

Effect of Indigenous probiotics supplementation in paddy straw silage and its effect on performance and blood lipids in Priangan ewe lambs

Diky Ramdani^{1*}, Wendry Setiyadi Putranto², Eulis Tanti Marlina², Jois Harsa¹, Aghnya Aulia Hanifa¹, Gunawan Jun Yizreel Manihuruk¹, Rina Agustina¹ and Ririn Siti Rahmatillah¹

¹University of Padjadjaran, Department of Animal Production, Faculty of Animal Husbandry, Sumedang, West Java, 45363 Indonesia

²University of Padjadjaran, Department of Animal Products Technology, Faculty of Animal Husbandry, Sumedang, West Java, 45363 Indonesia

*Corresponding author: diky.ramdani@unpad.ac.id

Abstract

Ramdani, D., Putranto, W. S., Marlina, E. T., Harsa, J., Hanifa, A. A., Manihuruk, G. J. Y., Agustina, R. & Rahmatillah, R. S. (2025). Effect of Indigenous probiotics supplementation in paddy straw silage and its effect on performance and blood lipids in Priangan ewe lambs. *Bulg. J. Agric. Sci.*, 31(1), 183–189

Paddy straw can be an affordable alternative fiber source to feed sheep. Ensiling can preserve and improve the quality of paddy straw silage (PSS). A completely randomized experimental design was used to compare the effect of 3 different doses of indigenous probiotics inclusion at 0% (iProbiotic-0), 4% (iProbiotics-4) and 8% (iProbiotics-8) in PSS based diet on dry matter intake (DMI, g/head/day), average daily gain (ADG, g/head/day), total cholesterol (mg/dL), triglycerides (mg/dL), low-density lipoprotein (LDL, mg/dL), and high-density lipoprotein (HDL, mg/dL) in the blood of Priangan ewe lambs during 70 days feeding trial using 6 replicates (n = 6). The results showed that Probiotics-8 treatment increased (P < 0.05) total DMI, but it did not have any impact (P > 0.05) on ADG, blood cholesterol, triglycerides, LDL, and HDL values of the lambs. Increased DMI of lambs consuming iProbiotic-treated PSS is a sign that indigenous probiotics inclusion during PSS preparation increases the palatability of PSS. The PSS can be an alternative and more affordable fiber source for sheep feeding in high rice producing countries.

Keywords: blood lipids; probiotics; paddy straw silage; performance; Priangan sheep

Introduction

Sheep is a potential livestock commodity to be developed in the rural communities of developing countries such as Indonesia. Indonesia's sheep population reached 17.794.344 heads in 2019 where West Java is one of the provinces that had the highest sheep population with a figure of 12.014.083 heads (Statistical Central Bureau, 2019). Priangan sheep (Decree of Indonesian Agricultural Minister No. 300/Kpts/SR.120/5, 2017) is the second most popular local breed in West Java after Garut sheep (Decree of Indonesian Agricul-

tural Minister No. 2914/Kpts/OT.140/6/2011). There is still an insufficient study on good feeding practices for Priangan sheep using available local feed sources.

Sheep development in West Java and other provinces is still dependent upon small-scale traditional farmers, which may be associated with poor farming management, low sheep performance, and inefficiency. As a highly populated province, sheep development in West Java has been threatened by the massive conversion of grasslands into plantations, residential, and industries leading to a significant reduce of high-quality forage availability for sheep feeding

especially during the dry season. In this situation, agricultural wastes are highly potential for their availability to be alternative feed sources in particular to paddy straw because most Indonesian people consume rice as the main staple food (Ramdani et al., 2020).

However, paddy straw has low nutritional values and high in crude fiber fractions includes lignin and silica making the straw is less digestible in the rumen compared with grasses (Ramdani et al., 2020). The ensiling process may preserve and improve the quality of paddy straw. Probiotics are likely required as a catalyst during the fermentation process so that the quality of paddy straw silage can be optimized. There are several commercial probiotics available in the markets, but local farmers can actually make their own. Probiotic liquid can be made by extracting old paddy straw silage and it may contain indigenous microbes that are more suitable for making a paddy straw silage.

Adding probiotics into a diet of ruminants has the potential to manipulate rumen fermentation and enhance animal productivity (Elghandour et al., 2015). Feed probiotics may control blood lipid profiles, reduce total cholesterol, Low-Density Lipoprotein (LDL), and increase High-Density Lipoprotein (HDL) in the blood, where the lipid profile in the blood may reflect the lipid profiles in the meat products. Increased levels of HDL will transport cholesterol from peripheral tissues to the liver, removing excess LDL deposits, where LDL plays a role in the delivery of cholesterol from the liver to all body tissues. The lipid profile in blood is one of the parameters that can be used to determine the health condition and productivity of the livestock. The lipid profiles include levels of total cholesterol, LDL cholesterol, HDL cholesterol, and triglycerides. Therefore, it is necessary to control the lipids in the blood of the livestock appropriately.

This experiment aimed to test the hypothesis that adding Indigenous probiotics (Iprobiotics) during the ensiling process of paddy straw silage preparation would not only increase dry matter intake (DMI, g/head/day), average daily gain (ADG, g/head/day), and HDL in blood (mg/dL), but also reduce the levels of blood cholesterols (mg/dL), LDL (mg/dL), and triglycerides (mg/dL) in Priangan ewe lambs over 70 days feeding trial.

Materials and Methods

Animals

The use of experimental animals in this study has been approved by Research Ethics Committee of Universitas Padjadjaran (Ethical Approval Number: 307/UN6.KEP/EC/2021). Eighteen Priangan ewes (about 10 months old) with an average initial body weight of 16.6 kg (coefficient of variation

12.5%) were used in this experiment and were divided into 3 different groups. Each lamb was randomly located in an individual pen (1.2 m long × 0.8 m wide × 0.9 m high) separated by wood panels through which each of them had visual and part-physical contact. Each lamb was initially subjected to an adaptation period of 14 days where each of them was fed 200 g of concentrate, 1.5 kg of experimental paddy straw silage (PSS) and ad libitum access to clean water. During adaptation, each lamb was sheared, cleaned, given anthelmintic orally (5 ml, Kalbazen-SG, PT. Kalbe Farma, Bekasi), and injected with B complex vitamins (2 ml, B-Kompleks, PT. Medion, Bandung).

Indigenous Probiotics Starter

Experimental PSS was prepared by ensiling paddy straw with the help of an Indigenous probiotic (iProbiotics) starter supplementation. The iProbiotics was in liquid form collected from more than 6 months old natural paddy straw silage. Briefly, chopped paddy straw (5–10 cm long) was adequately placed into 3 blue plastic barrels (120 L capacity each) as compact as possible to obtain oxygen-free condition before being closed tightly each using a lid with a metal fastener. Here, chopped paddy straw was naturally ensiled in each barrel without adding any probiotic starter. After 6 months, each barrel containing natural PSS was opened and the iProbiotics liquid was extracted and pooled from the PSS in all 3 barrels. The final iProbiotics starter was made by mixing iProbiotics liquid with molasses at a ratio 1:10 in a clean barrel and the mixture was diluted 10 times using clean tap water and kept in a room temperature for 7 days before being used as iProbiotic starter. Tabel 1 shows the total fungi, total lactic acid bacteria, and total yeast in the iProbiotic starter.

Table 1. Total Fungi, Total Lactic Acid Bacteria, and Total Yeast (CFU/mL) in iProbiotics Starter

	Total Fungi (CFU/mL)	Total Lactic Acid Bacteria (CFU/mL)	Total Yeast (CFU/mL)
iProbiotics starter	1.71×10^6	2.31×10^7	2.67×10^5

CFU: colony forming unit

Experimental Diets

Experimental diets consisted of PSS and a commercial concentrate. PSS was prepared by chopping fresh paddy straw using a chopper machine (5–10 cm long) adequately. A sufficient iProbiotics starter was also prepared and further diluted 10 times with clean tap water before being mixed thoroughly. After that, chopped paddy straw was gradually put into each blue plastic barrel (120 mL capacity) whilst, at the same time, diluted iProbiotics starter was sprayed evenly onto the straw. It was then to press the straw inside each

barrel to make it as compact as possible and to maximize oxygen-free condition. Finally, each barrel was closed tightly with a lid and metal fastener and kept it for 21 days before the experimental PSS can be ready to use.

There were 3 different doses (% or kg/100 kg) of diluted iProbiotic starter in Paddy straw during PSS preparation: (1) 0% (iProbiotic-0), 4% (iProbiotic-4), and 8% (iProbiotic-8). In addition, a commercial concentrate was purchased from a local feed mill located in the Wanaraja subdistrict, Garut regency. The nutrient contents of different PSS and concentrate can be seen in Table 2. During the 14-day adaptation and 70 days feeding trial, each lamb was fed 2 times a day in the morning at 08.00 (200 g concentrate and 700 g PSS) and in the afternoon at 16.00 (800 g PSS) while clean water drink was provided ad libitum. Any feed refusals were collected and weighed in the morning at about 07.00 (before morning feeding).

Total Fungi, Lactic Acid Bacteria, and Yeast Measurements

Total fungi, lactic acid bacteria, and yeast were measured in accordance with the procedure of Aerobic Plate Count (FDA, 2020). Briefly, a 1 ml starter sample was placed in a sterile tube and 9 ml of 0.9% NaCl solution was added and vortexed for homogenization for about 1 minute. This is a solution with 10^{-1} dilutions. One ml suspension with 10^{-1} dilution is transferred with the sterile pipette to solution 9 ml NaCl 0.9% to obtain 10^{-2} dilution. Dilutions were continued to 10^{-3} , 10^{-4} , 10^{-5} , 10^{-6} , 10^{-7} . About 0.1 ml of 10^{-4} , 10^{-5} , 10^{-6} , and 10^{-7} diluted solutions were cultivated in De Man Rogosa and Sharpe Agar (MRSA, Lactic Acid Bacteria), Potato Dextrose Agar (PDA, Fungi), and Malt Extract Agar (MEA, Yeasts) and incubated at 37°C for 24 h (MRSA) and 25°C for 48 h (PDA and MEA). The total colonies of lactic acid bacteria, fungi, and yeasts were calculated using Standard Plate Count method in a unit measurement of colony forming unit (CFU/ml) as referred to Indonesia National Standard (SNI 2897: 2008).

Chemical Analysis

Either PSS (in blue plastic barrels) or concentrate (in sacks) was randomly sampled in 3 or 5 different parts and pooled. Each sample was dried in an oven at 60°C for about 48 h. Each dried sample was then ground to pass through a 1-mm sieve in a sample disc mill before being subjected to various nutrient analyses using standard protocols of the Association of Official Analytical Collaboration (AOAC, 2005) to determine crude protein (CP, AOAC 990.03), ash (AOAC 942.05), ether extract (EE, AOAC 920.39), and crude fiber (CF, AOAC 962.09). All nutrient contents were expressed as a percentage DM except DM was expressed as a percentage of fresh sample. Nitrogen-free extract (NFE) was calculated using the following formula: $NFE = 100 - (CA + CP + CF + EE)$. Total digestible nutrients (TDN) for concentrate was calculated using the following formula: $TDN = 70.6 + (0.259 \times CP) + (1.01 \times EE) - (0.76 \times CF) + (0.0991 \times NFE)$, while TDN for PSS were predicted using the following formula: $TDN = (-26.685) + (1.334 \times CF) + (6.598 \times EE) + (1.423 \times NFE) + (0.967 \times CP) - (0.002 \times (CF^2)) - (0.67 \times (EE^2)) - (0.024 \times (CF \times NFE)) - (0.055 \times (EE \times NFE)) - (0.146 \times (CF \times CP)) + (0.039 \times ((CF^2) \times CP))$ (Hartadi et al., 1980; Ramdani et al., 2020). Gross energy (kcal/g) was measured by the help of a Bomb Calorimeter.

Cholesterol, triglycerides, HDL, and LDL measurements were initially begun with blood sample collections at the end of a 70-day feeding trial. Each blood sample (about 5 ml) of lamb was taken in the morning from the jugular vein using a syringe and transferred into an ethylene diamine tetra acetic acid (EDTA) tube. Each blood sample was then centrifuged at 3000 rpm for 15 min to separate plasma from the other blood components before each blood sample was ready to use for cholesterol and triglycerides analyses. Triglyceride concentration was calculated based on the absorbance values of the samples and the corresponding standard. Total cholesterol was measured by the enzymatic calorimetric test using the cholesterol oxidase p-amino phenazone (CHOD-PAP) method with the help of a spectrophotometer. Calculation

Table 2. Mean (n = 2) nutrient contents of the experimental diets

Contents	PSS with iProbiotics-0	PSS with iProbiotics-4	PSS with iProbiotics-8	Concentrate
Dry matter, %	39.5	35.5	38.4	80.0
Crude ash, %	18.7	18.2	18.2	12.6
Crude protein, %	5.81	6.05	6.05	8.19
Crude fiber, %	28.4	32.2	29.9	13.3
Crude fat, %	3.75	4.83	3.51	10.5
Nitrogen-free extract, %	43.3	38.7	42.3	55.4
Total digestible nutrients (TDN, %)	58.4	56.7	57.1	77.6
Gross Energi, Kcal/kg	3798	3910	3797	4336

PSS: Paddy straw silage

of cholesterol levels based on absorbance values of samples and the corresponding standards. The separated supernatant was then tested for HDL and LDL levels using the same method as measuring total cholesterol. The color absorbance was measured at a wavelength of 500 nm. Triglyceride, Cholesterol, HDL, dan LDL were calculated by the following formula:

$$\text{Triglyceride} = \frac{\text{Abs (assay)}}{\text{Abs (Standard)}} \times \text{Standard concentration}$$

$$\text{Cholesterol} = \frac{\text{Abs (assay)}}{\text{Abs (Standard)}} \times \text{Standard concentration}$$

$$\text{HDL (mg/dl)} = \frac{\text{Sample Absorbance} \times \text{Standard concentration}}{\text{Absorbance Standard}}$$

$$\text{LDL (mg/dl)} = \text{cholesterol total} - x - y$$

$$X = \text{triglyceride} / 5$$

$$Y = \text{HDL}$$

Data Collection and Measurements

Feed intake measurement was obtained from multiplying the amount of feed intake and the dry matter content obtained from the results of the proximate analysis of feed. Dry matter intake (DMI) was calculated with the following equation:

$$\text{DMI (g/head/day)} = \text{feed consumption (g)} \times \text{dry matter of feed (\%)}$$

Average daily gain (ADG) was known by calculating the final body weight minus the initial body weight. The lambs were weighed before morning feeding using a digital weighing scale at day 0 (initial weight), 14, 28, 42, 56, and 70. ADG was calculated with the following equation:

$$\text{ADG (g/head/day)} = \frac{\text{final weight (kg)} - \text{initial weight (kg)}}{\text{time (days)}}$$

Table 3. Mean (n = 6) Initial and final body weights (kg), ADG (g/head/day), Cholesterol (mg/dL), Triglycerides (mg/dL), LDL (mg/dL), and HDL (mg/dL) of experimental rearing ewes during 70 days feeding trial

Measurement	iProbiotics-0	iProbiotics-4	iProbiotics-8	SEM	P- value
Initial Body weight	15.9	17.4	16.5	0.85	0.503
Final Body Weight	16.2	18.7	16.9	1.03	0.248
ADG	4.05	19.8	5.71	7.15	0.307
Cholesterol	127.5	134.0	133.7	14.3	0.937
Triglycerides	51.6	54.4	34.4	11.3	0.424
LDL	53.2	52.8	58.6	11.3	0.921
HDL	64.0	70.3	68.2	3.74	0.493

Mean values were not significantly different at $P > 0.05$; SEM: standard error of mean; ADG: average daily gain; LDL: low-density lipoprotein; HDL: high-density lipoprotein

Statistical Analysis

Each chemical content of the feed materials was calculated as an average from duplicate analysis ($n = 2$). A one-way ANOVA in MINITAB 16 statistical software was used to compare the effect of 3 different doses of iProbiotics-0, iProbiotics-4, and iProbiotics-8 treatments in PPS based ration on performance and blood profiles in Priangan ewe lambs during 70 days feeding trial using 6 replicates ($n = 6$). In addition, a two-way ANOVA in the same software was also used to compare the main effect of 3 different doses of the above treatments at 5 different times (day 14, day 28, day 42, day 56, day 70) on performance of Priangan ewe lambs. Tukey's test was applied to compare means and statistical significance was assumed at $P < 0.05$. The residual data were analyzed for normality by passing the Anderson–Darling normality test at $P > 0.05$.

Results

Table 2 shows the nutrient compositions of the experimental diets. It seemed generally that iProbiotics supplementation during PSS preparation had no considerable effects on nutrient compositions of PSS.

Table 3 describes the means of performance and blood sample parameters of the experimental ewes during 70 days feeding trial. The iProbiotics treatment had no effect ($P > 0.05$) on ADG and blood lipid parameters of the ewes. Meanwhile, Table 4 shows the main effect of iProbiotics treatment and time on the performance of experimental ewes during the in vivo experiment. Across the time, the iProbiotics treatment had no effect ($P > 0.05$) on ADG of the ewes but iProbiotics-8 treatment increased ($P < 0.01$) total DMI through increased PSS intake. All the experimental ewes had a similar concentrate intake during the trial since they could consume all the offered concentrate without any refusals. Across the treatment, time had an impact ($P < 0.01$) on ADG and total DMI. The ADG of the ewes varied over the time

Table 4. Main effect of iProbiotics treatment (n = 30) and time (n = 18) on ADG (g/head/day) and DMI (g/head/day) of the experimental rearing ewe lambs during 70 days feeding trial, together with SEM and significances

Parameter	Treatment			Time					SEM and Significances		
	iProbiotics 0	iProbiotics4	iProbiotics8	Day 14	Day 28	Day 42	Day 56	Day 70	Treatment	Time	Treatment*Time
ADG	4.05	18.8	5.71	25.4 ^A	-15.1 ^B	34.5 ^A	-0.40 ^{AB}	3.17 ^{AB}	7.89 ^{NS}	14.9 ^{**}	17.35 ^{NS}
Total DMI	534.1 ^B	514.1 ^B	586.2 ^A	537.2 ^{AB}	548.4 ^{AB}	525.7 ^B	535.4 ^B	577.2 ^A	7.97 ^{**}	10.3 ^{**}	17.8 ^{NS}
PSS	374.0 ^B	354.0 ^B	426.1 ^A	377.1 ^{AB}	388.3 ^{AB}	365.6 ^B	375.3 ^B	417.1 ^A	3.15 ^{***}	4.07 [*]	7.07 ^{NS}
Concentrate	160.1	160.1	160.1	160.1	160.1	160.1	160.1	160.1	n/a	n/a	n/a

Mean values were not significantly different at $P > 0.05$ (^{NS}) and were significantly different at $P < 0.05$ (*), $P < 0.01$ (**), $P < 0.001$ (***) ; SEM: standard error of mean; n/a: not available; ADG: average daily gain; DMI: dry matter intake; PSS: paddy straw silage

of the feeding trial while the total DMI and PSS intake were higher at the end of the trial (day 70).

Discussion

The experimental Priangan ewes consumed a different DMI among treatments. DMI is strongly influenced by the nutritional contents and palatability of the diets. The better the nutritional contents and palatability the higher the DMI.

The iProbiotic-8 inclusion during PSS preparation increased the DMI of sheep significantly. This was a sign that iProbiotic inclusion increased the palatability of PSS. In prediction, the molasses content in iProbiotic plays an important role to increase the palatability in PSS. Molasses supplementation during silage preparation can increase the palatability because molasses is highly palatable (Saeed & Latif, 2008).

Paddy straw is a low-quality forage for sheep feeding (Ramdani et al., 2020). The addition of natural probiotics from old paddy straw silage extract is expected to improve the quality of PSS. Probiotics are explored as safer alternatives to antibiotics to improve the gastrointestinal microbiota balance and to enhance ruminant's health and productivity (Ford et al., 2014). Previous studies have shown that the use of probiotics (direct-fed microbial), such as lactic acid bacteria, can improve nutrient digestibility and growth performance (Salem et al., 2013; Adjei-fremah et al., 2018); also decrease pathogen colonization in the gut (Uyeno et al., 2015). Earlier studies reported that probiotics enhanced feed efficiency, increased weight gain, improved milk and meat production, reduced methane emission, and improved animal health (Elghandour et al., 2015; Sun et al., 2013). The animal health conditions can be checked through hematological tests including the levels of cholesterol, triglycerides, HDL, and LDL.

Dry matter intake is closely related to the weight gain of sheep, the more the amount of feed consumed, the more nutrients used by the body to increase the weight gain of sheep. ADG is an indicator of growth in livestock. The growth rate

of sheep is closely related to the amount of feed or nutrients used in their bodies. Sheep fed with additional probiotics in low-quality diet showed increased feed efficiency and daily gain (Elghandour et al., 2015).

Feeding sheep with PSS in this study did not give a significant change to the total blood cholesterol level, but the total blood cholesterol levels produced (127.4–133.9 mg/dL) were still in the normal range (50–140 mg/dL) (Meyer & Harvey, 2004). The results of sheep's blood LDL ranged from 53.1–58.6 mg/dL. The LDL contents were smaller than the HDL value of 64–70.3 mg/dL. LDL is often called less advantageous fat, so their contents are preferably lower than HDL (Faisal et al., 2017). According to Faisal et al. (2017), the LDL content of the blood in Garut sheep after weaning was 11–15.5 mg/dL. According to Sheikh et al. (2019), the LDL content of sheep on straw feeding without probiotics was 22.9 mg/dL and with probiotics was 20.7 mg/dL. This showed that blood LDL levels of sheep in the current study were averagely higher than the LDL levels of sheep in the previous two studies (Faisal et al., 2017; Sheikh et al., 2019).

The average blood HDL results of sheep in the current study were 64–70.3 mg/dL. The use of probiotics in the current study did not provide significant changes to blood HDL levels, but the blood HDL levels produced in the study were quite high. The results were higher than the study of (Faisal et al., 2017), where the HDL content of sheep-fed straw without probiotics was 41.3 mg/dL and with probiotics was 44.1 mg/dL. According to Sheikh et al. (2019), the blood HDL contents of weaned Garut Sheep were 22.2–46.2 mg/dL.

Feeding PSS in this sheep study did not provide a significant change in blood triglyceride level. The triglyceride levels were above normal ranging from 34.4 to 51.6 mg/dL. According to Hussein et al. (2022), the normal sheep blood triglyceride levels are between 28.6 and 34.5 mg/dL.

The insignificant results on total blood cholesterol levels were due to the role of lactic acid bacteria as probiotic start-

ers in the manufacture of PSS did not provide a maximum interaction. Lactic acid bacteria as a prebiotic starter stipulates the production of short-chain fatty acids. These short-chain fatty acids will compete with Hydroxy Methyl Glutamyl-CoA reductase (HMG CoA reductase), which plays a role in the formation of mevalonate in the cholesterol synthesis process so that cholesterol synthesis will be inhibited (Sudha et al., 2009; Adriani et al., 2019). The enzyme HMG-CoA reductase plays a role in cholesterol biosynthesis.

These conditions will inhibit the process of endogenous cholesterol synthesis, which is centered in the liver (Yang et al., 2013). So, the inhibition of HMG-CoA reductase has not been maximal in causing a decrease in cholesterol synthesis and also in increasing the number of HDL receptors (Singh, 2006). The age of the ewes used in the current study was about below 1 year old which was still in the growth phase. Cholesterol is needed for the formation of hormones and the repair of damaged cells. This can allow cholesterol homeostasis so that endogenous cholesterol levels are maintained (Luo et al., 2019).

The high level of LDL is caused by excessive energy or fat intake, which results in an increase in blood cholesterol levels, which reflects high levels of LDL in the blood. If atherosclerosis occurs in the blood vessels of the heart, it will cause coronary heart disease.

Energy and fat derived from feed will undergo a digestive process in the intestine into free fatty acids, triglycerides, phospholipids, and cholesterol, which are then absorbed into the form of chylomicrons. The rest of the breakdown of chylomicrons circulates to the liver and turns into cholesterol.

LDL formed by this receptor is important for controlling blood cholesterol, besides that in blood vessels, there are damaging cells that can damage LDL. Through this pathway of damaging cells, LDL molecules are oxidized, so they cannot enter the bloodstream again. Cholesterol that is abundant in LDL will accumulate in destroying cells, cholesterol will accumulate on the walls of blood vessels and form plaques. These plaques will mix with protein and be covered by muscle cells and calcium. This can then develop into atherosclerosis. Lactic acid bacteria as a probiotic starter during fermentation will produce short-chain fatty acids. These short-chain fatty acids will compete with Hydroxy Methyl glutamyl CoA reductase (HMG CoA reductase), which plays a role in the formation of mevalonate in the cholesterol synthesis process so that cholesterol synthesis will be inhibited (Sudha et al., 2009). The enzyme HMG-CoA reductase plays a role in cholesterol biosynthesis. These conditions will inhibit the process of endogenous cholesterol synthesis which is centered in the liver so that the inhibition of HMG-CoA reductase will not be maximized causing a decrease in cho-

lesterol synthesis and also in increasing the number of HDL receptors (Singh et al., 2006).

The provision of probiotics can also maintain the balance of microbial composition in the digestive tract of the livestock to increase digestibility and maintain health. Probiotics can play a role in helping the digestive function to digest and absorb feed nutrients. Increased digestibility of feed results in increased nutrient absorption.

The administration of various levels of PSS with probiotics is thought to not provide a significant difference to the consumption of organic matter for weaning ewes. This is because the pattern of consumption of organic matter follows the pattern of consumption of dry matter, which causes the sheep to have relatively the same metabolic processes and the resulting HDL and LDL will not be significantly different.

The mechanism of reducing triglyceride levels by statins begins when these inhibitors reduce cholesterol concentrations in hepatocytes and increase the performance of LDL-receptors which are closely related to VLDL components so that triglycerides will also be reduced (Scorve et al., 1993). The occurrence of inhibition of triglyceride synthesis in the liver and small intestine will result in a decrease in blood triglyceride levels. Scorve et al. (1993) explain that the decrease in fatty acid synthesis in the liver is the main factor causing the decrease in triglyceride synthesis in the liver which results in a further decrease in the concentration of triglycerides in the blood plasma.

Triglyceride levels are closely related to the activity and physiological needs of sheep. In addition, there are several factors that can affect triglyceride levels including consumption of crude fats in feed, age of livestock, sex of livestock, and genetic factors (Niza et al., 2015). Consumption of feed that contains a lot of fat will cause more fat absorption so that fat levels in the body will increase.

Conclusion

About 8% iProbiotics supplementation during PSS preparation is suggested to improve PSS intake by the sheep. Molasses content in iProbiotic plays an important role in increasing palatability and ensiling process of PSS. In addition, iProbiotic supplementation has no any harmful impact on the performance and blood profiles of the sheep.

Acknowledgments

Authors would like to thank Directorate Research and Community Services of Universitas Padjadjaran for funding and Mr. Gilang Satya Nugraha for editing the manuscript.

Author Contributions: Conceptualization, D.R. and W.S.P.; methodology, D.R., W.S.P., E.T.M., J.H.; validation, D.R.; formal analysis, J.H., A.A.H., G.J.Y.M., R.A., R.S.R.; funding resources, D.R.; writing—original draft preparation, D.R., A.A.H., G.J.Y.M., R.A., and R.S.R.; writing—review and editing, R.S.R. and D.R.; supervision, D.R., W.S.P. and E.T.M. All authors have read and agreed to the published version of the manuscript.

Funding: This study was partly funded by Directorate Research and Community Services of Universitas Padjadjaran through RPLK research grant 2021.

Informed Consent Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

References

- Adjei-fremah, S., Ekwemalor, K., Worku, M. & Ibrahim, S. (2018) Probiotics and Ruminant Health. *Intech*. Available from: <http://dx.doi.org/10.5772/intechopen.72846>.
- Adriani, L., Nurlaeni, L., Nurlailya, Z. & Mayasari, N. (2019). Effects of Supplementation of Cow's Milk and Soybean Milk Fermented with Probiotic Bacteria on Blood Lipid Levels and Meat Quality of Broiler Chicken. *Scientific Papers. Series D. Anim. Sci.*, 62(1), 116-121.
- AOAC (2005). Official Methods of Analysis of AOAC International. Gaithersburg, MD.
- Elghandour, M. M. Y., Salem, A. Z. M., Castañeda, J. S. M., Camacho, L. M., Kholif, A. E. & Chagoyán, J. C. V. (2015). Direct-fed microbes: a tool for improving the utilization of low quality roughages in ruminants. *J. Integr. Agric.*, 14, 526-533.
- Faisal, F., Rochana, A. & Kamil, K. A. (2017). Study on blood chemicals and average daily gain of female Garut sheep with different ratio of protein and energy. *Jurnal Ilmu Ternak*, 17(2), 92-96.
- FDA (US Food and Drug Administration). (2021). Bacteriology Analytical Manual (BAM) Chapter 3: Aerobic Plate Count. Available from: <https://www.fda.gov/food/laboratory-methods-food/bam-chapter-3-aerobic-plate-count>.
- Ford, A. C., Quigley, E. M. M., Lacy, B. E., Lembo, A. J., Saito, Y. A. & Schiller, L. R. (2014). Efficacy of prebiotics, probiotics, and synbiotics in irritable bowel syndrome and chronic idiopathic constipation: systematic review and meta-analysis. *Am. J. Gastroenterol.*, 109, 1547-1562.
- Hartadi, H., Reksohadiprojo, S., Lebdosukojo, S. & Tillman, A. D. (1980). Tables of feed ingredient compositions for Indonesia. Logan (US): International Feedstuffs Institute Utah Agricultural Experiment Station, Utah State University.
- Hussein, A. H., Patra, A. K., Puchala, R., Wilson, B. K. & Goetsch, A. L. (2022). Effects of Restricted Availability of Drinking Water on Blood Characteristics and Constituents in Dorper, Katahdin, and St. Croix Sheep from Different Regions of the USA. *Animals*, 12(22), 3167.
- Luo, J., Yang, H. & Song, B.-L. (2019). Mechanisms and regulation of cholesterol homeostasis. *Nat. Rev. Mol. Cell Biol.*, 21(4), 225-245
- Meyer, D. J. & Harvey, J. W. (2004). *Veterinary Laboratory Medicine Interpretation and Diagnosis*. Philadelphia: Saunders.
- Niza, R. S., Asni, E., Ade, W. & Isawati (2015). The correlation of atherogenic diet administration times on triglyceride levels of male Rattus Novergicus Wistar strain. *JOM FK.*, 2(2), 1-12.
- Ramdani, D., Budinuryanto, D. C. & Mayasari, N. (2020). The effect of paddy straw and concentrate containing green tea dust on performance and nutrients digestibility in feedlot lambs. *Turkish J. Vet. Anim. Sci.*, 44(3), 668-674.
- Saeed, A. A. & Latif, F. A. (2008). Effect of time of ensiling on the nutritive value of wheat straw treated with urea and molasses. *Al-Qadisiya J. Vet. Med. Sci.*, 7(2), 37-43.
- Salem, A. Z. M., Gado, H. M., Colombatto, D. & Elghandour, M. M. Y. (2013). Effects of exogenous enzymes on nutrient digestibility, ruminal fermentation and growth performance in beef steers. *Livest. Sci.*, 154, 69-73.
- Scorve, J., Al-Shurbaji, A., Asiedu, D., Bjorkheun, I., Berghund, L. & Berge, R. K. (1993). On The Mechanism of The Hypolipidemic Effect of Sulfur-Substituted Hexadecanedionic Acid (3-Thiadicarboxylic Acid) In Normolipidemic Rats. *J. Lipid Res.*, 34, 1117-1185.
- Sheikh, G. G., Masood, D., Ganai, A. M., Muzamil, S., Afzal, Y. & Ahmad, H. A. (2019). Effect of Probiotics Mix Supplementation on Haemato-Biochemical Parameters and Bacterial Faecal Shedding in Corriedale Lambs Fed Paddy Straw Based Complete Feed. *Indian J. Anim. Res.*, 53(8), 1049-1053
- Singh, D. K. (2006). Inhibition of Sterol 4Methyl Oxidase is The Principal Mechanism by Which Garlic Decrease Cholesterol Synthesis. *J. Nutr.*, 136, 7598-7648.
- Statistical Central Bureau (2019). Harvesting Area and Rice Production in Indonesia. Available from: <http://bps.go.id/>
- Sudha, M. R., Chauhan, P., Dixit, K., Babu, S. & Jamil, K. (2009) Probiotics as Complementary Therapy for Hypercholesterolemia. *Biol. Med.*, 1(4), 1-13.
- Sun, P., Wang, J. Q. & Deng, L. F. (2013). Effects of *Bacillus subtilis natto* on milk production, rumen fermentation and ruminal microbiome of dairy cows. *Animal*, 7(2), 216-222.
- Uyeno, Y., Shigemori, S. & Shimosato, T. (2015). Effect of probiotics/prebiotics on cattle health and productivity. *Microbes Environ.*, 30, 126-132.
- Yang, L., Han, G., Liu, Q. H., Wu, Q., He, H. J., Cheng, C. Z. & Duan, Y. J. (2013). Rice Protein Exerts a Hypocholesterolemic Effect Through Regulating Cholesterol Metabolism-related Gene Expression and Enzyme Activity in Adult Rats Fed a Cholesterol-Enriched Diet. *Int. J. Food Sci. Nutr.*, 64(7), 836-842.