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Effect of Feed Supplement Containing Dried Kratom Leaves on Apparent Digestibility, Rumen Fermentation, Serum Antioxidants, Hematology and Nitrogen Balance in Goats

Pin Chanjula [1,*], Juraithip Wungsintaweekul [2], Rawee Chiarawipa [3], Adirek Rugkong [3], Benjamad Khonkhaeng [4], Chanon Suntara [5] and Anusorn Cherdthong [5]

[1] Animal Production Innovation and Management Division, Faculty of Natural Resources, Hat Yai Campus, Prince of Songkla University, Songkhla 90112, Thailand
[2] Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Hat Yai Campus, Prince of Songkla University, Songkhla 90112, Thailand; juraithip.w@psu.ac.th
[3] Agricultural Innovation and Management Division, Faculty of Natural Resources, Hat Yai Campus, Prince of Songkla University, Songkhla 90112, Thailand; rawee.c@psu.ac.th (R.C.); adirek.r@psu.ac.th (A.R.)
[4] Department of Agricultural Innovation and Technology, Institute of Interdisciplinary Studies, Rajamangala University of Technology Isan, Nakhon Ratchasima 30000, Thailand; kbenjamad211223@gmail.com
[5] Tropical Feed Resource Research and Development Center (TROFREC), Department of Animal Science, Faculty of Agriculture, Khon Kaen University, Khon Kaen 40002, Thailand; chanon_su@kkumail.com (C.S.); anusornc@kku.ac.th (A.C.)
* Correspondence: pin.c@psu.ac.th

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Abstract: The objectives of the present study were to examine the influence of supplementation with dried kratom leaf (DKTL) on the performance, hematology, and nitrogen balance in goats. Four 12-month-old male crossbred (Thai Native x Anglo Nubian) goats with an initial body weight (BW) of 24.63 ± 0.95 kg were allocated randomly to receive four different levels of DKTL using a 4 × 4 Latin square design. The DKTL was added to a total mixed ration (TMR) diet with doses of 0, 2.22, 4.44, and 6.66 g/day to investigate the treatment's efficacy. The DKTL was high in secondary metabolites, including mitragynine, total phenolics, total tannins, flavonoids, and saponins. There were quadratic effects on total DMI in terms of kg/day ($p = 0.04$), %BW ($p = 0.05$), and g/kg BW.75 ($p = 0.02$). DKTL increased apparent digestibility with quadratic effects (DM; $p = 0.01$, OM; $p = 0.01$, CP; $p = 0.04$, NDF; $p = 0.01$, and ADF; $p = 0.01$). The pH value was within the rumen's normal pH range, whereas NH_3-N and BUN concentrations were lower with DKTL supplementation, and also reduced cholesterol (CHOL, $p = 0.05$) and low-density lipoprotein (LDL, $p = 0.01$). The protozoa population decreased linearly as DKTL levels increased ($p < 0.01$), whereas *Fibrobacter succinogenes* increased quadratically at 0 h ($p = 0.02$), and mean values increased linearly ($p < 0.01$). The average value of acetic acid (C2) and methane production (CH4) decreased linearly ($p < 0.05$) when DKLT was added to the diet, whereas the quantity of propionic acid (C3) increased linearly ($p = 0.01$). Our results indicate that DKTL could be a great alternative supplement for goat feed. We believe that DKTL could provide opportunities to assist the goat meat industry in fulfilling the demands of health-conscious consumers.

Keywords: kratom leaves; feed intake; blood chemistry; rumen function; small ruminant

1. Introduction

Herbs have been used as helpful materials to treat various diseases in livestock and humans. The benefits of their use include improved feed intake, nutrient digestibility, endocrine and immunological responses, and intermediate nutrient metabolism [1]. Many botanicals are rich sources of plant secondary compounds (PSCs), such as alkaloids,

flavonoids, polyphenolic components, condensed tannins (CT), saponins (SP), and antioxidants. Moreover, they might have antimicrobial activity, particularly in protozoal and methanogen populations. PSCs may influence digestion and rumen fermentation by modifying bacterial growth due to their capacity to interact with fiber and protein content. Antioxidants have high importance in terms of reducing oxidative stress, which is one of the causes of damage to biological molecules.

Interest is greatly increasing in the scientific community in regard to the effects of PSCs on animal nutrition. *Mitragyna speciosa* (Korth) Havil. is a native Southeast Asian tropical tree that is known as kratom in Thailand. It is a member of the Rubiacaeae plant family, and it contains high concentrations of bioactive plant compounds and PSCs. *M. speciosa* has a total phenolic content of 407.83 ± 2.50 mg GAE/g (Gallic acid equivalent, GAE), flavonoid content of 194.00 ± 5.00 mg QE/g (Quercetin equivalent, QE), and mitragynine concentration of 6.0–7.0% according to a previous study [2]. Kratom has been described as both a stimulant and a depressant for the central nervous system. PSCs have been shown to modulate ruminal fermentation [3], animal performance, animal health [4], and the quality of animal products [5]. So far, many hypotheses have been proposed about the possible mechanisms of interaction between PSCs, ruminal microorganisms, and ruminal metabolites. The supplementation of PSCs (tannins, saponins, phenolic, and flavonoids) at low to moderate dosages promotes ruminal fermentation and nitrogen metabolism while suppressing methanogen, resulting in increased animal performance [6]. Forage containing tannins has been shown to reduce the adverse impact of a heavy load of internal parasites and to have indirect effects through enhanced protein availability, which has a beneficial impact on animal production [7]. Saponins are other PSCs that have considerable potential as pharmaceutical or nutraceutical agents. They produce advantageous hematological and immune system effects [8]. Similarly, Matra et al. [9] reported that *Hylocereus undatus* peel powder contains CT and SP and could decrease CH_4 synthesis and protozoal population. The chemistry and pharmacology of PSCs from kratom have potential in this context, but they should be extensively examined before these compounds can be recommended for widespread use. Despite its therapeutic potential, *M. speciosa* has been associated with a number of cases of multiorgan toxicity, including cardiotoxicity [10]. However, the effectiveness of dried kratom leaf (DKTL) as an opium replacement in ruminant diets has yet to be determined, particularly in goat feed. There are still many scientific questions that need to be answered.

Thus, the current study was conducted to test the following hypothesis: whether DKTL can be included as an herbal feed additive in goat diets with no negative effects on the intake or apparent digestibility of dry matter and nutritional components. This study also examines whether the use of DKTL can improve the nitrogen balance and increase the microbial population, as well as its effects on ruminal fermentation parameters, metabolic profile, and feeding behavior. This research examined the influence of DKTL supplementation on digestibility, rumen fermentation, plasma metabolites, enzyme activities, serum immunity, serum antioxidants, and nitrogen balance in goats.

2. Materials and Methods

2.1. Dried Kratom Leaves

The fresh kratom leaves (green vein type) of *M. speciosa* (Rubiaceae) were collected from natural sources in Tambon Namphu, Ban Na San District, Surat Thani Province, Thailand during October, 2020. Authentication of plant material was carried out at the Department of Biology, Faculty of Science, Prince of Songkla University, Songkhla, Thailand, where the herbarium vouchers (Wungsintaweekul, J. N5/001 (PSU)) have been kept. The Ministry of Agriculture of Thailand granted only permitted PSU No. 10/2563 to use plant resources for research purposes. The preservation process of matured kratom leaves recommended by Hagerman and Butler [11] was conducted until being offered to the animals.

2.2. Location, Animals, Experimental Design and Diets

The research was conducted at the Experimental Goat Farm of the Prince of Songkla University, in Hat Yai City, Songkhla Province, Thailand, 90112. Four 12-month-old, male, crossbred (Thai Native x Anglo Nubian) goats with an initial body weight (BW) of 24.63 ± 0.95 kg (mean standard deviation) were allocated randomly to receive four different levels of DKTL using a 4 × 4 Latin square design. All animals were fed separately in pens (0.115 × 0.95 m) in well-ventilated facilities with free access to mineral salt blocks and water. The experiment was carried out through four periods of 21 days each. For the first 14 days, all animals were given their own TMR diets consisting of 30% Pangola grass hay (as roughage sources) and 70% concentrate diet. TMR was fed to the animals ad libitum before they were moved to metabolism crates for the final 7 days of the collection. During this time, they were limited to 90% of their previous voluntary TMR feed intake and supplemented with varying doses of DKTL to ensure overall feed intake. The following treatment sequences were allocated at random: daily DKTL supplementation at 0, 2.22, 4.44, and 6.66 g/goat/day. The animals were fed ad libitum with access to feed twice a day (50 g/kg refusals) (0700 and 1800). The ingredients and chemical compositions of total mixed rations (TMR) and Pangola grass hay (PGH) are shown in Table 1.

Table 1. Dietary ingredients and chemical composition of total mixed ration (TMR) and Pangola grass hay (PGH) fed to the goats during the trial.

Item (% of DM)	TMR [1]	
	Concentrate Diet	Roughage Source
Pangola grass hay (PGH)	-	30
Ground corn	36.2	-
Soybean meal	22.7	-
Fish meal	0.5	-
Leucaena leaf meal	4.0	-
Molasses	5.0	-
Dicalcium phosphate	0.3	-
Salt	0.3	-
Mineral and vitamin mix [2]	1.0	-
Chemical composition, %		
Dry matter	91.69	94.26
% of DM		
Crude protein	16.46	3.18
Ash	5.92	5.65
Organic matter	94.08	94.35
Ether extract	3	1.99
Non-fibrous carbohydrate [3]	31.92	14.77
Neutral detergent fiber	42.7	74.41
Acid detergent fiber	19.6	41.6
Acid detergent lignin	5.4	6.07
Gross energy, Mcal/kg DM	4.09	3.91
TDN, % [4]	76.06	55.6
Metabolizable energy, Mcal/kg DM [5]	2.75	2.01

[1] TMR diet was divided into four treatments depending on DKTL supplementation level: T1 = 0.00, T2 = 2.22, T3 = 4.44, T4 = 6.66 g/goat per day of dried kratom leaves (DKTL). [2] Minerals and vitamins (each kg contains): Vitamin A: 10,000,000 IU; Vitamin E: 70,000 IU; Vitamin D: 1,600,000 IU; Fe: 50 g; Zn: 40 g; Mn: 40 g; Co: 0.1 g; Cu: 10 g; Se: 0.1 g; I: 0.5 g. [3] Calculated as: NFC = 100 − (% NDF + % CP + % EE + % ash). [4] Estimated by the equation TDN = (%)DCP + DNFC) + DEE × 2.25 + (DNDF). [5] Estimated by the equation ME (Mcal/kg DM) = TDN × 0.04409 × 0.82).

2.3. Feed and Fecal Sampling Procedures

During the experiment, the amount of feed supplied and the number of refused samples were recorded on a daily basis. Feed, refusals, and fecal samples were collected from each individual goat at the end of each period using the complete collection technique. Dry matter (DM), ash, ether extract (EE), and crude protein (CP) were evaluated using AOAC methods [12]. Acid detergent fiber (ADF) and neutral detergent fiber (NDF) were determined according to Van Soest et al. [13]. Alkaloids were extracted and isolated from the plant using the procedures described by Jamil et al. [14]. The samples of DKTL were analyzed for CT and SP using the modified vanillin–HCL method [15]. An atomic absorption spectrophotometer was used to conduct a mineral analysis (AAS). The chemical composition of the DKTL is shown in Table 2.

Table 2. Chemical composition and nutritive values of dried kratom leaves (DKTL) used in the experimental diets (on a dry matter basis) for goats (*n* = 4/feed type).

Parameters	DKTL [1]
Dry matter [2] (%)	25.45
Chemical composition (% of DM)	
Dry matter	95.24
Crude protein	20.10
Ash	4.11
Organic matter	95.89
Ether extract	1.71
Neutral detergent fiber	44.49
Acid detergent fiber	27.31
Acid detergent lignin	8.25
Gross energy, Mcal/kg DM	4.63
Alkaloid profile (%)	
Mitragynine	4.14
Paynantheine	0.59
Speciogynine	0.26
Total condensed tannin content (%)	8.28
Total saponin content (%)	5.21
Flavonoids (%)	11.24
Phenolic acids (%)	4.10
Antioxidant activity	
DPPH [4] (IC_{50} (mg/mL)	1.04
FRAP [5] (%)	3.98
Mineral profile [3]	
Ca, %	0.84
P, %	0.20
K, %	1.53
Mg, %	0.30
S, %	1.26
Na, %	0.01
Fe, ppm	80.67
Cu, ppm	11.54
Mn, ppm	1862.30
Zn, ppm	32.14
B, ppm	69.71
Cr, ppm	3.23
Se, ppm	ND

[1] Tambon Namphu, Ban Na San, Surat Thani Province, Thailand. [2] Fresh matter basis. [3] P: phosphorus; K: potassium; Ca: calcium; Mg: magnesium; S: sulfur; Na: sodium; Fe: iron; Cu: copper; Mn: manganese; Zn: zinc; Cr: chromium; Se: selenium. ND: not determined. [4] DPPH: 2,2-diphenyl-1-picrylhydrazyl. [5] FRAP: ferric reducing antioxidant power.

2.4. Urine Sampling Procedures

To keep the final pH below 3 and avoid nitrogen (N) loss, total urine samples were collected on the same days as feces in a plastic container treated with sulfuric acid (10%).

To evaluate N use, urinary samples were obtained at roughly 100 mL of total urine volume, kept in a refrigerator, and pooled at the end of each session for total N analysis according to AOAC methods [12].

2.5. Rumen Fluid Sampling Procedures

Rumen fluid samples were obtained at 0 and 4 h after feeding on the last day of the data collection period. A stomach tube attached to a vacuum pump was used to collect approximately 50 mL of rumen fluid from the central area of the rumen each time. A portable temperature and pH meter was used to measure the pH and temperature of the rumen fluid immediately (HANNA Instruments HI 98153 microcomputer pH meter, Kallang Avenue, Singapore). After that, four layers of cheesecloth were used to filter the rumen fluid samples.

To interrupt the fermentation process of microbial activity, 30 mL of rumen fluid sample was collected and maintained in a plastic container with 5 mL of 1 M H_2SO_4. The mixture was centrifuged for 15 min at 16,000× *g*, and the supernatant was collected and evaluated for ammonia nitrogen (NH_3-N) using a Kjeltech Auto 2200 analyzer (Foss, Hilleroed, Denmark). Volatile fatty acids (VFAs) were evaluated using high-pressure liquid chromatography (HPLC; ETL Testing Laboratory, Inc., Cortland, NY, USA) [16].

2.6. Blood Sampling Procedures and Hematological and Serum Biochemical Analysis

Blood samples (about 10 mL) were taken from the jugular vein at 0 and 4 h post feeding in tubes containing 12 mg of EDTA on the last day of the data collection period. The plasma was kept at −20 °C until it was analyzed for hematocrit, erythrocytes, leucocytes, hemoglobin, differential leucocytic count (lymphocytes, basophils, eosinophils, monocytes, and neutrophils), and differential leucocytic count. During the study, hematological parameters such as the mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCHC) were determined. Another blood sample was taken, placed in a tube (without EDTA), and allowed to coagulate for 20 min at room temperature before being centrifuged for 10 min at 3000 rpm to separate the serum (Table Top Centrifuge PLC-02, Enfield, CT, USA). The collected serum was kept at −20 °C until it was analyzed (within one day) for mineral estimation and biochemical analysis.

2.7. Primers and Real-Time Polymerase Chain Reaction (Real-Time PCR)

Total anaerobic fungi, total bacteria, and cellulolytic bacteria such as *Fibrobacter succinogenes*, *Ruminococcus flavefaciens*, and *Ruminococcus albus* were studied using real-time PCR technique. An mcrA primer set was created to detect total methanogens using real-time PCR. Table 3 shows the sequences of all primer sets. Six sample-derived standards were established from the treatment pool set of community DNA. For each real-time PCR test, ordinary PCR was utilized to establish sample-derived DNA standards. The PCR product was then purified and measured using a spectrophotometer and a QIA quick PCR purification kit (QIAGEN, Inc., Hilden, Germany). The copy number concentration for each sample-derived standard was determined using the length of the PCR product and the mass concentration [17]. Five real-time PCR standards were created in all. In the assays of target genes, the following cycle settings were used: initial denaturation with one cycle of 50 °C for 2 min and 95 °C for 2 min, followed by 40 cycles of 95 °C for 15 s and 60 °C for 1 min for primer annealing and product elongation.

Fluorescence detection was carried out at the end of each denaturation and extension stage. The temperature was increased at a rate of 1 °C every 30 s from 60 to 95 °C to determine amplicon specificity using dissociation curve analysis of the PCR end products. The conditions for *R. albus* were as follows: 30 s at 94 °C for denaturing, 30 s at 55 °C for annealing, and 30 s at 72 °C (48 cycles) for extension, with the exception of 9 min of denaturation in the first cycle and 10 min of extension in the last cycle. A Chromo 4TM system was used to perform real-time PCR amplification and detection (Bio-Rad Laboratories,

Inc., Irvine, CA, USA). Real-time PCR amplification samples were measured in duplicate in a 20-μL reaction mixture including 4–6 mM $MgCl_2$, 10 μL of Mastermix (including Taq DNA polymerase, reaction buffer, dNTP mixture, $MgCl_2$, and SybrGreen), 2 μL of DNA template, and 0.8 L of μL of each primer (10 M/L).

Table 3. PCR primers for real-time PCR assay.

Microorganism	F/R	Primer Sequence (Sequence 5′–3′)	Amplicon (Base Pairs) (pb)	Annealing Temperature (°C)
Total bacteria	F	CGGCAACGAGCGCAACCC	130	55
	R	CCATTGTAGCACGTGTGTAGCC		
Total protozoa	F	CTTGCCCTCYAATCGTWCT	223	55
	R	GCTTTCGWTGGTAGTGTATT		
Total fungi	F	GAGGAAGTAAAAGTCGTAACAAGGTTTC	120	
	R	CAAATTCACAAAGGGTAGGATGATT		
Fibrobacter succinogen	F	GTTCGGAATTACTGGGCGTAAA	121	55
	R	CGCCTGCCCCTGAACTATC		
Ruminococcus albus	F	CCCTAAAAGCAGTCTTAGTTCG	175	55
	R	CCTCCTTGCGGTTAGAACA		
Ruminococcus flavefaciens	F	CGAACGGAGATAATTTGAGTTTACTTAGG	132	55
	R	CGGTCTCTGTATGTTATGAGGTATTACC		

2.8. Statistical Analysis

All of the data from the experiment was subjected to ANOVA for a 4 × 4 Latin square design using general linear model (GLM) approaches (Version 6.2.9200, Cary, NC, USA). The model Yijk = μ + Mi + Aj + Pk + ijk was used to analyze the data, where Yijk is the observation from animal j, receiving diet I in period k; μ is the overall mean; Mi is the effect of the different levels of DKTL (I = 1, 2, 3, 4); Aj is the effect of animal (j = 1, 2, 3, 4); Pk is the effect of period (k = 1, 2, 3, 4); and ijk is the residual effect.

Treatment effects on response variables were investigated using orthogonal polynomial contrasts (linear and quadratic). $p < 0.05$ and trends ($0.05 < 0.05 < 0.10$) were used to determine significance. The standard errors of the mean are calculated and given.

3. Results

3.1. Chemical Composition of DKTL

The chemical composition of the DKTL is presented in Table 2. DKTL had considerable amounts of CP, NDF, ADF, alkaloids, total phenols, total CT, and SP. The DKTL also had high amounts of CP (20.10%) and EE (1.71%), as well as moderate amounts of NDF (44.49%) and ADF (27.31%). It had high amounts of secondary plant metabolites, such as alkaloids, particularly mitragynine (4.10% DM), total phenolics 4.11% DM, total tannins# 8.28% DM, flavonoids 1.24% DM, and SP 52.14% DM. It also had high FRAP and DPPH-scavenging activity 3.98% and 1.04% DM. The results of the mineral constituents showed that the Ca, P, K, Mg, S, and Na contents were 0.84, 0.20, 1.53, 0.30, 1.26, and 0.01%, respectively, and the concentrations of Fe, Cu, Mn, Zn, B, and Cr were 80.67, 11.54, 1862.30, 32.14, 69.71, and 3.23 ppm, respectively.

3.2. Effects of DKTL on Nutrient Intake and Apparent Digestibility of Nutrients in Goats

The influences of different levels of DKTL on feed intake, nutrient intake, and apparent digestibility are presented in Table 4. There were quadratic effects on total DMI in terms of kg/day ($p = 0.04$), %BW ($p = 0.05$), and g/kg $BW^{0.75}$ ($p = 0.02$), with higher values

detected in the goats fed DKTL at 2.22–4.44 g/d. DKTL supplementation had no effect on nutritional intake (OMI, CPI, EEI, NDFI, and ADFI) ($p > 0.05$). Comparing treatment groups, there were quadratic effects on apparent digestibility of DM ($p = 0.01$), OM ($p = 0.01$), CP ($p = 0.04$), NDF ($p = 0.01$), and ADF ($p = 0.01$), with higher values for goats given DKTL at 2.22–4.44 g/d. However, EE was unaffected ($p = 0.27$). There was also a quadratic effect on ME intake in Mcal/d ($p = 0.03$) and ME in Mcal/kg DM ($p = 0.01$), with higher values for the goats fed DKTL at 2.22–4.44 g/d.

Table 4. Effects of supplement levels (g/d) of dried kratom leaves on feed intake, nutrient intake, and apparent digestibility of nutrients in goats.

Item	Supplement Levels of DKTL (g/d) [1]				SEM [2]	Contrast *p*-Value [3]	
	T1	T2	T3	T4		Linear	Quadratic
DMI, kg/d							
Total DMI, kg/d	0.903	0.982	0.964	0.855	0.02	0.40	0.04
DMI, %BW	3.13	3.47	3.39	2.94	0.13	0.44	0.05
DMI, g/kg $W^{0.75}$	72.45	79.87	78.12	68.23	2.57	0.34	0.02
OMI, kg/d	0.860	0.905	0.885	0.818	0.03	0.43	0.19
CPI, kg/d	0.153	0.158	0.155	0.143	0.01	0.35	0.26
EEI, kg/d	0.028	0.030	0.030	0.028	0.001	1.00	0.18
NDFI, kg/d	0.471	0.496	0.484	0.447	0.01	0.41	0.19
ADFI, kg/d	0.185	0.195	0.190	0.175	0.01	0.40	0.18
Apparent digestibility, %							
DM	60.82	66.27	66.60	55.30	1.37	0.19	0.01
OM	62.72	67.91	68.34	56.99	1.39	0.18	0.01
CP	58.50	63.86	64.24	51.92	1.59	0.28	0.04
EE	69.93	69.68	72.08	60.81	3.63	0.27	0.29
NDF	53.06	58.46	58.51	43.20	2.37	0.03	0.01
ADF	29.01	39.26	38.62	23.63	2.03	0.22	0.01
Estimated energy intake [4]							
ME Mcal/d	2.06	2.33	2.29	1.79	0.09	0.28	0.03
ME Mcal/kg DM	2.24	2.42	2.44	2.03	0.04	0.19	0.01

[1] T1 = 0.00, T2 = 2.22, T3 = 4.44, T4 = 6.66 g/goat per day of dried kratom leaves (DKTL). [2] SEM = standard error of the mean ($n = 4$). [3] Significant at $p < 0.05$ and were considered trends when $p < 0.10$. [4] 1 kg DOM = 3.8 Mcal ME/kg.

3.3. Effects of DKTL on Ruminal Fermentation and Blood Metabolites in Goats

The data from the rumen fermentation experiment are shown in Table 5, which includes ruminal temperature, pH, NH_3-N, and BUN levels. The temperature and pH of the rumen did not differ between the groups ($p > 0.05$). However, the mean value of rumen pH tended to increase ($p = 0.11$) when DKTL was added to the diet. The addition of DKTL to the diet reduced the concentrations of NH_3-N and BUN ($p < 0.05$ and $p < 0.01$, respectively). Plasma GLU at 0 h post feeding tended to respond quadratically ($p = 0.08$). Plasma GLU at 4 h post feeding and its mean values also increased with DKTL, and values were greater for the goats fed DKTL at 2.22–4.44 g/d (quadratic, $p = 0.01$). However, PCV was unaffected by the introduction of DKTL to the diet ($p > 0.05$). Plasma mitragynine at 0 and 4 h post feeding and its mean values were positive at all DKTL levels. The values linearly increased as DKTL levels were increased ($p < 0.01$), with levels that were higher for the goats fed DKTL at 2.22–6.66 g/d.

Table 5. Effects of supplement levels (g/d) of dried kratom leaves on rumen fermentation and blood metabolites in goats.

Item	Supplement Levels (g/d) of DKTL [1]				SEM [2]	Contrast *p*-Value [3]	
	T1	T2	T3	T4		Linear	Quadratic
Temperature, °C							
0 h post feeding	39.1	39.3	39.4	39.2	0.33	0.91	0.54
4	39.8	39.7	39.5	39.4	0.23	0.52	0.32
Mean	39.4	39.5	39.4	39.3	0.16	0.83	0.23
Ruminal pH							
0 h post feeding	6.76	6.85	6.92	6.85	0.11	0.50	0.47
4	6.35	6.46	6.52	6.47	0.36	0.41	0.27
Mean	6.55	6.65	6.72	6.66	0.06	0.11	0.97
NH_3-N, mg/dL							
0 h post feeding	14.63	11.77	9.72	10.01	1.25	0.03	0.25
4	18.55	16.05	13.21	14.61	0.91	<0.01	0.08
Mean	16.59	13.91	11.46	12.31	1.00	0.01	0.12
BUN, mg/dL							
0 h post feeding	21.76	16.44	16.98	16.48	0.73	<0.01	0.02
4	21.40	18.34	18.75	18.25	0.79	0.02	0.13
Mean	21.58	17.39	17.87	17.37	0.34	<0.01	<0.01
GLU, mg/dL							
0 h post feeding	63.00	67.75	67.75	65.00	1.96	0.50	0.08
4	61.75	73.00	74.50	67.25	1.47	0.20	0.01
Mean	62.38	70.38	71.13	66.13	1.17	0.21	0.01
PCV, %							
0 h post feeding	27.00	28.75	29.00	28.75	0.65	0.64	0.71
4	26.00	27.50	28.00	26.75	0.72	0.78	0.54
Mean	26.50	28.13	28.50	27.75	0.61	0.70	0.62
Cr, mg/dL							
0 h post feeding	1.19	1.29	1.14	1.17	0.06	0.70	0.78
4	1.26	1.22	1.23	1.16	0.02	0.57	0.90
Mean	1.23	1.25	1.18	1.16	0.04	0.62	0.84
Mitragynine, ng/ml							
0 h post feeding	0.00	0.54	0.51	0.62	0.17	0.04	0.24
4	0.00	0.81	1.06	1.21	0.10	<0.01	0.01
Mean	0.00	0.86	0.94	1.02	0.04	<0.01	0.01

[1] T1 = 0.00, T2 = 2.22, T3 = 4.44, T4 = 6.66 g/goat per day of dried kratom leaves (DKTL). [2] SEM = standard error of the mean (n = 4). [3] Significant at $p < 0.05$ and were considered trends when $p < 0.10$. NH_3-N = ammonia nitrogen; BUN = blood urea nitrogen; GLU = glucose; PCV, pack cell volume; Cr = creatinine; cortisol (Cr =< 1.00).

3.4. Effects of DKTL on Lipid Profile in Goats

The effects on lipid parameters are summarized in Table 6. DKTL supplementation reduced CHOL (p = 0.05) and LDL (p = 0.01) at 0 and 4 h post feeding, as well as the mean values. Goats fed DKTL at 2.22–4.44 g/d had higher values. The plasma concentration of triglycerides (TG) at 0 and 4 h post feeding and the mean values tended to respond quadratically (p = 0.10, 0.07, and 0.08, respectively) with increasing DKTL, with greater values for the goats fed DKTL at 2.22 g/d. The concentration of HDL at 0 and 4 h post feeding in the plasma tended to respond quadratically (p = 0.09, 0.11, and 0.10, respectively).

Table 6. Effects of supplement levels (g/d) of dried kratom leaves on lipid profile in goats.

Item	Supplement Levels (g/d) of DKTL [1]				SEM [2]	Contrast *p*-Value [3]	
	T1	T2	T3	T4		Linear	Quadratic
CHOL, mg%							
0 h post feeding	62.75	41.75	46.50	43.50	2.75	0.05	0.14
4	60.75	40.00	45.25	42.00	2.78	0.05	0.13
Mean	61.75	40.87	45.87	42.75	2.76	0.05	0.19
HDL-Chol, mg%							
0 h post feeding	31.25	35.75	36.75	32.25	1.16	0.72	0.09
4	27.75	34.75	36.50	32.50	2.35	0.30	0.11
Mean	29.50	35.25	36.63	32.38	1.74	0.44	0.10
LDL-Chol, mg%							
0 h post feeding	32.15	16.72	24.00	19.25	2.04	0.01	0.04
4	29.95	15.35	21.87	17.30	1.79	0.01	0.06
Mean	31.05	16.03	22.93	18.22	1.90	0.01	0.06
TG, mg%							
0 h post feeding	35.50	21.50	25.25	25.00	3.69	0.13	0.10
4	30.75	18.75	22.50	24.00	3.13	0.30	0.07
Mean	33.12	20.25	23.87	24.50	3.39	0.19	0.08

[1] T1 = 0.00, T2 = 2.22, T3 = 4.44, T4 = 6.66 g/goat per day of dried kratom leaves (DKTL). [2] SEM = standard error of the mean (n = 4). [3] Significant at $p < 0.05$ and were considered trends when $p < 0.10$. CHOL = cholesterol; HDL = high-density lipoprotein; LDL = Low-density lipoprotein; TG = triglyceride.

3.5. Effects of DKTL on Red Blood Profile (Complete Blood Count) in Goats

Red blood profiles and liver function are shown in Table 7. Red blood profiles (RBC, Hb, MCV, MCH, Plt, RDW, MCHC, TP, ALB, GLB, and A:G ratio) had similar values ($p > 0.05$), showing that DKTL had no effect.

Table 7. Effects of supplement levels (g/d) of dried kratom leaves on complete blood count in goats.

Item	Supplement Levels (g/d) of DKTL [1]				SEM [2]	Contrast *p*-Value [3]	
	T1	T2	T3	T4		Linear	Quadratic
RBC, 10^6/μL							
0 h post feeding	3.52	3.63	3.82	3.38	0.23	0.91	0.53
4	3.33	3.49	3.58	3.08	0.24	0.71	0.42
Mean	3.42	3.56	3.70	3.23	0.23	0.82	0.47
Hb, g/dL							
0 h post feeding	9.65	10.23	10.50	10.23	0.26	0.68	0.69
4	9.28	9.90	9.93	9.50	0.17	0.87	0.59
Mean	9.46	10.06	10.21	9.86	0.20	0.77	0.64
MCV, fL							
0 h post feeding	78.65	79.68	77.45	87.03	4.44	0.38	0.46
4	80.55	79.55	79.35	89.45	4.92	0.34	0.37
Mean	79.60	79.61	78.40	88.24	4.62	0.36	0.41
MCH, pg							
0 h post feeding	27.83	28.30	28.00	30.85	1.50	0.35	0.56
4	28.48	28.55	28.15	31.55	1.62	0.38	0.45
Mean	28.15	28.43	28.08	31.20	1.55	0.36	0.50
Plt, 10^3/uL							
0 h post feeding	709.00	940.00	895.00	867.00	93.89	0.39	0.25
4	791.00	792.00	813.00	868.00	120.16	0.58	0.79

Mean	750.00	866.00	854.00	867.50	96.86	0.44	0.60
RDW, %							
0 h post feeding	27.40	28.53	28.45	28.10	0.67	0.67	0.50
4	26.88	28.13	28.00	27.38	0.71	0.77	0.38
Mean	27.14	28.33	28.23	27.74	0.68	0.72	0.44
MCHC, g/dL							
0 h post feeding	35.45	35.40	36.28	35.45	0.51	0.85	0.70
4	35.48	35.83	35.45	35.35	0.39	0.87	0.83
Mean	35.46	35.61	35.86	35.40	0.31	0.99	0.76
TP, g%							
0 h post feeding	6.63	6.42	6.36	6.48	0.20	0.68	0.55
4	6.48	6.16	6.21	6.20	0.13	0.40	0.47
Mean	6.55	6.29	6.29	6.34	0.16	0.54	0.50
ALB, g%							
0 h post feeding	3.52	3.26	3.43	3.44	0.20	0.96	0.64
4	3.70	3.24	3.37	3.13	0.17	0.20	0.68
Mean	3.61	3.25	3.40	3.29	0.15	0.48	0.64
GLB, g%							
0 h post feeding	3.11	3.16	2.93	3.04	0.18	0.71	0.91
4	2.78	2.92	2.84	3.07	0.10	0.39	0.83
Mean	2.94	3.04	2.88	3.05	0.10	0.86	0.86
A:G ratio, g%							
0 h post feeding	1.16	1.06	1.23	1.16	0.10	0.77	0.90
4	1.35	1.14	1.22	1.05	0.09	0.24	0.91
Mean	1.25	1.10	1.23	1.11	0.06	0.62	0.90

[1] T1 = 0.00, T2 = 2.22, T3 = 4.44, T4 = 6.66 g/goat per day of dried kratom leaves (DKTL). [2] SEM = standard error of the mean (n = 4). [3] Significant at $p < 0.05$ and were considered trends when $p < 0.10$. RBC = red blood cell; Hb = hemoglobin; MCV = mean corpuscular volume; MCH = mean corpuscular hemoglobin; Plt = platelet; RDW = RBC distribution width; MCHC = mean corpuscular hemoglobin concentration; TP = total protein; ALB = albumin; GLB = globulin; A/G ratio = albumin to globulin ratio.

3.6. Effects of DKTL on White Blood Profile and Liver Function in Goats

Table 8 presents the white blood profile and liver function in goats fed different levels of DKTL. There were no differences ($p > 0.05$) among treatments with increasing DKTL levels. However, when supplementing DKTL linearly increased, there was also a tendency of increasing plasma levels of MONO at 4 h post feeding ($p < 0.01$) and its mean values ($p = 0.08$), which were highest when 6.66 g/d DKTL was supplemented. There were quadratic effects on the plasma levels of LYMP (p = 0.09, 0.04, and 0.04, respectively) among treatment groups (Table 7).

Table 8. Effects of supplement levels (g/d) of dried kratom leaves on white blood cell and liver function in goats.

Item [4]	**Supplement Levels (g/d) of DKTL** [1]				**SEM** [2]	**Contrast *p*-Value** [3]	
	T1	**T2**	**T3**	T4		**Linear**	**Quadratic**
WBC, $10^3/\mu L$							
0 h post feeding	11.58	9.40	10.39	12.25	1.36	0.68	0.22
4	11.63	9.35	11.02	11.86	1.21	0.72	0.31
Mean	11.61	9.37	10.71	12.05	1.27	0.70	0.26
NEU, %							
0 h post feeding	49.00	46.00	42.75	46.75	3.45	0.55	0.36
4	56.75	44.75	46.75	51.25	1.87	0.48	0.09
Mean	52.88	45.38	44.75	49.00	1.89	0.42	0.10

LYMP, %							
0 h post feeding	38.00	49.50	51.25	44.50	3.69	0.35	0.09
4	35.75	51.50	49.75	40.50	3.26	0.62	0.04
Mean	36.88	50.50	50.50	42.50	2.93	0.43	0.04
MONO, %							
0 h post feeding	3.25	2.75	2.75	2.50	0.70	0.54	0.88
4	0.25	1.75	1.25	4.00	0.28	<0.01	0.24
Mean	1.75	2.25	2.00	3.25	0.31	0.08	0.47
EOSIN, %							
0 h post feeding	9.75	1.75	3.25	6.25	3.96	0.68	0.27
4	7.25	2.00	2.25	4.25	2.59	0.55	0.28
Mean	8.50	1.88	2.75	5.25	3.26	0.63	0.27
AST, U/L							
0 h post feeding	108.00	111.75	97.75	99.00	10.44	0.45	0.92
4	104.25	103.75	99.25	95.75	10.33	0.55	0.89
Mean	106.13	107.75	98.50	97.38	10.11	0.49	0.90
ALT, U/L							
0 h post feeding	27.75	23.00	25.25	27.25	3.16	0.70	0.24
4	28.25	21.50	27.25	20.75	2.19	0.40	0.18
Mean	28.00	22.25	26.25	24.00	1.15	0.66	0.04
ALP, U/L							
0 h post feeding	323.25	212.75	321.00	271.00	60.30	0.84	0.57
4	348.75	222.25	305.25	233.00	55.43	0.22	0.56
Mean	336.00	217.50	313.13	252.00	56.49	0.47	0.55

[1] T1 = 0.00, T2 = 2.22, T3 = 4.44, T4 = 6.66 g/goat per day of dried kratom leaves (DKTL). [2] SEM = standard error of the mean (n = 4). [3] Significant at $p < 0.05$ and were considered trends when $p < 0.10$. [4] WBCs, white blood cells; NEU, neutrophil; LYPH, lymphocytes; MONO, monocytes, EOSIN, eosinophil; BASO, baso; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase. BASO; less than obvious, treatment of data below the detection limit.

3.7. Effects of DKTL on Ruminal Microorganism Population

The effects of DKTL on the number of microbial species in the rumen are shown in Table 9. The abundances of fungi and *R. albus* were not affected by increasing DKTL levels, but the values at 0 h post feeding and mean values showed that the protozoal population dropped linearly with rising DKTL levels ($p < 0.01$), whereas at 4 h post feeding, it tended to respond quadratically ($p = 0.08$). However, increased abundances were seen with increasing level of DKTL for *F. succinogenes* at 0 h post feeding and in the mean values (quadratically, $p = 0.02$ and linearly, $p < 0.01$, respectively), with greater values for the goats fed DKTL at 2.22–6.66 g/d. At all measurement intervals, bacteria and *F. succinogenes* responded quadratically to increasing DKTL ($p < 0.05$), with larger values for goats fed DKTL at 2.22 g/d.

Table 9. Effects of supplement levels (g/d) of dried kratom leaves on predominant cellulolytic bacterial, fungal, and protozoal population (copies/mL of rumen content) from in the rumen fluid of goats.

Item	Supplement Levels (g/d) of DKTL [1]				SEM [2]	Contrast *p*-Value [3]	
	T1	T2	T3	T4		Linear	Quadratic
Bacteria ($\times 10^{12}$)							
0 h post feeding	3.11	4.19	3.17	2.65	0.32	0.12	0.03
4	1.01	2.26	1.70	1.62	0.26	0.29	0.02
Mean	2.06	3.22	2.44	2.13	0.25	0.62	0.01
Fungi ($\times 10^{8}$)							
0 h post feeding	6.33	9.67	6.96	6.47	1.18	0.67	0.13
4	3.85	5.25	3.41	3.37	0.49	0.16	0.16
Mean	5.09	7.46	5.19	4.92	0.78	0.44	0.12
Protozoa ($\times 10^{9}$)							
0 h post feeding	7.32	3.90	2.37	1.95	0.72	<0.01	0.06
4	2.28	1.17	1.38	1.43	0.31	0.12	0.08
Mean	4.80	2.53	1.88	1.69	0.42	<0.01	0.03
Ruminococcus albus ($\times 10^{10}$)							
0 h post feeding	1.73	2.59	3.18	3.26	0.69	0.06	0.50
4	1.91	2.29	3.21	2.62	0.47	0.17	0.32
Mean	1.82	2.44	3.20	2.94	0.50	0.09	0.40
Ruminococcus flavefaciens ($\times 10^{9}$)							
0 h post feeding	2.11	4.02	2.59	2.37	0.32	0.66	<0.01
4	2.13	2.59	2.49	2.54	0.10	0.03	0.04
Mean	2.12	3.30	2.54	2.46	0.18	0.75	<0.01
Fibrobacter succinogen ($\times 10^{9}$)							
0 h post feeding	2.84	4.85	5.57	4.36	0.62	0.08	0.02
4	3.58	4.40	4.39	4.23	0.49	0.40	0.34
Mean	3.21	4.62	4.98	4.29	0.20	<0.01	<0.01

[1] T1 = 0.00, T2 = 2.22, T3 = 4.44, T4 = 6.66 g/goat per day of dried kratom leaves (DKTL). [2] SEM = standard error of the mean ($n = 4$). [3] Significant at $p < 0.05$ and were considered trends when $p < 0.10$.

3.8. Effects of DKTL on N Metabolism and Utilization

The N balance in goats given varied levels of DKTL is shown in Table 10. There were no differences ($p > 0.05$) among treatments for total N excretion, fecal N excretion, or urinary N excretion. Total N intake, absorbed N, and retained N tended to respond quadratically ($p = 0.10$, 0.09, and 0.08, respectively), although a decline in N intake was evident after DKTL inclusion exceeded 4.44 g/d, with higher values for the goats fed DKTL at 2.22–4.44 g/d.

Table 10. Effects of supplement levels (g/d) of dried kratom leaves on N balance in goats.

Item	Supplement Levels (g/d) of DKTL [1]				SEM [2]	Contrast *p*-Value [3]	
	T1	T2	T3	T4		Linear	Quadratic
N balance g/d							
Total N intake	23.05	25.01	24.36	23.21	0.48	0.97	0.10
N excretion g/d							
Fecal N	9.84	9.14	9.59	10.77	0.47	0.22	0.11
Urinary N	6.43	7.11	7.16	8.12	0.65	0.29	0.89
Total N excretion	16.28	16.25	16.75	18.89	0.74	0.21	0.45
Absorbed N	13.21	15.87	14.77	12.44	0.41	0.60	0.09
Retained N	6.77	8.76	7.61	4.32	0.63	0.39	0.08

N output (% of N intake)							
Absorbed	56.78	63.18	60.53	52.89	1.55	0.40	0.07
Retained	28.51	34.20	30.64	17.48	2.57	0.33	0.08
Total N loss	71.49	65.80	69.36	82.52	2.62	1.08	1.46

[1] T1 = 0.00, T2 = 2.22, T3 = 4.44, T4 = 6.66 g/goat per day of dried kratom leaves (DKTL). [2] SEM = standard error of the mean (n = 4). [3] Significant at $p < 0.05$ and were considered trends when $p < 0.10$.

3.9. Effects of DKTL on Volatile Fatty Acid and Methane Production

The volatile fatty acid (VFA) profile and methane production are shown in Table 11. There were similar results among treatments for TVFAs, butyric acid, and other VFAs. However, the average values of acetic acid and methane production linearly decreased in the goats fed DKTL at 2.22–6.66 g/d ($p < 0.05$). The propionic acid concentration was linearly improved when the level of DKTL rose in the goat diet ($p = 0.01$).

Table 11. Effects of supplement levels (g/d) of dried kratom leaves on volatile fatty acid profiles of goats.

Item	Supplement Levels (g/d) of DKTL [1]				SEM [2]	Contrast p-Value [3]	
	T1	T2	T3	T4		Linear	Quadratic
Total VFA, mmol/L							
0 h post feeding	67.63	72.06	74.01	70.42	8.13	0.83	0.72
4	66.01	68.22	87.63	80.69	6.14	0.10	0.58
Mean	66.82	70.14	80.82	75.56	4.38	0.18	0.49
Proportion of individual VFA, %							
Acetate (C_2)							
0 h post feeding	65.49	63.13	60.89	56.75	4.52	0.09	0.75
4	66.94	64.02	57.79	60.42	1.75	0.10	0.34
Mean	66.22	63.58	59.34	58.59	2.53	0.04	0.64
Propionate (C_3)							
0 h post feeding	19.09	21.15	22.3	28.55	3.43	0.08	0.47
4	18.99	21.23	27.36	27.45	2.11	0.03	0.71
Mean	19.04	21.19	24.83	28.00	1.61	0.01	0.64
Butyrate (C_4)							
0 h post feeding	12.53	12.63	13.68	11.59	2.18	0.86	0.50
4	11.99	11.97	12.97	10.00	1.06	0.59	0.47
Mean	12.26	12.30	13.33	10.80	1.21	0.69	0.37
Other VFA [4]							
0 h post feeding	2.43	2.43	2.61	3.42	0.58	0.27	0.51
4	2.07	2.74	1.79	2.12	0.39	0.71	0.71
Mean	2.25	2.59	2.20	2.77	0.49	0.43	0.69
Acetate:propionate ratio							
0 h post feeding	3.43	3.27	3.24	2.19	0.53	0.15	0.47
4	3.56	3.03	2.32	2.32	0.18	0.01	0.31
Mean	3.50	3.15	2.78	2.26	0.20	0.03	0.50
Methane, mol %							
0 h post feeding	29.32	27.73	26.91	22.61	2.60	0.09	0.67
4	29.69	27.75	23.67	23.64	1.50	0.03	0.66
Mean	29.51	27.74	25.29	23.13	1.25	0.01	0.61

[1] T1 = 0.00, T2 = 2.22, T3 = 4.44, T4 = 6.66 g/goat per day of dried kratom leaves (DKTL). [2] SEM = standard error of the mean (n = 4). [3] Significant at $p < 0.05$ and were considered trends when $p < 0.10$. [4] Sum of isobutyrate, isovalerate, valerate, and caproate.

4. Discussion

4.1. Chemical Composition of DKTL

Kratom is a tree that can grow in a variety of environments and has high contents of CP, mitragynine, flavonoids, phenolic acids, CT, and SP (Table 1). DKTL's nutritional values in our study were identical to those established by Phesatcha et al. [18], who reported that *M. speciosa* leaf powder (MSLP) contained 94.3, 21.2, 51.4, and 28.2% OM, CP, NDF, and ADF, respectively. The concentration of PSCs in DKTL, particularly mitraginine, paynantheine, speciogynine CT, and SP, were 4.10, 0.59, 0.26, 8.28, and 5.21%, respectively. The levels of mitragynine in our study were similar to those observed by Kikura-Hanajiri et al. [19], who revealed that mitragynine levels ranged from 1.2 to 6.3%, while 7-HMG levels ranged from 0.01 to 0.04%. However, our results were lower than those found by Goh et al. [2] and Phesatcha et al. [18]. Mitragynine is the most abundant active alkaloid in the leaves of *M. speciosa* [20], and we detected it as 82.7% of the overall alkaloid combination. DKTL contained a total phenolic content of 40.04 mg CE/g and flavonoid content of 112.42 mg RE/g. Its antioxidant activity in terms of DPPH and FRAP was 1.04 IC50 mg/mL and 39.87 mg CE/g, respectively, which were lower than those reported by Goh et al. [2]. However, a combination of genetic and environmental factors may change the nutritional content of DKTL [21].

The leaves of kratom are traditionally used in Malaysia and Thailand to treat intestinal infections, muscular soreness, coughing, and diarrhea [22]. There is a general effect of "cocaine-like" stimulation in modest doses, while large amounts cause "morphine-like" drowsiness and nausea [23]. They may enhance immunity, reduce blood pressure, and have antiviral, diabetes-suppressing, and appetite-suppressing properties [24]. Long-term usage at high dosages might result in anorexia, dry mouth, diuresis, and constipation [25]. Urine samples confirmed the presence of mitragynine and other *M. speciosa* alkaloids following kratom use, as well as synthetic pharmaceuticals, including O-desmethyltramadol [26].

4.2. Effects of DKTL on Nutrient Intake and Apparent Digestibility of Nutrients in Goats

DKTL is a novel feed additive that has functional components that are comparable to those found in *Cannabis indica* and might be utilized to suit livestock production requirements. However, its pharmacological and biochemical effects have yet to be identified in ruminants, particularly those of mitragynine and other PSCs in DKTL. The effects of DKTL supplementation on feed intake, nutrient intake, and apparent digestibility were investigated in this study. The treatments had no effect on nutritional intake, but there were quadratic effects on total DMI (kg/day, %BW and g/kg $BW^{0.75}$) and apparent DM, OM, CP, NDF, and ADF digestibility. Maximum responses were seen with 2.22–4.44 g/d of supplementation of DKTL in goats. The results show that mitragynine, CT, SP, and other PSCs in DKTL had a positive impact on nutrient digestibility and ruminal microorganism activity, as well as increased rumen fermentation efficiency, feed intake, and digestibility. Similar results were found by Vicknasingam et al. [22], who reported that kratom users had increased appetite (57.8–77.8%). DKTL appears to improve nutritional digestion in goats according to our data. The effect of DKTL supplementation on apparent digestibility was linear, and the lowest reaction was recorded at 6.66 g/d of DKTL supplementation. DKTL is considered to contain anti-nutritional chemicals such mitragynine, CT, SP, and phenolic acids, which may impact animal digestion, metabolism, and absorption of nutrients.

This result is consistent with those of Sultana et al. [27], and Su and Chen [28], who found that the high levels of CT and SP in *Moringa oleifera* leaves impaired nutritional digestibility, but only when supplemented with 100 mg of *Sanguisorba officinalis* [29]. Furthermore, CT and SP reduced the quantity of ruminal fibrolytic bacteria, which inhibited nutritional digestion [30]. Tannins can form hydrogen-bonding complexes with proteins and carbohydrates, which makes them less available for microbial digestion and

fermentation [31,32] because of CT and SP's ability to coat with fiber and protein [9]. Patra and Yu [33] discovered that increasing the amount of vanillin from plants affects the degradability of DM and NDF, pH, and NH_3-N in cultures, which is comparable to findings in the current study. However, the information on mitragynine's influence on rumen bacteria is limited, and the reasons remain unknown in the current investigation for the slightly lower nutritional digestibility identified when raising the concentration of DKTL over the threshold of 4.44 g/d. Mitragynine application to ruminants is difficult for monogastric animals because of rumen microbe digestion, mitragynine's antibacterial and antifungal properties, and PSCs in DKTL.

4.3. Effects of DKTL on Ruminal Fermentation and Blood Metabolites in Goats

pH, temperature, and NH_3-N were all measured in the rumen, and blood BUN was also measured to see whether there was a link between rumen NH_3-N and CP intake. The temperature, pH, NH_3-N, and BUN levels in the rumen are all essential indicators of rumen function and stability. The values of ruminal temperature, pH, PCV, and Cr were consistent across treatments, with ranges of 39.3 to 39.5 °C, pH 6.50 to 6.72, 26.50 to 28.50%, and 1.16 to 1.25 m g/dL, respectively. The rumen's temperature and pH were within the optimal range for microbial fiber and protein digestion. The typical rumen pH range is 6.3–6.8, which supports normal cellulolytic bacteria activity [34,35].

These results revealed that DKTL feeding at all levels resulted in great conditions. Strategic additions of CT and SP-containing feedstuffs may improve rumen efficiency by maintaining a higher pH and increasing microbial protein production [36,37]. Compared to the control group, supplementation with DKTL resulted in lower levels of NH_3-N and BUN. This is most likely due to the CT and SP in DKTL, which may have prevented protein degradation in the diet. This finding is supported by Wanapat et al. [37] and Patra and Yu [33] who revealed that CT provided nutritional advantages by producing a protein–tannin compound, which limited the availability of ruminal breakdown of dietary protein and reduced NH_3-N generation. Furthermore, as a result of tannin binding, more protein became inaccessible for transformation to NH_3-N by bacteria. These findings are also comparable to those of Calsamiglia et al. [38], who used essential oils to enhance NH_3 synthesis. Similarly, Wallace et al. [39] discovered that essential oils' major mode of action is the suppression of bacterial attachment to feed particles, which resulted in a reduction in NH_3 generation (deamination) from amino acids (AAs). The ideal ruminal NH_3-N concentration for effective digestion is 5.0–25.0 m g/dL. Additionally, when the NH_3-N levels in the rumen fluid increase from 9.7 to 21.4 m g/dL, higher breakdown rates of DM and CP in situ were noted [40]. There is much evidence that DKTL has an impact on the microbial fermentation of the rumen. Lower BUN values were also achieved by lowering the rumen levels of NH_3-N. The concentration of BUN in the rumen is closely associated with the concentration of NH_3 generation [41,42]. The level of blood urea N rises when the kidneys are injured and is one of the most important markers of renal function. A prior study found that *Panax ginseng* can lower BUN levels in the blood and minimize nephrotoxicity [43].

In ruminant animals, plasma GLU and Cr are the most significant indices of energy metabolism. In the current study, an increase in plasma glucose with additional DKTL supplementation revealed the glucogenic potential of this additive [44]. It was expected that DKTL supplementation would increase energy intake supply, which was confirmed in the current investigation, although the mechanism is unknown. The concentrations of Cr and PCV in plasma were not altered by DKTL supplementation. Plasma mitragynine levels were positive at all levels of DKTL and improved linearly as DKTL levels increased ($p < 0.05$), with higher values for the goats fed DKTL at 6.66 g/d. No signs of anorexia have been noticed. Vicknasingam et al. [22] found that kratom users had a 57.8–77.8% increase in appetite. Despite its medicinal advantages, usage of *M. speciosa* has been associated with many incidences of multiorgan toxicity, including cardiotoxicity [10]. Based on the

current study, supplementation with DKTL 2.22–6.66 g/d is considered safe for mature goats.

4.4. Effects of DKTL on Lipid Profile in Goats

There are few data on lipid profile parameters (such as CHOL, HDL, LDL, and TG) in goats following DKTL supplementation. Supplementing DKTL lowered the plasma concentrations of CHOL, LDL, and TG in the current trial, but it tended to enhance HDL concentrations. Because CHOL, LDL, and TG levels in the blood are significantly connected with heart disease, the findings are extremely important in preventing hyperlipidemia and contributing to the protection of the heart and liver [45]. HDL is also known as "good cholesterol", and several studies have shown that having a high HDL level is related to a lower risk of heart disease. These findings resemble those of recent research by Singh et al. [45], who found that kratom usage was linked to an increase in blood HDL levels. La-up et al. [46] recently published a study that found a link between kratom consumption and serum lipid profiles. They observed a correlation between kratom consumption and higher HDL levels (60 mg/dL) and lower triglyceride levels (90 mg/dL). In research including 100 kratom users and 100 healthy controls, Leong Bin Abdullah et al. [10] found that kratom users had a slightly reduced level of blood LDL and total cholesterol.

A prominent risk factor for coronary heart disease (CHD) and cerebrovascular disease (CVD) is lipid levels in the blood. Our findings support the notion that a variety of herbs might help raise HDL levels [47]. In normal mice, Boroujeni et al. [48] showed a decrease in plasma TG and CHOL within 3–6 h following injection of the intracellular polysaccharide (IPS) CS-F30. Li et al. [49] used *C. sinensis* in similar research and found that the treatment group had lower total cholesterol levels than the control group. Furthermore, Guo et al. [50] discovered that delivering a daily dose of cordycepin to male Syrian golden hamsters reduced the accumulation of total cholesterol (TC), TG, and LDL in their blood. Supplementing dairy cows with *Acremonium terricola* culture also lowered TG in a linear way according to Li et al. [49]. These findings imply that DKTL may affect the blood lipid metabolism of goats, but the explanation for the decrease in CHOL, LDL, and TG remains unknown. These effects might be linked to mitragynine or PSC effects, which are thought to be caused by the direct suppression of CHOL, LDL, and TG formation in the liver [51]. However, the results of the studies mentioned above indicate that plants may enhance HDL levels. Kratom includes more than 40 alkaloids in four categories, including mitragynine, pyran-fused mitragynine, oxindole, and pyran-fused oxindole [52]. Because compounds of oxindole might impact glucose and lipid metabolism, alkaloids of the oxindole class are expected to have a role in decreasing HDL [53]. Furthermore, antioxidants in kratom have been shown to help decrease triglycerides [54], and kratom has comparatively high antioxidant content [46].

4.5. Effects of DKTL on Red Blood Profile in Goats

Data on the red blood profile of goats (such as RCB, Hb, MCV, Plt, RDW, and MCHC) and TP (ALB and GLB) after DKTL supplementation are scarce. There were no significant variations in any blood parameters between the DKTL-supplemented and control groups in the current investigation. In particular, red blood profiles and TP did not change in goats consuming DKTL. All DKTL groups had serum biochemical characteristics that were within the normal range, indicating normal kidney and liver function. As a consequence, adding DKTL to goat diets at rates of up to 6.66 g/h/d had no negative effects on digestibility, blood hematology, or metabolites. These findings are consistent with previous evidence demonstrating that after the administration of *M. oleifera,* serum biochemical characteristics were within the normal range in all groups [55,56]. However, the biochemical mechanism behind this process is still unknown.

4.6. Effects of DKTL on White Blood Profile and Liver Function in Goats

The white blood profile and blood serum enzymes AST and ALP were not altered after DKTL treatments, but alanine aminotransferase (ALT) was lower in goats consuming 2.22 g/goat per day of DKTL (quadratic; $p = 0.04$). Antioxidant-rich herbal supplements may enhance liver tissue regeneration in animals. Plasma ALT activity, a measure of liver function, fell considerably in the DKTL diet group, suggesting that DKTL's antioxidants may protect goats' livers from injury. Previous research has also shown that Chinese herbal combinations lower CK activity in goats [57].

The anti-inflammatory and antioxidant actions of *M. oleifera*, which includes high quantities of phenolic acid and flavonoids, may be responsible for the considerable decrease in enzyme activity [2]. An animal's liver enzyme can be used as an indicator of its overall health [58]. Juhaimi et al. [54] discovered that adding 25% MF to the meals of nursing goats enhanced the animals' immunity greatly.

Gole and Dasgupta [59] found that in rats, the presence of mitochondrial enzymes and cytoplasm in plasma indicates liver damage. As a result of its placement in the plasma membrane, alkaline phosphatase receives much attention. This might be related to the stimulation of microbial growth at the same time, which increased digestibility.

4.7. Effects of DKTL on Ruminal Microorganism Population

The response of rumen microbial populations to DKTL is shown in Table 8. The findings revealed that total bacteria and fungi populations were not similar ($p > 0.05$). The CT and SP in the DKTL may explain why DKTL supplementation reduced protozoa populations linearly ($p < 0.01$). Ku-Vera et al. [60] reported that CT and SP could inhibit protozoa and methanogen production. By binding parts of the cell membrane, CT seems to have a significant impact on methanogenic archaea in the rumen. It has the ability to inhibit hydrogen transfer while also reducing the growth of methanogens [61].

Furthermore, Jayanegara et al. [32] theorized that SP could affect protozoa by building complexes with sterols on the protozoa's membranes, causing damage and disintegration to them. Polyorach et al. [62] discovered that feeding swamp buffaloes with *Garcinia mangostana* peel (contained CT and SP) reduced protozoa and methanogen populations as well as CH_4 generation. However, the response of protozoa to medicinal plant addition has produced mixed findings in the published literature.

In goats given Pakar leaves, Singh et al. [63] found that rumen protozoa levels decreased, but methanogen counts increased. Phesatcha et al. [36] found that tannins from *Leucaena leucocephala* reduced protozoa and methanogen populations *in vitro*. According to Baah et al. [64], quebracho tannins decreased ruminal protozoa in heifers. However, dietary tannins had no influence on the total number of protozoa and methanogens in the rumen.

Dietary interventions altered real-time PCR quantification of the ruminal microbe population, as shown in Table 9. The overall bacterial population was observed to differ considerably between the control and treatment groups. The population grew from 0 to 4 h after feeding, and the mean value was higher ($p < 0.05$). The highest values occurred for goats fed DKTL at 2.22–4.44 g/d. This result is similar to that of Phesatcha et al. [18], who explained that supplementing with *M. speciosa* leaf powder tended to increase microbial yield.

Quantitative PCR experiments revealed that supplementing 2.22–6.66 g/d of DKTL had no effect on the abundance of *R. albus*, *R. flavefaciens*, or *F. succinogenes*. Moreover, supplementation of DKTL at 2.22–6.66 g/d slightly tended to increase ($p > 0.05$) total fungi populations. The principal fibrolytic bacterial species in the rumen have been identified as *F. succinogenes*, *R. albus*, and *R. flavefaciens*, and an increase in cellulolytic bacteria populations may help ruminants digest fiber better [61]. In addition to the primary fibrolytic bacteria, the rumen also contains highly fibrolytic anaerobic fungi, which are thought to play a key role in fiber destruction in the rumen. However, the effect of DF on rumen

fungus was studied in this research, and real-time PCR demonstrated that it had no effect on ruminal fungal abundance. Most likely, none of these diets were of poor quality.

However, dietary treatments resulted in a linear decrease in protozoal populations. As previously noted, this might be related to CT and SP in DKTL and their effects on protozoan cell membranes. The presence of cholesterol in eukaryotic cell membranes is likely connected to CT's cholesterol affinity, but not in prokaryotic bacterial cells [48]. Dairy heifers given CT or SP showed lower populations of protozoa and fungus than non-supplemented heifers according to Poungchompu et al. [65]. Furthermore, supplementing with FMH increased the number of cellulolytic bacteria ($p < 0.05$), whereas amylolytic and proteolytic bacteria remained unaltered.

Hess et al. [66] discovered that adding saponin-rich fruits to a diet had no effect on overall bacterial counts. The ability of tannins to form compounds with bacteria's cell walls causes morphological changes in the walls and the release of extracellular enzymes, which has been linked to antibacterial action. However, Lan and Yang [67] found that supplementing with various herbs increased nutrient utilization by promoting cellulolytic bacterial activity in the rumen while limiting protozoa and methanogenic bacterial development, resulting in less energy loss through methane emission.

4.8. Effects of DKTL on N Metabolism and Utilization

Total nitrogen excretion, fecal nitrogen excretion, and urine nitrogen excretion were all unaffected by DKTL. The nitrogen absorption and retention were increased by 2.22 to 4.44 g/d of DKTL but decreased when 6.66 g/d of DKTL was added. The most nitrogen was maintained when DKTL was supplied at 2.22 to 4.44 g/d. DKTL supplementation increased N absorption and retention by increasing N intake and CP digestibility. Similarly, Viennasay et al. [68] found that increasing the digestibility of CP led to a rise in retained nitrogen. According to Phesatcha et al. [36], increasing CP and CT intake promoted protein transport from the rumen to the small intestine.

Chanjula et al. [69] found that supplementation with 100 to 200 g/d of spent mushroom (*Cordyceps militaris*) did not influence N intake, excretion, absorption, or retention, but supplementing with 300 g/d of *Cordyceps militaris* or 200–300 g/d of lemongrass powder did. Moreover, at adequate doses, the nitrogen absorption and retention were increased by including CT or SP [70–72]. In our investigation, adding DKTL to the diet could supply more protein to the lower stomach of the host ruminant.

Phesatcha et al. [36] observed that changes in N metabolism should be reflected in N excretion and N retention because N retention is the most relevant indicator for evaluating protein nutrition status in ruminants. In the current research, supplementation with 2.22 to 4.44 g/d of DKTL reduced N excretion to 8.76 and 7.61 g/d, while it enhanced N retention to 34.20% and 30.64% of N intake, respectively. This might be due to DKTL supplementation delaying N excretion and regulating the rate of N breakdown in the rumen in comparison to the control diet.

Excess nitrogen is quickly metabolized to NH_3 by ruminal bacterial urease, which is mostly eliminated in the form of urea via urine. Hünerberg et al. [73] found that NH_3 is a relatively volatile molecule that easily evaporates into the atmosphere. Excess nitrogen can also be lost during storage and application due to runoff and leaching, potentially polluting ground and surface water. Furthermore, bioactive components in PL cause a greater N_2 balance in sheep by reducing fecal and urine N_2 excretion, resulting in better whole-body protein synthesis rates. GL also hinders the deamination process in the rumen, resulting in greater N_2 turnover in sheep [74].

4.9. Effects of DKTL on Volatile Fatty Acid and Methane Production

In the current study, the addition of DKTC at 2.22–6.66 g/d enhanced TVFA and C3 ($p < 0.05$) in comparison to the control group. The H_2 shift from CH_4 generation, which is nutritionally favorable for goats, was linked to the predicted elevation in the VFA profile from C2 to C3 ($p < 0.05$). In the current investigation, adding DKTL to the diet lowered

CH_4 generation considerably. Providing CT and SP to ruminant animals has been demonstrated to reduce CH_4 levels in various studies [75–77]. Cieślak et al. [29] reported that methanogens such as *Methanopyrales*, *Methanocellales*, and *Methanomicrobiales* were shown to convert CO_2 and H_2 to CH_4. As a result, lower CH_4 moderation options are necessary, and energy consumption becomes more efficient [78,79].

5. Conclusions

Our results indicate that DKTL could be a great alternative supplement for goat feed. It has three features. First, it improves rumen fermentation by increasing digestibility and C3 concentration. Second, DKTL is a low-CH_4 additive that is environmentally beneficial. Finally, DKTL promotes goat health by reducing cholesterol (CHOL) and low-density lipoprotein (LDL) levels in the blood. However, the research on DKTL is still incomplete, and more perspectives are needed, particularly in the area of meat products. We believe that DKTL could provide opportunities to assist the goat meat industry in fulfilling the demands of health-conscious consumers.

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