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Yanza Y.R., Vincent Niderkorn, Anuraga Jayanegara. Fatty acid composition of Albizia, Calliandra, Leucaena and Sesbania as tropical forage legumes. 2nd International Conference on Animal Research for Eco-Friendly Livestock Industry, Aug 2023, Surakarta, Indonesia. <hal-04189499>

HAL Id: hal-04189499

<https://hal.inrae.fr/hal-04189499v1>

Submitted on 26 Jan 2026

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To cite this article: Y R Yanza *et al* 2024 *IOP Conf. Ser.: Earth Environ. Sci.* **1292** 012010

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Fatty acid composition of Albizia, Calliandra, Leucaena and Sesbania as tropical forage legumes

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Abstract. The objective of the present experiment was to determine fatty acids composition of *Albizia falcataria* (AF), *Calliandra calothyrsus* (CC), *Leucaena leucocephala* (LL), and *Sesbania grandiflora* (SG), both in the leaves and in the *in vitro* rumen fermentation system. Ground leaf samples of AF, CC, LL and SG were determined for their fatty acid (FA) profiles. The samples (200 mg DM each) were incubated *in vitro* in a rumen:buffer solution (1:2 v/v ratio) at 39°C for 24 h, either with or without the addition of linseed oil, conducted in four replicates. Following the incubation, the fermentation medium was subjected to fatty acid determination. Results revealed that the order of PUFA contents in the leaves from highest to lowest were CC>LL>SG>AF. Proportion of MUFA was particularly high in CC. Notably, the α -linolenic acid (ALA) concentration within the rumen after the *in vitro* incubation exhibited in the CC legume compared to the other observed legumes ($P<0.05$). Additionally, the *in vitro* incubation of CC resulted in the lowest concentration of stearic acid. The findings lead to the conclusion that CC exhibits more favorable FA profiles in the rumen when compared to the tropical forage tree-legumes in the present experiment.

1. Introduction

Currently, Indonesia imports a significant portion of its milk and milk products, amounting to approximately 78%, while only about 22% is produced locally [1]. This is largely due to the limited number of dairy animals compared to the country's human population, resulting in an insufficient domestic milk supply [2]. To address this issue and enhance local milk production, it is crucial to focus on developing the dairy goat industry [3]. Besides increasing goat's milk production, to improve milk production is promising, especially since the majority of consumers belong to the middle to upper class with higher purchasing power [4].

Milk quality is particularly important as it directly impacts human health in positive ways. Certain compounds found in milk, such as polyunsaturated fatty acids (PUFA), notably the C18:3n-3 (α -linolenic acid; ALA) and c9t11C18:2 (conjugated linoleic acids or rumenic acid, CLA), are believed to possess health-promoting properties [5,6]. These fatty acids (FA) concentrations in milk are influenced,



to some extent, by their proportion in the rumen, in which most of PUFA undergo biohydrogenation processes [7,8].

Several tropical legumes such as *Albizia falcataria* (AF), *Calliandra calothyrsus* (CC), *Leucaena leucocephala* (LL) and *Sesbania grandiflora* (SG), are rich in unsaturated composition such as the ALA and linoleic acids (C18:2n-6; LA) besides known for their high protein content [9]. Moreover, these tropical legumes may also inhibit biohydrogenation process in the rumen due to their bioactive compound mode of action [10]. In this context, the present study was executed to determine the fatty acid profiles of leaves from certain tree legume species such as i.e., *Albizia falcataria* (AF), *Calliandra calothyrsus* (CC), *Leucaena leucocephala* (LL) and *Sesbania grandiflora* (SG), after incubated in the rumen fluid through the batch culture *in vitro* experiment. Those legumes consisted of wide range of bioactive contents; hence the addition of linseed oil might assess the BH process in accumulation of t11-C18:1 (vaccenic acids; VA) and CLA composition, hence different tropical legumes may show different steps of the microbial BH pathway [10].

2. Materials and methods

Leaves of four tree legume species such as AF, CC, LL, and SG, were gathered at IPB University, Dramaga Bogor, Indonesia. Approximately 3 kg of fresh matter leaves from each species was sampled from multiple individual plants. All collected samples were withered for two days in a greenhouse, followed by drying at 50°C for 24 h [9]. Subsequently, dried samples were ground utilizing a hammer mill, ultimately passing through a 1 mm sieve. Grounded materials were then used for the determination of their respective fatty acid (FA) profiles.

In addition, 200 mg of dry matter of each tropical legume was subjected to *in vitro* fermentation (four replication) using the Hohenheim gas test. The incubation process was carried out at 39°C for 24 hours with the 30 ml buffered rumen fluid (rumen:buffer solution, 1:2 v/v). Some incubations were conducted without linseed oil, while others had 5% linseed oil of plant dry matter added. After 24 h of anaerobic incubation, the fermentation effluent was extracted from the syringe in preparation for fatty acid analysis following previous experimental protocol [9]. The analytical trajectory involved the extraction and conversion of fatty acids (FA) into their corresponding fatty acid methyl esters (FAME), a preparatory stage preceding their subsequent determination via employment of a gas chromatographic instrument [10]. The identification of individual FA was achieved through alignment with a FAME standard. The quantification of FA concentrations within the fermentation medium was conducted by means of comparative evaluation vis-à-vis a predefined quantity of an internal standard, specifically for C19:0 FA category.

The collected data were subjected to statistical analysis using factorial analysis of variance (Factorial ANOVA), where distinct legume species and the variable of linseed oil supplementation were considered as treatment factors. In determining significant differences between treatment means, the Duncan's -test was employed

3. Results and discussion

The FA profile of tropical legumes is presented in Table 1, whereas the FA from *in vitro* incubation either with and without the addition of linseed oil is presented in Table 2. Obtained results indicate no interactions between the type of legume species and the linseed oil supplementation.

Linseed oil supplementation significantly increased the vaccenic acid (VA) concentration in all legumes, except for *Albizia falcataria* after the *in vitro* incubation ($P < 0.05$). Moreover, linseed oil supplementation also increased stearic acid concentration ($P < 0.01$). Nevertheless, the addition of linseed oil did not significantly influence the α -linolenic acid (ALA), linoleic acid (LA), and conjugated linoleic acid (CLA) concentrations in the rumen.

Upon diverse leguminous tree species, *Calliandra calothyrsus* consisted the most ALA fatty concentration and significantly higher compared to the other tree legumes ($P < 0.05$). A comparable pattern was consistently noted in the case of LA concentration. On the other hand, the concentration of stearic acid was lowest in the incubation of *Calliandra calothyrsus*.

Moreover, the incubation of *Sesbania grandiflora* reduced ruminal ALA and LA concentrations, but synchronously produced an excessive SA concentration. This response can be explained by the tannin

contents in the respective plant species [6,11], where *Calliandra calothyrsus* had the highest tannin level of 8.1% DM, while the *Sesbania grandiflora* had the lowest total tannin contents, about 0.2% DM, among all tested tropical forage tree-legumes. Hence, it is conceivable in the present study that tannins presence in tropical tree-legumes might responsible for the inhibition of biohydrogenation processes of ALA and LA within the rumen environment [6].

Table 1. The FA composition (% total FA) of different tropical legumes.

Fatty acid	<i>Albizia falcata</i>	<i>Calliandra calothyrsus</i>	<i>Leucaena leucocephala</i>	<i>Sesbania grandiflora</i>
C16:0	21.9	5.1	21.2	24.2
C18:0 (SA)	6.8	1.1	9.6	8.6
C18:1n-9 (OA)	3.3	3.0	4.9	4.9
C18:2n-6 (LA)	11.0	16.2	8.5	14.6
C18:3n-3 (ALA)	29.8	37.6	36.7	28.7
SFA	50.5	16.7	43.6	45.6
MUFA	6.2	28.2	8.3	7.6
PUFA	43.4	55.1	48.0	46.8

FA: fatty acids; SA: stearic acid; OA: oleic acid; LA: linoleic acid; ALA: α -linolenic acid; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids

Table 2. Ruminal fatty acid composition (% total FA) of incubated Albizia, Calliandra, Leucaena and Sesbania leaves without or with linseed oil addition.

Plant	Linseed oil (% DM)	C18:3n-3 (ALA)	C18:2n-6 (LA)	c9t11C18:2 (CLA)	t11C18:1 (VA)	C18:0 (SA)
<i>Albizia falcata</i>	0	1.30 ^a	3.06 ^a	0.052	4.25 ^{ab}	46.9 ^c
	5	1.42 ^a	3.25 ^a	0.080	5.16 ^{abc}	51.5 ^d
<i>Calliandra calothyrsus</i>	0	4.57 ^c	6.13 ^b	0.152	4.71 ^{ab}	37.0 ^a
	5	3.34 ^b	5.12 ^b	0.226	8.37 ^d	43.0 ^b
<i>Leucaena leucocephala</i>	0	1.85 ^a	2.76 ^a	0.084	4.78 ^{ab}	47.1 ^c
	5	1.49 ^a	2.81 ^a	0.099	7.30 ^{cd}	51.4 ^d
<i>Sesbania grandiflora</i>	0	1.31 ^a	1.72 ^a	0.061	3.24 ^a	48.8 ^{cd}
	5	1.17 ^a	2.65 ^a	0.092	5.71 ^{bc}	55.8 ^e
SEM		0.28	0.29	0.015	0.40	1.20
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

DM: Dry matter; C18:3n-3: α -linolenic acid (ALA); C18:2n-6: linoleic acid (LA); c9t11C18:2: conjugated linoleic acid (CLA); t11C18:1: vaccenic acid (VA); C18:0: stearic acid (SA); SEM: standard error of means. Different superscripts within the same column are statistically different at $P < 0.05$.

4. Conclusion

Calliandra calothyrsus exhibits more favorable ruminal FA profiles when compared to the other tropical forage legumes in this experiment. However, no significant effect either the interaction with tropical legume plants in the use of linseed oil in this experiment.

Acknowledgments

All authors are grateful to the Ministry of Education, Culture, Research and Technology, Republic of Indonesia, for funding the present study through the PHC-NUSANTARA scheme, year 2023, Grant Number: 15819/IT3.D10/PT.01.02/P/T/2023.

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