Buletin Peternakan 46 (4): 216-221, November 2022

ISSTAP
Indonesian Society for Sustainable Tropical Animal Production

Bulletin of Animal Science

ISSN-0126-4400/E-ISSN-2407-876X Accredited: 36a/E/KPT/2016
http://buletinpeternakan.fapet.ugm.ac.id/

Doi: 10.21059/buletinpeternak.v46i4.77328

Effect of The Combination of Protected and Non-Protected Soybean Oil (*Glycine max* L.) Supplementation on Characteristics of Rumen Fermentation, Nutrient Digestibility, and Nitrogen Balance in Garut Sheep

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Article history
Submitted: 26 August 2022
Accepted: 20 October 2022

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ABSTRACT

Soybean oil is a high source of unsaturated fatty acids which if given to sheep have the potential to accumulate in the meat. However, in the rumen unsaturated fatty acids undergoes biohydrogenation by rumen microbes, and the addition of fat in the feed has the potential to reduce fiber fermentation in the rumen which can have an impact on animal performance. This study aimed to determine the effect of the combination of protected and non-protected soybean oil supplementation on the characteristics of rumen fermentation, nutrient digestibility, and nitrogen balance of Garut sheep. Twelve male Garut lambs aged 13 months and weighing 29±3.23 kg were kept in a metabolic cage and divided into three groups. The basal diet of 60% King grass and 40% pollard bran was supplemented with protected and non-protected soybean oil with the ratio of 3%:0%, 1.5%:1.5%, and 0%:3%, respectively, based on the dry matter of ration. The data obtained were analyzed by One-Way ANOVA, followed by the Duncan Multiple Range Test (DMRT). The results showed that supplementation had no effect on rumen fatty acids profile, microbial protein, ammonia concentration, total volatile fatty acids, acetat, propionate, butirat, and pH. The digestibilities of dry matter, organic matter, crude protein, crude fibre, and crude fat were not affected. Supplementation also had no effect on nitrogen balance. Hence supplementation of different levels of protected fats did not influence animal performance in Garut sheep.

Keywords: Fat protection, Fat supplementation, Nitrogen balance, Nutrient digestibility, Rumen fermentation characteristics

Introduction

Soybean oil contains around 58% of polyunsaturated fatty acids (PUFA) (de Almeida Chuffa *et al*., 2014). In the rumen digestion process, PUFA undergoes biohydrogenation by rumen microbes into saturated fatty acids, which diminishes the benefits of PUFA for ruminants by reducing the supply of PUFA in the circulation system (Sudibya *et al*., 2009). Essential unsaturated fatty acids are useful in reducing the risk of heart disease in consumers (Harris, 2010).

To decrease biohydrogenation, a formaldehyde-based technique of protection can be employed. Encapsulating fat with protein-bound compounds such as formaldehyde can provide protection (Riyanto *et al.,* 2015). This complex protects fats because formaldehyde forms cross-links with amino acids in proteins, known as methylene bridges (Kiernan, 2000). With this protection, it is believed that unsaturated fatty acids can bypass the rumen and be used by ruminants.

Riyanto *et al.* (2015) examined the use of formaldehyde as PUFA protection. They concluded that formaldehyde content of 37% with a level of 2% could be utilized to protect a mixture of soybean groats and lemuru fish oil in a ratio of 4:1 and can provide both protein and PUFA. Tiven *et al*. (2015) evaluated the protection of oil with formaldehyde *in vivo* in thin-tailed sheep and found that the protection of crude palm oil with formaldehyde is advantageous in terms of feed conversion and has no negative impact on ruminants. However, there are no studies regarding the use of formaldehyde as protection of soybean oil added to Garut sheep.

Nutrient digestibility was measured to know the effect of fat supplementation on feed palatability because fatty acids supplementation of more than 5% may interfere with rumen fermentation (Wina and Susana, 2013). Nitrogen balance was also measured because unsaturated fatty acid supplementation could increase the nitrogen efficiency of Garut sheep, as reported by Kandi *et al.* (2020) that supplemented calsium soap of linseed oil improved the performance in

young lambs which is attributed to improvement in nitrogen efficiency. Hence in this study, nutritional digestibility and nitrogen balance were also assessed to highlight the advantages of soybean oil protection.

Therefore, this study aimed to examine the effect of the combination of protected and non-protected unsaturated fatty acids of soybean oil supplementation on the characteristics of rumen fermentation, nutrient digestibility, and nitrogen balance in Garut sheep. In addition, the results of this study can serve as a reference for breeders or readers considering the addition of protected soybean oil in feed.

Materials and Methods

Animals and treatments

The animal used in this study were 12 Garut male lambs aged 13 months with an average body weight of 29.11±3.23 kg. The twelve sheep were divided into three groups so that four sheep served as replicates in each group. The basal diet of 60% King grass and 40% bran pollard was supplemented with protected and non-protected soybean oil with the ratio of 3%:0% (T1), 1.5%:1.5% (T2), and 0%:3% (T3), respectively, based on the dry matter of ration.

Sample analyses

Proximate analysis of feed ingredients was carried out. First, King grass was chopped and dried in an oven at 55ºC. Next, King grass and pollard bran were ground using a Wiley Mill and filtered using a 1 mm diameter screen. The chemical composition of the feed ingredients was then analyzed using the AOAC method (2005), including the contents of dry matter (DM), organic matter (OM), crude protein (CP), crude fat (CF), and crude fiber (CFb). Afterward, the nitrogen-free extract (NFE) and total digestible nutrient (TDN) were calculated (Hartadi *et al.,* 1997). The chemical composition of feed ingredients is presented in Table 1.

Samples of king grass, pollard bran, and soybean oil were analyzed for saturated and unsaturated fatty acid profiles using the gas chromatography method at Laboratorium Penelitian dan Pengujian Terpadu (LPPT) of Universitas Gadjah Mada, Yogyakarta. The soybean oil protection procedure adhered to the Tiven (2011) procedure. First, soybean oil was weighed and mixed evenly with milk powder in a ratio of 1:2, then 37% technical formaldehyde was added at a level of 2% of the weight of the mixture, then mixed again evenly to form encapsulated soybean oil. The treatment model is presented in Table 2.

Subsequently, the sheep were weighed to determine the initial weight, and then the ration was made according to Kearl (1982). Sheep were kept in metabolic cages and adapted first. Adaptation was carried out for two weeks, during which time consumption and excretion became stable. During the collection period, fecal samples, urine samples, feeding and refusal samples, and rumen fluid samples were taken. All information was recorded, including the amount of refusal, feeding samples, the total amount of feces and samples, and the total amount of urine and samples. Feces samples were collected as much as 10%, placed in black plastic, and then refrigerated. The sampling of refusal and given feed were carried out where the grass was 300 g, and the pollard bran was 100 g. The grass samples were stored in a newsprint and then stapled and identified. Pollard bran samples were stored in transparent and sealable plastic.

The feed samples were stored in an oven at 105°C. Every afternoon, 40 mL of sulfuric acid was added to the urine collection bucket to maintain the content in the urine. In the morning, sulfuric acid was added to the urine sub-sample until the pH reached 3, then 100 mL was taken and stored in the refrigerator. The collection period was seven days. At the end of the period, rumen fluid collection and body weight were measured. When taking rumen fluid, feed particles are filtered with gauze. The rumen fluid was brought to the laboratory and then prepared and analyzed for saturated and unsaturated fatty acid profiles. After that, samples of feed, refusal, feces, and urine were composited. Meanwhile, samples for proximate analysis of DM, OM, CP, CF, and CFb (AOAC, 2005) were samples of feed, refusal, and feces. Samples for analysis of saturated and unsaturated fatty acid profiles were samples of feed, soybean oil, and rumen fluid. Finally, urine

Table 1. Chemical composition of basal feed ingredients (% of DM)

Feedstuff	DM*	OM*	CP*	CF*	CFb*	NFE*	TDN
King grass	20.12	87.25	9.86	2.09	31.42	43.88	56.63
Pollard bran	87.17	93.67	15.45	4.59	9.30	64.33	82.91

* The results of the analysis at the Nutritional Biochemistry Laboratory of the Faculty of Animal Husbandry, Universitas Gadjah Mada.
DM: dry matter, OM: organic matter, CP: crude protein, CF: crude fat, CFb: crude fiber, NFE: nitrogen-free extract, TDN: total digestible nutrient.

Table 2. Treatment model

Treatment	Treatment level (% of DM ration)	
	Protected soybean oil	Non-protected soybean oil
T1	3.0	0.0
T2	1.5	1.5
T3	0.0	3.0

T1: 3%:0%, T2: 1.5%:1.5%, T3: 0%:3%.

samples were used for nitrogen content analysis following the Kjeldahl method (AOAC, 2005).

To determine the microbial protein levels, the rumen fluid was centrifuged at 3,000 rpm for 15 minutes to separate the feed particles. Then, 3 mL of the supernatant was centrifuged again 10,000 rpm. The precipitate obtained was rumen microbes. Determination of the amount of biomass was based on the protein content of microbes using the Lowry method by spectrophotometer. Protein standard curves were made using bovine serum albumin (BSA) as a standard.

To determine the NH_3 levels, the rumen fluid was centrifuged at 3,000 rpm for 15 minutes to separate the feed particles. The filtrate obtained was then used for the analysis of NH_3 levels. NH_3 levels are known by looking at the indophenol reaction, namely the reaction between ammonia and sodium phenate, which is catalyzed to produce a stable blue compound. The solution was then read using a spectrophotometer at 630 nm (Anam, 2020). Volatile fatty acid (VFA) were measured using the Shimadzu GC-8A series gas chromatography method based on Filípek and Dvořák (2009). The pH of the rumen fluid was measured using a calibrated pH meter (Hanna benchtop pH meter).

Statistical analysis

The data were analyzed by One-Way ANOVA. Significant differences continued by Duncan's New Multiple Range Test (Astuti, 1981).

Results and Discussion

Rumen fermentation characteristics

Data on the effect of supplementation with a combination of protected soybean oil and non-protected soybean oil on the fatty acid profile are presented in Table 3. The results showed no significant difference (P>0.05).

Insignificant difference of fatty acid profile among treatment group might because the added oil did not interfere with microbial protein synthesis, this was evidenced by the no difference in the amount of microbial protein in each treatment, shown in Table 4. The lower the microbial protein synthesis, the lower the rumen fatty acids produced in the rumen, because the fat in the rumen will be broken down by microbes. Therefore, the protection of soybean oil using formaldehyde as much as 3% dry matter ration did not change the fatty acid profile of the rumen fluid.

The result of statistical analysis in Table 4 showed no significant difference (P>0.05) in each treatment with supplementation of a combination of protected and non-protected soybean oil on all rumen fermentation characteristic parameters. Microbial protein and ammonia concentration was not affected by supplementation. Anam (2020) reported that *in vitro* supplementation treatment with a combination of pure and protected corn oil with a ratio of 5%:0% did not affect microbial protein compared to controls. Anam (2020) also reported that supplementing a combination of pure and protected corn oil had no effect on rumen NH_3 fermented in vitro. Costa *et al*. (2017) explained that most studies showed no different results when oil supplementation was carried out on rumen NH_3 levels.

Total of VFA, acetate, propionate, and butyrate were not affected. These results are similar to those reported by Hartati (2014), who reported that formaldehyde-protected fat supplementation did not affect rumen volatile fatty acids, acetate, propionate, and butyrate. Anam (2020) reported that the total VFA from supplementation of 5% pure corn oil and 0% protected corn oil was not different from the control. Comparing to Anam (2020) the range of VFA was from 134 to 181 mM, which means it was in the normal range.

Rumen pH was not affected. The proportion of non-structural carbohydrates in the treatment was quite the same. The rumen pH value will change or decrease if the soluble carbohydrates contained in the feed are high. Van Soest (1994) stated that feed with high energy

Table 3. Profile of fatty acids in rumen fluid for each treatment

Fatty acids[ns] (% relative)	Treatment level (% of DM ration)		
	3:0	1.5:1.5	0:3
Butyrate	8.21±3.55	14.58±2.42	13.31±6.23
Caproic	1.70±0.88	2.85±1.83	2.40±1.22
Tridecanoate	2.91±1.24	3.50±0.40	3.25±0.97
Myristate	5.10±2.88	8.55±1.09	7.54±2.47
Mirostoleic	0.00±0.00	0.00±0.00	1.30±0.76
Pentadecanoate	0.29±0.28	0.75±0.74	0.73±0.73
Margarate	3.34±3.33	10.12±3.62	15.25±2.29
Heptadecenoic	0.16±0.07	0.31±0.03	0.28±0.12
Elaidate	0.00±0.00	0.00±0.00	17.15±17.15
Oleate	8.20±3.55	14.58±2.42	13.31±6.23
Linolelaidate	66.46±7.04	54.62±4.40	36.95±13.44
Eicosadienoate	0.42±7.74	0.85±0.40	0.74±0.43
Arachidonate	0.00±0.00	0.00±0.00	0.84±0.50
DHA	0.76±0.46	0.97±0.67	0.20±0.19
Saturated fatty acids	21.54±7.32	40.34±6.01	42.49±7.81
Unsaturated fatty acids	26.17±9.08	34.89±6.79	44.88±7.52
Monounsaturated fatty acids	0.16±0.07	0.30±0.03	18.73±17.63
Polyunsaturated fatty acids	67.64±6.92	56.43±4.18	38.72±13.35

ns = Not significant.

Tabel 4. The levels of microbial protein, NH_3, volatile fatty acid, and rumen fluid fatty acid of Garut sheep supplemented with a combination of protected and non-protected soybean oil (mean±SE)

Parameter [ns]	Treatment level (% of DM ration)		
	3:0	1.5:1.5	0:3
Microbial protein (mg/mL)	0.76±0.08	0.73±0.03	0.99±0.31
NH_3 (mg/100 mL)	7.18±0.85	5.51±0.75	7.60±0.81
VFA total (mM)	134.46±29.95	161.80±15.51	181.89±15.91
Acetate (mM)	82.34±16.63	103.36±10.10	118.52±9.39
Propionate (mM)	37.24±11.96	40.09±3.92	45.37±5.11
Butyrate (mM)	14.87±3.10	18.34±2.17	17.99±1.64
pH	6.15±0.30	6.09±0.08	6.13±0.15

ns = Not significant (P>0.05).

content will be followed by a decrease in rumen pH. The pH value of all treatments ranged from 6.09 to 6.15. According to McDonald *et al*. (2002), typical rumen pH values ranged from 6.0 to 7.0, meaning that the rumen pH values of sheep in this study were within the normal range.

Nutrient digestibility

Data on the effect of supplementation with a combination of protected soybean oil and non-protected soybean oil on nutrient digestibility are presented in Table 5. Statistical analysis showed that protected fat did not significantly affect the digestibility of dry matter, organic matter, crude protein, crude fiber, crude fat, NFE, and TDN. This is consistent with studies conducted by Behan *et al.* (2019) who found that supplementation of rumen-protected fat (RPF) did not influence DM, OM, CP, NDF and ADF digestibilities in Dorper sheep. Naik *et al*. (2009) used long-chain fatty acid calcium soap and did not have a significant effect on dry matter digestibility, organic matter digestibility, crude protein digestibility and crude fiber digestibility. Hartati (2014) reported adding protected fat in feed did not affect the digestibility of feed nutrients except for crude fat digestibility. This is in accordance with

Schroeder *et al*. (2004), that fat supplementation did not affect fiber digestibility in the rumen. The fat that escapes the protection in the rumen may still be able to be properly degraded by lipolytic microbes in the rumen so that it does not stick to the feed fiber which can disrupt the digestibility of the fiber in the rumen.

Nitrogen balance

Statistical analysis showed that protected fat supplementation did not affect N consumption, fecal N excretion, urinary N excretion, digested N, N digestibility, and N balance at various levels. Soybean oil has the potential to reduce microbial protein synthesis because fat can basically interfere with rumen fiber fermentation (Wina and Susana, 2013), so there is a possibility of a negative impact on N balance.

However, in this study, the N balance was positive, meaning that soybean oil had no negative impact on microbial protein synthesis. Retnani *et al*. (2019) reported that male Indonesian local sheep weighing an average of 13.93±1.63 kg with forage:concentrate ratio of 30:70 had a N digestibility of 60.58%. It appears that the digestibility of N sheep in this study was higher possibly due to the differences in the rations given. In addition, there was no significant difference in the amount of N in the ration, so that the N balance was not significantly different. This is in line with the report of McDonald *et al.* (2002), revealed that the value of nitrogen balance was influenced by nitrogen consumed, nitrogen in urine and feces, and nitrogen in livestock products.

Table 5. Nutrient digestibility of Garut sheep supplemented with a combination of protected and non-protected soybean oil (mean±SE)

Digestibility (%)[ns]	Treatment level (%)		
	3:0	1.5:1.5	0:3
Dry matter	75.96±1.66	72.79±2.83	73.61±1,66
Organic matter	77.69±1.86	74.69±2.66	76.77±2,85
Crude protein	71.28±2.56	70.45±4.23	71.81±1,95
Crude fiber	74.69±1.14	66.77±4.82	69.44±3.57
Crude fat	90.58±0.97	87.97±2.72	91.58±0.47
Nitrogen-free extract	79.67±2.44	78.21±1.60	80.18±4.17
Total Digestible Nutrient	73.70±1.71	70.71±2.49	72.76±2.74

ns = Not significant (P>0.05).

Table 6. N consumption, fecal N excretion, urinary N excretion, digested N, N digestibility, and N balance for each treatment (mean±SE)

Parameters[ns]	Treatment level (%)		
	3:0	1.5:1.5	0:3
N consumption (g/day)	16.42±1.00	15.08±0.50	14.61±0.95
Faecal N excretion (g/day)	4.71±0.46	4.48±1.42	4.15±0.48
Urinary N excretion (g/day)	5.80±3.07	4.63±0.69	1.03±0.15
Digested N (g/day)	11.70±0.55	10.60±0.48	10.45±0.54
N digestibility (%)	71.28±2.56	70.45±4.23	71.81±1.95
N balance (g/day)	5.90±3.34	5.96±1.04	9.42±0.45

ns = Not significant (P>0.05).

Conclusions

This study concluded that different levels of combined supplementation of protected soybean oil and non-protected soybean oil did not influence performance of Garut sheep.

Acknowledgment

The author would like to thank all parties who took part in this research, especially the Faculty of Animal Science UGM, which has provided fund research through the final assignment recognition program (RTA) in 2021 so that this research can be conducted.

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