Tannin Anthelmintic Doses, Metabolizable Energy and Undegraded Protein Contents of Rubber Leaves (*Hevea brasiliensis*) as Herbal Nutrition for Goats

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ABSTRACT: Analysis of feed chemical composition, total tannins and condensed tannin contents of rubber leaves carried out in previous research showed that rubber leaves have a potency as herbal nutrition for goats. In this current research, rubber leaves were analyzed further to determine the metabolizable energy (ME) and undegraded protein (UDP) contents, and also to determine the tannin anthelmintic doses of rubber leaves. The materials used were fresh and 1d-withered rubber leaves and branches (edible portion) taken from 4 types of rubber tree clones. The contents of ME and UDP were in sacco analysed according to Ørskov and Mc. Donald (1979). The determination of tannin anthelmintic doses was carried out by analysing the effects of tannins extract against Hemonchus contortus nematodes in vitro. The results showed that the average values of UDP and ME contents in fresh rubber leaves and branches were 40.88% and 8.07 MJ ME/Kg DM, and in 1d-withered form were 37.15% and 8.09 MJ ME/Kg DM. The use of tannin doses in vitro which was equivalent to 2 g (contained in ± 213.6 g fresh rubber leaves), 4 g, 6 g and 8 g tannins doses in vivo, showed that 100 % worms in all doses tested were dead after 4 hours immersing in tannin extracts, and the worms death were preceded by paralysis process. The Speed of the worm paralysis and death increased with the increasing doses tested. It can be concluded that rubber leaves have a low content of ME but a high content of UDP, and hence, it can be used primarily as a high UDP forage source for ruminants, and the tannins compound contained in the rubber leaves with the dose of ≥ 2 g tannins per a head of goat has a strong anthelmintic effect against adult female *Haemonchus contortus* worms.

Keywords: *Hevea brasiliensis*, Metabolizable Energy, Undegraded Protein, Tannins, Anthelmintic

INTRODUCTION

Analysis of rubber leaf (*Hevea brasiliensis*) potency as herbal nutrition (Nutritive Herbs) for ruminants has been carried out previously by quantitatively analyzing the feed chemical composition, the content of total tannins and condensed tannin of the leaves and branches (edible portion) of rubber tree (Wigati, *et al.,* 2014a). The average of feed chemical composition of the fresh rubber leaves were 40.4% DM, 87.9% OM; 19.5% CP; 6.2% EE and 27.0% CF. The branches part of fresh rubber had a lower quality than the leaves part. Withering rubber leaves and branches increase the quality of the feed. The average of total tannin and condensed tannins contents (DM base) of rubber leaves and branches in the fresh form were 2.71%, and 2.37 %.- and in the 1-d withered form were 2.05%, and 2.52% (Wigati, *et al.,* 2014a). The results of the previous research showed that rubber leaves and branches have a good quality as a feed source for goats. The contents of total tannins and condensed tannins in rubber leaves and branches indicated a nutritive level of tannins content, and also indicated a potency as herbal medicine for goats. According to Makkar (2003), tannins within certain limits (2-4% DM basis) can improve the efficiency of microbial protein synthesis and degradation and can protect the protein in the rumen, thereby increasing the

flow of essential amino acids to the small intestine and increase the absorption of amino acid into the blood which in turn will be able to increase the productivity of livestock. In ruminants, tannin, particularly condensed tannin has been reported to have an effect as a medicinal herb, which is as anti-parasitic gastro-intestinal (natural anthelmintic) as reported by Paolini *et al.* (2003). The objective of the current research was to further explore the potency of rubber leaves and branches (edible portion) as herbal nutrition for ruminant, especially for goats, by in sacco analysis to determine the contents of undegraded protein (UDP) and metabolizable energy (ME), and by in vitro analysis to analyse the effects of tannins extract against adult female Haemonchus contortus nematodes to determine the tannin anthelmintic doses.

MATERIALS AND METHODS

Research Materials

The feed samples used in the study were fresh and 1-d withered rubber leaves with its branches (edible portion) taken from four types of rubber clones growing in several locations of the rural community rubber plantation in Jambi province, Indonesia. The experiment used nylon bags with ± 12 µm porosities, and a fistulated non pregnant female Bali cattle which was fed King grass (*Panisetum purpureum*), rice bran and soya bean cake (rumen pH were 6.7 to 6.9). For the determination of tannin anthelmintic doses were used tannins extract from fresh rubber leaves and its branches, and adult female *Haemonchus contortus* nematodes obtained from abomasums of local goats.

Research Methods

In sacco analysis. Feed samples in nylon bags were incubated in rumen with incubation time of 2, 4, 8, 16, 24, 48 and 72 hours to measure in sacco feed degradation. The quantity of DM, OM and CP lost from the previous samples were degraded in the rumen, and were then used to measure the values of a, b and c by using the exponential equation according to Orskov and Mc Donald (1979) as followed : $P = a + b (1- e\text{-}ct)$, where P is the fraction degraded at time t, a is the intercept (soluble fraction), b is the potentially degraded fraction (the insoluble but fermentable fraction), e is natural log, c is the rate constant of b, and t is incubation time. Subsequently, the obtained values of a, b and c were used to calculate effective degradability (ED) of DM, OM and CP by using the following equation: $ED = a + (bc/ c + k)$, where ED is the amount degraded during the time its spends in the rumen (effective degradability), and a, b, and c are as above and k is the outflow rate.

The Metabolizable Energy (ME) contents of the feed samples were measured by using the value of Degradability of Organic Matter (DOM) at 48 hour incubation (Actual Degradation of Organic Matter) by calculation and its conversion from several equations according to NRC (1981), i.e. 1 Kg DOM = 4.620 Mcal DE = 3.787 Mcal ME; = 16184 KJ ME = 16.20 MJ ME, and 1 MJ ME = 0.234 Mcal ME.

In vitro analysis. The tannin anthelmintic doses of fresh rubber leaves with its branches were determined in vitro by using tannin extracts of the feed samples. The extraction of tannins used ether/aquades solvents. The doses of tannins extract used were 3.5%, 7.0%, 10.5% and 14.0% (dry weight rubber leaves and branches), those doses were equivalent to 2g, 4 g, 6 g and 8 g in vivo for a head of goat. The calculation of anthelmintic tannin doses used data obtained from the previous reseaches, i.e. total volume of rumen fluid of goat were ± 13% of body weight, dry matter, dry weight and total tannin contents of the fresh rubber leaves and its branches were 31.77%, 34.04% and 2.95% respectively (Wigati, *et al*., 2014a). In this experiment, each of twelve adult female *H. contortus* worms were directly taken from fresh goat abomasum (<3 hours after

slaughtering) and were inserted into tannin extract in petri dish at a dose that was tested. The signs of activity, paralyse and death of the worms in tannin extract were observed using observation times of 30 min, 60 min, 90 min, 120 min, 150 min, 180 min and 240 min.

Research Parameters and Data Analysis

Parameters taken were the contents of Undegraded Protein (UDP) and Metabolizable Energy (ME) of fresh and 1-d whithered rubber leaves and its branches, and the anthelmintic doses of tannin extracts from the fresh rubber leaves and its branches. All data obtained in the study were analyzed descriptively.

RESULTS AND DISCUSION

Feed chemical composition of rubber leaves and its branches

Feed chemical composition of rubber leaves and its branches was presented in Table 1. The results showed that rubber leaves and its branches have high contents of dry matter (DM), organic matter (OM) and crude Protein (CP) (> 20%), and also high contents of ADF and NDF (> 35%).

Table 1. Feed chemical composition of rubber leaves and its branches in the fresh and 1-d withered forms

Sample Type	DM (%)	OM (%)	CP (%)	ADF (%)	NDF (%)
Fresh	31.77	95.26	20.21	38.76	55.76
1-d Withered	36.89	95.20	23.07	38.62	57.68

NDF = *neutral detergent fibre* ADF = *acid detergent fibre*

The contents of undegraded protein (UDP) of the rubber leaves and its branches

The contents of UDP in rubber leaves and its branches in the fresh and 1-d withered forms were presented in Table 2. The UDP content was higher in the fresh form than that in the 1-d withered form. Previous research showed that fresh rubber leaves and its branches had total tannins contents higher than those of 1-d withered rubber leaves and its branches (Wigati, *et al.*, 2014a).

Table 2. The content of UDP of rubber leaves and its branches

Sample Type	a	b	c	ED of CP (%)	UDP (%)
Fresh	41.61	31.20	0.08	59.12	40.88
1-d Withered	45.18	29.35	0.09	62.85	37.15

It is suggested that there is a close relationship between total tannin content with the value of UDP in a feedstuff. Tannins has been reported to have effect in protecting the feed ingredients from degradation in the rumen (Makkar, 2003). The value of UDP content of fresh rubber leaves and its branches was higher than that of groundnut haulm, King grass, rice bran, dried cassava tubers and soya bean meal, but was lower than that of cassava leaves hay (Wigati, *et al.,* 2014b). Based on the values of DM, OM, CP and UDP of the fresh rubber leaves and its branches (edible portion), it can be concluded that the forage can be used as a high protein feed source. Withering the forage has been reported to improve the quality of the forage (Wigati, *et al.,* 2014a), but slightly lowered its UDP content.

The content of Metabolizable Energy (ME) of the rubber leaves and its branches

Metabolizable Energy calculation was based on the values of the effective degradability of OM, the content of OM and the actual degradation of OM in the feed. Metabolized energy content (Metabolizable Energy/ME) in the rubber leaves and its branches was presented in Table 3. The result showed that the ME content of the fresh rubber leaves and its branches was almost similar to that of the 1-d withered form.

Table 3. The content of Metabolizable Energy (ME) of the rubber leaves and its branches.

Sample Type	EDOM (%)	OM (%)	OM (g)/kg DM	DOM (%) at 48 h	DOM (g) at 48 h	ME (MJ/kg DM)
Fresh	41.43	95.26	952.63	52.56	500.73	8.11
1-d Withered	40.35	95.20	952.02	52.01	495.17	8.02

The ME contents in both fresh and 1-d withered rubber leaves and its branches were relatively lower than ME contents of other feedstuffs resulting in the previous study (Wigati, *et al.,* 2014b) such as King grass (10.45 MJ ME), cassava leaves hay (11.45 MJ ME), rice bran (10.15 MJ ME) and soybean meal (14.73 MJ ME). Lower content of ME in both fresh and 1-d withered rubber leaves and its branches was suggested to be caused by high crude fiber contained in the feed material as indicated by the value of its ADF and NDF (Table 1). High crude fiber content in the feeds would cause the rate of degradation of organic material was slower and produced less substrate that was degraded to produce energy needed by the animal. Since, rubber leaves and its branches contained a low ME content, hence, the use of rubber leaves and its branches for feeding ruminants should be added with other high energy feed sources.

In vitro analysis of tanin anthelmintic effects of rubber leaves and its branches

The observation results of anthelmintic effect of tannins contained in fresh rubber leaves and its branches were presented in Table 4.

Table 4. Tannin anthelmintic Effects against adult female *Haemonchus contortus*

Observation Time	Percentage (%) of active/paralysis/dead worms							
	2 g Tannin dose		4 g Tannin dose		6 g Tannin dose		8 g Tannin dose	
09:00	100	active	100	active	100	active	100	active
10:00	100	active	100	active	100	active	100	active
11:00	83.33	active	75	active	16.67	active	16.67	active
	16.67	paralysis	25	paralysis	83.33	paralysis	83.33	paralysis
11:30	41.67	active	33.33	active	16.67	active	8.33	active
	58.33	paralysis	66.67	paralysis	83.33	paralysis	91.77	paralysis
12:00	100	paralysis	100	paralysis	100	paralysis	91.67	paralysis
							8.33	dead
12:30	100	paralysis	91.67	paralysis	91.67	paralysis	83.33	paralysis
			8.33	dead	8.33	dead	16.67	dead
13:00	100	dead	100	dead	100	dead	100	dead

The results showed that the use of tannin doses in vitro which was equivalent to 2 g (contained in ± 213.6 g fresh rubber leaves), 4 g, 6 g and 8 g tannins doses in vivo, showed that 100 % worms in all doses tested were dead after 4 hours immersing in tannin extracts, and the worms death were preceded by paralysis process. The Speed of the worm paralysis and death increased with the increasing doses tested. It can be concluded that the tannins compound contained in the rubber leaves and its branches with the dose of ≥ 2 g tannins per a head of goat has a strong anthelmintic effect against adult female *Haemonchus contortus* worms.

CONCLUSIONS

Based on these results it can be concluded that both fresh and 1-d withered rubber leaves and its branches have high protein and undegraded protein contents but have a relatively low metabolizable energy content, and that the tannins contained in the rubber leaves and its branches have a strong anthelmintic effect in all doses tested. The results of the study are expected to be the basis of widely use of rubber leaves and its branches as a source of forage and herbal medicine for ruminant, particularly in the development of goats or beef cattle reared in an integrated farming system with rural community rubber plantation.

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