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by Bambang Prasetyono

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B.W.H.E Prasetiyono[1], A. Subrata[1], B.I.M. Tampoebolon[1], Surono[1] and Widiyanto[1]

[1]Faculty of Animal and Agricultural Sciences, Diponegoro University, Semarang, Central Java, Indonesia.

Corresponding E-mail: bambangwhep@ymail.com

Abstract

The influence of tannin from tea waste and *gambier* as natural tannin sources on ruminal protein degradability was studied in this investigation. The soybean meal was used as protein source in this investigation. There were three treatments in this investigation mainly without protection (NT); protection with tea waste (Tt); and protection with *gambier* (Tg). The measured parameters consisted of in vitro dry matter digestibility (IVDMD), in vitro organic matter digestibility (IVOMD), and the ruminal fermentation characteristics. Results of this experiment showed that protection with tannin from tea waste as well as *gambier* increased ($p<0.05$) the IVDMD (77.0 vs 85.10 and 86.20%) and IVOMD (75.70 vs 83.40 and 84.40%). The highest IVDMD and IVOMD ($p<0.05$) was found in Tg treatment group. The Ruminal Undegradable Protein (RUP) in Tt and Tg group was higher than that in NT group (66.29 and 69.20 vs 51.10%). The ruminal protozoa population decreased ($p<0.05$) as a result of tannin protection (6690 vs 3790 and 5480 microorganism/ml rumen fluid). The Tt treatment group showed the lowest ($p<0.05$) ruminal protozoa population. The ammonia concentration in Tt and Tg tend to be lower compared to NT (4.67 and 4.69 vs 5.00 mM). The soybean meal protein protection with tea waste and *gambier* tannin increased IVDMD, IVOMD, RUP. The ruminal protozoa population and ammonia concentration, on the other hand, were decreased by tannin protection from those tannin natural sources. The natural tannin from *gambier* was the most effective protection agent for soybean meal protein.

1. Introduction

Various protein supplementation techniques in ruminant ration formulations have been tested, but it was still necessary to improve efficiency, both in terms of process and increase of added value to meet high-quality proteins that can be absorbed in the small intestine. Selection of feed ingredients as a source of protein supplements is very necessary and important, especially related to material quality, procurement sustainability, and economic value. Feed ingredients of protein sources are still relatively expensive, so it is necessary to improve the efficiency of nutrients that have high biological value to increase the production of ruminant livestock. Soybean meals have a good biological value, and the crude protein with the content of about 41% can be used as a protein supplement. This is, however, still a high level of degradation in the rumen, which is about 50-80% [1]. Therefore, protection techniques were needed to decrease soybean meal protein degradation in the rumen [2], so the percentage of soybean meal protein intake and digestibility in the small intestine can be increased. Protection techniques in soybean meal protein with a natural materials have not been carried out, so that the research has been tested in vitro, via utilization of natural tannin extracted from tea waste of a beverage company of "PT Sosro Indonesia" and *gambier* (*Uncaria gambir Roxb*). This

study aimed to evaluate the ruminal protein degradability of soybean meal protein products protected with natural tannins from tea waste or *gambier*.

2. Materials and Methods

2.1. Preparation of natural tannin extract and treatments

The tannin extraction from tea waste and *gambier* were studied by immersing them in a container using 95% alcohol [3,4] with a ratio between the ingredients extracted (tea waste and *gambier*) and alcohol ie 1: 3 for 12 hours. The obtained extract was filtered using a clean cloth, then evaporated until the extract solution was more concentrated and then dried in an oven at 40^0C to obtain tannin crystals. The concentration of condensed tannins was determined by using the drying extract oven. The condensed tannin concentration was calculated by the following equations:

(1) Tannin total = (weight of extract (crystal tannin) / sample weight) x 100%
(2) Tannin condensation to total tannin = (weight of sediment / weight of extract (tannin extract)) x 100%
(3) Tannin condensation= tannin total (%) x tannin condensation to total tannin (%)

The tannin concentration obtained from the extraction was used for soybean meal protection. The soybean meal protection was conducted by means of tannins from tea waste or *gambier* with 0.4% each (equivalent to 2.2 mg tannin / 0.55 g soybean meal) mixed on soybean meal. Then tannin extract was sprayed on soybean meal by using sprayer. The samples of soybean meal were divided into 3 treatment groups, namely Nt= the soybean meal without protection; Tt= protected soybean meal with tea waste; and Tg= protected soybean meal with *gambier*.

2.2. Chemical analyses

The rumen fermentation characteristic parameters were tested by in vitro fermentation technique according to Tilley and Terry [5]. The sources of inoculum were taken from the goat rumen that was cut in the slaughter house of Semarang, Central Java, Indonesia. The rumen fluid of the goat was taken and filtered and then inserted into the thermos, to be immediately taken to the laboratory for analysis.

A 3-g sample was put into the reaction tube and then 24 ml of inoculum liquid and 36 ml of McDougal buffer solution were added. Before being used McDougall solution was saturated with CO_2 so that the pH ranges from 6.8-6.9. Before being fermented the fermenter contents are also fed with CO_2 to create an anaerobic condition and then the fermenter is sealed with plastic rubber. Next they are incubated into shaker bath at temperature of 40^0C. The fermentation process is stopped with saturated HgCl2. The samples of the fermented rumen fluid were then analyzed for VFA, NH3, Protein total, Rumen Undegradable Protein (RUP) and Microbial Protein Synthesis [5]. In vitro dry matter digestibility (IVDMD) and In vitro organic matter digestibility (IVOMD) were also analized according to Tilley and Terry [5]. Ruminal protozoa population were calculated with method of Kurniawati and Supadmo [6]. Methan production was calculated according to Menke and Steingass [7]

2.3. Statistical analysis

The data were examined by Analyzed of Varians (ANOVA). The means differences between treatment were analyzed by Duncan's Multiple Range Tests with the general linear model procedures of SAS [8].

3. Results and Discussion

Several parameters were analyzed to show the influence of protein protection by tannin from tea waste and *gambier* on feed protein utility. Those parameters included the dry matter digestibility, and organic matter digestibility (IVDMD and IVOMD), ruminal ammonia concentration, VFA of rumen fluid, total protein production, Ruminal undegradable protein, protozoa population and microbial protein synthesis (Table 1).

Table 1. Characteristics of rumen fluid fermentation with respect to soybean meal protein protected with tannin from tea waste and *gambier*.

Parameters	Treatments			SEM	Significance
	NT	Tt	Tg		
VFA (mM)	110.01[a]	91.70[b]	93.30[b]	3.64	♦
Proportion of VFA (%):					
Acetate (C2)	68.10	66.60	67.00	0.29	NS
Propionate (C3)	19.10	19.59	18.90	0.07	NS
Butyrate (C4)	12.70	13.82	14.10	0.27	NS
C2:C3 ratio	3.59	3.40	3.49	0.02	NS
NH3 (mM)	5.00	4.67	4.69	0.05	NS
Protein Total (g)	86.00[c]	123.10[b]	147.50[a]	1.66	♦
RUP (%)	51.10[b]	66.29[a]	69.20[a]	1.87	♦
IVDMD (%)	77.00[c]	85.10[b]	86.20[a]	0.14	♦
IVOMD (%)	75.70[c]	83.40[b]	84.40[a]	0.02	♦
Microbial Protein Synthesis (mg/ml)	0.30	0.20	0.24	0.04	NS
Protozoa pupulation	6690[a]	3790[c]	5480[b]	200.68	♦
(microorganism/ml rumen fluid					
Methane (ml/ mg)	4.80[a]	3.90[b]	4.10[b]	0.12	♦

♦ $p<0.05$.
NS = non significant.
NT= Non Tannin.
Tt= Tannin from tea waste.
Tg= Tannin from *gambier*.
SEM = Standard Error of Means.

3.1. In vitro ruminal fermentability

In vitro dry matter and organic matter digestibility increased ($p<0.05$) due to protein protection with tannin from tea waste as well as *gambier*. The increasing of digestibility resulted from protein protection with tannin from *gambier* was higher than that resulted from protein protection with tannin from tea waste. The IVDMD and IVOMD in without protection treatment group, protection

with tea waste tannin and protection with *gambier* tannin were 77.00 and 75.70; 85.10 and 83.40; 86.20 and 84.40, respectively. The increasing of IVDMD as well as IVOMD resulted from protein protection with tannin might occur because of the increasing of rumen bacteria population along with the decreasing ($p<0.05$) of protozoa population in tannin treatment groups (Tt and Tg) compared to Nt (6690 vs 3790 and 5480 microorganism/ml rumen fluid). The decreasing of protozoa population reduced the rumen bacteria predation by protozoa [9, 10] so that rumen bacteria population increased, including *cellulolytic* bacteria.

Fermentation constrain in soybean meal resulted from cell wall and seed shell especially the one containing fiber. The increase of bacteria population in the rumen would increase rumen microbial capacity to degrade the fiber, so that the IVDMD as well as IVOMD increased. The primary product of organic matter of soybean carbohydrate in the rumen was volatile fatty acids (VFA). The interesting phenomenon found was the significant decrease ($p<0.05$) of rumen VFA production in tannin treatment groups (Tt and Tg) compared to Nt (110.01 vs 91.70 and 93.30 mM) although its IVDMD and IVOMD increased compared to Nt. That case could occur because of the increasing of carbon skeleton use mainly an alpha keto acid (intermediary compound in VFA production) in Tt and Tg for microbial protein biosynthesis. That phenomenon was reflected in the increase ($p<0.05$) of total protein production in Tt and Tg treatment groups compared to Nt (123.10 and 147.50 vs 86.00 mg/g).

3.2. Energy efficiency of ruminal metabolism

Soybean protein protection by tannin from tea waste as well as *gambier* increased the ruminal energy metabolism efficiency. That case reflected on the decrease ($p<0.05$) of methane production with resulted from protein protection with tannin. The methane production in Tt and Tg treatment groups were lower ($p<0.05$) than that in Nt group (3.90 and 4.10 vs 4.80ml/mg) in (Tt and Tg vs Nt), respectively. The decrease of methane production in tannin protection treatment groups could occur due to using of hydrogen and de novo fatty acids biosynthesis which reflected in the tendency of butyric acids increase in Tt and Tg treatment groups compared to Nt treatment groups. This experiment result was in line with previous finding that tannin could decrease the gas production [11,12,13].

3.3. Efficiency of ruminal protein metabolism

The increase of IVOMD as result of soybean protein protection with tannin was not accompanied with the increase of protein degradability because of the decrease of soybean meal protein solubility due to the protein bounding by tannin. Table 1 showed that soybean meal protein protection with *gambier* tannin was more effective than that with tea waste. That was reflected by ruminal ammonia concentration [14] in Tt and Tg treatment groups which tent to be lower than that in Nt group although IVDMD and IVOMD in Tt and Tg treatment group were higher than that in Nt treatment group. That phenomenon also showed the increase of protein bypass due to bounding of that compound by tannin. The increase of that protein bypass was showed by the increase ($p<0.05$) of RUP in tannin treatment group (Tt and Tg) compared to Nt (51.10 vs 66.29 and 69.20%). The microbial protein synthesis in Tt and Tg treatment groups, on the one hand, were maintained because of the increase of an alpha keto acid availability as carbon skeleton for amino acid synthesis (reflected in the decrease of VFA production) so that the use of ammonia for microbial protein synthesis was more efficient. Portion of RUP, on the other hand, increased due to soybean meal protein protection by tannin [11]. The combination between the increasing of microbial protein synthesis efficiency and the increasing of RUP (bypass protein), resulted in the increase of ruminal protein metabolism efficiency [14, 15]. That result was showed by the increase of total protein production. The total protein production was the combination between bypass protein and microbial protein [16].

4. Conclusion

Soybean meal protein protection by tannin of tea waste as well as *gambier* improved the ruminal fermentability which reflected in the increase of IVDMD and IVOMD. Soybean meal protein protected by tannin increased the ruminal energy metabolism efficiency which reflected in the decrease of methane production. The use of tea waste and *gambier* as natural tannin sources for soybean meal protein protection increased the protein utility which reflected in the increase of RUP. The natural tannin from *gambier* was the most effective protection agent for soybean meal protein.

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