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d200718 Fatty acids composition and DOI: 10.13057/biodiv/ biohydrogenation reduction agents of tropical forages Malik MakMur1,?, Mardiati Zain2,??, Yetti Marlida2, Khasrad2,

ANURAGA JAYANEGARA3 1Faculty of Animal Science, Universitas Andalas. Jl. Unand, Kampus Limau Manis,

Padang 25163, West Sumatra, Indonesia. Tel./fax. +62-751-71464, ?email: malikmakmur27@gmail.com

2Department of Animal Nutrition, Faculty of Animal Science,

Universitas Andalas.

Tel./fax. +62-751-71464. ??email:

Manis, Padang 25163, West Sumatra, Indonesia.

mardiati@ansci.unand.ac.id 3Department of Animal Nutrition, Faculty of Animal Science, Institut Pertanian Bogor. Jl.

Agatis, Kampus IPB Dramaga, Bogor 16680, West Java, Indonesia Manuscript received: 13 May 2019. Revision accepted: 21 June 2019. 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

composition of the 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 mus	supplied <u>through</u>	a feed. The
provision of forage-based feeds (grass	s and legumes) can significantly $incr$	rease the omega-3 content in	ruminant
meat when compared to	concentrate-based feed (Daley et al.	. 2010; Ruechel, 2012; <u>Vahmani</u>	<u>et al.</u>
2015). With the increase in the	proportion of <u>PUFA</u> in	meat, it reduces the level of satu	<u>urated fatty</u>
acids (SFA), and the ratio of PU	FA: SFA in meat increa	ases. Poulson et al. (2004), sugg	ested that the
conjugated linoleic acid (CLA) content of	<u>f</u> musculus longissimus <u>of</u>	Angus crossing cattle increa	sed 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

vaccenic acid (trans-11 C18: 1).

Extensive

aspect

a small portion of

acids, especially stearic acid (C18: 0) and

biohydrogenation activity causes 90% of PUFA to be ineffectively deposited in meat and milk (Jayanegara et al. 2010; Lourenco et al. 2017). Forage as diets for 2011a; Shingfield et al. 2010; Enjalbert et al. ruminants, contains PUFA which is dominant in <u>a-linolenic (C18: 3n-3) and linoleic</u> 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 2011a; Jafari et al. 2016; Vasta et al. 2019). Studies on the increase of PUFA content in livestock

<u>al.</u> 2011a; Jafari <u>et al.</u> 2016; Vasta <u>et al.</u> 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

and the content of biohydrogenation reduction agents is crucial. The objective of this study was to evaluate

composition of fatty acids (SFA, MUFA, and PUFA) and the composition of biohydrogenation the 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

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 <u>dry matter (DM).</u> Analysis of <u>crude protein</u> and <u>crude fat</u> 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 <u>fatty acid composition</u> Determination of the fatty acid					
<u>composition of the</u> forage <u>samples</u> 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					
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 <u>x 0.25 mm x 0.2 µm)</u> to					
separate the methyl ester; and was <u>detected</u> by <u>a flame</u> ionized <u>detector (FID)</u> . 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 (N2) was used as a carrier gas with a flow rate of 18 cm/sec and split of 1:					
100. Identification offatty acid in the sample was achieved by matching the retentiontimes with					
FAME standards. The fatty acid concentration was interpreted as a percentage (%) of the total					
identified <u>fatty acids. Data</u> analysis Data analyses <u>were</u> done according to <u>the technique for order of</u>					
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 <u>of a</u> normalized <u>decision matrix</u> from <u>a</u> predetermined <u>decision matrix</u> . (iv)					
Determination of the positive ideal solution and the ideal negative solution from the weighted					
<u>normalized decision matrix by</u> 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. MAKMUR et al. – Fatty acids composition of tropical forages 1919 RESULTS AND DISCUSSION conservation, and application of nitrogen fertilization. Khan et al. (2015), revealed that there was a large variation Fatty acid composition in PUFA content in tropical forage species but are Forage					
type <u>had a significant</u> influence <u>on the</u> relatively similar to <u>fatty acid</u> composition. <u>The</u> selection composition of the fatty acids. In grass species (Table 1), of forage species is one of the effective strategies in the average					
composition <u>of C18: 3n-3</u> (29%) <u>was higher</u> improving <u>the</u> quality <u>of</u> fatty acid profiles in meat					
and than C16: 0 (28%) and C18: 2n-6 (23%). With the milk by taking into account the fatty acid					

content, PUFA composition of C18: 3n-3, the highest was B. decumbens composition, and polyphenol content (Guerra-Rivas et al. (40.52%) and the lowest was P. maximum (8.98%). The 2013; Patino et al. 2015). The vegetative growth stage is highest PUFA composition in the grass was B. decumbens the optimal condition in harvesting forages where the (60.81%), higher than P. purpuphoides (49.67%) and the highest PUFA and crude protein content is reached at this lowest was P. purpureum (34.05%). stage and will decrease when entering the generative stage Likewise, legume species (Table 2)

where C18: 3n-3 (Dewhurst et al. 2001; Buccioni et al. 2012). Forage (42%) significantly dominated,

followed by C16: 0 (17%) conservation such as hay and ensilage causes a decrease in and C18: 2n-6 (17%). The

highest C18-3n-3 composition essential fatty acids in plants due to the endogenous was C. calothyrsus (53.60%) and the lowest was S. lipolysis of PUFA (Dewhurst et al. 2006). The treatment of guaianensis (29.61%). The highest PUFA composition was N fertilizer in forages was able to influence the fatty acid found in the legume species C. calothyrsus concentration by increasing the leaf/stem ratio where the (74.58%),higher than I. zollingeriana (63.25%) and the leaf component was richer in the C18: 3n-3 content when lowest was L. leucocephala (49.85%). compared to other plant

components (Witkowska et al. <u>The results of this study are in</u>	accordance with	2008). <u>The result</u>	ts of			
this study have implications that Clapham $et al. (2005)$,	Jayanegara <u>et al.</u>	(2011a), <u>Khan et</u>	tropical			
forage feed has a high composition of essential <u>al. (2015)</u> , who fatty acids C18: 3n-3. Therefore, it can increase the repor	Sultana et al. (2015) ted that the composition of		. ,			
biosynthesis of omega-3 long chain fatty acids such as	forage fatty <u>acid</u> p	rofile <u>and</u> was t	followed by			
C16: 0 and eicosapentaenoic acid (EPA) and docosahexaenoic	acid C18: 2n <u>-6.</u>	There are	four main			
factors that influence the (DHA) in animal tissues. composition of fatty acids in forage (Clapham et al. 2005; Khan et						
al. 2012): plant species, growth stages Table 1.	Fatty acid profile	of the grasses s	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 <u>C18: 2n-6: C18: 3n-3</u>, s.e.m <u>.-standard error of</u> the <u>mean Table</u> 2. <u>Fatty acid profile</u>

of the legumes species (%) Fatty acids G. sepium C. calothyrsus S. guaianensis L. leucocephala I.

zollingeriana s.e.m. C14: 0 C16: 0 C18: 0 C18: 1n-9 C18: 2n-6 C18: 3n-3 C20: 0 Total SFA Total MUFA Total

 PUFA
 n-6:
 n-3
 1.1
 0.3
 21.1
 6.4
 8.7
 2.6
 5.0
 4.6
 11.6
 20.8
 43.2
 53.6
 2.4
 2.0
 39.2
 19.0
 5.1
 6.4
 55.6
 74.6
 0.3
 0.4
 1.6

 2.3
 18.4
 19.6
 6.9
 8.0
 7.0
 8.5
 22.6
 13.6
 29.6
 36.3
 1.9
 2.4
 40.4
 41.4
 7.4
 8.7
 52.2
 49.9
 0.8
 0.4
 1.5
 17.3
 4.2
 4.3
 15.4
 47.9

 1.8
 32.4
 4.3
 63.3
 0.3
 0.7
 5.9
 2.6
 1.8
 4.7
 9.4
 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 <u>et al. 2017).</u>

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

<u>C18: 2</u> (Vasta <u>et al. 2010;</u> Andres <u>et al.</u> 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 <u>et al. 2005;</u> He <u>et al. 2006;</u> Carreño <u>et al.</u> 2015). <u>In</u> 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: <u>3n-3</u>,

<u>C18: 2n-6 and C18: 1n-9;</u> also appearance of C18: 0 in the rumen system (Jayanegara et al. 2011a;

Jafari <u>et al.</u> 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 <u>improve the quality of ruminant products</u>

through improved fatty acid profiles. Table

crude fat, neutral detergent fiber, total phenols and 3.Contens of crude protein, total tannins (g/100g DM) Forage species P. maximum C. plectostachyus P. purpurephoides P. purpureum B. decumbens G. sepium C. calothyrsus S. guaianensis L. leucocephala zollingeriana s.e.m. Sample Crude Crude type NDF Total Total protein fat phenols tannins Grass 7.55 1.41 66.05 0.46 0.12 Grass 9.64 1.78 68.72 0.45 0.02 Grass 13.07 2.42 66.02 0.5 0.03 Grass 7.02 2.51 64.01 1.98 0.94 Grass 17.50 2.70 53.67 1.19 0.94 Legume 25.20 3.96 35.73 1.11 0.19 Legume 28.46 4.11 50.72 0.66 0.16 Legume 17.91 2.92 41.45 1.98 0.86 Legume 25.15 4.80 31.63 2.65 1.15 Legume 31.90 3.64 21.91 2.46 1.13 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 MAKMUR et al. - Fatty acids composition of tropical forages 1921 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 (H2) and carbon dioxide (CO2) 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 <u>et al.</u> 2018). Study <u>of</u> 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 <u>et al. 2014</u>). Zhang <u>et al.</u> (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

<u>conditions.</u> 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. <u>ACKNOWLEDGEMENTS This study was</u>

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