TÜBİTAK

Turkish Journal of Veterinary and Animal Sciences

http://journals.tubitak.gov.tr/veterinary/

Research Article

Turk J Vet Anim Sci
(2021) 45: 21-27

doi:10.3906/vet-2007-51

The effect of *Jatropha multifida* L. as a natural dietary additive on anthelmintic activity and performance in lambs infected by *Trichostrongylus* spp.

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Received: 14.07.2020 • **Accepted/Published Online:** 31.01.2021 • **Final Version:** 23.02.2021

Abstract: *Jatropha multifida* leaf powder (JMLP) is rich in phenolic tannins and it has the potential to be a natural dietary additive for ruminants. A 4 × 5 factorial design was used to study the effects of 4 different doses of JMLP treatment (dry matter, DM basis) at 0% (JMLP-0), 0.5% (JMLP-0.5), 0.75% (JMLP-0.75), and 1% (JMLP-1) in the diet of ewe lambs infected by *Trichostrongylus* spp. at 2332 ± 248 eggs/g feces on fecal egg counts (FEC, eggs/g feces) of 5 different measurement times (0, 14, 28, 42, and 84 days) using 5 replicates. Average daily gain (ADG, g/head/day) and dry matter intake (DMI, g/head/day) of the lambs were also recorded at 14, 28, 42, and 84 days of the feeding trial. The results showed that JMLP treatments, measurement times, and their interactions had significant effects ($P < 0.05$) on FEC, ADG, and DMI. Based on the averages of all the measurement times, the lambs fed JMLP-0.5 had the lowest ($P < 0.05$) FEC and DMI, and the highest ($P < 0.05$) ADG compared with other JMLP treatments. Based on the averages of all the JMLP treatments, day 42 had the lowest ($P < 0.05$) FEC and DMI, and the highest ($P < 0.05$) ADG in comparison with other measurement times. It is suggested that 0.5% of JMLP can be included in the diet of growing lambs as an antiparasitic to improve their performances and its efficacy can be seen optimally after 42 days.

Key words: *Jatropha multifida* leaves, anthelmintic, dietary additive, lambs

1. Introduction

The prohibition of growth-promoting antibiotic usage in livestock production has been applied in The European Union since 2003 (EC Regulation No. 1831/2003) and followed by other countries such as Indonesia in 2017 (Indonesian Ministry of Agriculture Regulation No. 14/2017). This has led researchers to investigate the potential use of medicinal plants containing plant secondary metabolites such as phenolic tannins as natural anthelmintic and dietary additive in the diets of ruminants [1,2]. Tannins are able to improve not only productivity [1–4] but also health [5] of the ruminant animals. Tannins can decrease the solubility and degradability of most feed proteins by rumen microbes because of their binding ability to the plant proteins resulting in decreased protein digestibility and ammonia output in the rumen but increasing potential by-pass protein to be absorbed in the small intestine [6–8].

A previous study involving sheep, goats, and deer suggested that about 3-4% condensed tannins in the diets of ruminants should be reached to have an antiparasitic activity [9]. *Jatropha multifida* leaf powder (JMLP) consists of 34.5% total phenols and 13.2% total tannins [10]. These bioactive compounds are nontoxic materials and are potentially used as a natural anthelmintic and dietary additive [10,11]. Therefore, this study aimed to test the hypothesis that an appropriate amount of JMLP inclusion into a diet of ewe lambs infected by *Trichostrongylus* spp. would provide antiparasitic activity and improve average daily gain (ADG, g/head/day) of growing lambs during 84 days of a feeding trial. The antiparasitic activity and ADG improvement due to JMLP inclusion would be dependent upon dose and duration.

2. Materials and methods

2.1. Animals

The use of experimental lambs in this study has been approved by the research ethical committee of Universitas Padjadjaran No. 1434/UN6.KEP/EC/2018. Twenty ewe lambs that are about 8 months old (of Priangan breed, Decree of Indonesian Agricultural Minister No. 300/Kpts/SR.120/5/2017) were used in this experiment. Their initial average body weight was 16.4 kg (8.78 coefficient of variation, CV). After 1 week of adaptation and 3 weeks of worm infecting processes, the average body weight of lambs became 15.3 kg (5.86% CV) and the experiment

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was started here. Each ewe was randomly placed in an individual pen (1.5-m long × 0.8-m wide × 0.9-m high) parted by wood panels through which each of individuals had eye and part-physical contacts. Each lamb had free access to experimental diet and ad libitum clean water. Each lamb was given a commercial anthelmintic (Ivomec, at 1 mL/kg body weight) until worm-free as seen from the FEC analysis. After that, about 10,000 third-stage larvae (L_3) of Trychostrongylidae were orally given to each lamb. These larvae were obtained from eggs (Figure) of 4 infected donor lambs. Larvae infesting was done every week for 3 weeks. In the 3rd week, each lamb was measured for FEC (2332 ± 248 eggs/g feces) and the day-0 of the experiment was begun.

2.2. Animal feeding

During the feeding trial, each lamb was fed a basal diet consisting of elephant grass (GRS) (*Pennisetum purpureum* cv. Mott) and a commercial concentrate (CON). Each lamb was offered a basal diet at 4% (DM basis) of the body weight (656 g) containing 80% GRS (524.8 g) and 20% CON (131.2 g). JMLP treatments were included in the CON at 0% (JMLP-0), 0.5% (JMLP-0.5 = 3.28 g), 0.75% (JMLP-0.75 = 4.92 g), and 1% (JMLP-1 = 6.56 g). All lambs were fed 2 times daily: full CON and halved GRS at 07.00 AM and other halved GRS in the late afternoon at 03.00 PM. Refusal of CON and GRS were collected and weighed each day before morning feeding (06.00 AM). Chemical compositions of GRS and CON along with JMLP can be seen in Table 1.

2.3. *Jatropha multifida* leaf powder (JMLP)

Jatropha multifida leaves (mature, dark green) were picked and collected from Agriculture Development Polytechnic (Polbangtan, Bogor) in the morning at 07.00–08.00 AM. The leaves were washed with tap water and dried in a drying oven at 60 °C until the dried weight of the leaves was constant. The dried leaves were then mashed up using a blender until they reached powder form (JMLP). During the feeding experiment, each JMLP treatment was given to the corresponding lamb in the morning by mixing it with the CON diet.

2.4. Infective larvae (L_3)

Infective larvae (Figure) were initially prepared by collecting fresh feces from 4 worm-infected ewes. The feces were then pooled and broken down, mixed with vermiculate, and stored in a dark place at 22 °C with 70–80% humidity for 10 days. Finally, infective larvae (L_3) were harvested using the Baermann technique.

2.5. Data collection and measurements

Feces were collected with the grab sampling method from rectum of each lamb at 0, 14, 28, 42, and 84 days after larvae infection. The examination of FEC was done using the floating and Whitlock counting slide methods [12]. Briefly, about 3 g of each feces sample was weighed and put into a beaker glass where 17 mL of water was added and stirred. After that, about 40 mL of saturated salt solution was also added into the beaker glass and mixed until homogeneous. About 0.5 mL of the mixed solution was then taken using a pipette into a Whitlock counting slide and the worm eggs were identified and counted under a microscope with 40× magnification. The FEC result as eggs per gram feces was calculated by multiplying the number of discovered worm eggs with 40 [13]. The identification of eggs and worm larvae type was based on the RVC method [14,15].

Daily intake was measured by counting the difference between offered and refused feeds correspondingly in a gram of DMI (g/head/day). The lambs were weighed before morning feeding using a digital weighing scale at day 0 (initial weight), 14, 28, 42, and 84, and ADG (g/head/day) was calculated with the following equation:

$$ADG = \frac{final\ weight\ (kg) - initial\ weight\ (kg)}{time\ (days)}$$

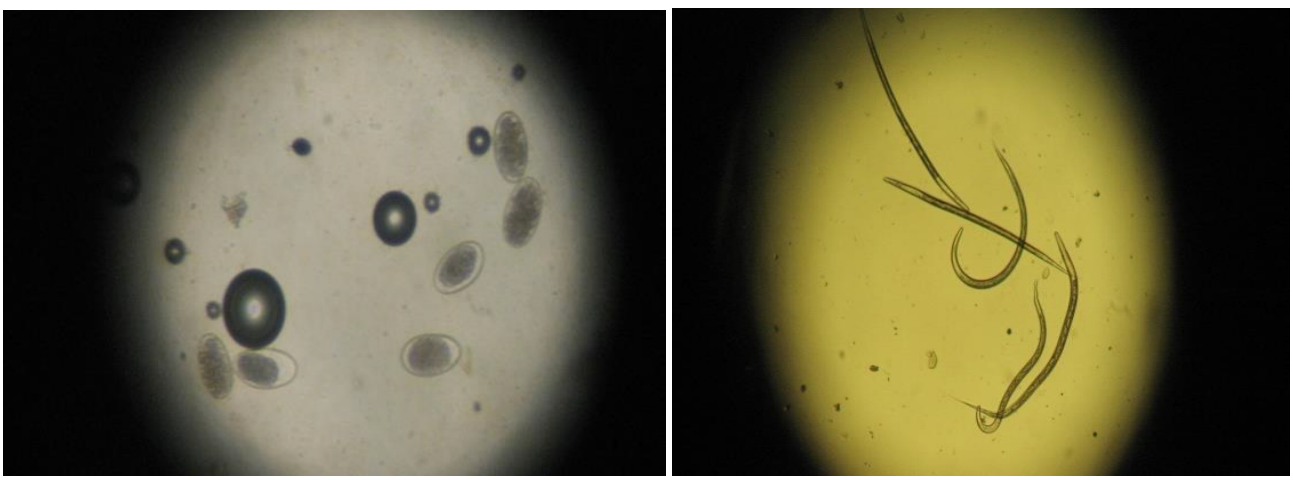

Figure. Microscopic eggs (left) and infective larvae (right) of *Trichostrongylus* spp.

Table 1. Chemical compositions (% DM or otherwise stated) of feed materials.

Contents	GRS	CON	JMLP
DM (% sample)	21.7	66.5	98.0
Ash	13.4	8.86	10.8
CP	10.8	8.56	26.4
CF	27.8	10.7	16.0
NFE	44.94	68.2	42.1
EE	3.12	4.68	3.75
TDN	58.8	71.4	71.3
Ca	0.24	0.56	1.10
P	0.21	0.20	0.21
TP	4.71	0.58 – 0.63[4,18]	36.5
TT	2.97	0.18 – 0.57[4,18]	13.2

DM: dry matter, GRS: grass, CON: concentrate, JMLP: *Jatropha multifida* leaf powder, CP: crude protein, CF: crude fiber, NFE: nitrogen-free extract, TDN: total digestible nutrients, Ca: calcium, P: phosphorus, TP: total phenols, TT: total tannins.

2.6. Chemical analyses

Dried and ground feed samples were examined using standard methods of the Association of Official Analytical Collaboration (AOAC, 2005) [16] to determine DM, crude protein (CP, AOAC 990.03-2002), crude ash (CA, AOAC 942.05-1942), ether extract (EE, AOAC 920.39-1920), and crude fiber (CF, AOAC 962.09-1971). Similar methods of AOAC [16] were also utilized to determine calcium (Ca, AOAC 927.02-1945) and phosphor (P, AOAC 965.17-1966) based on atomic absorption spectroscopy (Perkin Elmer AAnalyst 400) and spectrophotometer (Multimode Reader Infinite 200 Pro Nanoquant) analyses, respectively.

The sample preparation for Ca analysis was as follows: about 5 g of each dried sample was weighed in a porcelain plate and put into a furnace at a gradually increased temperature of 100 °C every 30 min until it reached 600 °C for about 6 h. The samples were then removed from the furnace and cooled at room temperature before adding 5 mL of 65% HNO_3 and drying it on a stove. After cooling at a room temperature, each sample was then transferred into a 50-mL flask with the help of distilled water (mili-Q) and the water was added until the limit mark. Each solution was diluted using distilled water when necessary.

Making a standard curve for Ca analysis was initially done with pipetting 1 mL of standard solution of Ca 1000 ppm and putting it in a 10-mL flask to make a standard solution of Ca 100 ppm. This 100-ppm solution was pipetted as much as 0.2, 0.4, 0.6, 0.8, and 1 mL and put into10-mL flask; distilled water was added until the mark line and the solution was homogenized to obtain a standard solution of Ca: 2, 4, 6, 8, and 10 ppm. Finally, the standard solution of Ca, along with the sample solutions, was measured using the Atomic Absorption Spectrophotometer (AAS) at λ 422.7 nm. Distilled water was also measured as a blank sample.

Phosphorus analysis was begun with dissolving 2 g of ammonium molybdite in 40 mL of warm distilled water (50 °C). Separately, 0.1 g of ammonium vanadate was put into a 30-mL flask and dissolved with boiled distilled water, cooled, and added 14 mL of HNO_3 while being stirred. The two solutions were combined and added distilled water to a volume of 100 mL. Standard phosphate solution was initially prepared by weighing potassium dihydrogen phosphate 0.1918 g and dissolving it in 100 mL of distilled water (1000 ppm). From a standard phosphate solution of 1000 ppm, pipetted 0.05, 0.1, 0.2, 0.3, 0.4, and 0.5 mL into a 10-mL flask, and 2 mL of vanadat-molibdat reactor and distilled water until the mark line were added. The solution was homogenized to obtain the standard of 0, 5, 10, 20, 30, 40, and 50 ppm. It was the kept at room temperature for 10 min before measuring the absorbance values with UV-Vis Spectrophotometer at λ 400 nm.

The Folin–Ciocalteu procedure was used to quantify total phenols (TP) and total tannins (TT) in GRS and JMLP as explained in Makkar [17] with tannic acid (Sigma–Aldrich, Germany) as the reference standard. The total phenol and total tannin tests were conducted using the Folin-Ciocalteu method based on a spectrophotometer (Varian Cary 50 UV-Vis Spectrophotometer, Agilent) with an absorbance value reading at a wavelength of 725 nm. The samples were extracted using 70% (v/v) acetone. The TP and TT contents in CON were referred to the data in Ramdani et al. [4] and Gerlach et al. [18]. Nitrogen-free extract (NFE) was calculated using the following equation: NFE = 100 – (CA + CP + CF + EE). Total digestible nutrients (TDN) for CON was predicted using the following equation: TDN = 70.6 + (0.259 × CP) + (1.01 × EE) – (0.76 × CF) + (0.0991 × NFE), while TDN for GRS and JMLP were estimated using the following equation: TDN = (–26.685) + (1.334 × CF) + (6.598 × EE) + (1.423 × NFE) + (0.967 × CP) – (0.002 × (CF2)) – (0.67 × (EE2)) – (0.024 × (CF × NFE)) – (0.055 × (EE × NFE)) – (0.146 × (CF × CP)) + (0.039 × ((CF2) × CP)) [4]. All chemical contents were expressed as % DM except DM was expressed as % fresh sample.

2.7. Statistical analysis

Each chemical content of the feed materials was calculated as an average from duplicate analysis (n = 2). A 4 × 5 factorial design was used to compare 4 different doses of JMLP treatments (JMLP-0, JMLP-0.5, JMLP-0.75, and JMLP-1) in a diet of ewe lambs infected by *Trichostrongylus* spp. and 5 different measurement times (0, 14, 28, 42, and

84 days) on FEC, ADG, and DMI using 5 replicates. The data were statistically analyzed using multiway ANOVA in SPSS 21 statistical software in which the Duncan multiple range test was applied to compare means. Statistical significance was assumed at $P < 0.05$. The residual data were analyzed for normality by passing the Shapiro–Wilk normality test at $P > 0.05$.

3. Results

Table 1 presents the chemical contents (% or otherwise stated) of GRS, CON, and JMLP as feed materials used in this experiment. As expected, JMLP had higher DM, CP, TP, and TT contents in comparison with GRS and CON.

Table 2 presents mean body weights (kg/head) and FEC (eggs/g feces) of the infected lambs for the main effects of JMLP treatments and times along with SEM and significances. Based on the averages of all the times, JMLP-1 resulted in the lowest ($P < 0.05$) body weight and the greatest ($P < 0.05$) FEC compared with the other treatments while JMLP-0.5 resulted in the lowest ($P < 0.05$) FEC and it tended to result in higher body weight along with JMLP-0.75 in comparison with JMLP-0 although they were not statistically different. Based on the averages of all the JMLP treatments, day 84 had the highest ($P < 0.05$) body weight in comparison with the other measurement times.

Table 3 shows mean ADG (g/head/day) and DMI (g/head/day) of the infected lambs for the main effects of JMLP treatments and times along with SEM and significances. Based on the averages of all the times, JMLP treatments from 0.5% to 1% resulted in higher ($P < 0.05$) ADG than JMLP-0 but JMLP-0.5 resulted in the lowest ($P < 0.05$) DMI compared to the other treatments. The lambs on JMLP-0.5 consumed the lowest ($P < 0.05$) grass but they consumed the highest ($P < 0.05$) CON. Although there was no significant difference ($P > 0.05$) among JMLP treatments in ADG, JMLP-0.5 was better. Based on the averages of all the JMLP treatments, day 42 had the highest ($P < 0.05$) ADG but day 84 had the greatest ($P < 0.05$) DMI. Day 28 had the lowest ($P < 0.05$) DMI of grass and CON in comparison with the other treatments.

4. Discussion

Phenolic tannins offer several advantageous impacts to ruminant animals such as providing antiparasitic properties [19]. The beneficial effect of tannins on reducing gastrointestinal nematodes in ruminants can act indirectly by enhancing the host's response to parasites. The ability to bind to proteins by tannins can protect proteins from rumen degradation but increase protein flow and absorption of amino acids in the small intestine. Increased intestinal protein supply is known to increase host homeostasis and immune responses to worms [9].

The body weight on day 14 of treatment is the lowest bodyweight along with the highest number of FEC compared to treatment on other days. The current study found that moderate and high *Trichostrongylus* spp. infection in lambs could decrease body weights but an appropriate dose of JMLP inclusion in the diet would improve ADG. Iqbal et al. [20] observed that sheep fed with 2% and 3% tannin extract showed higher body weight gains of 56.6 and 68.3 g/head/day, respectively compared with those fed without tannin extract. Nematollahi et al. [12] concluded that severe anemia and loss of body weight are the potential clinical signs of nematode worm infection in sheep. Williams et al. [21] also found that Trichostrongylidae infection caused acute diarrhea. Trichostrongylidae worm eggs are commonly found in the feces about 21 days after oral infection and the adult worms could be found in the small intestine [22]. As a consequence of parasitism, the infected lambs presented reduced serum albumin concentrations and demonstrated severe small intestine lesions, such as villous atrophy and epithelial erosion, which impaired the digestion and absorption of nutrients, causing a significant loss in performance [23]. Sheep infected with *T. colubriformis* also showed severe lesions in the small intestine mucosa, such as generalized villous atrophy and erosion in the duodenal epithelium. These lesions would likely cause a decrease in the efficiency of nutrient digestion and absorption, causing significant impairment in their productive performance [24].

FEC on day 14 for all treatments with the addition of JMLP has increased and FEC without JMLP administration has decreased, this is due to the level of immunity of the host after receiving a severe infection response. After infection, the larvae can become metabolically inactive for a period which may last several months, and then continue development. Although the immune status of the host also influences the degree of hypobiosis, most larvae usually stop at some time of the year, when environmental conditions are least favorable for the development and survival of eggs and larvae [25].

On day 28, all treatments with the addition of JMLP decreased and FEC in the treatment without JMLP increased, this is because the consumption of tannins given to the feed was able to reduce worms. Research on various plants rich in tannins (*Acacia pennatula*, *Lysiloma latisiliquum*, and *Leucaena leucocephala*) as anthelmintic using the Larval Migration Inhibition Assay method by Alonso-Díaz et al. [26] concluded that tannin extracts could be used as an anthelmintic for the control of *H. contortus*. The administration of quebracho extracts containing 73% tannins at 16% w/w of the feed intake had a significant effect on reducing the number

Table 2. Effects of JMLP treatments on mean body weights (g/head/day) and FEC (eggs/g feces) of ewe lambs infected by *Trichostrongylus* spp. during 84-day feeding trial together with SEM and significances[a].

Measurement	JMLP treatments (n = 25)_				Times (n = 20)					SEM and significance		
	JMLP-0	JMLP-0.5	JMLP-0.75	JMLP-1	Day 0	Day 14	Day 28	Day 42	Day 84	Treatment	Time	Treatment × time
Body weight	15.7^{b}	16.5^{b}	16.2^{b}	14.3^{a}	15.3^{A}	14.9^{A}	15.2^{A}	16.1^{AB}	16.8^{B}	0.48^{*}	0.36^{*}	0.93^{NS}
FEC	2645^{bc}	1166^{a}	2147^{ab}	3603^{c}	2332^{A}	4102^{B}	1724^{A}	1294^{A}	2499^{A}	507.8^{*}	479.1^{*}	862.7^{*}

[a]Mean values were significantly different at $P < 0.05$ (*), $P < 0.01$ (**), $P < 0.001$ (***) and not significantly different at $P > 0.05$ (^{NS}), NS: not significant, JMLP: *Jatropha multifida* leaf powder, FEC: fecal egg count (eggs/g feces), n: number of replicates, SEM: standard error of mean, JMLP-0: JMLP in the diet at 0%, JMLP-0.5: JMLP in the diet at 0.5%, JMLP-0.75: JMLP in the diet at 0.75%, JMLP-1: JMLP in the diet at 1%.

Table 3. Effects of JMLP treatments and times of measurement on mean ADG (g/head/day) and DMI (g/head/day) of ewe lambs infected by *Trichostrongylus* spp. during 84-day feeding trial together with SEM and significances[a].

Measurements	JMLP treatments (n = 20)				Times (n = 20)				SEM and significance		
	JMLP-0	JMLP-0.5	JMLP-0.75	JMLP-1	Day 14	Day 28	Day 42	Day 84	Treatment	Time	Treatment × time
ADG	-15.40^{a}	41.07^{b}	38.75^{b}	18.04^{b}	-29.10^{A}	21.25^{B}	69.10^{C}	21.30^{B}	13.06^{*}	20.05^{*}	20.22^{*}
DMI	584.1^{c}	495.9^{a}	552.8^{bc}	568.3^{b}	556.0^{B}	522.8^{A}	530.9^{A}	591.4^{C}	7.90^{*}	14.16^{*}	15.88^{*}
DMI grass	466.7^{c}	384.9^{a}	462.4^{c}	452.1^{b}	440.1^{B}	413.2^{A}	432.5^{B}	480.2^{C}	19.35^{*}	13.80^{*}	7.09^{*}
DMI CON	117.4^{b}	126.1^{c}	103.1^{a}	104.0^{a}	115.8^{B}	107.9^{A}	115.63^{B}	111.18^{AB}	5.53^{*}	1.91^{*}	3.29^{*}

[a]Mean values were significantly different at $P < 0.05$ (*), $P < 0.01$ (**), $P < 0.001$ (***) and not significantly different at $P > 0.05$ (^{NS}), NS: not significant, JMLP: *Jatropha multifida* leaf powder, ADG: average daily gain, DMI: dry matter intake, CON: concentrate, n: number of replicates, SEM : standard error of mean, JMLP-0: JMLP in the diet at 0%, JMLP-0.5: JMLP in the diet at 0.5%, JMLP-0.75: JMLP in the diet at 0.75%, JMLP-1: JMLP in the diet at 1%.

of *Trichostrongylus colubriformis* worm eggs [27]. Athanasiadou et al. [27] found the observed anthelmintic activity of quebracho extract in vitro could be attributed to tannin capacity to bind to proteins and could operate via several mechanisms. Tannins could bind to the free protein in the wells for larvae nutrition; reduced nutrient availability in the wells could have resulted in larvae starvation and death. Condensed tannins may also bind to the cuticle of larvae, which is high in glycoprotein. During the transcuticular diffusion process, bioactive compounds such as alkaloids, flavonoids, saponins, and tannins move into the body of larvae so that the process of energy formation and absorption of nutrients is disrupted [28]. In this condition, the larvae will die because of the reduced amount of energy and electrolytes inhibit larval development [29].

Consumption of dry ingredients is influenced by palatability where JMLP has a pungent aroma and bitter taste because it contains higher phenols and tannins. The proportion of JMLP-0.5 could be estimated to have no negative effect on the palatability since CON with JMLP-0.5 intake by lambs was higher than the other treatments. Meanwhile, the lambs fed JMLP-1 treatment reduced their CON consumption. This might be caused by higher JMLP inclusion in the CON.

Decreased palatability due to high tannin inclusion can be caused by a reaction between tannins and saliva or a direct reaction with taste receptors triggering the sense of taste [30]. Frutos et al. [30] discovered that high tannin content in a diet of sheep (>50 g/kgDM) can reduce feed intake while low tannin content (<50 g/kg DM) does not affect feed intake. Iqbal et al. [20] found that sheep fed with a diet containing tannin showed less DMI (854 g/head/day) compared with those without tannin addition (964.4 g/head/day). A decrease in feed consumption is also influenced by parasitic investment. Subclinical parasitic infections by *Trichostrongylus* spp. can reduce feed consumption by 10–30% and this nematode can impair the absorption of nutrients for growth [31].

Intestinal inflammation occurs in sheep infected with helminths, causing an alteration in vascular permeability,

with the diffusion of liquids into the abdominal cavity, especially in cases of chronic infections, electrolyte imbalance, and protein loss [24].

It can be concluded that JMPL can be considered a potential natural dietary additive and anthelmintic as it contains considerable amounts of protein, phenols, and tannins. A proper dose of JMLP treatment can reduce the numbers of *Trichostrongylus* spp. worm eggs, leading to increased ADG in growing lambs. JMLP utilization at 0.5% in the diet (dry matter basis) of growing lambs is recommended as a dietary additive and antiparasitic and its efficacy can be seen optimally after 42 days.

Acknowledgments
The authors would like to thank the Indonesian Ministry of Agriculture for funding this research through BPPSDMP scholarship program 2017. The first author received a full postgraduate scholarship from the Indonesian Ministry of Agriculture to study at the Faculty of Animal Husbandry, Universitas Padjadjaran.

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