intensity can affect seaweed photosynthesis which indirectly affects the content of bioactive compounds in it [6]. Consumption of crude fiber in R3 treatment was higher ($P < 0.05$) than other treatments, while R1 and R2

treatments resulted in the same crude fiber consumption ($P > 0.05$). This shows that the crude fiber and carbohydrate content of *Gracilaria sp.* increased the total consumption of crude fiber because the DM dry matter consumption was the same between treatments (Table 2). The highest consumption of crude fiber (R3) is related to

the ability of organic Cr to increase glucose absorption into cells so that livestock will increase their consumption of crude fiber to meet microbial energy needs in the rumen.

Meanwhile, the digestibility of crude fiber did not differ ($P>0.05$) between treatments. This shows that the crude fiber and carbohydrate contents of *Gracilaria sp.* easily fermented in the rumen, but its anti-methanogenic compounds did not affect the microbial population as one of the factors affecting nutrient digestibility [13]. Halogen analog compounds *Gracilaria sp.* used in this study was thought to be low enough so that it could not suppress the development of methanogens. Meanwhile, the combination of *Gracilaria sp.* and organic Cr resulted in high crude fiber digestibility related to the ability of organic Cr to increase carbohydrate metabolism. Organic Cr can increase the activity of the insulin hormone which plays a role in increasing the use of carbohydrates and fats so that the digestibility of crude fiber can increase [15, 16].

Protein consumption and digestibility were not affected ($P>0.05$) by *Gracilaria sp.* flour supplementation. and organic Cr. This is not in accordance with the statement of Machado et al. [17] that the bromoform content in seaweed can reduce the concentration of $N-NH_3$ through decreasing protein degradation and amino acid deamination. Based on these results it is assumed that the content of halogen analog compounds of seaweed *Gracilaria sp.* used in this study was low so that it was not optimal to reduce methanogens and increase the digestibility of sheep. Other antimicrobial and antiprotozoal compounds such as tannins and saponins were also low and caused no increase in the development of microbial populations. Saponins can inhibit the development of methanogens indirectly by inhibiting the symbiosis of methanogens with protozoa. Saponins act as defaunation agents that can inhibit the development of protozoa so that it is expected that digestive bacteria will increase and methanogens will decrease [14]. This result is different from the study of Prayitno et al. [9] showed that *Gracilaria sp.* 4% can reduce methanogens up to 84% *in vitro*. This difference can occur due to differences in the content of bioactive compounds in seaweed which are influenced by environmental factors [6].

The duration of rumination in R3 treatment was lower ($P<0.05$) than R2 and R1 treatments, while R1 and R2 treatments did not differ ($P>0.05$). These results showed that organic Cr supplementation decreased rumination time, while *Gracilaria sp.* did not affect rumination activity. Similar results were shown in the study of Kargar et al. [18] that calves supplemented with Cr under heat stress conditions decreased feeding frequency, rumination frequency, and rumination time. The mechanism of Cr on rumination behavior is not clear, but it may be related to the ability of Cr to stimulate insulin

hormone which plays a role in increasing glucose absorption into cells as well as reticulo-rumen motility [19]. Based on this statement, Cr supplementation can increase regurgitation activity due to high reticulo-rumen movement which in turn increases the frequency of rumination of livestock. This is different from the results of this study which showed a decrease in rumination behavior in the treatment of organic Cr supplementation. Meanwhile, the crude fiber content of *Gracilaria sp.* relatively low so it did not affect the length of rumination. High crude fiber content can increase rumination time because it requires more intensive mastication. The length of rumination of local sheep from the study was 311.43-329.72 minutes/day or about 5-6 hours/day, which was higher than the results of Subhan et al. [20] which is about 3 hours/day. The length of rumination of the research result is still within the normal range according to the statement of previous study [21] that the duration of rumination of small ruminants ranges from 6-8 hours/day with a maximum time of 10 hours/day. The length of rumination is influenced by the levels of NDF [22], such as the higher the levels of NDF feed will increase the time of rumination. The study basal feed (R0) was thought to have high levels of NDF, especially those contained in field grass and indigofera, while supplementation with *Gracilaria sp.* did not affect NDF content because it contains low fiber content. The frequency of rumination was not affected ($P<0.05$) by supplementation with *Gracilaria sp.* and organic Cr flour. Giving *Gracilaria sp.* although it increased the total nutrient content of the feed, it was given in the form of flour so that it did not affect rumination behavior because it was easy to swallow and digest. In addition, the digestibility of feed in each treatment was the same (Table 2) so it did not affect the frequency of rumination. One that affects the length of rumination is the level of digestibility. If the food is more difficult to digest in the rumen it will increase the rumination time per unit of digested feed [8]. The difference in the digestibility of DM and OM for each treatment (Table 2) caused the rumination time between the two treatments to be no different. In addition, the results showed that the duration and frequency of rumination occurred mostly at night. Lighting affects chewing behavior which determines the rumination rhythm for 24 hours [23]. This result is different from that of Keskin et al. [24] on Awassi sheep and Shami goats that rumination time is not affected by light. However, in general, small ruminants eat in the morning and ruminate at night. *Gracilaria sp.* flour supplementation treatment. In this study it was the best from other treatments because it did not affect the duration and frequency of rumination but could increase crude fiber consumption.

4. CONCLUSION

The research could be concluded that supplementation of *Gracilaria sp.* Meal increasing crude fiber consumption without disturb duration rumination.

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