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RESEARCH ARTICLE

The Effect of Spirulina Supplementation into the Basal Diet on the Productivity and Environmental Cost of Thin-tailed Lambs Fattening

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ABSTRACT

Spirulina platensis is a supplement that has a good impact on the growth performance of lambs, but information about the amount of this substance in feed efficiency and environmental impact is still required to be determined. The study aimed to examine the effect of the supplementation level of Spirulina platensis in the basal diet on the productivity and environmental costs of Thin-tailed lambs fattening. Twenty-four male lambs (aged 4 months; weighing 14.99 kg) were allocated to a completely randomized design with 4 treatments and six replications in each treatment. The treatments were 1) 100% basal diet without Spirulina supplementation (T0); 2) 99.5% basal diet + 0.5% Spirulina platensis supplementation (T1); 3) 99.0% basal diet + 1.0% Spirulina platensis supplementation (T2); and 4) 98.5% basal diet + 1.5% Spirulina platensis supplementation (T3). The parameters observed were dry matter intake (DMI), nutrient utilization, rumen fluid and blood profile, productivity, feed cost and nitrogen excretion and methane emission. The results showed that DMI of T3 was the highest among the treatments (985.66 g/d; $p < 0.05$). Retained protein of T2 (70.82 g/d) and T3 (72.49) was similar ($p > 0.05$) and higher than that of others ($p < 0.05$). Acetic, propionic and butyric acids 3h after feeding in T3 were higher ($p < 0.05$) than those of the others (178. 21 mMol, 69.82 mMol and 12.42 mMol, respectively). Lambs fed T2 and T3 were similar (averaged 186.7 g/d; $p > 0.05$) in average daily gain (ADG) and higher than that of the others. Feed conversion ratio (FCR) of T2 (4.74) was not significantly different from T3 ($p > 0.05$) but higher ($p < 0.05$) than that of T0 and T1, while T0, T1 and T3 were not significantly different ($p > 0.05$). Feed cost per gain (FC/G) of T2 (Rp 31,996/kgADG) was the lowest ($p < 0.05$) among the others. Nitrogen excretion per kg ADG of T2 and T3 was similar ($p > 0.05$; averaged 0.035 g/kgADG) and lower ($p < 0.05$) than that of T0 and T1. Methane emission per kg ADG of T2 (11.52 L/ADG) was the lowest ($p < 0.05$) among the other treatments. In conclusion, the supplementation of Spirulina platensis up to 1% (T2) increased productivity, reduced feed cost and environmental cost of Thin-tailed lambs production.

Keywords: Diet, Environmental cost, Lambs, Nutrient utilization, Productivity, Spirulina

Introduction

In the lamb feedlot system, feeding is always aimed at achieving maximum feed efficiency and high productivity. [1,2] On the other hand, sheep have a negative impact on the environment because they produce methane (CH_4) as part of enteric fermentation and N_2O emissions from faeces and urine. In general, livestock contributes 14.5% of global GHG emissions. [3] Livestock in Indonesia contributed 26,826 Gg CO_2^{-e}

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per year from 2017–2021, of which CH_4 emissions were dominated by 85.71% from enteric fermentation and 0.46% N_2O from manure. Sheep farming in Indonesia contributed 8.04% of CH_4 and 12.56% N_2O manure emissions.[4] CH_4 gas produced in the rumen is calculated as energy loss, accounting for approximately 8–12% of digestible energy. In ruminant fattening programs, CH_4 also reflects feed nutrient utilization. This is because feed fermentation in the rumen produces volatile fatty acids (VFAs), consisting of acetic, propionic, and butyric acids. CH_4 gas is a by-product of VFA formation during the rumen fermentation process by microbes in the rumen. During VFA synthesis, methanogenic bacteria utilize hydrogen (H_2) and carbon dioxide (CO_2) produced in the formation of acetic acid in the rumen to produce CH_4 gas.[5,6] Meanwhile, N_2O emissions from livestock manure are significantly influenced by the deposition of ruminant waste into the environment through faeces and urine. Thin-tailed lambs retain 8–10% of their nitrogen in their bodies, while the remainder is excreted through faeces and urine.[2] Nitrogen output through urine increases with increasing nitrogen intake, while the proportion of nitrogen output through faeces remains relatively constant.[7]

From the explanation above, this is a challenge for the sheep fattening business; in addition to increasing production and profits, it has to pay attention to the environmental impacts that will arise. Therefore, an effort is needed to improve the feeding system, especially to reduce CH4 from enteric fermentation and increase protein retention in the body so that nitrogen excretion into the environment is reduced, in order to increase animal productivity and reduce the environmental cost of production.[5] One strategy in an effort to increase feed utilization, feed efficiency, and reduce emissions of pollutants is supplementation of feed that is not only rich in amino acids, but also in bioactive compounds.[8,9] It was due to bioactive materials, such as tannins, essential oils, and saponins, that significantly impact ruminal fermentation by modulating microbial activity, which can lead to reduced methane emissions, altered nutrient digestion, and improved animal health and product quality.[10]

One of the feed additives that has the potential to increase productivity and reduce environmental pollution is Spirulina platensis. This material is a source of amino acids, fatty acids, carotenes, minerals, vitamins, and xanthophyll phytopigments, as well as phycocyanin, phenolic acids, chlorophyll, and gamma linoleic acid.[11,12] Spirulina platensis is a microalgae and is found in various types of environments, both in brackish, marine and fresh waters.[13,14] Spirulina platensis dominates the global biomass market with estimated production ranging from 5 to 10 kton/year.[15,16] Baliarti et al.[17] found that 0.95% supplementation of Spirulina platensis in the diet of Thin-tailed lambs did not affect sheep productivity. On the other hand, Rabee et al.[18] found that supplementation of 1.3% Spirulina platensis in the diet of rams resulted in a decrease in acetic acid and an increase in propionic acid. The lower the acetic propionic acid ratio, the more it indicates a lower the methane production and the better efficiency of energy usage to increase body weight in ruminants.[19] However, there have been no studies on the appropriate level of Spirulina platensis supplementation in feed, not only to increase feed efficiency and production but also to reduce pollution generated by the thin-tailed lamb production process. This study aimed to examine the effect of the level of Spirulina platensis supplementation in the diet on increasing feed efficiency, lamb productivity and reducing methane emission and nitrogen excretion per body weight gain of Thin-tailed lamb in Indonesia.

Materials and methods

Animals, diet and experimental design

This study was approved by the ethical committee of Animal and Agricultural Sciences, Universitas Diponegoro (Approval No. 60-11/A-20/KEP-FPP). This study used 24 male Thin-tailed lambs aged 4 months, with an average initial body weight of 14.99 kg (CV = 1.26%) as experimental units. The feed given was basal feed supplemented with Spirulina platensis. Spirulina platensis contained 96% DM, 54.90% crude protein (CP), 2.53% ether extract (EE), 5.10% Ash, and 0.71% crude fiber (Cfi).

The feed was given ad libitum (available at all times) in the form of mash. The basal feed consisted of 30% corn cobs, 15% wheat bran, 15% pollard flour, 12% biscuit flour (waste from biscuit), 10% palm kernel meal, 10% cassava flour and 3% organic protein (monosodium glutamate waste). The nutritional content of feed is presented in Table 1.

Table 1. Nutritional content of the diet.

Nutrient (%)	T0	T1	T2	T3
Moisture	11.4	8.5	11.7	12.2
Crude Protein	18.6	18.8	19.0	19.3
Crude Fiber	15.2	15.2	15.3	15.3
Extra Ether	2.6	2.7	1.9	2.5
Ash	5.3	4.4	4.8	4.6
NFE	58.3	60.1	61.0	60.0
NDF	50.2	48.3	48.6	47.9
ADF	30.2	31.7	30.8	30.2
TDN	74.2	75.4	74.6	74.1

Abbreviation: NFE = Nitrogen-free extract; NDF = Neutral detergent fiber; ADF = Acid detergent Fiber; TDN = Total digestible nutrients.

This study used a completely randomized design (CRD) with 4 treatments and 6 replications. The treatments applied were levels of Spirulina platensis supplementation in the basal diet, namely:

1) 100% basal diet without Spirulina platensis supplementation (T0);
2) 99.5% basal diet + 0.5% Spirulina platensis supplementation (T1);
3) 99.0% basal diet + 1% Spirulina platensis supplementation (T2);
4) 98.5% basal diet + 1.5% Spirulina platensis supplementation (T3).

The study was conducted in 4 stages, namely preparation (2 weeks), adaptation (2 weeks), preliminary treatment (2 weeks) and treatment (9 weeks). In the preparation stage, the cage, feed and experimental lambs were prepared. The cage was cleaned, and the lambs were dewormed. In this stage, the lambs were adapted to the cage environment and the basal diet to eliminate the effect of the previous diet. In the preliminary stage, the lambs were randomly placed in the cage and their treatment feed. In this stage, diet was given according to the treatment to eliminate the effect of the previous feed.

The treatment stage began with weighing the lambs to determine their initial body weight. The provision and remaining feed were weighed and recorded every day to calculate feed intake. Lambs were weighed and recorded every week during rearing time. The feed was offered ad libitum (always available) starting at 7 am. The refusals were weighed every day at 07.00 am before feeding. Drinking water was provided ad libitum.

Every week, the diet samples and refusals were dried in an oven at 105˚C for 24 hours until the weight was constant to determine the dry matter content. Total collection of feces and urine was carried out in the fourth week of rearing time for 7 consecutive days to determine nutrient digestibility and retention. The amount of feces and urine of each lamb was collected every day and then weighed. Diet samples, refusals and sub-samples of feces at an amount of 10% were taken every morning during the total collection and dried in an oven at 105˚C for 24 hours to determine the dry matter content. Diet samples and sub-samples of feces were dried in an oven at 65˚C for 72 hours, then ground and filtered to particles measuring 1 mm for chemical analysis (energy, protein, ash, fat and fiber). Urine sub-samples were taken every morning at an amount of 10% during the total collection and stored in a freezer at –5˚C before being analyzed. Urine sub-samples of 10 ml were taken to analyze protein levels, then 1 ml of the urine sub-samples was dried in the oven at 60˚C for 4 days until the weight was stable to determine the energy content in urine. Feed and feces samples were analyzed according to AOAC standard procedures; namely, dry matter (DM; method 934.01), ash (method 942.05) and crude fat (EE; method 920.39). Nitrogen in feed, feces and urine was analyzed using the Kjeldahl method.[20]

The parameters observed in this study were daily dry matter intake (DMI), organic matter intake (OMI), crude protein intake (CPI), feed digestibility, nutrient digested and retained, ammonia and VFA profile in the rumen, rumen microbial protein production, blood ammonia and glucose, potential methane gas output, nitrogen excretion in the feces and urine, eating behavior, productivity, feed conversion, feed cost per gain.

The procedures to obtain nutrient utilization were as follows:

- Nutrient Intake (g/day) = Weight of feed nutrients given – Weight of remaining feed nutrients
- Fecal nutrients (g) = Nutrient content in feces (%) × fecal output (g)
- Digestible nutrients (g) = Dietary nutrient intake (g) – Fecal nutrients (g)
- Nutrient digestibility (%) = Dietary nutrient intake (g) minus fecal nutrients (g) divided by feed nutrient intake (g) multiplied by 100%
- TDN (%) = (% digestible crude protein + % digestible crude fiber + % digestible NFE + (2.25 x % digestible extract ether))
- Urinary nitrogen (g) = Urinary nitrogen content (%) × Urinary output
- Urinary energy (kcal) = Urinary energy content (kcal) × Urinary output (g)
- Nitrogen retention (g) = Nitrogen consumption (g) – Nitrogen in feces and urine (g)

Rumen fluid profile and methane emission

Rumen fluid samples were taken using a pump and tube. The tube was inserted into the mouth and rumen cavity of the sheep. The pump was turned on to suck 30 ml–40 ml of rumen fluid. The pH value of the rumen fluid was measured, then the fluid was added with H_2SO_4 (20% concentration) until the pH reached 3 or less.[5,21] Then, the sample was filtered to extract the fluid and stored in the freezer before being analyzed. Rumen fluid was taken twice, namely 0 h before feeding and 3 h after feeding. The VFA concentrations were analyzed using gas chromatography as described by Horwitz.[22] The ammonia concentration of rumen fluid was analyzed spectrophotometrically as described by Weatherburn.[23] The partial results of volatile fatty acids (acetate, propionate and butyrate)

were used to calculate the potential methane output in lambs. Calculation of methane emission was estimated using the Moss[24] formula, namely:

$$CH_4 = 0.45\ C_2 - 0.275\ C_3 + 0.40\ C_4$$

Where:
C2 = acetic acid
C3 = propionic acid
C4 = butyric acid

Microbial nitrogen production

The calculation of microbial N production in small ruminants was based on the equation of Chen and Gomes[25] as follows:

$$Y = 0.84\ X + \left(0.150\ BW0^{75}e^{-0.25X}\right)$$

Microbial N production (g N/ d) = (X × 70)/(0.116 × 0.83 × 1000) = 0.727x Microbial N production efficiency (g N/kg DOMI) = (Microbial N Production)/(DOM intake).

Where:
X (mmol/d) = amount of microbial purine absorbed
Y (mmol/d) = amount of microbial purine derivatives excreted through urine
$BW^{0.75}$ = metabolic body weight
e = 2.71828
0.83 = Microbial purine digestibility coefficient
70 = Amount of N in purine (mg/mmol)
0.116 = ratio of purine to total N in microbial mixture

Blood metabolites

Blood samples were collected via the jugular vein at 0 h before feeding and 3 h after feeding. Blood was put into a silicone vacuum tube (Vacutainer®) with anticoagulant (sodium fluoride/EDTA), to obtain serum and plasma. Then, the samples were centrifuged at 3000 rpm for 15 minutes. Aliquots of serum and plasma were then stored in previously identified 2 mL polyethylene tubes and frozen in a freezer for further glucose and ammonia concentration analysis.

Behavior

All lambs were visually observed to evaluate eating behavior at the 5th week for 3 consecutive days. Observations were made every five minutes during 24 hours to determine the time spent on activity, standing, eating, drinking, rumination, urination, defecation and lying down. On the same day, all animal behavioral parameters were calculated according to the procedures described in Nugroho et al.[26,27]

Data analyze

The data obtained were analyzed using analysis of variance at levels of 5%. If there was significance, it would be continued to be analyzed using the Duncan test.

Results and discussion

Nutrient utilization

The nutrient utilization of Thin-tailed Lambs is presented in Table 2. The DMI and OMI of T3 were higher than those of T0, T1 and T2 ($p < 0.05$). It indicated that supplementing the basal diet with Spirulina platensis increased DMI. The higher the supplementation, the higher the DMI. This study was in line with the previous meta-analysis by Orzuna-orzuna et al.[28] The supplementation of Spirulina platensis 28 g/kg DM into the diet increased DMI.

The CPI of Thin-tailed lambs in this study was similar ($p > 0.05$) among the treatments (averaged 154.55 g/d). This was because the supplementation of Spirulina platensis slightly reduced the protein content of the basal diet and was replaced by the protein content in Spirulina platensis. Therefore, despite the increase in DMI, the total protein intake did not alter significantly.

Rumen fluid profile

The rumen fluid profile in this study is presented in Table 2. The supplementation of Spirulina did not significantly change ($p > 0.05$) the concentration of ammonia in the rumen at 0 h and 3 h after feeding. This was due to the fact that the protein intake in this study was similar among the treatments. This indicated that the concentration of rumen ammonia was stable. Manoni et al.[29] and Luthfi et al.[5] claimed that there were benefits that could be obtained by ruminants when the concentration in the rumen did not dramatically change. This allowed the pH conditions in the rumen to remain stable and prevented the ruminant from bloating caused by drastic and high changes in ammonia concentration.

This study also found that there were no significant differences in acetic, propionic and butyric acids at 0h before feeding ($p > 0.05$). Then, supplementation of Spirulina platensis in the basal diet significantly increased the acetic, propionic and butyric acids at 3 h after feeding ($p < 0.05$). Lambs fed on T3 had higher acetic, propionic and butyric acids at 3 h after feeding. This result was caused by the high OM intake as an increase in the supplementation of Spirulina platensis in the basal diet. It might also be due to the supplementation of Spirulina platensis in the basal

Table 2. Nutrient utilization of thin-tailed lambs fed basal diet supplemented by spirulina platensis.

Parameters	T0	T1	T2	T3	SEM	*p* value[1)]
Initial Weight (kg)	14.74	15.16	14.57	15.50	0.33	0.85
Intake						
DMI (g/d)	817.05^{c}	793.43^{c}	893.01^{b}	985.66^{a}	24.04	0.04
DMI (% BW)	4.37	4.12	4.36	4.65	0.07	0.15
OMI (g/d)	773.75^{c}	758.52^{c}	850.14^{b}	924.91^{b}	21.84	0.05
CPI (g/d)	151.97	140.44	156.28	169.53	4.03	0.16
Rumen fluid Profile						
Ammonia (mg/100 mL)						
Ammonia 0 h (mg/100 mL)	8.70	10.39	8.91	9.74	0.34	0.41
Ammonia 3 h (mg/100mL)	17.46	18.27	17.92	17.22	0.46	0.91
Diff. ammonia (0–3)	8.76	7.88	6.10	7.48	0.37	0.21
VFA 0 h (mMol)						
Acetic	54.13	50.77	43.88	40.56	2.36	0.28
Propionic	19.68	18.82	16.30	16.39	0.78	0.46
Butyric	4.38	3.92	3.62	3.58	0.32	0.87
A/P ratio	2.77	2.63	2.72	2.47	0.06	0.38
VFA 3 h (mMol)						
Acetic	99.88^{c}	116.17^{c}	141.84^{b}	178.21^{a}	5.99	0.00
Propionic	40.27^{c}	43.81^{c}	56.13^{b}	69.82^{a}	2.46	0.00
Butyric	10.52^{c}	9.06^{c}	9.72^{b}	12.42^{a}	0.52	0.00
A/P ratio	2.48	2.69	2.53	2.58	0.04	0.52
The different of VFA at 0–3 h						
Diff. Acetic	45.75^{c}	65.40^{c}	97.96^{b}	137.65^{a}	7.50	0.00
Diff. Propionic	20.59^{c}	25.00^{c}	39.83^{b}	53.43^{a}	2.88	0.00
Diff. Butyric	6.13^{b}	5.14^{c}	6.10^{b}	8.84^{a}	0.47	0.02
Microbial N Production (g/d)	3.01^{c}	2.98^{c}	3.33^{ab}	3.77^{a}	0.10	0.05
Microbial Protein Production (g/d)	18.82^{c}	18.64^{c}	20.81^{b}	23.53^{a}	0.62	0.05
Microbial N efficiency (g/kgDOMI)	24.34	24.43	24.68	25.86	0.71	0.87
Digestibility						
DMD (%)	64.92	66.64	66.32	65.33	0.84	0.92
OMD (%)	80.52	81.12	79.75	79.26	0.48	0.61
CPD (%)	83.93	83.18	80.63	81.07	0.50	0.08
DMD (g/d)	664.88	643.64	712.56	785.99	18.05	0.18
OMD (g/d)	627.67	616.27	678.81	734.39	19.55	0.23
CPD (g/d)	128.34	116.96	126.16	137.74	3.72	0.42
Retention						
Protein (%)	68.19^{c}	81.72^{b}	87.71^{ab}	89.43^{a}	1.98	0.00
Protein (g/d)	57.22^{d}	68.00^{c}	70.82^{b}	72.49^{a}	1.56	0.00

SEM = Standard error of the mean; DMI= Dry matter intake; OMI= Organic matter intake; CPI= Crude protein intake; DMD = Dry matter digestibility; OMD= Organic matter digestibility; CPD= Crude protein digestibility; DOMI = Digested organic matter intake; VFA= Volatile fatty acids; [1)]The different superscripts in the same row indicate significant differences among treatments ($p < 0.05$).

diet, which improved the cellulolytic bacteria that can digest the feed entering the rumen, resulting in high volatile fatty acids. Studies by Christodoulou et al.[30] and Tsiplakou et al.[31] found that the addition of microalgae in the feed of sheep increased rumen bacteria *Ruminococcus albus*, *Ruminococcus flavefaciens*, and *Fibrobacter succinogenes*. Koike and Kobayashi[32] stated that these bacteria have a very important role in cellulose degradation. Greater cellulose degradation increased the rate of feed passage in the digestive tract and resulted in higher DMI.[4,33] Therefore, this finding also explained why DMI increased with an increase in supplementation levels. Gumilar et al.[34] found that the range of total VFA that can be used to synthesize microbes in the rumen was 70–150 mMol. Since there was a significant impact on VFA concentration in the rumen, supplementing the basal diet with Spirulina platensis significantly improved the change in VFA concentration from 0h to 3h after feeding in the rumen ($p < 0.05$). Lambs fed on T3 had the highest increase in VFA at 3 h after feeding compared to the others. The differences in VFA from 0h to 3 h after feeding of T3 were 137.65 mMol of acetic acid, 53.43 mMol of propionic acid, and 8.84 mMol of butyric acid.

The microbial protein production in this study Table 2 was improved by supplementing the basal diet with Spirulina platensis ($p < 0.05$). The higher the supplementation level of Spirulina platensis, microbial protein production led to an increase in the rumen of Thin-tailed lambs. The lambs fed T3 had the highest microbial protein production compared to

T0, T1 and T2. It indicated that the supplementation of Spirulina platensis improved the population of microbes in the rumen. This finding also confirmed the hypothesis that VFA in this study increased with an increase in DMI and Spirulina platensis supplementation levels. This study was in line with the findings by Panjaitan et al.[35] that supplementation of microalgae in beef cattle feed improved the microbial protein production. Microbial N efficiency of all treatments was similar ($p > 0.05$). The average microbial N efficiency in this study was 24.82 g/kgDOMI. It was due to the high microbial N protein balanced with high digested OM, thus producing a similar efficiency in all treatments. Previous studies found that the extent of ammonia production and VFA utilization in the rumen are important factors to determine the efficiency of utilization of protein and energy for higher ruminal microbe growth.[2,35–37]

Digestibility and retention

The feed digestibility is presented in Table 2. There were no significant differences ($p > 0.05$) among the treatments in DMD, OMD, and CPD. The supplementation of Spirulina platensis also did not change the digested nutrients ($p > 0.05$). The similar digestibility across all treatments was due to the non-extreme increase in feed intake. A previous study by Luthfi et al.[6] found that lambs had similar digestibility even though DMI increased from 4% to 7.9% of body weight. The difference in feed intake did not alter feed digestibility when there was no change in the rate of feed passage through the digestive tract. The finding of Carvalho et al.[18] showed that Spirulina platensis was a type of microalgae, and the cells did not have cell walls, so they were easily digested. The cell walls of Spirulina platensis did not have thick cell walls like other microalgae. Therefore, it was easily damaged[38,39] and had high potential to increase the bio-accessibility of its components in the rumen.

The retained protein of lambs fed T2 and T3 was higher than that of T0 and T1 ($p < 0.05$). It was due to the fermentation in the rumen, which resulted in high microbial N production Table 2. The high microbial N production, as an increase in the supplementation of Spirulina platensis, provided amino acids that can be easily metabolised. Costa et al.[40] claimed that the high microbial production in sheep enhanced the flow rate of amino acid absorption in the duodenum. It was because microbial protein provided all the amino acids required by ruminants. Cao et al.[41] found that some amino acids (valine, leucine, and isoleucine) could recruit signal transduction pathways that increased protein synthesis in skeletal muscle, which could result in higher ADG in small ruminants. Parvar et al.[36] found that dietary protein was degraded to ammonia, peptides and amino acids by rumen microbial enzymes. As a result, these compounds were converted into microbial protein. Microbial protein synthesis in ruminants provides a part of the amino acids required for growth, maintenance and animal production.

Blood ammonia and glucose concentrations

The concentrations of blood ammonia and glucose are presented in Table 3. Supplementing the basal diet with Spirulina platensis did not alter ammonia and glucose concentrations in the blood. Blood ammonia concentration of all treatments was similar both at 0h and at 3h ($p > 0.05$). The non-significant blood ammonia concentration among the treatments was due to the CPI in this study being similar among treatments, resulting in similar ammonia concentration in the rumen Table 2. The concentration of ammonia in the serum of lambs was within normal ranges for healthy ruminants. Radostits[42] and Izadi et al.[37] found the normal ranges of urea-N of sheep are 8 to 20 mg/dL. Because there was no significant effect of Spirulina platensis supplementation in the basal diet on ammonia and glucose

Table 3. Blood profile of thin-tailed lambs fed basal diet supplemented by spirulina platensis.

Parameters	T0	T1	T2	T3	SEM	*p* value[1)]
Ammonia (mg/dL)						
Ammonia 0 h	1.23	1.74	1.31	1.14	0.85	0.12
Ammonia 3 h	1.56	2.07	1.83	1.72	1.27	0.69
Diff. ammonia (0–3 h)	0.33	0.33	0.52	0.58	0.89	0.78
Glucose (mg/dL)						
Glucose 0 h	42.40	44.80	44.40	43.19	2.03	0.70
Glucose 3 h	63.19	64.44	63.99	64.09	3.01	0.63
Diff. Glucose (0–3 h)	20.78	19.64	19.59	20.90	1.05	0.51

SEM = Standard error of the mean; [1)]The different superscripts in the same row indicate significant differences among treatments ($p < 0.05$).

Table 4. Eating behavior of thin-tailed lambs fed basal diet supplemented by spirulina platensis.

Parameters (min/d)	T0	T1	T2	T3	SEM	*p* value[1)]
Drinking	51.67^{c}	60.83^{c}	260.00^{b}	596.67^{a}	13.19	0.03
Eating	266.67^{b}	260.00^{b}	558.33^{a}	517.50^{a}	34.49	0.00
Ruminating	317.50^{bc}	340.00^{b}	391.67^{b}	479.17^{a}	27.73	0.04
Standing	596.67^{c}	495.00^{c}	922.50^{a}	815.00^{b}	46.94	0.00
Laying	955.00^{b}	1062.50^{b}	1328.33^{a}	1390.83^{a}	52.76	0.00
Urinating	65.83^{c}	65.83^{c}	120.83^{a}	83.33^{b}	8.48	0.05
Defecating	30.00^{c}	29.17^{c}	59.17^{a}	49.17^{b}	4.37	0.02

SEM = Standard error of the mean; [1)]The different superscripts in the same row indicate significant differences among treatments ($p < 0.05$).

concentration at 0h and 3h Table 3, the difference in ammonia concentration in blood was also similar among the treatments. The average blood ammonia changes were 4.41 mg/L.

There was no significant difference in blood glucose concentration among the treatments($p > 0.05$), either at 0h or at 3h. Glucose is absorbed from the alimentary tract, including the absorption of VFA from the rumen into the blood, and used as an energy source. At 0 h before eating, the concentration of VFA in the rumen of all treatments was similar, resulting in similar blood glucose in all treatments. The increase at 3 h after eating was not linear with the increase in VFA as a result of supplementation of Spirulina platensis in the basal diet. It was suspected that lambs have a homeostatic system for blood glucose levels at 3h after feeding. Luthfi et al.[43] stated that if the blood glucose concentration is above the livestock threshold, insulin will automatically be secreted to store glucose in the form of glycogen, which is then released and transported into cells in the liver or muscles as glycogen reserves. Radostits[42] and Izadi et al.[37] found that the normal range of glucose levels is 50–80 mg/dL.

Eating behavior

The effect of Spirulina platensis supplementation in the basal diet of Thin-tailed lambs is presented in Table 4. Supplementing the basal diet with Spirulina platensis had a significant effect on Thin-tailed lamb activities ($p<0.05$). The lambs fed T2 and T3 had the highest eating activity. The lambs fed T2 had the highest standing, urinating and defecating activities compared to the others. The highest eating activity was in line with the higher DMI in lambs fed a basal diet supplemented with Spirulina platensis up to 1% and 1.5%. It also caused the higher standing and defecation in this study. Da Silva et al.[44] found that the higher the DMI, the more eating and standing activity is required to reach the feed to be eaten and then excreted through faeces. Dietary composition can influence the ingestive behaviour and physiological responses of animals as they adapt to nutritional changes.[45] Therefore, it alters the metabolism of the microbial population in the rumen, consequently affecting thermoregulation and the feeding behaviour of animals.[46]

On the other hand, the lambs fed T3 had the highest drinking, ruminating and lying activities compared to the others Table 4. In this case, as DMI increased, the more digestible cell wall portion tended to be less digested due to the faster digestion rate. This caused lambs fed T3 to have a longer drinking, lying, and ruminating time. Lima-Montelli et al.[47] claimed that DMI was negatively correlated with digestibility. In this study, the higher DMI observed in T3 lambs resulted in an increased digestion rate, and as a result, nutrient digestibility was not higher than the other treatments throughout the digestive tract.

Production and feed cost per gain

The final weight and average daily gain (ADG) of lambs fed T2 and T3 were higher ($p < 0.01$) than those of T0 and T1 Table 5. This is because supplementation of Spirulina platensis in the basal diet greatly increased DMI, VFA concentrations and utilized nutrient intakes to increase body weight gain. The higher amount of VFA absorbed through the rumen wall was sent to the liver and converted into glucose as an energy source for the animal's body metabolism. Sujani et al.,[48] claimed that the concentration of VFAs such as acetic, propionic and butyric acids in the rumen is the most important fermentation end product of ruminants and provides 70%–80% of the total energy requirements of the host animal. Luthfi et al.[2] found that Thin-tailed lambs had high productivity by increasing feed nutrients, quantity and utilization. This study was higher than the findings of Orzuna-Orzuna et al.[28] that the supplementation (around 6–30g/kg DM) of Spirulina platensis increased ADG up to 0.024 kg/d and decreased FCR −0.283 kg/kg. Rabee et al.,;[18]

Table 5. Nitrogen excretion and methane emission, productivity, feed and environmental cost of thin-tailed lambs fed basal diet supplemented by spirulina platensis.

Parameters	T0	T1	T2	T3	SEM	*p* value[1)]
Emissions						
Nitrogen excretion (g/d)	8.05^{a}	6.18^{c}	6.39^{bc}	6.46^{b}	0.26	0.03
Methane production (L/d)	1.83^{c}	2.26^{b}	2.15^{b}	2.63^{a}	0.06	0.00
Productivity						
Final Weight (kg)	22.44^{b}	23.43^{b}	26.61^{a}	26.98^{a}	0.60	0.00
ADG (g/d)	122.17^{b}	131.35^{b}	191.15^{a}	182.24^{a}	7.83	0.00
Cost						
Feed conversion ratio	7.35^{b}	6.24^{b}	4.74^{a}	5.41^{ab}	0.33	0.05
Feed cost per gain (Rupiah)	37.351^{bc}	35.653^{b}	31.996^{a}	41.194^{c}	1549	0.03
Nitrogen exc./ADG (g/gADG)	0.08^{c}	0.05^{b}	0.03^{a}	0.04^{a}	0.005	0.00
Methane prod./ADG (L/gADG)	16.77^{d}	15.54^{c}	11.52^{a}	13.98^{b}	0.001	0.02

SEM = Standard error of the mean; ADG = Average daily gain; [1)]The different superscripts in the same row indicate significant differences among treatments ($p < 0.05$).

Orzuna-orzuna et al.[28] and Allak et al.[49] claimed supplementation of microalgae enhanced the health of goat and sheep and improved ADG and FCR by fixing the nutrient utilization, reducing the percentage of DNA damage and improving the oxidative status in blood serum of goats and sheep.

Nitrogen excretion through feces and urine was decreased as Spirulina platensis supplementation level in the basal diet increased ($p < 0.05$). In this study, the higher the supplementation of Spirulina platensis in the basal diet, the lower the nitrogen excretion through feces and urine Table 5. This result confirmed that the microbial protein production of lambs fed a basal diet with supplemented Spirulina platensis was highly metabolized and retained high protein, which could be used to synthesize muscle cells and resulted in ADG Table 5.

The methane emission of lambs fed a basal diet supplemented with Spirulina platensis was significantly different ($p < 0.01$). Lambs fed T3 had the highest methane production compared to others Table 5. It was due to the supplementation of Spirulina platensis in the basal diet that resulted in higher VFA. The fermentation of feed in the rumen produced VFA consisting of acetic, propionic and butyric acids, and methane gas is a by-product of the formation of VFA during the fermentation process in the rumen. Microbes in the rumen break down feed and produce volatile fatty acids (VFA), carbon dioxide (CO_2), ammonia (NH_3) and methane (CH_4). During VFA synthesis, methanogenic bacteria utilize hydrogen (H_2) and carbon dioxide (CO_2) produced in the formation of acetic acid in the rumen to produce methane gas.[5,6]

The study also showed that supplementing the basal diet decreased the feed and environmental cost for body weight gain ($p < 0.01$). Lambs fed T2 had the lowest FCR and FC/G Table 5. The FCR in this study was also lower than the finding by Luthfi et al.[2] that the FCR of Thin-tailed lambs fed high feed intake (ad libitum; 7.9% BW) was 6.8. The high body weight gain of T2 implied that supplementation up to 1% was more efficient and cheaper than the other treatments. This study was comparable to the findings of Junior et al.[50] that Dorper x Santa inez lambs fed a high-energy diet with Orange (*Citrus sinensis*) essential oil inclusion resulted in 4.01 FCR. Rianto et al.[51] and Luthfi et al.[2] found that FCR increased as feed intake utilization and lamb productivity increased, resulting in higher feed efficiency, and the cheaper feed cost per gain achieved. This study indicated that supplementation of Spirulina platensis into the basal diet up to 1% was very efficient for increasing Thin-tailed lamb productivity. The supplementation of Spirulina platensis in the basal diet not only reduced FC/G cost but also significantly reduced ($p < 0.01$) nitrogen excretion and methane emission for increasing one unit of body weight gain. The study showed that the lambs fed T2 had the lowest nitrogen excretion (0.035 g/gADG) and methane emission (0.12 L/gADG).

Conclusion

It was evident that supplementation of Spirulina platensis into the basal diet increased DMI, nutrient utilization, rumen fluid, productivity, feed cost and nitrogen excretion and methane emission in sheep without altering their blood profile. The use of Spirulina platensis reduced methane and nitrogen emissions into the environment. It was concluded that supplementation of Spirulina platensis into the basal diet at 1% of the diet is optimum to improve the efficiency of feed, productivity and reduce the environmental cost of Thin-tailed lambs production.

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Authors' declaration

- Conflicts of Interest: None.
- We hereby confirm that all the Tables in the manuscript are ours.
- No human studies are present in the manuscript.
- The author has signed an animal welfare statement.
- Ethical Clearance: The project was approved by the local ethical committee at Universitas Diponegoro, Semarang, Indonesia.

Authors' contributions statement

Conceptualization: L. N., R.E., Data curation: L. N., R. E.,Formal analysis: S. H.F., Methodology: L. N., R. E., Software: L. N., S.H.F., Validation: L. N., R. E., S. S., P. E., Investigation: P. E., S. S., R. E., Writing - original draft: L. N., R. E., Writing - review & editing: L. N., R. E., S. S., P. E.

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تأثير إضافة طحالب السبيرولينا إلى النظام الغذائي الأساسي على إنتاجية الحملان ذات الذيل النحيف وتكلفتها البيئية في عملية التسمين

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الخلاصة

السبيرولينا (Spirulina platensis) هي مكمل غذائي له تأثير جيد على أداء النمو لدى الحملان، إلا أن المعلومات المتعلقة بمستوى هذه المادة في كفاءة الأعلاف وتأثيرها البيئي لا تزال بحاجة إلى تحديد. هدفت الدراسة إلى فحص تأثير مستوى إضافة السبيرولينا (Spirulina platensis) إلى النظام الغذائي الأساسي على الإنتاجية والتكاليف البيئية لتسمين الحملان ذات الذيل الرقيق. تم استخدام أربعة وعشرين حملًا ذكراً (بعمر 4 أشهر ووزن 14.99 كجم) وفق تصميم عشوائي كامل مع 4 معاملات و6 مكررات لكل معاملة. كانت المعاملات كالتالي: 1) نظام غذائي أساسي 100% بدون إضافة السبيرولينا (T0)؛ 2) نظام غذائي أساسي 99.5% + 0.5% سبيرولينا (T1)؛ 3) نظام غذائي أساسي 99.0% + 1.0% سبيرولينا (T2)؛ و4) نظام غذائي أساسي 98.5% + 1.5% سبيرولينا (T3). كانت المتغيرات المقاسة هي تناول المادة الجافة (DMI)، استخدام المغذيات، سائل الكرش وملف الدم، الإنتاجية، تكلفة العلف، وإفراز النيتروجين وانبعاث الميثان.

أظهرت النتائج أن DMI في المعاملة T3 كان الأعلى بين المعاملات (985.66 غ/يوم؛ $p<0.05$). كان البروتين المحتجز في T2 (70.82 غ/يوم) و T3 (72.49 غ/يوم) متشابهاً ($p>0.05$) وأعلى من المعاملات الأخرى ($p<0.05$). كانت الأحماض الأسيتيك والبروبيونيك والبيوتيريك بعد 3 ساعات من التغذية في T3 أعلى ($p<0.05$) من باقي المعاملات (178.21 ملي مول، 69.82 ملي مول، و12.42 ملي مول على التوالي). كانت زيادة الوزن اليومية (ADG) في الحملان المغذاة على T2 و T3 متشابهة (بمتوسط 186.7 غ/يوم؛ $p>0.05$) وأعلى من باقي المعاملات. لم يكن هناك فرق معنوي في معامل تحويل العلف (FCR) بين T2 و T3 ($p>0.05$)، لكنه كان أعلى ($p<0.05$) من T0 و T1، بينما لم تختلف T0 و T1 و T3 معنوياً ($p>0.05$). كانت تكلفة العلف لكل زيادة وزن (FC/G) في T2 (31,996 روبية/كغ ADG) هي الأقل ($p<0.05$) بين المعاملات. كان إفراز النيتروجين لكل كغ ADG في T2 و T3 متشابهاً ($p>0.05$؛ بمتوسط 0.035 غ/كغ ADG) وأقل ($p<0.05$) من T0 و T1. كان انبعاث الميثان لكل كغ ADG في T2 (11.52 لتر/ADG) هو الأقل ($p<0.05$) بين باقي المعاملات. في الختام، أدت إضافة السبيرولينا (Spirulina platensis) حتى مستوى 1% (T2) إلى زيادة الإنتاجية، وتقليل تكلفة العلف، والتكلفة البيئية لإنتاج الحملان ذات الذيل الرقيق.

الكلمات المفتاحية: النظام الغذائي، التكلفة البيئية، الحملان، استخدام المغذيات، الإنتاجية، السبيرولينا.