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The Kielanowski Institute of Animal Physiology and Nutrition, Polish Academy of Sciences, Jabłonna

Manila palm extract supplementation improves rumen fermentation and eliminates gastro-intestinal parasites in goats

T. Norrapoke[1], T. Pongjongmit[1,*] and C. Supapong[2]

[1] Kalasin University, Faculty of Agricultural Technology, Department of Animal Production Technology, Kalasin Province, 46000, Thailand
[2] Rajamangala University of Technology Srivijaya, Faculty of Agriculture, Department of Animal Science, Nakhon Si Thammarat, 80240, Thailand

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* Corresponding author:
e-mail: tpongjongmit@hotmail.com

ABSTRACT. The purpose of the study was to investigate the impact of Manila palm supplementation on rumen fermentation and eradication of gastrointestinal nematodes in goats. Sixteen native crossbred goats, with an average weight of 20 ± 3.1 kg were utilised in a randomized complete block design experiment. Group 1 (control) received ivermectin at a dose of 3.03 µg/kg body weight. Groups 2, 3 and 4 were treated with Manila palm seeds, peel and whole Manila palm extract at a rate of 5 ml/kg goat weight, respectively. The results revealed that Manila palm supplementation did not have a significant impact on feed conversion ratio, feed intake, digestibility, blood urea nitrogen, haematocrit, ruminal pH, ammonia-nitrogen, temperature, total volatile fatty acids, butyric acid or microbial population ($P > 0.05$). However, the concentration of acetic acid was reduced by Manila palm supplementation, whereas propionic acid was increased, particularly in the Manila palm whole extract group, compared to the ivermectin-treated goats. Additionally, methane (CH_4) production was decreased in the Manila palm-supplemented group when compared to the ivermectin group. Moreover, the faecal egg count decreased after 7 and 21 days of treating the goats with Manila palm extracts. Therefore, the study concluded that supplementation with Manila palm whole extract at a rate of 5 ml/kg goat weight resulted in an increase in propionic acid, a decrease in methane production and faecal egg count, and did not affect microbial populations.

Introduction

Currently, the profession of goat farming is gaining popularity among Thai farmers and goats are considered animals of major economic significance in Thailand. According to the Planning Division Department of Livestock Development (2018), in 2017, there were 46478 goats, compared to 17209 in 2012, representing an annual increase of 170.08%. However, data from the Veterinary Research and Development Center, Eastern Region (VRD-EP) in 2020 demonstrated that the goat population was gaunt, anaemic and decreasing due to excessive deaths, the main cause being infection with external and internal parasites or infections with blood protozoa (Department of Livestock Development, 2020). As a result of helminthiasis and other diseases, the farming profession is still facing challenges in meeting consumer demand, particularly due to parasites that cause significant problems for farmers. Large numbers of internal helminths carried by goats can result in acute diseases and deaths, and even smaller infestations can lead to decreased productivity. To address this, farmers can use deworming drugs, but they can increase costs and may result in residue deposition, which is against the principle of halal feed. Therefore, it is advisable to use clean feed ingredients in goat diets that are free from residues and do not contain meat mixtures such as brain, blood, and bones. In addition, the use of hormonal substances to accelerate growth (growth promoter) and antibiotics is prohibited (Semae, 2007). In line with the traditional wisdom of Thai villagers, many herbs such as neem tree, cassod tree, papaya, tamarind, betel nut or Manila palm are used as antiparasitic drugs (Limcharoen et al., 2013).

The use of herbs in ruminant feeds is mainly aimed at stimulating feed intake, digestion and eliminate parasites (Korosec et al., 2009). Herbs contain essential active ingredients that can enhance feed utilization and help treat animal injuries. These active ingredients contained in herbs are secondary substances present in plants, with a wide range of applications, involving the functioning of several body systems (Wachtel-Galor and Benzie, 2011).

Manila palm (*Adonidia merrillii* Becc.) is a palm species from the family *Arecaceae*. Manila palm fruits have a dry matter (DM) content of 63.15%, and contain around 4.31% crude protein (CP), 68.63% neutral detergent fibre (NDF), 38.98% acid detergent fibre (ADF), and 13.54% condensed tannin (CT). The seeds of Manila palm contain arecoline, which has antiparasitic properties against helminthiasis caused by tapeworms and nematodes. The fruit of Manila palm also contains approximately 20.16 mg/100 g of tannins, which are mainly hydrolysable gallotannins (Bassey et al., 2017). These tannins can form stable protein-tannin complexes and interact with a wide variety of protein targets in microbes and animals (Van Wyk et al., 2015a; b). Gallotannins, which can also condense with catechins, contain a large number of phenolic hydroxyl groups, allowing them to form stable protein-tannin complexes and thus interact with a wide variety of protein targets in microbes and animals (Van Wyk et al., 2015a; b).

Our hypothesis was that Manila palm supplementation could be effective in modifying rumen fermentation by stimulating the rumen microbial growth and reducing the number of gastrointestinal nematodes. Therefore, the aim of the study was to investigate the effect of Manila palm supplementation on production performance, rumen fermentation, haematology and eradication of gastrointestinal nematode control in goats. The work presents an interesting approach that utilises locally available herbs and reduces the use of antibiotics in goats, ultimately increasing the productivity of the animals' diet.

Material and methods

All experiments were conducted in accordance with the principles and guidelines approved by the Animal Ethics Committee of Kalasin University. Manila palm used in the experiment was obtained from Kalasin Province.

Herbal extract preparations

Manila palm fruits were prepared by selecting half-soft, half-mature, and whole fruits (aged between 4–6 weeks) in fresh condition. The fruits were then baked at 60 °C, ground and sieved through a 1 mm sieve. A total of 1 kg of the ground fruits were mixed with 1 l of water, boiled, and simmered until 200 ml of extracted water was obtained.

Animals, diets and experimental design

Eight male and eight female native crossbred goats, with an initial body weight (BW) of 20 ± 3.1 kg and age between 1–2 years were used in this experiment. All animals were housed in individual pens and offered a commercial concentrate diet (16% CP) at 2% BW. Rice straw, water and mineral salt block were offered *ad libitum*. The experiment was a randomized complete block (based on sex) design (RCBD) trial planned to compare 4 forms of ivermectin and Manila palm supplementation. Group 1 received ivermectin at a dose of 3.03 μg/kg body weight. Groups 2, 3 and 4 were treated with seeds from Manila palm, Manila palm peel and Manila palm whole extract at a dose of 5 ml/kg body weight, respectively. The experiment was conducted for 35 days.

Data collection and samples analysis

Animals were weighed at the beginning and at the end of each period to determine changes in body weight. Feed intake was recorded throughout the experimental period and was calculated by subtracting the amount of feed refused from the feed offered to the animals; Uneaten feed was discarded before morning feeding. Spot sampling method was used to collect feed offered and feed refused.

Faeces were collected by rectal method in the morning in the last 5 days of the trial. The dry matter (DM) content of feed and rice straw was determined by oven-drying at 100 °C to a constant weight. The feed and faeces were dried at 60 °C for 48 h, then ground and sieved through a 1 mm screen (Cyclotech Mill, Tecator, Hoganas, Sweden) and used for chemical analysis. The diets and faeces were chemically analysed for the content of organic matter (OM), ash, ether extract (EE) and CP according to AOAC (2012) guidelines; NDF and ADF were determined according to the method of Van Soest et al. (1991); acid insoluble ash (AIA) according to Schnieder and Flat (1975); CT according to the method described by Burns (1971), modified by Wanapat and Poungchompu (2001).

Ten millilitres of blood were sampled from the jugular vein of each animal at 0, 3 and 6 h after feeding on day 35 of the experiment. Plasma was separated by centrifugation at 5000 *g* for 10 min and stored at −20 °C for further analysis. Blood urea nitrogen (BUN) was determined according to the method of Crocker (1967), and haematocrit (HCT) according to the method of Kaneko et al. (1997). Approximately 45 ml of ruminal fluid samples were collected (also at 0, 3 and 6 h after feeding) on day 35 of the experiment through a stomach tube connected to a vacuum pump. Ruminal fluid pH and temperature were immediately determined using a portable pH and temperature meter. Approximately 45 ml of rumen fluid was collected and divided into two portions. The first portion was mixed with 5 ml of 1 M H_2SO_4, centrifuged at 16000 *g* for 15 min, and subsequently used for NH_3-N analysis using an Kjeltech Auto 1030 Analyzer and the Kjeldahl methods according to AOAC (2012) guidelines. The total volatile fatty acids (VFA) and VFA profiles were analysed using HPLC (Waters 600E Multisolvent Delivery System, Waters 484 UV detector, Nova-Pak C18 columns, 4 × 150 mm, mobile phase 10 mM H_2PO_4 (pH 2.5); ETL Testing Laboratory, Inc., Cortland, NY, USA) according to the method of Samuel et al. (1997). The second portion of the ruminal fluid was mixed with formalin solution and used to count the population of bacteria, protozoa, and fungi using a microscope with a haemocytometer (Boeco, Hamburg, Germany). Methane (CH_4) production was calculated using the equation of Moss et al. (2000):

$$CH_4 \text{ production} = 0.45 \text{ (acetate)} - 0.275 \text{ (propionate)} + 0.4 \text{ (butyrate)}.$$

Faecal sample collection

Faecal samples were collected by rectal method on day 0, 1, 3, 7, 14, 21, 28 and 35 after treatment. The samples were transferred to a plastic box, tightly closed and labelled with goat number and date of collection and stored at 4 °C. The faecal egg count was determined using the modified McMaster technique described by Whitlock (1948).

Statistical analysis

The data were analysed using a randomized complete block design (RCBD) and the ANOVA procedure of SAS (2013). Differences between treatment means were determined using Duncan's new multiple range test (Steel and Torrie, 1980). Differences among means were assumed as statistically significant at $P \leq 0.05$.

Results and discussion

Based on the data presented in Table 1, it can be observed that Manila palm products had lower DM, ash, CP and AIA contents, but higher OM content in comparison to rice straw and concentrate. Additionally, Manila palm peel had a higher crude protein content than seeds and whole fruits. The chemical analysis showed that concentrate and rice straw compositions were consistent with those reported by Gunun et al. (2018).

Table 2 shows that the addition of the studied supplements did not have a significant effect on ADG and FCR ($P > 0.05$), and no differences were observed between treatments ($P > 0.05$). Our results were consistent with those reported by Thongpea (2015), who investigated tamarind seed supplementation at different levels (2.5, 5 and 7.5 g/kg BW) and found no significant effect on ADG and FCR ($P > 0.05$). Similarly, there was no significant effect of the studied supplements on total feed intake, and rice straw and concentrate intake in terms of % BW and kg, g/kg $BW^{0.75}$ ($P > 0.05$). However, opposite results were reported by Khuttiyo et al. (2014), who found that the addition of a supplement to the diet of nursery pigs significantly increased feed intake and average daily feed intake ($P < 0.05$). Wongnen et al. (2019) also reported no significant effect of tannin sources on feed intake, ADG, FCR, or bodyweight change in goats.

Table 1. Chemical composition of concentrates, rice straw and Manila palm *(Adonidia merrillii* Becc.) products, %

Items	Concentrates	Rice straw	Manila palm		
			Manila palm peel	Manila palm seeds	Manila palm whole fruit
DM, %	90.48	91.45	72.92	71.15	63.15
% DM					
Ash	11.76	12.04	5.24	1.77	3.26
OM	88.23	87.96	94.75	98.22	96.73
CP	16.84	2.69	6.49	3.88	4.31
AIA	2.13	11.33	0.76	0.09	0.39
NDF	53.22	82.20	72.84	57.26	68.63
ADF	20.43	43.90	45.48	22.90	38.98
CT, %	–	–	16.64	6.98	13.54

DM – dry matter, OM – organic matter, CP – crude protein, AIA – acid insoluble ash, NDF – neutral detergent fibre, ADF – acid detergent fibre, CT – condensed tannin

Table 2. Effect of Manila palm (*Adonidia merrillii* Becc.) product supplementation on production performance and voluntary feed intake in goats

Items	Group				SEM	*P*-value
	T1	T2	T3	T4		
ADG, kg/d	0.13	0.14	0.13	0.14	0.02	0.95
FCR	6.52	6.82	6.78	6.80	0.64	0.98
Total DM intake						
kg/day	0.68	0.77	0.73	0.75	0.03	0.42
% BW	2.73	2.56	2.57	2.57	0.04	0.08
g/kg $BW^{0.75}$	61.10	60.15	59.21	59.89	1.38	0.81
Rice straw intake						
kg/day	0.27	0.28	0.26	0.27	0.02	0.88
% BW	1.10	0.94	0.94	0.93	0.04	0.08
g/kg $BW^{0.75}$	24.66	22.01	21.56	21.88	1.13	0.26
Concentrate intake						
kg/day	0.41	0.48	0.46	0.47	0.02	0.10
% BW	1.63	1.62	1.63	1.63	0.002	0.07
g/kg $BW^{0.75}$	36.43	38.13	37.65	38.01	0.43	0.08

T1 – group received ivermectin 3.03 μg/kg body weight, T2 – group received seeds in Manila palm extract 5 ml/kg body weight, T3 – group received Manila palm peel extract 5 ml/kg body weight, T4 – group received Manila palm whole extract 5 ml/kg body weight; ADG – average daily gain, FCR – feed conversion ratio, BW – body weight, DM – dry matter, SEM – standard error of the mean; $P > 0.05$

The inclusion of Manila palm products in the goat diet did not significantly affect nutrient digestibility (Table 3). The apparent digestibility of dry matter, organic matter, crude protein, neutral detergent fibre, and acid detergent fibre was not significantly different between treatments ($P > 0.05$). These findings were similar to the results of Srisaikham (2014), who found that feeding animals with Manila palm did not affect the digestibility of dry matter, crude protein, or fibre ($P > 0.05$). Neem leaves contain substances that have also been found to eliminate parasites, similar to Manila palm. Mulisa et al. (2019) reported that supplementing goat diets with 300 g of neem leaves resulted in an increase in apparent DM digestibility in comparison to groups that received pigeon pea.

Table 3. Effect of Manila palm (*Adonidia merrillii* Becc.) product supplementation on nutrient digestibility in goats

Items	Group				SEM	*P*-value
	T1	T2	T3	T4		
AD, %						
DM	60.84	61.30	58.47	60.83	1.34	0.48
OM	61.84	62.15	58.67	61.62	1.72	0.49
CP	65.59	66.53	64.99	67.01	1.34	0.71
NDF	53.93	53.78	57.94	55.94	3.66	0.83
ADF	39.13	38.01	34.81	39.37	1.99	0.40

T1 – group received ivermectin 3.03 μg/kg body weight, T2 – group received seeds in Manila palm extract 5 ml/kg body weight, T3 – group received Manila palm peel extract 5 ml/kg body weight, T4 – group received Manila palm whole extract 5 ml/kg body weight; AD – apparent digestibility, DM – dry matter, OM – organic matter, CP – crude protein, NDF – neutral detergent fibre, ADF – acid detergent fibre, SEM – standard error of the mean; $P > 0.05$

The effect of Manila palm product supplementation on blood urea nitrogen and haematocrit in goat is presented in Table 4. The results showed that supplementation with Manila palm and ivermectin did not influence blood urea nitrogen and haematocrit at hour 0 and 4 ($P > 0.05$). Consequently, the amount of NH_3-N absorbed from the rumen did not differ significantly between the treatments, and the observed BUN levels were within the normal range (10.38–10.93 mg). According to Lloyd (1982), normal BUN levels in goats range from 11.2 to 27.7 mg/dl, which varies depending on several factors such as age, feed, and the amount of edible protein, especially NH_3-N levels in the rumen.

Table 4. Effect of Manila palm (*Adonidia merrillii* Becc.*)* product supplementation on blood urea nitrogen (BUN) and haematocrit (HCT) in goats

Items	Group				SEM	*P*-value
	T1	T2	T3	T4		
BUN, mg/dl						
0 h post feeding	10.93	10.55	10.43	10.53	0.24	0.51
4 h post feeding	10.83	10.60	10.38	10.75	0.20	0.47
mean	10.88	10.58	10.40	10.64	0.18	0.40
HCT, %						
0 h post feeding	28.50	28.75	28.50	29.50	0.96	0.86
4 h post feeding	28.00	26.00	27.75	27.25	0.84	0.40
mean	28.25	27.38	28.13	28.38	0.54	0.59

T1 – group received ivermectin 3.03 μg/kg body weight, T2 – group received seeds in Manila palm extract 5 ml/kg body weight, T3 – group received Manila palm peel extract 5 ml/kg body weight, T4 – group received Manila palm whole extract 5 ml/kg body weight; SEM – standard error of the mean; $P > 0.05$

Therefore, an increase in NH_3-N levels in the rumen can elevate BUN levels in the bloodstream, indicating nitrogen consumption and the assimilable nitrogen levels (Wanapat, 1990). HCT levels observed in goats ranged from 26 to 29%, indicating normal health status without anaemia. Jain (1993) reported that the normal range of haematocrit was 22–38%. The HCT value is an important indicator that is used to diagnose and assess the overall health of goats. A lower-than-normal HCT value indicates signs of anaemia, which in turn may indicate blood disorders in the animal. Conversely, a higher-than-normal HCT value can be a sign of polycythaemia, which is caused by the formation of abnormally large red blood cells (Jain, 1993).

Table 5 presents the effect of Manila palm products on rumen fermentation and CH_4 production in goats. There was no effect of Manila palm products on rumen pH, NH_3-N, temperature, total VFA and butyric acid ($P > 0.05$). However, acetic acid concentration was significantly reduced by Manila palm supplementation ($P < 0.05$), whereas propionic acid level

Table 5. Effect of Manila palm (*Adonidia merrillii* Becc.) product supplementation on rumen fermentation and methane production in goats

Items	Group				SEM	*P*-value
	T1	T2	T3	T4		
Ruminal pH	6.90	6.87	6.90	6.91	0.02	0.60
Ruminal temperature, °C	39.45	39.02	38.90	39.45	0.32	0.54
NH_3-N, mg/dl	16.05	15.47	14.30	15.76	1.02	0.65
Total VFA, mmol/l	127.31	119.40	129.53	130.62	6.33	0.61
VFA profiles, mol/100 mol						
acetic acid	68.28[a]	64.11[ab]	65.06[ab]	60.63[b]	1.55	0.04
propionic acid	21.82[b]	26.46[ab]	26.21[ab]	29.96[a]	1.45	0.02
butyric acid	9.89	9.42	8.72	9.40	0.66	0.68
CH_4 production[A]	28.68[a]	25.34[ab]	25.56[ab]	22.80[b]	1.05	0.02

T1 – group received ivermectin 3.03 µg/kg body weight, T2 – group received seeds in Manila palm extract 5 ml/kg body weight, T3 – group received Manila palm peel extract 5 ml/kg body weight, T4 – group received Manila palm whole extract 5 ml/kg body weight; [A] calculated according to Moss et al. (2000): CH_4 production = 0.45 (acetate) − 0.275 (propionate) + 0.4 (butyrate); VFA – volatile fatty acid, CH_4 – methane, NH_3-N – ammonia nitrogen, SEM – standard error of the mean; $P > 0.05$; [ab] – means within a row with different superscripts are significantly different at $P < 0.05$

was increased ($P < 0.05$), especially in the group of goats fed whole Manila palm extract (5 ml/kg goat weight) compared to the ivermectin T1 group. In addition, CH_4 production was significantly reduced ($P < 0.05$) as a result of supplementation of Manila palm compared to ivermectin, possibly because of its CT content, which can reduce generation of this gas. These findings suggest that Manila palm can be used as a feed supplement for goat without any negative effects on rumen fermentation.

Methane production calculated using volatile fatty acids as a variable varied among treatments, and the group of goats fed Manila palm whole extract had the lowest methane production compared to the group receiving ivermectin. This could be attributed to the presence of condensed tannins in Manila palm that can reduce protozoan population. Protozoa are known to produce methane, thus a reduction in their population could lead to a decrease in methane gas production. Moreover, Moss et al. (2000) reported that prediction of methane production using the proportion of volatile fatty acid could help explain changes in hydrogen pathway in rumen ecology. Beef cattle fed CT produced less CH_4, which was consistent with a previous study of Kongmun et al. (2009). The combination of *Samanea saman* and *Pennisetum purpureum* (Valencia et al., 2018) in cattle diets has been shown to contribute to the reduction of CH_4 production up to 50.9%.

As shown in Table 6, the total direct counts of bacterial, protozoal and fungal populations were not affected in goats fed ivermectin or Manila palm ($P > 0.05$). It is possible that the observed reductions were only a result of a single, first administration of ivermectin and Manila palm at the start of the experiment. However, Russell and Rychlik (2001) found that microbial ecology in ruminants could be altered by feeding them different rations. Previous study of

Table 6. Effect of Manila palm (*Adonidia merrillii* Becc.) product supplementation on microbial population in the rumen of goats

Items	Group				SEM	*P*-value
	T1	T2	T3	T4		
Total direct count (cells/ml)						
protozoa (× 10^5)	4.75	4.80	4.73	4.65	0.09	0.69
anaerobic fungi (× 10^7)	6.65	7.05	7.08	6.64	0.13	0.09
bacteria (× 10^{11})	5.68	5.76	5.69	5.61	0.11	0.82

T1 – group received ivermectin 3.03 µg/kg body weight, T2 – group received seeds in Manila palm extract 5 ml/kg body weight, T3 – group received Manila palm peel extract 5 ml/kg body weight, T4 – group received Manila palm whole extract 5 ml/kg body weight; SEM – standard error of the mean; $P > 0.05$

Poungchompu et al. (2009) reported that mangosteen peel pellet and soapberry fruit significantly reduced the number of rumen protozoa, similarly as plants containing tannins (Bhatta et al., 2009).

Table 7 presents the influence of supplementation with Manila palm products, i.e. seeds, peel and

Table 7. Effect of Manila palm (*Adonidia merrillii* Becc.) product supplementation on roundworm egg counts in goats

Items	Group				SEM	*P*-value
	T1	T2	T3	T4		
Before transfer						
day 0	325	325	337.5	325	14	0.89
After transfer						
day 1	212.5	225	200	225	16.53	0.67
day 3	150	150	162	162	8.83	0.59
day 7	100[b]	100[b]	125[a]	100[b]	0	<0.0001
day 14	50	50	62.5	62.5	8.83	0.59
day 21	0[b]	0[b]	25[a]	0[b]	0	<0.0001
day 28	50[b]	50[b]	75[a]	50[b]	0	<0.0001
day 35	125[a]	100[b]	125[a]	100[b]	0	<0.0001

T1 – group received ivermectin 3.03 µg/kg body weight, T2 – group received seeds in Manila palm extract 5 ml/kg body weight, T3 – group received Manila palm peel extract 5 ml/kg body weight, T4 – group received Manila palm whole extract 5 ml/kg body weight; SEM – standard error of the mean; $P > 0.05$; [ab] – means within a row with different superscripts are significantly different at $P < 0.05$

whole Manila palm extract on the number of roundworm eggs in goat's faeces. The results showed a significant decrease in the number of roundworm eggs in goat faeces from days 1 to 7 and 7 to 21 after supplementation with Manila palm products ($P < 0.001$). This effect can be attributed to the life cycle of roundworms in goats, which takes approximately 3–4 weeks (Thedford, 1986). These findings are consistent with the study of Khongsen and Limcharoen (2014), who found no significant difference in the efficacy of the deworming drug vamizole and water extract from betel palm and neem bark at 21–28 days after deworming; they also found that the number of helminth eggs increased on days 28 and 35.

Conclusions and recommendations

Supplementation with Manila palm whole extract at a rate of 5 ml/kg body weight resulted in increased propionic acid level, decreased methane production and reduced faecal egg counts. Based on these findings, feeding whole Manila palm extract at a dose of 5 ml/kg body weight is recommended because it is improves rumen fermentation and eliminates gastro-intestinal parasites in goats. However, further research is necessary to investigate the impact of Manila palm extract on the diversity of methanogenic bacteria.

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Conflict of interest

The Authors declare that there is no conflict of interest.

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