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Fatty acids composition and biohydrogenation reduction agents of tropical forages

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Abstract. *Makmur M, Zain M, Marlida Y, Khasrad, Jayanegara A. 2019. Fatty acids composition and biohydrogenation reduction agents of tropical forages. Biodiversitas 20: 1917-1922.* The study was conducted to determine the composition of fatty acids, measured rumen biohydrogenation reduction agents (total phenols and total tannins) content and selected promising plants in various species of tropical forages. Ten species of tropical forages, namely, *Panicum maximum*, *Cynodon plectostachyus*, *Pennisetum purpurephoides*, *Pennisetum purpureum*, *Brachiaria decumbens*, *Glyricidia sepium*, *Calliandra calothyrsus*, *Stylosanthes guaianensis*, *Leucaena leucocephala* and *Indigofera zollingeriana* were used in this study. The fatty acids composition (% of total identified fatty acids) which were dominant in grasses were C18: 3n-3 (29%), C16: 0 (28%) and C18: 2n-6 (23%). Whereas in legumes, the significantly higher composition of fatty acids was C18: 3n-3 (42%) followed by C16: 0 (17%) and C18: 2n-6 (17%). The average poly-unsaturated fatty acids (PUFA) composition in grasses was relatively lower (44.6%) than legumes (59%). Likewise the content of total phenols and total tannins (g/100g DM) of grasses (0.91 and 0.41) and legumes (1.72 and 0.70). The selection of the forage plant species was based on the criteria of PUFA composition and biohydrogenation reduction agents using TOPSIS method. The results obtained show that *B. decumbens* (grass) and *I. zollingeriana* (legume) had the highest preference value of 0.74 and 0.87, respectively. In conclusion, *B. decumbens* and *I. zollingeriana* are forage species that have potential to provide healthier ruminant products.

Keywords: Biohydrogenation reduction agents, fatty acids, tropical forages

INTRODUCTION

Nutrition manipulation is needed to produce healthier meat by increasing the content of polyunsaturated fatty acids (PUFA). PUFA are a category of essential fatty acids, and its availability must be supplied through a feed. The provision of forage-based feeds (grasses and legumes) can significantly increase the omega-3 content in ruminant meat when compared to concentrate-based feed (Daley et al. 2010; Ruechel, 2012; Vahmani et al. 2015). With the increase in the proportion of PUFA in meat, it reduces the level of saturated fatty acids (SFA), and the ratio of PUFA: SFA in meat increases. Poulson et al. (2004), suggested that the conjugated linoleic acid (CLA) content of *musculus longissimus* of Angus crossing cattle increased five times that of CLA (*cis*-9, *trans*-11 C18: 2) isomer during the pasture-based finisher period. Where CLA intake plays an important role in maintaining human health through its role as anticarcinogenic. PUFA consumed by ruminants most times undergo metabolic processes by rumen microbes that originate from the genus *Butyrivibrio* sp. The lipolysis and biohydrogenation processes that occur in the rumen system make unsaturated fatty acids to be converted to saturated fatty acids, especially stearic acid (C18: 0) and a small portion of vaccenic acid (*trans*-11 C18: 1). Extensive biohydrogenation activity causes 90% of PUFA to be ineffectively deposited in meat and milk (Jayanegara et al. 2011a; Shingfield et al. 2010; Lourenco et al. 2010; Enjalbert et al. 2017).

Forage as diets for ruminants, contains PUFA which is dominant in α-linolenic (C18: 3n-3) and linoleic forms (C18: 2 n-6). Although the lipid content in forage is relatively small, it plays a central role in forming the composition of fatty acids in ruminant meat. Furthermore, the components of the secondary metabolite plant such as phenols and tannins which have antibiotic effects on rumen biohydrogenation microbes, bound complexes with macronutrients, and increase PUFA accumulation (Dewhurst et al. 2003; Lourenco et al. 2007; Jayanegara et al. 2011a; Jafari et al. 2016; Vasta et al. 2019). Studies on the increase of PUFA content in livestock products through forage-based feed are still limited to feed crop species that grow in sub-tropical regions. While in the tropical forage, this study tends to be forgotten because forage is still considered a source of fiber and its strategic function as a source of quality feed that is able to improve the quality of livestock products is not yet known. Therefore, further verification is needed. An in vitro study conducted by Jayanegara et al. (2011a), in 27 species of tropical forages revealed the potential of tropical forage in modulating biohydrogenation and increasing flow of C18: 3n-3 and C18: 2n-6 through the rumen. However, several plant

species which are used as the main source of tropical forage is still lacking information. Considering the high biodiversity of forage species in tropical areas, forage-based systems is the most inexpensive, sustainable and adaptable method for small farmers in the tropical region. Therefore, a strategic study of the exploration of tropical forage species in terms of the fatty acid composition aspect and the content of biohydrogenation reduction agents is crucial.

The objective of this study was to evaluate the composition of fatty acids (SFA, MUFA, and PUFA) and the composition of biohydrogenation reduction agents (total phenols and total tannins) found in each tropical forage species (grass and legume), which is valuable information for determining promising species to modify biohydrogenation and improve fatty acid profile.

MATERIALS AND METHODS

Location

The forage sample collection was carried out at BPTU-HPT Padang Mengatas, Indonesia during September 2018 where the average rainfall is 1800 mm/year, the temperature range from 18-28ºC and average air humidity is about 70%. The station is located at an altitude between 700-900 meters above sea level. The soil type is podzolic (red-yellow), with pH of 5.6, and clay texture.

Forages samples collection

Samples of 10 tropical forages were collected from pasture area in BPTU-HPT Padang Mengatas, Indonesia. The collected forages are species that are generally used as sources of forage in the tropics. The 10 species which consisted of five grass species (*Panicum maximum*, *Cynodon plectostachyus*, *Pennisetum purpurephoides*, *Pennisetum purpureum*, *Brachiaria decumbens*) and five legume species (*Glyricidia sepium*, *Calliandra calothyrsus*, *Stylosanthes guaianensis*, *Leucaena leucocephala*, *Indigofera zollingeriana*). Each species collected weighed 3 kg of biomass and consisted of leaves and edible parts. They were stored indoors for 3 days and dried in an oven at 60ºC for 3 h. They were then mashed by pressing them through a filter (of 1 mm mesh size). The smashed samples were put into an air-tight plastic pack and were stored until they were analyzed.

Extraction of samples and their chemical analysis

Extraction and quantification of total phenols and total tannins were estimated according to the procedure of Makkar (2003). Test tubes that contained 1 g of each sample received 10 mL of the solution, before they were put in a beaker that had been filled with distilled water and was placed in a water bath; and ultrasonicated for 20 min at room temperature. Each sample was centrifuged for 10 min at 3000 rpm and 4ºC. The resulting supernatant was poured into another test tube. The remaining residues were extracted again using 2.5 mL of acetone 70% (v/v) using the same extraction procedure. Analysis of total phenols and total tannins was done using a standard solution of 0.1 $mgmL^{-1}$ tannins acid. As well as adding polyvinylpolypyrrolidone to separate tannins from non-tannin phenols and the resultant was then read using UV-Vis spectrophotometer (U-1800-5930482, High-Technologies Corporation, Tokyo, Japan) with a wavelength of 724 nm. The total phenols and total tannins are expressed in g/100g dry matter (DM). Analysis of crude protein and crude fat content was done following the standard procedure of AOAC (2005). Determinations of neutral detergent fiber were quantified according to Van Soest et al. (1991).

Determination of fatty acid composition

Determination of the fatty acid composition of the forage samples was preceded by preparation of a standard solvent based on AOCS (1993) method, and the extraction of lipid and preparation of fatty acid methyl esters (FAME) was done through transmethylation of FAME using gas chromatography, according to the procedure of AOAC (2000). The prepared FAME was then analyzed using gas chromatography (model Agilent 7890B, Agilent Technologies, Inc., USA), equipped with Supelco SPTM 2560 capillary column (100m x 0.25 mm x 0.2 μm) to separate the methyl ester; and was detected by a flame ionized detector (FID). Ramping temperature setting up to 30ºC/min with 3 running ramps. The injectors and detectors were set at 225 and 240ºC, respectively. High purity nitrogen (N_2) was used as a carrier gas with a flow rate of 18 cm/sec and split of 1: 100. Identification of fatty acid in the sample was achieved by matching the retention times with FAME standards. The fatty acid concentration was interpreted as a percentage (%) of the total identified fatty acids.

Data analysis

Data analyses were done according to the technique for order of preference by similarity to ideal solution (TOPSIS) method (Yoon and Hwang 1981). The stages of data analyses are as folows: (i) Establishment of assessment attributes in determining forage species that have the potential to reduce biohydrogenation activity based on literature investigations. (ii) Establishment of the attribute weight (%) with details of 50% PUFA composition and 50% biohydrogenation reduction agent (25% total phenols and 25% total tannins). (iii) Determination of a normalized decision matrix from a predetermined decision matrix. (iv) Determination of the positive ideal solution and the ideal negative solution from the weighted normalized decision matrix by identifying the maximum value or the minimum value based on the criteria for PUFA composition and the content of the biohydrogenation reduction agent for each species. (v) Determination of the separation or distance approach between the values of each alternative with a positive ideal solution and a negative ideal solution. (vi) Determination of the preference value for each species by combining the calculation between the distance of the alternative approach of the positive ideal solution and the alternative distance from the negative ideal solution. (vii) Ranking of forage species based on the preference value.

RESULTS AND DISCUSSION

Fatty acid composition

Forage type had a significant influence on the composition of the fatty acids. In grass species (Table 1), the average composition of C18: 3n-3 (29%) was higher than C16: 0 (28%) and C18: 2n-6 (23%). With the composition of C18: 3n-3, the highest was *B. decumbens* (40.52%) and the lowest was *P. maximum* (8.98%). The highest PUFA composition in the grass was *B. decumbens* (60.81%), higher than *P. purpuphoides* (49.67%) and the lowest was *P. purpureum* (34.05%).

Likewise, legume species (Table 2) where C18: 3n-3 (42%) significantly dominated, followed by C16: 0 (17%) and C18: 2n-6 (17%). The highest C18-3n-3 composition was *C. calothyrsus* (53.60%) and the lowest was *S. guaianensis* (29.61%). The highest PUFA composition was found in the legume species *C. calothyrsus* (74.58%),higher than *I. zollingeriana* (63.25%) and the lowest was *L. leucocephala* (49.85%).

The results of this study are in accordance with Clapham et al. (2005), Jayanegara et al. (2011a), Khan et al. (2015), Sultana et al. (2015) and Dias et al. (2017) who reported that the composition of C18: 3n-3 most dominated forage fatty acid profile and was followed by C16: 0 and C18: 2n-6. There are four main factors that influence the composition of fatty acids in forage (Clapham et al. 2005; Khan et al. 2012): plant species, growth stages conservation, and application of nitrogen fertilization. Khan et al. (2015), revealed that there was a large variation in PUFA content in tropical forage species but are relatively similar to fatty acid composition. The selection of forage species is one of the effective strategies in improving the quality of fatty acid profiles in meat and milk by taking into account the fatty acid content, PUFA composition, and polyphenol content (Guerra-Rivas et al. 2013; Patino et al. 2015). The vegetative growth stage is the optimal condition in harvesting forages where the highest PUFA and crude protein content is reached at this stage and will decrease when entering the generative stage (Dewhurst et al. 2001; Buccioni et al. 2012). Forage conservation such as hay and ensilage causes a decrease in essential fatty acids in plants due to the endogenous lipolysis of PUFA (Dewhurst et al. 2006). The treatment of N fertilizer in forages was able to influence the fatty acid concentration by increasing the leaf/stem ratio where the leaf component was richer in the C18: 3n-3 content when compared to other plant components (Witkowska et al. 2008). The results of this study have implications that tropical forage feed has a high composition of essential fatty acids C18: 3n-3. Therefore, it can increase the biosynthesis of omega-3 long chain fatty acids such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in animal tissues.

Table 1. Fatty acid profile of the grasses species (%)

Fatty acids	*P. maximum*	*C. plectostachyus*	*P. purpurephoides*	*P. purpureum*	*B. decumbens*	s.e.m.
C14: 0	2.3	2.2	1.0	1.8	2.7	0.6
C16: 0	24.0	27.1	20.6	23.0	22.2	2.4
C18: 0	4.4	3.5	3.3	4.7	3.0	0.7
C18: 1n-9	11.7	8.9	7.9	5.0	5.8	2.7
C18: 2n-6	24.8	24.1	15.7	12.8	20.3	5.2
C18: 3n-3	9.0	17.0	34.0	20.1	40.5	12.9
C20: 0	1.5	1.9	3.2	6.8	1.0	2.3
Total SFA	50.0	50.0	42.4	61.0	33.4	10.2
Total MUFA	12.4	8.9	7.9	5.0	5.8	2.9
Total PUFA	37.6	41.1	49.7	34.0	60.8	10.7
n-6: n-3	2.8	1.4	0.5	0.6	0.5	1.0

Note: SFA-saturated fatty acid, MUFA- monounsaturated fatty acid, PUFA-polyunsaturated fatty acid, n-6: n-3- ratio C18: 2n-6: C18: 3n-3,s.e.m.-standard error of the mean

Table 2. Fatty acid profile of the legumes species (%)

Fatty acids	*G. sepium*	*C. calothyrsus*	*S. guaianensis*	*L. leucocephala*	*I. zollingeriana*	s.e.m.
C14: 0	1.1	0.3	1.6	2.3	1.5	0.7
C16: 0	21.1	6.4	18.4	19.6	17.3	5.9
C18: 0	8.7	2.6	6.9	8.0	4.2	2.6
C18: 1n-9	5.0	4.6	7.0	8.5	4.3	1.8
C18: 2n-6	11.6	20.8	22.6	13.6	15.4	4.7
C18: 3n-3	43.2	53.6	29.6	36.3	47.9	9.4
C20: 0	2.4	2.0	1.9	2.4	1.8	0.3
Total SFA	39.2	19.0	40.4	41.4	32.4	9.3
Total MUFA	5.1	6.4	7.4	8.7	4.3	1.8
Total PUFA	55.6	74.6	52.2	49.9	63.3	10.0
n-6: n-3	0.3	0.4	0.8	0.4	0.3	0.2

Note: SFA-saturated fatty acid, MUFA- monounsaturated fatty acid, PUFA-polyunsaturated fatty acid, n-6: n-3- ratio C18: 2n-6: C18: 3n-3, s.e.m.-standard error of the mean

Biohydrogenation reduction agents

The total phenols content of tropical forages ranges from 0.45 to 2.65 g/100 g DM (Table 3). Among the grass species studied, the content of total phenols was highest in *P. purpureum* (1.98) and lowest in *C. plectostachyus* (0.45). In legume species, *L. leucocephala* (2.65) had the highest total phenols content and the lowest was recorded in *C. calothyrsus* (0.66). In this study, phenols concentrations tend to be lower but concentration was not the main factor in suppressing biohydrogenation activity; rather it is the phenols composition itself (Jayanegara et al. 2011a).

In tropical plants, phenolic components have a more massive concentration than plants in temperate climates.This is caused by exposure to ultraviolet rays in high intensity (Berli et al. 2011). In vitro studies have shown convincing results that the phenolic component can reduce C18: 0 accumulation in rumen fluid and increase conjugated linoleic acid (CLA) isomers (Vasta et al. 2009; Ishlak et al. 2015; Buccioni et al. 2017). The same results were shown in an *in vivo* study where polyphenol supplementation affected the biohydrogenation of PUFA and the composition of the rumen microbiota by increasing intermediate fatty acids such as *cis*-9, *trans*-11 C18: 2 (Vasta et al. 2010; Andres et al. 2016; Yusuf et al. 2017). More specifically, one form of phenolic components such as condensed tannins and hydrolyzable tannins each plays a role in inhibiting various stages of biohydrogenation (Costa et al. 2018).

The formation of the tannin-protein complex has also been reported to reduce the effects of negative lipolysis and rumen PUFA metabolism (Cabiddu et al. 2010). The phenolic compounds have been shown to modify the biohydrogenation and methanogenesis pattern of rumen through anti-microbial ability and the formation of phenols-lipid complexes (Smith et al. 2005; He et al. 2006; Carreño et al. 2015). In vitro rumen fermentation studies revealed the potential of the phenols component as a biohydrogenation reduction agent capable of modifying ruminal lipid metabolism by suppressing the disappearance of essential fatty acid groups such asC18: 3n-3, C18: 2n-6 and C18: 1n-9;also appearance of C18: 0 in the rumen system (Jayanegara et al. 2011a; Jafari et al. 2016). The implication is that phenolic components contained in tropical forages can increase the transfer of PUFA in a feed to livestock products more effectively.

Determination of the preferred forage species

Figure 1.means that the forage of *B. decumbens* grass species has the greatest relative preference distance, which is equal to 0.74, followed by *P. purpureum* 0.64, *P. purpurephoides* 0.25, *C. plectostachyus* 0.12, and *P. maximum* 0.09. These results suggest that *B. decumbens* is the best solution for selecting tropical grass species which suggest that *B. decumbens* is the best solution for selecting tropical grass species which is appropriate as a forage-based ration material which is expected to reduce biohydrogenation and improve the quality of ruminant products through improved fatty acid profiles.

Table 3. Contens of crude protein, crude fat, neutral detergent fiber, total phenols and total tannins (g/100g DM)

Forage species	Sample type	Crude protein	Crude fat	NDF	Total phenols	Total tannins
P. maximum	Grass	7.55	1.41	66.05	0.46	0.12
C. plectostachyus	Grass	9.64	1.78	68.72	0.45	0.02
P. purpurephoides	Grass	13.07	2.42	66.02	0.5	0.03
P. purpureum	Grass	7.02	2.51	64.01	1.98	0.94
B. decumbens	Grass	17.50	2.70	53.67	1.19	0.94
G. sepium	Legume	25.20	3.96	35.73	1.11	0.19
C. calothyrsus	Legume	28.46	4.11	50.72	0.66	0.16
S. guaianensis	Legume	17.91	2.92	41.45	1.98	0.86
L. leucocephala	Legume	25.15	4.80	31.63	2.65	1.15
zollingeriana	Legume	31.90	3.64	21.91	2.46	1.13
s.e.m.		9.0	1.0	16.6	0.9	0.5

Note: s.e.m.-standard error of the mean, NDF-neutral detergent fiber

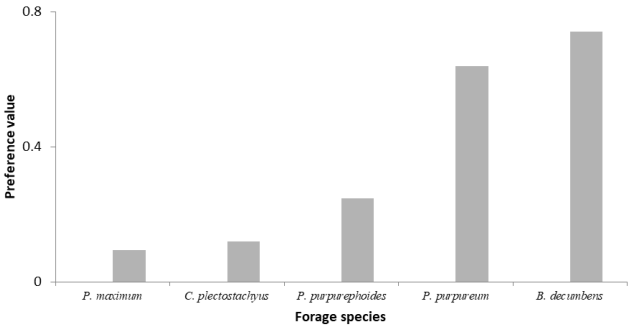

Figure 1. Preference value of grass species

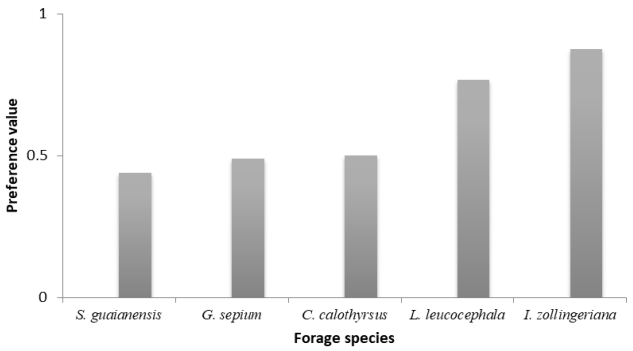

Figure 2. Preference value of legume species

Figure 2 presents data on preference values among tropical legume species where the greatest preference value was achieved by species *I. zollingeriana* which was equal to 0.87, followed by *L. leucocephala* 0.77, *C. calothyrsus* 0.50, *G. sepium* 0.49 and *S. guaianensis* 0.44. These results indicate that *I. zollingeriana* promises the right tropical

legume species to reduce the negative effect of biohydrogenation and increase PUFA bypass flow. Until now, there have been no studies that measure the extent to which these selected species are able to modulate rumen lipid metabolism. However, the study of Suharlina et al. (2016) revealed a strong indication in *I. zollingeriana* where supplementation in the range of 20-80% of rations was able to suppress methane production and total production of rumen gas which positively correlated with the biohydrogenation process. The utilization of hydrogen (H_2) and carbon dioxide (CO_2) substrates simultaneously is the main relationship between the process of methanogenesis and biohydrogenation (Lourenco et al. 2010). Interestingly, biohydrogenation reduction agents have the same inhibitory characteristics of the rumen methanogenesis pathways (Jayanegara et al. 2011b). Furthermore, the ability of based feed *I. zollingeriana* is able to modify fermentation and rumen degradation activities more efficiently and improve the performance of ruminant livestock (Ginting et al. 2010; Tarigan et al. 2017; Tarigan et al. 2018). Study of determining plant species based on the TOPSIS method has proven its accuracy in identifying plant species on various assessment indicators (Alavi et al. 2012; Arabameri et al. 2014; Ariapour et al. 2014). Zhang et al. (2018), stated that TOPSIS based decision analysis is able to accurately identify forage species that can produce optimal forage quality and biomass in varied land conditions.

We concluded that among tropical grass species, the PUFA composition of *B. decumbens* is the highest when compared to other grass species. While among tropical legume species, the PUFA composition of *I. zollingeriana* is the highest when compared to other legume species. In the content of biohydrogenation reduction agents, *P. purpureum* has the highest content in grasses and *L. lecocephala* species has the highest content in legumes. Whereas the best determination of tropical grass species and legumes, based on the criteria for PUFA composition and the content of biohydrogenation reduction agents are *B. decumbens* and *I. zollingeriana*. Both grass-legume species are expected to be the basis of diet which has potential to delivering PUFA more effectively into livestock products.

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