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Fermentation Characteristics, Digestibility, and Estimation of Ruminant Methane from Saponin: A Quantitative Study

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ABSTRACT

The effect of using saponins on ruminants' performance differed from several published research data based on the level of saponins added to the feed. This research was conducted to analyze the effect of saponins on fermentation characteristics, digestibility, and estimation of methane in ruminants with a mixed model approach from published journal articles—a total of 127 studies from 32 journals, national and international. The variable measured included the level of saponins (%), dry matter intake, Average Daily Gain (ADG), Dry Matter Digestibility (DMD), Organic Matter Digestibility (OMD), Crude protein (CP), Neutral Detergent Fiber (NDF), Acid Detergent Fiber (ADF), protozoa population, bacterial population, Volatile Fatty Acid (VFA), acetate/C_2, propionate/C_3, butyrate/C_4, valerate/C_5, acetate/propionate (C_2/C_3), NH_3, pH, and methane gas production. The results showed that using saponins in ruminants increased ADG, CP, ADF, and NDF degradation, Total VFA, and proportion of propionate. The addition of saponins level reduced the protozoa population, acetate proportion, and the ratio of acetate: to propionate (C_2/C_3). In contrast, feed intake and digestibility decreased with the administration of saponin. The bacterial population were similar among treatments, and methane production increased by increasing saponins. In conclusion, the administration of saponin level recommended is 0.3-3.1% of the total ration to improve performance and increase feed efficiency.

Keywords: digestibility, methane, rumen fermentation, ruminant *in vivo*, saponin

INTRODUCTION

The success of a livestock business is primarily determined by production efficiency and feed utilization for animal growth, development and reproduction (Yanuartono et al., 2017). Saponin is an anti-nutritional substance that can be added to feed as protozoa defunct agents to ruminants that can be given in specific doses to maximize the degradation process in the rumen and reduce methane emissions to the environment (Krisnawan et al., 2015). Saponins have a broad diversification of structures. Certain saponin compounds with surfactant properties can cause the protozoa cell wall to lysis so that they can be used as defunct protozoa agents (Yanuartono et al., 2017).

Several observations of saponins administration to ruminants in vivo showed inconsistent results where growth inhibition occurred in livestock. Administration of saponins at low doses increased ration consumption and digestibility (Sliwinski et al., 2014) but had no significant effect on high doses administration of saponins (Anantasook et al., 2015). It was probably caused by the bitter taste of saponins, which reduces palatability and feed consumption. For other reasons, saponins can irritate the lining of the mouth and digestive tract, affecting the absorption of nutrients in livestock (Yanuartono et al., 2017). Therefore, from previously published research data, it is necessary to study the dose of saponins administered to ruminants in vivo and their effect on performance, rumen fermentation, and methane production.

This study aims to quantitatively examine the effect levels of saponin administration on fermentation characteristics, digestibility, and methane production estimation ruminant in vivo using a regression analysis approach from published journal data. This study aims to provide information regarding the effect of saponin level administration on fermentation characteristics, digestibility, and methane estimation of ruminants in vivo. This study hypothesizes that using saponins at optimal levels in ruminant feed in vivo can increase feed efficiency by increasing the total VFA concentration and the proportion of propionate and reducing the production of acetate and butyrate in the rumen.

MATERIALS AND METHODS

The research involved collecting and processing data which was carried out for five months, from March to July 2021. Data were collected by searching for journals from various sources, such as science direct and google scholar, regarding the addition of steroid and triterpenoid saponin extracts to the performance of ruminants *in vivo*. Journal criteria included in the database are national and international accredited journals with the addition of saponins to research parameters. The database consisted of 127 studies from 32 journal articles (Table 1). Furthermore, various parameter data are recorded on a Microsoft Excel 2013 worksheet, and the units are equated by converting them to predetermined units. Then, the data were analyzed using SAS software version 9.2 and interpreted in tabular form.

Table 1. Quantitative test studies of the effectiveness of using saponins on fermentation characteristics, digestibility, and estimation of ruminant methane *in vivo*

No	References	Types of livestock	Body Weight	Feed used	Saponin source	Saponin dosage (%)
1	Sliwinski et al. (2002)	Sheep	35.1	Concentrate, Hay	*Yucca Schidigera*	0, 0.002, 0.3
2	Santoso et al. (2006)	Sheep	55.8	Concentrate, timotius silage	*Yucca Schidigera*	0, 0.024
3	Santoso et al. (2004)	Sheep	55	Concentrate	*Yucca Schidigera*	0, 0.012
4	Wang et al. (2009)	Sheep	38.9	Concentrate, hay	Yucca *Schidigera*	0, 0.17
5	Wang et al. (2016)	Friesian Holstein	550	Forage, Concentrate	Tea Saponin	0, 0.73, 1, 1.5
6	Thalib et al. (1995)	Sheep	15	Concentrate	*Sapindus rarak extract*	0, 0.011
7	Pen et al. (2007)	Sheep	60.9	Concentrate	*Quillaja saponaria,Yucca Schidigera*	0, 0.048, 0.118
8	Hess et al. (2004)	Sheep	30.1	Concentrate	*Sapindus saponaria*	0, 0.036
9	Abreu et al. (2004)	Sheep	40.3	Legume	*Sapindus saponaria*	0, 0.96
10	Aazami et al. (2009)	Sheep	48	Concentrate	*Quillaja saponaria*	0, 0.036, 0.054, 0.1, 0.2
11	Mao et al. (2009)	Sheep	14.2	Concentrate	*Tea Saponin*	0, 0.39
12	Nasri et al. (2011)	Sheep	18.6	Concentrate	*Quillaja saponaria*	0, 0.03, 0.06, 0.09
13	Zhou et al. (2012)	Goat	25	Concentrate	*Tea Seed Saponin*	0, 0.28, 0.42, 0.56
14	Anantasook et al. (2014)	Dairy cow	405	Concentrate	*Samanea saman extract*	0, 2.28
15	Lovett et al. (2006)	Dairy cow	585	Total mixed ration	*Yucca Schidigera extract*	0, 1.8, 3, 3.5, 6
16	Wu et al. (1994)	Dairy cow	650	Concentrate	*Yucca Schidigera extract*	0, 0.03, 0.06, 0.09, 0.12
17	Hussain et al. (1994)	Bull	574, 658	Concentrate	*Yucca Schidigera extract*	0, 0.075
18	Benchaar et al. (2008)	Dairy cow	730	Total mixed ration	*Yucca Schidigera extract*	0, 0,33
19	Holtshausen et al. (2009)	Dairy cow	627	Total mixed ration	*Quillaja saponaria,Yucca Schidigera*	0, 0.6
20	Hristov et al. (1999)	Beef cattle	443	Concentrate	*Yucca Schidigera*	0, 0.04, 0.116
21	Suharti et al. (2009)	Beef cattle	186	Concentrate	*Sapindus rarak extract*	0, 2,5, 5
22	Suharti et al. (2015)	Beef cattle	187.7	Concentrate	*Lerak extract*	0, 0.1, 0.2
23	Liu et al. (2019)	Beef cattle	60	Concentrate	*Tea Saponin*	0, 0.43
24	Lila et al. (2005)	Beef cattle	248	Concentrate	*Sarsaponin (Yucca Schidigera)*	0, 0.5, 1
25	Guyader et al. (2017)	Dairy cow	617	Concentrate	Tea *Saponin*	0, 0.14
26	Li et al. (2012)	Dairy cow	354, 429, 400	Corn silage	*Quillaja saponaria,Yucca Schidigera, Tea Saponin*	0, 0.07, 0.08, 0.5, 0.7
27	McMurphy et al. (2014)	Beef cattle	523	Hay, Concentrate	*Micro-Aid Yucca S*	0, 0.18, 0.36
28	McMurphy et al. (2014)	Beef cattle	289	Forage, Concentrate	*Micro-Aid Yucca S*	0, 0.18, 0.36
29	Nasri et al. (2011)	Sheep	18.6	Concentrate	*Quillaja Saponaria*	0, 0.03, 0.06, 0.09
30	Liu et al. (2018)	Sheep	22.3	Forage, Concentrate	*Alfalfa Saponin Extract*	0, 0.3, 0.6, 1.2, 2.4
31	Carlos et al. (2016)	Beef cattle	364	Forage, Concentrate	*Tea Seed Saponin*	0, 1.3, 2.25
32	Yuan et al. (2007)	Sheep		Hay, Concentrate	*Tea Saponin*	0, 0.42

The database obtained was then analyzed using a meta-analytic approach based on the mixed model method (St-Pierre 2001). Mixed model analysis (PROC MIXED) was performed with SAS software version 9.2. The study was taken as a random effect, while the level of addition of saponins was a fixed effect. The mathematical model is as follows:

$$Y_{ij} = B_0 + B_1 X_{ij} + s_i + e_i$$

Information:
Y_{ij} : Dependent variable
B_0 : Coefficient of linear regression Y to X
X_{ij} : Continuous Variable
B_1 : Linear regression
S_i : Random effect research i
e_i : Residual error

RESULTS AND DISCUSSION

A total of 127 data on the use of saponins were used to study the effectiveness of using saponins on fermentation characteristics, digestibility, and estimation of ruminant methane. The mixed model regression equation results can be seen in Table 2.

The administration of saponins significantly reduced the consumption of forage and ruminant concentrate ($p<0.001$). The result is consistent with previous research that adding saponins can reduce animal feed consumption due to the bitter taste in saponins, thereby reducing palatability and feed consumption (Yanuartono et al., 2017; Suharti, 2010). In addition, saponins also have soap-like properties so that they can irritate the mouth and digestive tract lining, affecting the absorption of nutrients (Gee et al., 1997). Dry matter digestibility (DMD) and organic matter digestibility (OMD) of feed decreased significantly ($p<0.001$) with the administration of saponins, following previous research that saponin administration at levels of 11.2g and 22.4g/day DM reduced the digestibility of livestock (Lila et al., 2005). The same results were reported: digestibility in the rumen in vitro and in vivo in sheep decreased when given saponins from lerak fruit methanol extract at 8g and 12g/day BK (Wina et al. 2005, 2006).

The degradation of crude protein (CP), acid detergent fiber (ADF), and neutral detergent fiber (NDF) in the feed increased significantly with the administration of higher saponin levels ($p<0.001$). Previous studies have shown varying results, and Nasri et al. (2011) found that adding QS did not affect the digestibility of OMD and crude protein. Santoso et al. (2007) found that fiber digestibility (NDF) decreased with increasing levels of QS supplementation in goat feed. Hess et al. (2004) saw decreased fiber degradation in the rumen of sheep receiving *S. saponaria*. In contrast, Pen et al. (2007) concluded that saponins from Q. Saponaria increased NDF digestibility in sheep fed Italian ryegrass and concentrate. In dairy cows, the administration of *Y. schidigera* and *Q. saponaria* did not affect the digestibility of NDF and ADF (Holtshausen et al., 2009). Variations in the results obtained in previous studies indicate that the response of livestock to saponins depends on feed composition, type and source of saponins, and level of use of saponins in feed.

The average daily gain of livestock increased significantly ($p <0.001$). According to Suharti (2011), there was an increase in live weight growth in cattle given rations containing Lerak flour, presumably because saponins worked as defaunation agents (saponins), which can suppress the growth of rumen protozoa. The low protozoa population allows bacteria to degrade feed ingredients and increase live weight growth optimally.

Administration of saponins to the rumen microbial population significantly reduced the rumen protozoa population ($p<0.001$) but did not significantly affect the rumen bacterial population. A decrease in the rumen's protozoa populations has been reported in vitro (Wang et al., 1998; Lu et al., 1987) and in vivo (Santoso, 2006; Hristov et al., 1999; Sliwinski et al., 2014), where the effect of the reduction is very significant. Depending on the dose used (Lovett et al., 2006), the type of ration, and the feeding amount (Pen et al., 2007). Hristov et al. 1999 described a 42% lower protozoa population in the rumen fluid of heifers receiving 20 g/day of YS compared to fluids from control heifers. A decrease in the protozoa population was also observed in the study by Suharti et al. (2010), that administration of lerak extract to a level of 0.8 mg/ml reduced the number of protozoa populations *in vitro* but not significantly *in vivo*.

In vitro, saponins from different sources had antiprotozoal activity. They were used as defaunation agents, but in several in vivo studies, saponin supplementation did not affect the number of protozoa populations (Benchaar et al., 2008). The antiprotozoal activity of saponins is due to cholesterol in the membranes of eukaryotic cells (including protozoa) but not in prokaryotic bacterial cells, so rumen protozoa are susceptible to saponins because saponins show an affinity for cholesterol (Klita et al., 1996). The bacterial population did not increase significantly but was higher than the control. The decline in protozoa populations in several studies has also suggested that saponins have a toxic effect on other microorganisms in the rumen, including bacteria (Lu and Jorgensen, 1987; Wu et al., 1994). Navas Camacho et al. (1993) added that the bacterial population did not change in the rumen by feeding the ration with *E. cyclocarpum*, the same result was found that YSE supplementation in vivo did not affect the total concentration of bacteria and fungi in the rumen contents (Eryavuz et al., 2004).

Table 2. The mixed model regression equation results

Response parameter	n	Model	Intercept	SE intercept	Slope	SE slope	P value	Root MSE	R square
DMI (kg/day)	93	Q	0,030	0,002	-1,42,E-07	0	<0,0001	0,005	0,969
					3,06,E-12	0			
ADG (g/day)	36	Q	2,727	0,386	5,10,E-05	1,50,E-04	<0,0001	1,155	0,875
					-8,20,E-09	0			
Digestibility									
DMD (%)	56	Q	64,998	1,341	-1,20,E-04	1,20,E-04	<0,0001	5,659	0,863
					2,34,E-09	0			
OMD (%)	46	Q	65,879	1,675	-2,00,E-05	7,50,E-05	<0,0001	2,836	0,971
					6,03,E-10	0			
CP (%)	61	Q	61,676	1,815	7,90,E-05	1,56,E-04	<0,0001	7,094	0,900
					-1,30,E-09	0			
NDF (%)	61	Q	55,516	1,812	6,87,E-06	1,82,E-04	<0,0001	7,874	0,889
					1,48,E-10	0			
ADF (%)	48	Q	48,169	1,930	1,33,E-03	1,16,E-03	<0,0001	10,499	0,798
					2,41,E-07	0			
Rumen Microorganisms									
Protozoa (10^4/ml)	44	Q	178,030	78,678	-2,35,E-02	1,06,E-02	<0,0001	244,543	0,901
					6,95,E-07	0			
Bacteria (10^9/ml)	14	L	6,545	3,009	1,83,E-02	1,98,E-02	0,382	3,091	0,950
Rumen Fermentation									
pH	81	Q	6,405	0,054	-5,82,E-06	9,20,E-06	<0,0001	0,246	0,879
					2,84,E-10	0			
NH_3 (mmol/ml)	76	Q	3,768	0,447	-6,00,E-05	1,23,E-04	<0,0001	2,590	0,804
					1,02,E-09	0			
Total VFA (mmol/l)	80	Q	97,781	5,674	2,29,E-04	5,71,E-04	<0,0001	14,628	0,958
					1,04,E-08	0			
C_2 (%)	76	Q	68,363	1,481	-2,20,E-04	1,77,E-04	<0,0001	4,649	0,939
					6,01,E-09	0			
C_3 (%)	77	Q	19,543	0,782	1,88,E-04	1,32,E-04	<0,0001	3,551	0,887
					7,82,E-09	0			
C_4 (%)	77	Q	10,321	0,510	7,90,E-05	8,70,E-05	<0,0001	2,287	0,885
					2,24,E-09	0			
$IsoC_4$ (%)	45	Q	0,935	0,134	-1,00,E-05	2,70,E-05	<0,0001	0,537	0,817
					2,31,E-10	0			
C_5 (%)	42	Q	1,526	0,265	-4,46,E-06	1,80,E-05	<0,0001	0,356	0,978
					7,92,E-11	0			
$IsoC_5$(%)	46	Q	1,386	0,190	2,97,E-06	5,30,E-05	<0,0001	1,113	0,654
					7,80,E-11	0			
A: P	77	Q	3,552	0,174	-3,00,E-05	2,30,E-05	<0,0001	0,619	0,920
					1,18,E-09	0			
Methane (g/d)	103	Q	84,001	6,662	5,11,E-04	1,16,E-04	<0,0001	3,487	0,999
					-1,44,E-08	0			

Description: n: the amount of data; L: linear model, Q: quadratic model; The model is very significant at $P \leq 0.01$, significant at $P<0.05$, tends to be significant at $P<0.10$, not significant at $P>0.10$; ADG: Average Daily Gain, DMD: Dry Matter Digestibility, OMD: Organic Matter Digestibility, CP: Crude Protein, NDF: Neutral Detergent Fiber, ADF: Acid Detergent Fiber, VFA: Volatile Fatty Acid, C_2: Acetate, C_3: Propionate, C_4: butyrate; $IsoC_4$: isobutyrate, C_5: valerate, $IsoC_5$: isovalerate, C_2/C_3: acetate/propionate ratio.

The addition of saponins was able to lower the pH significantly ($p<0.001$). According to previous research, the rumen pH decreased drastically when fed with saponin-treated feed from an average value of 6.66 to an average of 6.12 at 1-2 hours after eating (Santoso et al., 2004). Wu et al. (1994) observed a decrease in rumen pH when YS was given 0–8 g/day to dairy cows and observed the most significant effect at 6 g YS/day. Reducing rumen pH due to adding saponins is thought to mediate the decline in protozoa populations (Eryavuz & Dehority, 2004).

The administration of saponins significantly reduced ammonia concentration ($p<0.001$) due to ammonia binding to saponin compounds. Saponins bind ammonia when the ammonia concentration is high and release ammonia when the concentration is low in the rumen (Makkar et al., 1998). The concentration of ammonia in the rumen can also be reduced when the growth of protozoa is inhibited (Williams and Coleman, 1991). Reducing ammonia release with additives can increase the amount of released protein flowing into the duodenum, which is associated with the body's nitrogen retention. Saponin supplementation consistently reduced rumen ammonia concentrations based on several previous studies, namely ruminal fluid N ammonia concentrations decreased in livestock given QSE and YSE compared to controls (Hussain & Cheeke, 1995; Sliwinski et al., 2002; Lila et al., 2005; Pen et al., 2005; Pen et al., 2007).

Saponin supplementation in feed showed an increase in VFA concentration ($p<0.001$), an increase in the proportion of C_3, C_4 and $IsoC_5$ fatty acids, and a decrease in the ratio of C_2, C_5 and $IsoC_4$ ($p<0.001$). In contrast, the proportion of acetate and propionate decreased significantly ($p<0.001$). Several previous in vivo studies obtained mixed results, namely the concentration of VFA decreased, and the ratio of acetate to propionate did not change (Edwards et al., 2005); VFA concentration increased, and the ratio of acetate to propionate increased (Lila et al., 2005); VFA concentration increased slightly, but the fatty acid composition and ratio of acetate to propionate in the rumen did not change (Alert et al., 1993).

Rumen fermentation parameters were not affected by additives (Flachowsky & Richter, 1991). The results varied due to differences in the use of rations, saponin levels, and administration methods. The increase in total VFA production and the proportion of propionate with the addition of lerak extract (saponins) showed an increase in the efficiency of fermentation by rumen microbes. In addition, lerak extract can also modify rumen microbial activity by directing propionate formation and reducing butyrate production. Increased propionate production is also expected to minimize H_2 supply because propionate production in rumen metabolic pathways uses H_2, which competes with methanogenic bacteria to form methane. Therefore, using saponins has excellent potential to reduce methane production in the rumen.

The use of saponins in feed decreased methane production (g/day) ($p<0.001$) due to increased digestibility of NDF and ADF fiber in the feed, described in a study by Hess et al. (2004) that methane production decreased with decreased NDF and ADF digestibility of feed supplemented with *S. saponaria*. Although the effect of saponin extract on methane production is not always associated with fiber digestibility. The results of previous studies indicated that methane production in the rumen was lower in the saponin treatment than in the control. The lower output of CH_4 is probably caused by a decrease in the number of protozoa by saponins, thereby reducing the population of methanogen-producing microbes in the rumen.

CONCLUSION

Administration of saponins to ruminants in vivo can improve livestock performance in terms of increased average daily gain (ADG), degradation of crude protein (CP), ADF and NDF, total VFA and the proportion of propionate in the rumen. In addition, adding saponins can reduce protozoa populations, acetate production, and the ratio of acetate: to propionate in the rumen, as well as methane production. The recommended level of saponin administration is 0.3-3.1% of the total feed for ruminants to improve performance and increase feed use efficiency.

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