Ruminal Fatty Acid Profiles of Leaves from Some Leguminous Tree Species as Incubated in an *In Vitro* Fermentation System

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Abstract The present experiment was aimed to investigate ruminal fatty acid profiles of leaves from some leguminous tree species when they are incubated in buffered rumen fluid *in vitro*. Ground leaf samples from four leguminous tree species namely *Albizia falcataria*, *Calliandra calothyrsus*, *Leucaena leucocephala* and *Sesbania grandiflora* (200 mg DM each) were incubated *in vitro* in four replicates with 30 ml of rumen:buffer solution (1:2 v/v) by using the Hohenheim gas test method, conducted at 39ºC for 24 h. Incubation was performed without or with linseed oil addition with a dose of 50 mg/g plant DM. After incubation, the fermentation fluid (containing feed residues) was withdrawn from the syringe and subjected to fatty acid analysis by using a gas chromatograph device. Results showed that no significant interactions were found between plant species and linseed oil addition, suggesting that both factors act independently from each other. Ruminal concentration of α-linolenic acid was highest when incubating *C. calothyrsus* and significantly higher than that of other plants ($P<0.05$), while concentration of stearic acid was lowest in the incubation of *C. calothyrsus*. It can be concluded that *C. calothyrsus* has better ruminal fatty acid profiles as compared to the other plants.

Keywords Legume, Tree, Fatty acid, Rumen

1. Introduction

Indonesia currently is a net importer country for milk and milk products; approximately 70% of these products is obtained from foreign countries while only 30% is originated from local production. A main reason of such problem is concerning the limited population of dairy animals in comparison to total human population in the country, thus insufficiency of domestic milk supply. Strengthening dairy goat industry is important to increase milk and milk products supply locally. Apart from increasing goat's milk production, improving the quality of milk produced appears to be potential as well since majority of such consumers are middle to upper class people in term of purchasing power. Milk quality is of interest especially when there is a direct relationship with beneficial health effects on human. Accordingly, health promoting properties of milk are assigned, but not limited, to polyunsaturated fatty acids (PUFA) particularly α-linolenic acid (C18:3n-3) and rumenic acid (*c*9*t*11C18:2) [1]. Concentrations of both fatty acids in milk depend on, to a certain extent, their concentrations in the rumen where a large amount of PUFAs undergo biohydrogenation processes [2]. The present experiment was aimed to investigate ruminal fatty acid profiles of leaves from some leguminous tree species when they are incubated in buffered rumen fluid *in vitro*.

2. Materials and Methods

Leaf samples from four leguminous tree species namely *Albizia falcataria*, *Calliandra calothyrsus*, *Leucaena leucocephala* and *Sesbania grandiflora* were collected from the area of Bogor Agricultural University, Darmaga, Indonesia. For each species, samples were collected from several individual plants in the amount of approximately 3 kg (fresh matter). The samples were stored indoors in a greenhouse for two days, dried at 50ºC overnight and ground by using a hammer mill to pass through a 1 mm sieve. Samples were subjected to crude protein and total tannin analyses. Further, an amount of 200 mg DM of each plant was incubated *in vitro* in four replicates with 30 ml of rumen:buffer solution (1:2 v/v) by using the Hohenheim gas test method [3], conducted at 39ºC for 24 h. Incubation was performed without or with linseed oil addition

with a dose of 50 mg/g plant DM. After incubation, the fermentation fluid (containing feed residues) was withdrawn from the syringe and subjected to fatty acid analysis. Fatty acids were extracted and transesterified into fatty acid methyl esters (FAME) prior to determination in a gas chromatograph device [4]. Identification of individual fatty acid was conducted by running a FAME standard. Quantification of fatty acid concentrations in the fermentation fluid was based on the concentration of a pre-determined amount of an internal standard, i.e. C19:0. Data obtained were analyzed by factorial analysis of variance (ANOVA), in which various plant species and addition of linseed oil served as factors. Duncan's multiple range test was employed to ascertain the differences among treatment means.

3. Results and Discussion

Ruminal fatty acid profiles, i.e. α-linolenic acid (C18:3n-3), linoleic acid (C18:2n-6), rumenic acid (*c*9*t*11C18:2), vaccenic acid (*t*11C18:1) and stearic acid (C18:0), of leaves from *in vitro* incubation of some leguminous tree species either without or with linseed oil addition are presented in Table 1. For all fatty acids, no significant interactions were found between plant species and linseed oil addition, suggesting that both factors act independently from each other.

Table 1. Fatty acid profiles (mg/g FAME) of leaves from some leguminous tree species without or with linseed oil addition

Plant	Linseed oil (mg/g DM)	C18:3n-3	C18:2n-6	*c*9*t*11C18:2	*t*11C18:1	C18:0
A. falcataria	0	13.0^{a}	30.6^{a}	0.52	42.5^{ab}	469^{c}
	50	14.2^{a}	32.5^{a}	0.80	51.6^{abc}	515^{d}
C. calothyrsus	0	45.7^{c}	61.3^{b}	1.52	47.1^{ab}	370^{a}
	50	33.4^{b}	51.2^{b}	2.26	83.7^{d}	430^{b}
L. leucocephala	0	18.5^{a}	27.6^{a}	0.84	47.8^{ab}	471^{c}
	50	14.9^{a}	28.1^{a}	0.99	73.0^{cd}	514^{d}
S. grandiflora	0	13.1^{a}	17.2^{a}	0.61	32.4^{a}	488^{cd}
	50	11.7^{a}	26.5^{a}	0.92	57.1^{bc}	558^{e}
SEM		2.80	2.90	0.15	4.01	12.0
P-value						
Plant		0.012	0.001	0.147	0.165	<0.001
Linseed		0.158	0.904	0.124	<0.001	<0.001
Plant*Linseed		0.356	0.294	0.819	0.301	0.557

Different superscripts within the same column are statistically different at P<0.05

Addition of linseed oil increased ruminal vaccenic acid concentration significantly (P<0.05) for most of the leguminous tree species incubations, except *A. falcataria*. Such concentration increase was also observed for stearic acid. No significant differences were found for α-linolenic acid, linoleic acid and rumenic acid concentrations on linseed oil addition. Comparing among various leguminous tree species, ruminal concentration of α-linolenic acid was highest when incubating *C. calothyrsus* and significantly higher than that of other plants (P<0.05). Such pattern was consistently observed for linoleic acid concentration. Concentration of stearic acid was lowest in the incubation of *C. calothyrsus*. In contrast to *C. calothyrsus*, incubation of *S. grandiflora* resulted in low α-linolenic and linoleic acid concentrations at simultaneously high stearic acid level. Apparently an explanation to such a response is related to tannin contents in the respective plant species; total tannin contents of *C. calothyrsus* and *S. grandiflora* were 8.1 and 0.2% DM, respectively. Those were the highest and the lowest concentrations among the plant tested, respectively. It is quite plausible that tannins may inhibit biohydrogenation steps of α-linolenic and linoleic acids in the rumen.

4. Conclusions

Incubations of leaves from leguminous tree species containing substantial amounts of tannins lead to higher concentrations of beneficial fatty acids to human health, i.e. α-linolenic and linoleic acids in the rumen. Among the plants investigated in the present study, *C. calothyrsus* has better ruminal fatty acid profiles as compared to the others.

References

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