BIO Web of Conferences **167**, 04006 (2025) https://doi.org/10.1051/bioconf/202516704006
ICoSIA 2024

The effect of combination of protected and non-protected soybean oil (*Glycine max* L.) supplementation on *in vitro* nutrient digestibility

Bayu Prasetyo[1], *Dimas Hand Vidya* Paradhipta[1], *Yogi Sidik* Prasojo[1], *Muhlisin*[1], *Lies Mira* Yusiati[1], and *Chusnul* Hanim[1*]

[1]Department of Animal Feed and Nutrition, Faculty of Animal Science, Universitas Gadjah Mada, Yogyakarta, Indonesia

Abstract. This study aims to determine the effect of the combination of protected (PSO) and non-protected (SO) soybean oil supplementation on nutrient digestibility in vitro. Fatty acid protection using rejected milk and formaldehyde. Feed substrate consists of 60% elephant grass and 40% bran pollard, then supplemented with PSO and SO with various levels of balance, namely T1 (0%:5%), T2 (1.25%:3.75%), T3 (2.5%:2.5%), T4 (3.75%:1, 25%), and T5 (5%:0%) based on dry matter (DM). This study consisted of three replications. In vitro, the digestibility of dry matter (IVDMD), organic matter (IVOMD), crude protein (IVCPD), and crude fiber (IVCFD) was determined using a two-stage in vitro technique (Tilley and Terry 1963, modified by Utomo 2012). Data were analyzed using one-way ANOVA, followed by Duncan's test. Results revealed that supplementation with PSO and SO at T4 and T5 significantly increased total and rumen IVDMD and total IVOMD ($P<0.05$), decreased rumen IVCPD, and had no effect on rumen IVOMD, total IVCPD, and both rumen and total IVCFD. In conclusion, 5% PSO supplementation did not negatively affect IVDMD, IVOMD, and IVCFD but reduced IVCPD in ruminants.

1 Introduction

Oil is one of the feed ingredients as an energy source needed by livestock with high production levels, maintaining a balance between structural and non-structural carbohydrates and optimizing the utilization of digestible energy [1]. Oil supplementation rich in polyunsaturated fatty acids has a positive impact, which can be used as a methane gas production reducing agent that captures hydrogen (hydrogen sink) in the biohydrogenation process used in methane gas formation [2]. In addition, oil can suppress toxicity effects on protozoa and methanogens, and some rumen microbes that digest fiber [3].

In addition to the positive impact, oil supplementation rich in unsaturated fatty acids in ruminant feed has a negative effect, which can be a barrier to digestibility in the rumen. This is because the oil will envelop the feed particles so that the work of enzymes in the rumen is not maximized [4]. Oil supplementation of more than 5% of the dry matter of the ration has a negative impact by coating the feed and physically blocking access for digestion or binding to the surface of microbial cell membranes, which can inhibit enzyme function and microbial enzyme production. Therefore, excess oil supplementation in feed can inhibit microbial activity in the rumen [5]. During the digestive process in the rumen, unsaturated fatty acids will undergo a biohydrogenation process by rumen microbes into saturated fatty acids [6].

One way to reduce the negative impact of oil supplementation for ruminants is to protect feed sources of fatty acids, especially unsaturated fatty acids with formaldehyde. Oil protection with formaldehyde in oil supplementation in ruminant feed is one way to reduce the negative effects of fat for ruminants so that feed sources of fatty acids are not degraded in the rumen [7]. The principle of using formaldehyde in protecting fatty acids is that formaldehyde will form chemical bonds with proteins that are stable at neutral pH, which is the pH in the rumen, and will be labile at acidic pH, which is the pH of the abomasum [8].

Soybean is a high-protein feed ingredient [9,10] and an oil producer rich in unsaturated fatty acids. Soybean oil contains 85% unsaturated fatty acids, the majority of which is 53.86% linoleic acid [11]. Unsaturated fatty acids contained in soybean oil supplemented in ruminant feed can improve the chemical quality of meat and milk [12, 13]. However, previous studies have neither focused on nor verified the evaluation of the effects of combining protected and non-protected soybean oil supplementation on the efficiency of nutrient digestibility in ruminants under in vitro conditions. Therefore, this study was conducted to determine the effect of the combination of protected and non-protected soybean oil (*Glycine max* L.) on nutrient digestibility *in vitro*.

* Corresponding author: c.hanim@ugm.ac.id

2 Materials and methods

2.1 Materials

Materials used in this study include soybean oil, skim milk powder, 37% technical formaldehyde, rumen fluid of fistulous Balinese cattle, and feed substrates including elephant grass (*Pennisetum purpureum*) and bran pollard (60:40), thoroughly homogenized and ground to pass 1 mm screen.

2.2 Capsulation of soybean oil

The procedure for capsulation of unsaturated fatty acids from soybean oil follows the method described by Tiven et al. [7]. The preparation of protected fatty acids using soybean oil begins with soybean oil and discarded milk powder as a source of aldehyde-binding protein weighed in a ratio of 1:2 based on DM sample rations and then mixed until evenly distributed. The mixture was then added with 37% technical formaldehyde at a dose of 2% of the total weight of the mixture. The mixture was then stirred again until homogeneous to form protected soybean oil. There were five treatments of the combination of PSO:SO, which was evaluated using *in vitro* nutrient digestibility: T1 (0%:5%), T2 (1.25%:3.75%), T3 (2.5%:2.5%), T4 (3.75%:1, 25%), and T5 (5%:0%).

2.3 Incubation and analytic methods

Fistulous Balinese cattle were fed an adapted diet for one week, and then rumen fluid was collected before feeding them in the morning. The rumen fluid was then filtered using six-layer gauze and put into an insulated bottle with a temperature of 39ºC. Determination of nutrient digestibility values using in vitro method [14] modified by Utomo [15]. In vitro nutrient digestibility was analyzed in two stages, namely stage one (48 h) and stage two (96 h). The ratio of rumen fluid and buffer medium was 1:4 (v/v). Twenty-five milliliters of rumen buffer liquid were put into 50 ml glass tubes to determine IVDMD and IVOMD, and 50 ml of rumen buffer liquid was put into 100 ml glass tubes to determine IVCPD and IVCFD. Fermenters were duplo for each treatment and incubated at 39ºC for 48 hours and 96 hours. Chemical content analysis included IVDMD, IVOMD, IVCPD, and IVCFD using [16] method.

2.4 Statistical analysis

One-way analysis of variance (ANOVA) design was used to analyze the data. The differences found among means were tested by Duncan's Multiple Range Test (DMRT) using SPSS version 25.0.

3 Result and Discussion

The results showed that the T5 combination treatment significantly (P<0.05) increased DMD in the rumen and total when compared to T1, T2, T3, and T4 combinations (Table 1). The results further showed that there was no significant difference (P>0.05) with the supplementation of a combination of protected and non-protected soybean oil at all levels of the combination of IVOMD in the rumen (Table 1). In contrast to IVOMD in the rumen, T5 significantly (P<0.05) increased total IVOMD when compared to T3 and T4 (Table 2).

The highest IVDMD and IVOMD results were oil supplementation in T5 given full protection treatment. This shows that the negative impact of soybean oil supplementation can be minimized such as toxic effects on rumen microbes. This is in accordance with the opinion of Wina and Susana[17], which states that unprotected oil can reduce feed digestibility higher when compared to protected oil. Oil protection is a solution to prevent the negative effects of oil through direct toxic effects on rumen microbes. Research conducted by Widianingrum et al. [18] showed that adding 250 µL plus 2% formalin increased IVDMD and IVOMD both in the rumen and post-rumen. This is in line with the opinion of Anam et al. [19], who stated that a greater level of corn oil protection gave higher IVDMD and IVOMD values. The positive impact of supplementation of protected soybean oil can be seen in Hanim's research (2021-unpublished) which shows that supplementation of protected soybean oil with an increasing ratio in feed has a positive effect on the fatty acid profile, namely higher unsaturated fatty acid levels when compared to saturated fatty acids which are 58%-67% and 28%-38% respectively.

Table 1. IVDMD in the rumen (48 h) and total (96 h) of feed supplementation of protected (PSO) and non-protected (SO) soybean oil in several ration.

Treatments[1]	IVDMD(%)[2]	
	48 h	96 h
T1	37,72^{a}±1,88	50,92^{a}±0,82
T2	40,28^{a}±0,70	49,52^{a}±1,69
T3	40,94^{a}±4,00	50,14^{a}±5,14
T4	40,95^{a}±4,14	51,84^{a}±2,27
T5	48,12^{a}±2,35	57,41^{b}±0,37

[1]T1: PSO 0% and SO 5%; T2: PSO 1,25% and SO 3,75%; T3: PSO 2,5% and SO 2,5%; T4: PSO 3,75% and SO 1,25%; T5: PSO 5% and 0%.
[2]IVDMD: in vitro dry matter digestibility
[abcd]superscripts within the same row indicate significant difference (P<0.05)

The results of rumen IVOMD, which showed no significant difference (P>0.05), were caused by the high portion of forage feed in the ration used (Table 2). In accordance with the opinion of Faizah et al. [20], the absence of a significant difference is due to the high ratio of the accompanying feed contained in the feed ration used. A high proportion of fiber in feed can create a stable rumen atmosphere for microbes in digesting feed. Previous research [21-23] stated that the proportion of feed fiber as much as the portion of fiber in the ration of 60% can create ideal environmental conditions for rumen microbial activity. Agree with Adeyemi et al. [24] who stated that Corotino oil supplementation in rations with a balance of forage and concentrates 50:50 also did not have a significant effect on feed digestibility.

Table 2. IVOMD in the rumen (48 h) and total (96 h) of feed supplementation of protected (PSO) and non-protected (SO) soybean oil in several ration.

Treatments[1]	IVOMD(%)[2]	
	48 h[ns]	96 h
T1	39,60±0,46	51,97[ab]±0,52
T2	39,51±0,38	50,58[ab]±1,51
T3	38,40±4,01	48,59[a]±4,93
T4	38,99±4,04	47,32[a]±1,60
T5	44,95±0,34	55,01[b]±0,33

[1]T1: PSO 0% and SO 5%; T2: PSO 1,25% and SO 3,75%; T3: PSO 2,5% and SO 2,5%; T4: PSO 3,75% and SO 1,25%; T5: PSO 5% and 0%.
[2]IVOMD: in vitro organic matter digestibility
[abcd]superscripts within the same row indicate significant difference (P<0.05)
[ns]not significant

The results showed that T3, T4, and T5 significantly (P<0.05) decreased IVCPD in the rumen when compared to T1(Table 3). However, T2 did not show any decrease in IVCPD in the rumen. In contrast to IVCPD in the rumen, the results showed no significant difference with the supplementation of a combination of protected and unprotected soybean oil at all combination balances on total IVCPD (Table 3).

The highest IVCPD results were seen in T1. This is thought to be due to the absence of formaldehyde addition in the feed used in protecting soybean oil. The use of formaldehyde is thought to exceed the needs in protecting the oil of skimmed milk powder, so that free formaldehyde does not bind to skimmed milk powder. The use of aldehyde as a protection agent may have a negative impact, namely being one of the defaunation agents for the presence of protozoa in the rumen, so that it can reduce the population of protozoa that are responsible for degrading proteins in the rumen. McDonald et al. [25] stated that protein in feed will be hydrolyzed into peptides and amino acids by rumen microbes, one of which is protozoa. Protozoa in the rumen are one of the main proteolytic organisms that play a role in protein degradation. Gebeyehu and Mekasha [26] stated that defaunation is the removal of protozoan microfauna from the rumen in various ways. One of the methods used for defaunation is using chemicals. Wandra et al. [27] stated that formaldehyde is a chemical that can kill protozoa cells. The use of formaldehyde in ruminant feed is sufficient with 1% formaldehyde only. Increasing the level of formaldehyde is not necessary, considering that formaldehyde is a hazardous chemical that in large quantities can reduce microbial activity [28], especially the protozoa population [29]. This is in accordance with the results of research on T3, T4, and T5 which show that the higher the level of formaldehyde protection in soybean oil, the IVCPD value will decrease as well. In line with research conducted by Yusuf et al. [30] which showed that sheabutter fat supplementation at the 5% level resulted in the highest IVCPD then decreased at the 10% and 0% levels. Wulandari et al. [31] showed that supplementation of 0.4% protected CPO caused a decrease in crude protein digestibility in the rumen.

Table 3. IVCPD in the rumen (48 h) and total (96 h) of feed supplementation of protected (PSO) and non-protected (SO) soybean oil in several ration.

Treatments[1]	IVCPD(%)[2]	
	48 h*	96 h[ns]
T1	52,84[c]±7,25	67,71±14,82
T2	45,32[bc]±6,68	56,49±15,46
T3	42,90[b]±3,40	50,71±18,79
T4	33,61[a]±0,41	61,39±3,01
T5	37,58[ab]±1,99	54,50±16,48

[1]T1: PSO 0% and SO 5%; T2: PSO 1,25% and SO 3,75%; T3: PSO 2,5% and SO 2,5%; T4: PSO 3,75% and SO 1,25%; T5: PSO 5% and 0%.
[2] IVCPD: in vitro crude protein digestibility
[abcd]superscripts within the same row indicate significant difference (P<0.05)
[ns]not significant
*superscripts within the same row indicate highly significant difference (P<0.01)

IVCPD total results that show no significant differences are due to the strength of the protection in the rations used, and presumably the composition of nutrients, especially protein, which is not much different between treatments. Anam et al. [19] stated that formaldehyde has the ability to bind to proteins found in skim milk which in turn will protect corn oil in feed rations. Another factor that causes no difference in crude protein digestibility is the low proportion of protein from skim milk added to the ration. Another factor that causes no significant difference in total IVCPD is the rate of protein digestibility that occurs in the abomasum, which is fairly short so that the protein in the feed, especially the protected protein (skim milk), is not completely released and causes incomplete protein degradation. In line with the opinion of Riyanto et al. [32], protein degradation can be influenced by the rate of digestibility and the length of feed in the digestive tract. The highly digestible crude protein will produce more crude protein that the livestock's body can utilize. Anam et al. [19] showed that supplementation of protected corn oil with a balance of 1.25%, 2.5%, 3.75%, and 5% was not significantly different in total crude protein digestibility. In line with the research of [33] showed that supplementation of 2.99% soybean oil and 2.99% protected unsaturated fatty acids did not give a significant difference to total IVCPD.

Table 4. IVCFD in the rumen (48 h) and total (96 h) of feed supplementation of protected (PSO) and non-protected (SO) soybean oil in several ration.

Treatments[1]	IVCFD(%)[2]	
	48 h[ns]	96 h[ns]
T1	36,54±2,24	40,81±5,73
T2	33,15±6,84	42,01±7,95
T3	40,17±1,81	35,59±18,79

T4	38,25±11,82	40,41±2,86
T5	35,71±1,22	38,04±5,03

[1]T1: PSO 0% and SO 5%; T2: PSO 1,25% and SO 3,75%; T3: PSO 2,5% and SO 2,5%; T4: PSO 3,75% and SO 1,25%; T5: PSO 5% and 0%.
[2] IVCFD: in vitro crude fibre digestibility
[ns]not significant

IVCFD results showed that there was no significant difference with the supplementation of a PSO and SO at all combination balances on IVCFD in the rumen and total (Table 4). This is thought to be the impact of supplementing non-protected soybean oil in the ration at a level of ≤5%. Oil supplementation with a level of ≤5% of the ration DM is still within normal limits, so it does not have a negative effect on the digestibility of feed crude fiber in the rumen. In line with the opinion of [28] stated that the use of fat in ruminant rations is very limited, namely a maximum of 5% of the ration BK because it can inhibit the activity of rumen microbes in degrading crude fiber. This statement is supported by the opinion of Anam et al. [19] stated that the addition of oil >5% of the ration DM can suppress the ability and inhibit the metabolism of rumen microbes because oil can wrap feed and close the surface access of microbial cell membranes in contact with feed which in turn can interfere with enzyme production to degrade feed.

The main reason for this is the protection of the oil, which does not or suppresses the negative impact on rumen microbes. In line with Tanuwiria et al. [34] who stated that protection is a form of feed manipulation in the rumen that aims to maximize the supply of nutrients for livestock. Protection treatment of oil causes rumen microbial activity to be normal because the protected oil will go directly to the abomasum. Normal microbial activity indicates the amount of feed consumed, and the rate of feed in the rumen is no different. The faster flow rate of feed particles leaving the rumen causes the potential for feed ingredients to be degraded by rumen microbes to be shorter so that it does not interfere with fiber digestibility.

In line with the research of [1], which showed that soybean oil supplementation of as much as 2.6% had no significant difference (P>0.05) in crude fiber digestibility both in the rumen and total. In line with Jalc et al. [35], the supplementation of different unsaturated fatty acids, namely oleic, linoleic, and linolenic acids, of 3.5% in the ratio, did not reduce NDF degradation. This indicates that oil supplementation ≤5% of ration BK does not have a negative impact on crude fiber digestibility. The opposite results in the research of Alexander et al. [36] which showed that supplementation of 10% sunflower oil significantly decreased crude fiber digestibility.

In conclusion, supplementing protected soybean oil and soybean oil at a certain ratio on feed can affect the nutrient digestibility of the rumen and post-rumen fermentation. The supplementation of a combination of protected and non-protected soybean oil 5%:0% doesn't have a negative effect on dry matter digestibility, organic matter digestibility, and crude fiber digestibility, but it does negatively affect crude protein digestibility in ruminants.

References

1. G. Fiorentini, J. Messana, P. Dian, R. Reis, R. C. Canesin, A. Pires, and T., Berchielli, Digestibility, fermentation, and rumen microbiota of crossbred heifer fed diets with different soybean oil avaibilities in the rumen. Animal Feed Science and Technology. **181**, 26-34 (2013). https://doi.org/10.1016/j.anifeedsci.2013.01.011
2. N. Toprak, Do fats reduce methane emission by ruminants? - a review. Anim. Sci. Pap. and Rep. **33**(4) (2015).
3. K. Beauchemin, S. Mcginn, C. Benchaar, and L. Holtshausen, Crushed sunflower, flax, or canola seeds in lactating dairy cows diets: effects on methane production, rumen fermentation, and milk production. J. Dairy Sci. **92**, 2118-2127 (2008). https://doi.org/10.3168/jds.2008-1903
4. A. Hristov, M. Pol, M. Angle, S. Zaman, C. Schneider, P. Ndegwa, V. Vaddella, K. Johnson, K. Shingfield, and S. Karnati, Effect of lauric acid and coconut oil on ruminal fermentation, digestion, ammonia losses from manure, and milk fatty acid composition in lactating cows. J. Dairy Sci. **92**, 5561-5582 (2009). https://doi.org/10.3168/jds.2009-2383
5. M. Johnson, Understanding rumen fermentation: I. Effect of high DHA algal oil on microbial biohydrogenation and II. Monitoring microbial shifts in response to antibiotics and oil using T-RFLP analysis, Master Thesis, North Carolina State University, California, 2009
6. G. Vilela, H. Parente, R. Bessa, S. Alves, H. Cavalcante, A. Zanine, L. Bezerra, D. Ferreira, G. Oliveira, F. Dos Santos, R. Oliveira, M. Parente, Effect of replacing babassu oil with sunflower oil on nutrient utilization, rumen fermentation, and biohydrogenation estimate in sheep. Small Ruminant Research. **237**, 107321 (2024) https://doi.org/10.1016/j.smallrumres.2024.107321
7. N. Tiven, L. Yusiati, Rusman, and U. Santoso, Ketahanan asam lemak tidak jenuh dalam crude palm oil terproteksi terhadap aktivitas mikrob rumen domba *in vitro*. Media Peternakan. **34**, 42-49 (2011) http://dx.doi.org/10.5398/medpet.2011.34.1.42
8. T. Scott, and J. Ashes, Dietary lipids for ruminants: protection, utilization and effects on remodelling of skeletal muscle phospholipids. Australian Journal of Agriculture Research. **44**(3) (1993) https://doi.org/10.1071/AR9930495
9. Y. Prasojo,M. Niimi, M. Hashiguchi, M. Muguerxa, and G. Ishigaki, The effects of the different sowing methods and planting density on the yield components of soybean (*Glycine max*) under intercropping condition with rhodes grass

(*Chloris gayana* Kunth.). Australian Journal of Crop Science. **17,** 10 (2023) http://doi.org/10.21475/ajcs.23.17.10.p3880

10. Y. Prasojo, G. Ishigaki, M. Hashiguchi, M. Muguerza, R. Akashi, Evaluation of regrowth ability of soybean for forage utilization under two-cutting system. Australian Journal of Crop Science. **15**, 1452-1458 (2021) http://doi.org/10.21475/ajcs.21.15.12.p3315
11. I. Isa, Penetapan asam lemak linoleat dan linolenat pada minyak kedelai secara kromatografi gas. Saintek. **6**(1)((2011)
12. H. Sultana, T. Ishida, T. Shintaku, S. Kanda, and H. Itabashi, Effect of feeding ca-salts of fatty acids from soybean oil and linseed oil on c9,t11-cla production in ruminal fluid and milk of holstein dairy cows. Asian-Australian Journal of Animal Science. **21**(9) (2008) https://doi.org/10.5713/ajas.2008.60730
13. P. Patriani, J. Sumarmono, and W. Suryapratama, Kualitas karkas domba lokal yang diberi pakan jerami padi fermentasi dengan suplementasi minyak kedelai. Jurnal Agripet. **10**(2) (2010) http://dx.doi.org/10.17969/agripet.v10i2.645
14. J. Tilley, and R. Terry, A two stage technique for in vitro digestion of forage crop. J. Brit. Grass. Soc. **18**, 104-111 (1963) http://dx.doi.org/10.1111/j.1365-2494.1963.tb00335.x
15. R. Utomo, Modifikasi metode penetapan kecernaan in vitro bahan kering atau bahan organik. Sintesis, **5**, 1-11 (2010)
16. AOAC, Official Methods of Analysis. 12th ed., (Association of Official Analytical Chemist, Washington D. C., 2005)
17. E. Wina, and I. Susana, Manfaat lemak terproteksi untuk meningkatkan produksi dan reproduksi ternak ruminansia. WARTAZOA. **23**(4)(2013)
18. D. Widianingrum, S. Salasia, dan C. T. Noviandi., Kecernaan dan karakteristik fermentasi rumen *in vitro* ransum ruminansia dengan suplementasi virgin coconut oil terproteksi, in Proceeding National Seminar on Animal Husbandry and Veterinary Technology. Jember, Indonesia, October 14-17 (2019)
19. M. Anam, L. Yusiati, C. Hanim, Z. Bachruddin, and A. Astuti, Effect of combination of protected and non-protected corn oil supplementation on in vitro nutrient digestibility. 4th Animal Production International Seminar, Malang, Indonesia, October 24-27 (2019). **478** http://dx.doi.org/10.1088/1755-1315/478/1/012026
20. L. Faizah, Widiyanto and A. Muktiani, The effect of total or partial protected vegetable oil supplementation on in vitro digestibility, feed fermentability and energy efficiency. Buletin Peternakan. **3**, 171-178 (2019)
21. T. Jenkins, Lipid metabolism in the rumen. Journal of Dairy Science. **76**(12) (1993) https://doi.org/10.3168/jds.S0022-0302(93)77727-9
22. K. Ueda, A. Ferlay, J. Chabrot, K. Loor, Y. Chilliard, and M. Doreau, Effect of linseed oil supplementation on ruminal digestion in dairy cows fed diets with different forage:concentrate ratios. Journal Dairy Science. **86**(12) (2003) https://doi.org/10.3168/jds.S0022-0302(03)74011-9
23. J. Messana, T. Berchielli, P. Arcuri, R. Reis, R. Canesin, A. Ribeiro, G. Fiorentini, and J. Fernandes, Rumen fermentation and rumen microbes in Nellore steers receiving diets with different lipid contents. Revista Brasileira de Zootecnia. **42**(3) (2013) http://dx.doi.org/10.1590/S1516-35982013000300008
24. K. Adeyemi, M. Ebrahimi, A. Samsudin, A. Alimon, R. Karim, S. Karsani, and A. Sazili, Influence of carotino oil on in vitro rumen fermentation, metabolism and apparent biohydrogenation of fatty acids. Animal Science Journal. **86**(3) (2015) https://doi.org/10.1111/asj.12279
25. McDonald, P., R. A. Edward, J. F. D. Greenhalgh, C. A. Morgan, L. A. Sinclair, and R. G. Wilkinson, Animal Nutrition Seventh Edition (Longmans Scientific and Technological, New York, 2010)
26. A. Gebeyehu, and Y. Mekasha, Defaunation: effects on feed intake, digestion, rumen metabolism and weight gain. Wudpecker Journal of Agricultural Research. **2**(5) (2020)
27. F. Wandra, A. Pranowo, I. Hernaman, U. Tanuwiria, dan B. Ayuningsih, Fermentabilitas ransum yang mengandung ampas bir dalam cairan rumen (in vitro). Jurnal Sains Peternakan Indonesia. **15**(2) (2020) https://doi.org/10.31186/jspi.id.15.2.227-235
28. X. Sun, Y. Wang, X. Ma, S. Li, and W. Wang, Producing natural functional and low-carbon milk by regulating the diet of the cattle—The fatty acid associated rumen fermentation, biohydrogenation, and microorganism response. Frontier in Nutrition. **9**, 955846 (2022) https://doi.org/10.3389/fnut.2022.955846
29. L. Thanh, N. Phakachoed, W. Suksombat, J. Loor, and T. Hang, Partial substitution of fish oil for linseed oil enhances beneficial fatty acids from rumen biohydrogenation but reduces ruminal fermentation and digestibility in growing goats. Translational Animal Science. **5**(3), 1-13 (2021) https://doi.org/10.1093/tas/txab116
30. A. Yusuf, O. Olafadehan, C. Obun, M. Inuwa, M. Garba, and S. Shagwa, Nutritional evaluation of sheabutter fat in fattening of yankasa sheep. Pakistan Journal of Nutrition. **8**, 7 (2009) https://doi.org/10.3923/pjn.2009.1062.1067

31. Wulandari, B. Widyobroto, C. Noviandi, and A. Agus, In vitro digestibility and ruminal fermentation profile of ruminant diet in response to substitution of mixture feedstuff protected. Livestock Research for Rural Development. **32**(12) (2020)
32. J. Riyanto, S. Widyawati, dan Sudibya, Pengaruh perbedaan rasio menir kedelai proteksi dan tanpa proteksi terhadap konsumsi, kecernaan dan nilai nutrien pakan domba ekor gemuk. Livestock and Animal Research. **18**(3) (2020) https://doi.org/10.20961/lar.v18i3.45995
33. F. Renno, J. Junior, J. Gandra, M. Filho, L. Verdurico, R. Barletta, and F. Vilela, Effect of unsaturated fatty acid supplementation on digestion, metabolism and nutrient balance in dairy cows during the transition period and early lactation. Revista Brasileira de Zootecnica. **43**(3) (2014) https://doi.org/10.1590/S1516-35982014000400008
34. U. Tanuwiria, D. Budinuryanto, S. Darodjah, dan W. Putranto, Studi suplemen kompleks mineral minyak dan mineral-organik dan pengaruhnya terhadap fermentabilitas dan kecernaan ransum in vitro serta pertumbuhan pada domba jantan. Jurnal Protein. **14**(2) (2006)
35. D. Jalc, M. Certik, K. Kundrikova, and P. Namestkova, Effect of unsaturated C18 fatty acids (oleic, linoleic and α-linolenic acid) on ruminal fermentation and production of fatty acid isomers in an artificial rumen. Veterinarni Medicina. **52**(3) (2007) http://dx.doi.org/10.17221/2012-VETMED
36. G. Alexander, Z. Rao, and J. Prasad, Effect of supplementing sheep with sunflower acid oil or its calcium soap on nutrient utilization. Asian-Australasian Journal of Animal Sciences. **15**(9) (2002) https://doi.org/10.5713/ajas.2002.1288