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Consumption of Purple Neem Foliage Rich in Anthocyanins Improves Rumen Fermentation, Growth Performance and Plasma Antioxidant Activity in Growing Goats

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Abstract: This study aimed to investigate how the consumption of purple neem foliage rich in anthocyanins improves rumen fermentation, growth performance and plasma antioxidant activity in growing goats. In total, 25 Anglo-Nubian Thai native male goats (about 20 ± 2 kg body weight; mean standard deviation (SD)) were assigned to 2 × 2 + 1 factorial in a completely randomized study design. There were five treatments: (1) control, (2) 3% normal neem foliage in concentrate, (3) 6% normal neem foliage in concentrate, (4) 3% purple neem foliage in concentrate and (5) 6% purple neem foliage in concentrate. The results show that the goats that were fed 6% purple neem foliage in concentrate had a higher ($p < 0.01$) feed intake gDM/d, %BW, g/kgBW$^{0.75}$, nutrient intake, nutrient digestion, final weight, weight change and ADG than did the goats that were fed 3% purple neem foliage in concentrate, 3% normal neem foliage in concentrate, 6% normal neem foliage in concentrate and control treatment. The feeding of 6% purple neem foliage in concentrate had higher ($p < 0.01$) N intake, N urine, N digestion, N digestion (%), N retention and N retention (%) than the other treatments. The goats receiving 6% purple neem foliage in concentrate had no negative effect ($p < 0.01$) on pH but had a higher ($p < 0.01$) level of ammonia nitrogen, BUN, acetic acid, propionic acid, ratio of acetic acid to propionic acid and total VFA at 2 and 4 h after feeding compared to the other treatments. The effect of anthocyanin-rich 6% purple neem foliage was shown to be higher than the other treatments ($p < 0.01$) for total bacteria, *Butyrivibrio fibrisolvens*, *Fibrobacter succinogenes*, *Ruminococcus albus*, *Ruminococcus flavefaciens* and *Streptococcus bovis* at 2 and 4 h after feeding. The goats fed 6% purple neem foliage displayed higher ($p < 0.01$) levels of total antioxidant (TAC), superoxide dismutase (SOD), glutathione peroxidase (GPX), 2,2-diphenyl-1-picrylhydrazyl (DPPH) and catalase (CAT) antioxidant activity in plasma at 2 and 4 h after feeding. The goats fed 6% purple neem foliage had lower ($p < 0.01$) protozoa methanogen and malondialdehyde (MDA) at 2 and 4 h after feeding. In developing growing goats, the introduction of anthocyanin-rich purple neem foliage boosted plasma antioxidant capacity, improved rumen volatile fatty acids and caused a shift in the structure and relative abundance of the ruminal microbial community.

Keywords: purple neem foliage; normal neem foliage; rumen fermentation; microbial population; growth performance; antioxidant activity; growing goats

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1. Introduction

Thailand is located in the tropics, where animals endure high temperatures all year; these temperatures cause oxidative stress to the animals, leading to oxidative damage to the animal body [1]. Roughage must be fed to ruminants during the dry season since it is not accessible during that time. Purple neem foliage, which is high in anthocyanin, may

be fed to animals to minimize environmental effects and production costs. Furthermore, its phytochemicals, such as polyphenols and vitamins, may increase the quality of meat and milk as well as their shelf-life stability. As a result, they may eliminate free radicals to reduce oxidative stress in ruminants [2]. Overall, anthocyanin from purple neem foliage has the potential to influence ruminal digestibility and fermentation, as well as animal production behavior, hence minimizing the consequences of oxidative stress in ruminants.

Purple neem foliage (*Azadirachta indica* A. Juss var. siamensis Valeton) is a popular crop in Thailand, particularly in Asian nations. Because of its potential biological and pharmacological benefits, neem shoots and leaves are regarded as one of the most important factors in improving animal production. Neem's therapeutic uses as an antihelminthic agent have been the main focus of attention [3]. Purple neem is a native fodder that contains anthocyanins. The addition of purple neem leaves to ruminant diets boosts the plant's functionality and helps reduce the severe feed deficiency observed in the drier tropics during the dry season; it can also improve palatability, digestibility and animal production, and exhibits potent antioxidant activity and reduces oxidative stress in ruminants [4]. Anthocyanin is a collection of flavonoid compounds that are natural colorants that belong to the bioflavonoids category. It is classified into two categories of chemical compounds: anthocyanins (ANS) and anthocyanidins (ACN) [5]. Anthocyanins are water-soluble plant pigments found in the flesh, skin and roots of a variety of brightly colored fruits and vegetables, including purple neem leaves, plums and berries [6]. In human, animal and in vitro research, anthocyanins have been linked to reactive oxygen and nitrogen species (ROS and RNS) [7].

To the best of our knowledge, there is no information on the feeding of anthocyanin-rich purple neem foliage consumption to growing goats. Feeding purple neem foliage to animals can minimize both economic and environmental expenses. Because of the presence of bioactive substances such as phytochemicals and vitamins, purple neem foliage is a native fodder and may be fed to animals. Natural antioxidants may be found in purple neem foliage (*Azadirachta indica* A. Juss var. siamensis Valeton). Anthocyanins and phenolic compounds are abundant in purple neem foliage. They have an antioxidant role in that their structure may donate electrons to free radicals with unpaired electrons and decrease agents in the electron transfer reaction route [8]. The information of anthocyanin in purple neem foliage can exhibit potent antioxidant activity and reduce oxidative stress in ruminants; thus, purple neem foliage in balanced ruminant diets does not have a negative effect on animal productivity and also improves rumen fermentation, rumen digestibility in ruminants and the plasma antioxidants of ruminants [9]. In this study, we establish that the consumption of purple neem foliage rich in anthocyanins improves rumen fermentation, growth performance and plasma antioxidant activity in growing goats.

2. Materials and Methods

2.1. Preparation of Plant Harvested

Samples of forage species such as purple neem foliage planted on a Suranaree University of Technology (SUT) farm in Nakhon Ratchasima, Thailand were prepared by cutting tips of about 30 cm from the youngest leaves from several trees. The harvested samples were immediately brought back to the laboratory, where all leaves of the forages were collected and dried at 65 °C for 48 h, processed into a powder, and kept in sealed plastic bags until the extraction time [10]. After grinding through a 1.0 mm sieve, the samples were stored at 4 °C in an airtight container in darkness until analysis.

2.2. Pant Extraction

Five grams of crude or residue content was extracted with 20 mL of solvents on the basis of their polarity. Methanol was used in this section. The extraction process was run with Soxhlet apparatus for 3–4 h. The extract was filtered and kept. The remaining solid residue on the filter paper was reextracted three times with the fresh solvent, Soxhlet, and then filtered. All the filtrates were pooled and combined based on the solvent, followed by

evaporation using a Rotary evaporator. The extracts were finally filtered through a 0.45 µm PVDF syringe filter, and the volume was increased to 10 mL using the same solvent, then stored at −20 °C until the analysis of the anthocyanin content [11].

2.3. Determination of Anthocyanin Content by HPLC: Isolate Anthocyanin Content

The HPLC-grade solvents, including acetonitrile, methanol, and chloroform used in the extraction process, were purchased from Labscan (Bangkok, Thailand) and Duksan (Gyunggido, Korea) for Acetonitrile. Standard chemicals with anthocyanin content (total anthocyanin, cyanidin, delphinidine, petunidine, pelagonodine, peonidinea and malvidine) were purchased from Sigma Chemical Co. (Sigma, Saint Louis, Missouri, USA). All water used in all the preparations was of the double distillated water grade (Millipore, Illkirch-Graffenstaden, France). A standard stock solution (1 mg/mL) was created by diluting 1 mg of selecting standard with 0.5 mL of HPLC-grade methanol, followed by sonication for 15 min in ice and vortex. The standard stock solution was then adjusted to 1 mL with the mobile phase solution (1:9, HPLC-grade acetonitrile:1% acetic acid). HPLC analyses were performed with HPLC Agilent Technologies 1260 Infinity, Santa Clara, CA, USA with four solvent delivery system quaternary pumps (61311B), including a diode array detector (DAD 61315D) with a 10 mm flow cell, an automatic sample injection valve equipped with a 100 loop and Agilent Open LAB CDS 1.8.1 system manager as the data processor. The separation was achieved using a reversed-phase Zorbax SB-C18 column (3.5 µm particle size, i.d. 4.6 mm × 250 mm). The method for chromatographic analysis followed that in [12] with modifications.

2.4. Experimental Design, Animal Diets and Managements

The experiment was conducted according to recommendations by the Animal Care and Use Committee of Suranaree University of Technology, Nakhon Ratchasima province, Thailand. The treatments were 2 × 2 + 1 factorial in a completely randomized design. A total of 30 Anglo-Nubian Thai native male goats had five treatments that comprised T1, control; T2, 3% normal neem foliage in concentrate; T3, 6% normal neem foliage in concentrate; T4, 3% purple neem foliage in concentrate; and T5, 6% purple neem foliage in concentrate. During the 60-day experimental period, the goats were fed with 1.5% of BW DM/day containing Pangola (*Digitaria eriantha*) hay and 16% crude protein at a ratio of 60:40. All goats received feed supplemented with their respective treatment. The goats were fed in the morning and afternoon at approximately 07.00 and 16.00. During the adjusting period (14 days before starting each experimental period), all animals were fed with the same feed. The nutrient composition and anthocyanin composition in the two types of Neem foliage are shown in Tables 1–3. All goats were kept in individual feeding pens during the 60-day experimental period. Mineral blocks and clean water were available ad libitum for all animals. Feed refusals were recorded on a daily basis, and DM consumption was determined (DMI). The goats' weight was measured in order to establish their growth performance under normal feeding.

Table 1. Anthocyanin composition (mg/g DM) in Purple Neem foliage.

Anthocyanin Composition (mg/g DM)	Purple Neem Foliage (mg/g DM)
Total Anthocyanin Cyanidin	132.89
	39.96
Delphinidine	26.12
Petunidine	32.60
Pelagonodine	10.93
Peonidine	9.492
Malvidine	19.67

Table 2. Chemical composition of Normal Neem foliage and Purple Neem foliage.

Ingredient	Purple Neem Foliage	Normal Neem Foliage
Chemical composition (%DM)		
Dry matter	57.37	51.10
Crude protein	9.24	9.20
Ash	7.45	6.20
Ether extract	1.80	1.70
Non-fibrous carbohydrate	25.00	31.90
Neutral detergent fiber	56.51	51.00
Acid detergent fiber	44.85	37.50

Calculated as: NFC = 100 − (%NDF + %CP + %EE + %ash).

Table 3. Feed ingredients and chemical composition of experimental diets.

	Diet						
Items	**Control**	**3% Normal Neem Foliage**	**6% Normal Neem Foliage**	**3% Purple Neem Foliage**	**6% Purple Neem Foliage**	**SEM**	***p*-Value**
Ingredients (%air-dry basis)							
soybean meal	18.00	19.00	17.00	19.00	17.00		
Rice bran	27.00	21.00	20.80	23.00	22.00		
cassava chip	27.00	30.00	32.30	35.00	33.10		
corn	26.40	22.40	22.80	18.40	20.80		
salt	0.40	0.40	0.40	0.40	0.40		
limestone	0.20	0.20	0.20	0.20	0.20		
premix	1.00	4.00	0.50	1.00	0.50		
Purple Neem	0.00	3.00	6.00	3.00	6.00		
Chemical composition (%DM)							
Dry matter	74.06	75.32	74.57	75.37	74.84	0.24	0.43
Ash	6.17 ^{d}	6.95 ^{a}	6.19 ^{b}	6.10 ^{cd}	6.42 ^{cb}	0.008	0.01
Crude protein	16.71 ^{a}	16.01 ^{e}	16.29 ^{c}	16.27 ^{d}	16.62 ^{b}	0.07	0.01
Ether extract	4.27 ^{d}	4.59 ^{c}	5.57 ^{a}	4.57 ^{c}	5.08 ^{b}	0.13	0.01
Non-fibrous carbohydrate	28.21	31.12	38.13	38.55	34.49	2.49	0.69
Neutral detergent fiber	44.64	41.33	33.82	34.51	37.39	2.50	0.67
Acid detergent fiber	30.70	33.22	30.87	32.60	32.15	2.83	1.00
TDN, %	88.69 ^{a}	84.77 ^{c}	87.18 ^{ab}	87.37 ^{ab}	86.91 ^{b}	0.39	0.005
Metabolizable energy, Mcal/kg DM	3.21 ^{a}	3.06 ^{b}	3.15 ^{a}	3.16 ^{a}	3.14 ^{ab}	0.01	0.005

Contains per kilogram premix: 10,000,000 IU vitamin A; 70,000 IU vitamin E; 1,600,000 IU vitamin D; 50 g iron; 40 g zinc; 40 g manganese; 0.1 g cobalt; 10 g copper; 0.1 g selenium; 0.5 g iodine; Calculated as: NFC = 100 − (%NDF + %CP + %EE + %ash); Estimated by the equation TDN = (%)DCP + DNFC) + DEE × 2.25 + (DNDF); Estimated by the equation ME (Mcal/kg DM) = (TDN × 0.04409 × 0.82); a, b, c, d, e in the same row there is a statistically significant difference ($p < 0.05$).

2.5. Chemical Composition

In total, 500 g of basal feed was dried in a vacuum oven at 65 °C for 72 h before being ground in a Wiley mill (Retsch SM 100 mill; Retsch Gmbh, Haan, Germany) using a 1 mm sieve. Chemical and nutritional analyses were performed on dried samples. The Neutral Detergent Fiber (NDF) and Acid Detergent Fiber (ADF) content was appropriate [13] using an FT 122 FibertecTM analyzer (Foss, Hillerød, Denmark). The feed's hemicellulose content was estimated as NDF minus ADF. Nitrogen content was also determined in two experimental diets and dietary refusals using a KjeltecTM 8400 fully automated Kjeldahl analyzer (FOSS, Hillerød, Denmark); 6.25 was used as the conversion factor to

obtain crude protein (CP) values. Furthermore, the second sub-sample was extracted at 50 °C for 24 h with 0.01 N hydrochloric acid (HCl) dissolved in an 80% methanol solution, and the supernatant was collected and transferred into a 50 mL volumetric flask for HPLC determination of anthocyanin [11]. The chromatographic separation was performed on a reversed-phase column Zorbax SB-C18 column (3.5 µm particle size, i.d. 4.6 mm × 250 mm, Agilent Technologies, Santa Clara, CA, USA) for 65 min at 28 °C. The injection volume was fixed at 20 µL. The mobile phase was composed of HPLC-grade acetonitrile and 10% acetic acid (1:9). A binary gradient of (A) 10% acetic acid, 5% acetonitrile, and 1% phosphoric acid and (B) acetonitrile was used for chromatographic separation at a flow rate of 0.8 mL/min. The absorption of the chemicals tested and assessed was measured with a photodiode array UV detector set at 520 nm.

2.6. Feed and Fecal Sampling

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 the period using the complete collection technique. Dry matter (DM), ash, ether extract (EE) and crude protein (CP) were evaluated using AOAC methods [13]. Acid detergent fiber (ADF) and neutral detergent fiber (NDF) were determined according to [14].

2.7. Urine Sampling Procedures

Total urine samples were collected on the same days as feces in a plastic container treated with sulfuric acid (10%) to keep the final pH below 3 and avoid nitrogen (N) loss. To assess N usage, urine samples were collected at about 100 mL of total urine volume, refrigerated and pooled at the end of each session for total N analysis using AOAC techniques [15].

2.8. Apparent Digestibility

The acid-insoluble ash (AIA) technique was used to assess apparent nutrient digestibility (%) as follows: apparent nutritional digestibility (%) = 100 − ((100 × %AIA in diet × %AIA in fecal)/(%AIA in fecal × %AIA in diet) [16]. After being oven dried at 65 °C for 72 h, all fecal samples were crushed and passed through a 1 mm filter before being kept at 4 °C until analysis.

2.9. Blood Biochemical Indicators

Blood samples were collected through jugular venipuncture into a single 10 mL heparin-containing vacuum tube at 0, 2 and 4 h after morning feeding on the penultimate feeding week at 08.00. The blood sample was transferred to a 1.5 mL tube after centrifugation (Allegra R X-30R Centrifuge, Beckman Coulter, Life Sciences Division Headquarters 5350 Lakeview Parkway S Drive Indianapolis, In 46268, United States) at 3500× *g* for 20 min at 4 °C and it was kept at −20 °C until the antioxidant activity enzymes in the plasma were analyzed. The levels of total antioxidant (TAC), superoxide dismutase (SOD), glutathione peroxidase (GPX), malondialdehyde (MDA), 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging capacity and catalase (CAT) in the plasma were determined using commercial kits (Sigma-Aldrich, Darmstadt, Germany). The product codes were MAK187, MAK379, MAK437, MAK085, MAK088 and MAK381. All measurement techniques were performed in line with the manufacturer's specifications. We employed an automated enzymatic colorimetric approach on a microplate (96 wells, UV plate), quadruplicate, built into a microreader (Varioskan-LUX multimode microplate reader, Thermo Fisher Scientific, Waltham, MA, USA), as mentioned in a past study [17].

2.10. Rumen Fermentation Parameters

At the end of the experiment, at 0 and 4 h after feeding on the last day of the data collection period, approximately 30 mL of ruminal fluid was collected. A portable pH

meter was used to rapidly determine the pH of the ruminal fluid (Oakton 700, Cole-Parmer, Vernon Hills, IL, USA). Ammonia nitrogen (NH_3-N) was measured using the technique of [18]. The strained rumen fluid was then placed in a sterile thermos flask and brought immediately to the laboratory. When the filtered rumen fluid arrived at the laboratory, it was separated into two aliquots. The first aliquot (5 mL) of the filtrates was treated with 0.5 mL of 50% (v/v) HCl, 0.5 mL of a metaphosphoric acid solution (187.5 g/L) and 0.5 mL of a formic acid (250 mL) solution. The concentrations of VFAs in the filtrates were determined using gas chromatography (Agilent 6890 GC, Agilent Technologies, Santa Clara, CA, USA), silica capillary column (30 m × 250 µm × 0.25 µm). The initial temperature was 40 °C for 2 min, increasing to 100 °C at a rate of 3.5 °C/min and then to 249.8 °C at a rate of 10 °C/min. The total run time was 30 min. The temperature of the boil room was 250 °C; the carrier gas was He (99.99%). The pressure before columniation was 31.391 psi; the carrier gas flow rate was 3.0 mL/min, and the solvent delay time was 3 min.

2.11. DNA Extraction and Real-Time PCR Quantification

The second aliquot of filtrates (5 mL) was homogenized for microbiological detection and kept at 80 °C until the relative abundances of specified rumen bacteria were determined. A quantitative real-time PCR (qPCR) procedure was used to extract DNA from the homogenized filtrates after they had been thawed. The QIA amp DNA Stool Mini Kit was used to extract total genomic DNA from 1 mL of homogenized filtrates (Qiagen, Hilden, Germany). Nano Drop 2000 was used to assess DNA purity and concentration (Thermo Fisher Scientific, Waltham, MA, USA), and the absorbance ratio was set to 260:280. The extracted DNA's integrity was then tested using an Image Quant LAS 500 imager (GE Healthcare Bio-Sciences, Pittsburgh, PA, USA) by electrophoresis on a 1 percent agarose gel with a voltage of 5 V/cm and a run period of 20 min. The DNA yield was eluted with suitable dilutions (volume of nuclease-free water) and kept at 20 °C until further analysis. The relative abundances of selected primers in genomic DNA extracted from rumen fluids were determined using a Quanti Tect SYBR Green RT–PCR Kit (full master mix; Qiagen, Inc., Hilden, Germany) fitted with the selected primer set and a Roche Light cycler 480-II (Roche Applied Science, Basel, Switzerland), using previously reported amplification and qPCR settings [19]. The materials for relative abundances of total bacteria, *Butyrivibrio fibrisolvens*, *Fibrobacter succinogenes*, *Ruminococcus albus*, *Ruminococcus flavefaciens*, *Streptococcus bovis*, Protozoa and Methanogen were obtained from Vivantis Technologies Sdn Bhd (Selangor Darul Ehsan, Malaysia). The primers used for qPCR were forward primer 5′-CGGCAACGAGCGCAACCC-3′ and reverse primer 5′-CCATTGTAGCACGTGTGTAGCC-3′, forward primer 5′-ACACACCGCCCGTCACA-3′ and reverse primer 5′-TCCTTACGGTTGGGTCACAGA-3′, forward primer 5′-GGTATGGGAT GAGCTTGC-3′ and reverse primer 5′-GCCTGCCCCTGAACTATC-3, forward primer 5′-CCCTAAAAGCAGTCTTAGTTCG-3′ and reverse primer 5′-CCTCCTTGCGGTTAGAACA-3, forward primer 5′-TCTGGAAACGGATGGTA3′ and reverse primer 5′-CCTTTAAGACAG GAGTTTACAA-3′, forward primer 5′-TTCCTAGAGATAGGAAGTTTCTTCGG-3, forward primer 5′-ATGATGGCAACTAACAATAGGGGT-3, forward primer 5′-CTTGCCCCTCYAATC GTWCT-3′ and reverse primer 5′-GCTTTCGWTGGTAGTGTATT-3′ and forward primer 5′-TTCGGTGGATCDCARAGRGC-3′ and reverse primer 5′- GBARGTCGWAWCCG-TAGAA TC-3′. A standard curve was created prior to commencing qPCR assays using a six-fold serial dilution of pooled DNA. To guarantee consistency, each selected species or group of bacteria had qPCR assays performed in quadruplicate using both standards and genomic DNA samples. Each sample was conducted in triplicate, and the PCR results were combined and evaluated by electrophoresis using 2% agarose gel. An Axy Prep DNA Gel Extraction Kit was used to purify the PCR results (Axy gen Biosciences, CA, USA). Each sample was mixed in an equal ratio and uniformly stirred according to the sequencing amount, and the PCR products were measured using a Quantus TM Fluorometer. Using the Light Cycler 480 software version 1.2.9.11 (Roche Applied Science, Basel, Switzerland), the Ct values were translated into normalized relative numbers, which compensated for PCR efficiency.

2.12. Statistical Analyses

All results were analyzed as 2 × 2 + 1 factorial in a completely randomized design using the general linear model procedure of SAS version 9.1.3 (SAS Inst. Inc., Cary, NC, USA). Differences between treatment means were determined by Duncan's New Multiple Range Test [20]. The model Yijk = μ + Ai + Bj + (AB)ij + εijk was used to analyze the data, where Yijk, all dependent variables, μ = the overall mean, Ai, the effect of cultivar of Normal Neem foliage and Purple Neem foliage, Bj, the effect of level of Normal Neem foliage and Purple Neem foliage, ABij = the interaction of cultivar× level of Neem foliage, and εijk, residual effect. The relative mRNA abundance was determined using the average abundance of the gene in the data as the calibrator, and the data were analyzed by ANOVA. At ($p < 0.05$), differences were considered statistically significant.

3. Results

3.1. Feed Intake, Nutrient Intake, and Growth Performance

The influences of different levels of purple neem foliage rich in anthocyanins on feed intake and nutrient intake are presented in Table 4. There was a significant difference in feed intake and nutrient intake in terms of (gDM/d, %BW and g/kgBW$^{0.75}$) ($p < 0.01$) with high values detected in the goats fed 6% purple neem foliage. The effect of purple neem foliage rich in anthocyanins had a higher nutrient intake (OM, CP, EE, NDF and ADF) ($p < 0.01$) in the goats fed the 6% purple neem foliage diet.

Table 4. Effect of purple neem foliage rich in anthocyanins on feed intake of growing goats.

Items	Control	Normal Neem Foliage		Purple Neem Foliage		SEM	*p*-Value			
		3%	6%	3%	6%		Control vs. Treatment	Cultivars	Levels	C × L
Feed intake										
gDM/d	642.00 ^{e}	755.20 ^{d}	809.60 ^{c}	853.00 ^{b}	949.00 ^{a}	20.94	0.01	0.01	0.01	0.001
%BW	3.03 ^{e}	3.18 ^{d}	3.24 ^{c}	3.34 ^{b}	3.49 ^{a}	0.03	0.01	0.01	0.01	0.0001
g/kgBW$^{0.75}$	49.17 ^{e}	60.27 ^{d}	64.53 ^{c}	70.07 ^{b}	75.20 ^{a}	1.82	0.01	0.01	0.01	0.0162
Nutrient intake g DM/d										
OMI	802.97 ^{e}	1105.33 ^{d}	1393.81 ^{c}	1550.82 ^{b}	1676.60 ^{a}	64.99	0.01	0.01	0.01	0.0008
CPI	31.27 ^{d}	35.40 ^{c}	37.41 ^{bc}	39.55 ^{ab}	41.60 ^{a}	0.79	0.01	0.01	0.01	0.977
EEI	7.54 ^{e}	11.34 ^{d}	11.84 ^{c}	14.43 ^{b}	14.79 ^{a}	0.53	0.01	0.01	0.01	0.40
NDFI	683.33 ^{e}	1039.03 ^{d}	1242.31 ^{c}	1365.72 ^{b}	1548.44 ^{a}	60.77	0.01	0.01	0.01	0.38
ADFI	447.35 ^{e}	739.73 ^{d}	812.74 ^{c}	847.66 ^{b}	873.49 ^{a}	31.71	0.01	0.01	0.01	0.01

SEM, standard error of the mean; g DM/d, daily intake of dry matter; a, b, c, d, e in the same row, there is a statistically significant difference $p < 0.05$. Calculated formula: %BW = ((g DM intake × 100)/(body weight (kg) × 1000)); g/kgBW$^{0.75}$ = g DM intake/(kg body weight)$^{0.75}$.

Goats fed the 6% purple neem foliage diet had a higher apparent digestion of DM, OM, CP, EE, NDF and ADF ($p < 0.01$) than that in the other treatments presented in (Table 5).

There was a substantial difference in the final weight, weight change and average daily gain (ADG) (Table 6). The goats fed 6% purple neem foliage had the highest final weight, weight change and ADG ($p < 0.01$) than those fed the other treatments.

3.2. Nitrogen Utilization

The data from nitrogen utilization as shown in Table 7, there was a significant difference in the interaction between cultivars' levels of nitrogen utilization parameters. The goats fed 6% purple neem foliage had the highest levels of N intake (g/d), N excretion from urine (g/d), N digestion, N digestion (%) and N retention (%) ($p < 0.01$) of all the treatments.

3.3. Rumen Fermentation Parameters

The effects of purple neem foliage rich in anthocyanins are summarized in Table 8. The goats fed purple neem foliage rich in anthocyanins had no effect on pH, ammonia nitrogen (NH_3-N), blood urea nitrogen (BUN), acetic acid (C_2), propionic acid (C_3), butyric acid (C_4),

acetic acid: propionic (C_2/C_3) and total VFA (TVFA) at 0 h ($p > 0.05$) post feeding. The goats receiving 6% purple neem foliage had no effect on pH values ($p > 0.05$). There were the highest in NH_3-N, BUN, C_2, C_3, C_4, C_2/C_3 and TVFA levels after feeding (2 to 4 h) $p < 0.01$ in the goats fed 6% purple neem foliage diet compared to the other groups (Tables 8 and 9).

Table 5. Effect of purple neem foliage rich in anthocyanins on nutrient digestion in growing goats.

Items	Control	Normal Neem Foliage		Purple Neem Foliage		SEM	*p*-Value			
		3%	6%	3%	6%		Control vs. Treatment	Cultivars	Levels	C × L
Apparent Digestibility, % of intake										
DDM	49.47 ^{e}	50.45 ^{d}	51.61 ^{c}	53.54 ^{b}	55.62 ^{a}	0.45	0.01	0.01	0.01	0.002
DOM	64.57 ^{e}	65.54 ^{d}	66.42 ^{c}	68.18 ^{b}	70.52 ^{a}	0.43	0.01	0.01	0.01	0.01
DCP	47.36 ^{e}	55.48 ^{d}	56.89 ^{c}	58.02 ^{b}	63.20 ^{a}	1.06	0.01	0.01	0.01	0.01
DEE	50.39 ^{e}	53.14 ^{d}	55.59 ^{c}	57.21 ^{b}	60.51 ^{a}	0.71	0.01	0.01	0.01	0.004
DNDF	55.07 ^{e}	63.01 ^{d}	64.94 ^{c}	73.81 ^{b}	75.35 ^{a}	1.53	0.01	0.01	0.0004	0.64
DADF	52.21 ^{e}	53.99 ^{d}	62.06 ^{c}	67.45 ^{b}	68.68 ^{a}	1.39	0.01	0.01	0.01	0.01

SEM, standard error of the mean; DDM, digestibility of dry matter; DOM, digestibility of organic matter; DCP, protein digestibility; DEE, fat digestibility; DEE, digestibility fatty digestibility; DNDF, the digestibility of the crude fiber that cannot be dissolved in neutral solution; DADF, the digestibility of the fiber that cannot be digested in an acidic solution; a, b, c, d, e in the same row, there is a statistically significant difference $p < 0.05$.

Table 6. Effect of purple neem foliage rich in anthocyanins on performance in growing goats.

Items	Control	Normal Neem Foliage		Purple Neem Foliage		SEM	*p*-Value			
		3%	6%	3%	6%		Control vs. Treatment	Cultivars	Levels	C × L
Body weight										
Initial weight, kg	20.14	20.40	20.40	20.89	20.80	0.14	0.18	0.17	0.89	0.89
Final weight, kg	30.03 ^{e}	31.10 ^{d}	31.60 ^{c}	32.45 ^{b}	33.05 ^{a}	0.22	0.01	0.01	0.01	0.03
Weight change, kg	9.73 ^{c}	10.39 ^{bc}	10.55 ^{bc}	10.80 ^{b}	11.95 ^{a}	0.17	0.01	0.0002	0.0036	0.02
ADG, g/d	162.13 ^{c}	173.23 ^{b}	175.77 ^{bc}	180.00 ^{b}	199.17 ^{a}	2.81	0.01	0.0002	0.00	0.02

SEM, standard error of the mean; a, b, c, d, e in the same row there is a statistically significant difference $p < 0.05$.

Table 7. Effect of purple neem foliage rich in anthocyanins on nitrogen utilization in growing goats.

Items	Control	Normal Neem Foliage		Purple Neem Foliage		SEM	*p*-Value			
		3%	6%	3%	6%		Control vs. Treatment	Cultivars	Levels	C × L
N intake	7.83 ^{e}	8.26 ^{d}	8.51 ^{c}	8.65 ^{b}	9.25 ^{a}	0.10	0.01	0.01	0.01	0.01
N Faces	3.75 ^{a}	2.14 ^{b}	1.81 ^{c}	1.77 ^{c}	1.62 ^{d}	0.16	0.01	0.01	0.01	0.01
N Urine	0.16 ^{d}	0.11 ^{e}	0.17 ^{c}	0.21 ^{b}	0.23 ^{a}	0.01	0.01	0.01	0.01	0.01
N digestion	2.42 ^{d}	3.56 ^{c}	3.45 ^{c}	3.90 ^{b}	4.90 ^{a}	0.12	0.01	0.01	0.48	0.01
N digestion (%)	46.65 ^{e}	59.37 ^{c}	54.71 ^{d}	65.71 ^{b}	75.14 ^{a}	1.98	0.01	0.01	0.01	0.01
N retention	2.59 ^{d}	3.34 ^{b}	3.07 ^{c}	3.30 ^{b}	3.82 ^{a}	0.08	0.01	0.01	0.01	0.01
N retention (%)	48.12 ^{d}	48.04 ^{d}	54.65 ^{c}	61.26 ^{b}	72.34 ^{a}	1.87	0.01	0.01	0.01	0.01

SEM, standard error of the mean; a, b, c, d, e in the same row there is a statistically significant difference $p < 0.05$.

3.4. Microbial Population

As the effect of purple neem foliage rich in anthocyanins that was shown in Table 10 shows, dietary treatments had no effect on the microbial population ($p > 0.01$). At 0 h, total bacteria, Protozoa, Methanogen, *Butyrivibrio fibrisolvens*, *Fibrobacter succinogenes*, *Ruminococcus albus*, *Ruminococcus flavefaciens* and *Streptococcus bovis* showed a significant change ($p < 0.01$) after feeding (2 to 4 h). When compared to other treatments, the goats receiving 6% purple neem foliage at 2 and 4 h after feeding had the highest ($p < 0.01$) levels of total bacteria, *Butyrivibrio fibrisolvens*, *Fibrobacter succinogenes*, *Ruminococcus albus*, *Ruminococcus flavefaciens* and *Streptococcus bovis*. When compared to other treatments, the goats receiving 6% purple neem foliage had lower levels of Protozoa and Methanogen at 2 and 4 h after feeding ($p < 0.01$).

Table 8. Effect of purple neem foliage rich in anthocyanins on pH, ammonia nitrogen and blood urea nitrogen in growing goats.

Items	Control	Normal Neem Foliage		Purple Neem Foliage		SEM	*p*-Value			
		3%	6%	3%	6%		Control vs. Treatment	Cultivars	Levels	C × L
					pH					
0-h	6.94	6.95	6.96	6.97	6.99	0.01	0.30	0.27	0.62	0.89
2-h	6.75	6.77	6.78	6.79	6.80	0.01	0.04	0.07	0.64	0.88
4-h	6.82	6.87	6.83	6.86	6.85	0.01	0.03	0.82	0.05	0.27
Mean	6.83	6.86	6.85	6.88	6.88	0.006	0.06	0.20	0.77	0.67
					Ammonia nitrogen mg/dL					
0-h	12.38	12.40	12.14	12.36	12.11	0.05	0.24	0.71	0.01	0.98
2-h	12.41 ^{e}	13.42 ^{d}	14.12 ^{c}	15.25 ^{b}	16.60 ^{a}	0.30	<0.0001	<0.0001	<0.0001	0.001
4-h	13.50 ^{e}	14.40 ^{d}	15.14 ^{c}	16.40 ^{b}	17.20 ^{a}	0.27	<0.0001	<0.0001	<0.0001	0.71
Mean	12.76 ^{e}	13.41 ^{d}	13.80 ^{c}	14.67 ^{b}	15.30 ^{a}	0.19	<0.0001	<0.0001	<0.0001	0.04
					BUN mg/dL					
0-h	10.11	10.14	10.15	10.16	10.18	0.01	0.03	0.21	0.54	0.70
2-h	11.44 ^{e}	12.46 ^{d}	13.60 ^{c}	14.28 ^{b}	15.08 ^{a}	0.27	<0.0001	<0.0001	<0.0001	0.12
4-h	15.58 ^{c}	16.32 ^{b}	16.34 ^{b}	17.36 ^{a}	17.50 ^{a}	0.16	<0.0001	<0.0001	0.55	0.65
Mean	12.38 ^{e}	12.97 ^{d}	13.36 ^{c}	13.93 ^{b}	14.25 ^{a}	0.14	<0.0001	<0.0001	<0.0001	0.55

SEM, standard error of the mean; a, b, c, d, e in the same row there is a statistically significant difference ($p < 0.05$).

3.5. Antioxidant Activity in Plasma

As shown in Table 11, there was no impact ($p > 0.01$) of dietary treatments on total antioxidant (TAC), superoxide dismutase (SOD), glutathione peroxidase (GPX), malondialdehyde (MDA), 2,2-diphenyl-1-picrylhydrazyl (DPPH) or catalase (CAT) antioxidant activity in the plasma at 0 h. However, there was a significant difference ($p < 0.01$) in the antioxidant activity in plasma of TAC, SOD, GPX, MDA, DPPH and CAT in the goats receiving dietary treatments after feeding at 2 and 4 h. The total antioxidant, superoxide dismutase, glutathione peroxidase, 2,2-diphenyl-1-picrylhydrazyl and catalase levels were significantly higher ($p < 0.01$) in the goats receiving 6% purple neem foliage than in the other treatments. In terms of the antioxidant activity in plasma, the goats receiving 6% purple neem foliage had reduced ($p < 0.01$) levels of malondialdehyde after feeding at 2 and 4 h compared to the other groups.

Table 9. Effect of purple neem foliage rich in anthocyanins on Volatile fatty acid in growing goats.

Items	Control	Normal Neem Foliage		Purple Neem Foliage		SEM	*p*-Value			
		3%	6%	3%	6%		Control vs. Treatment	Cultivars	Levels	C × L
					Acetic acid (%molar)					
0-h	56.02	56.06	56.03	56.08	56.04	0.01	0.15	0.40	0.10	0.85
2-h	57.51 ^{d}	59.14 ^{c}	59.43 ^{c}	60.29 ^{b}	61.45 ^{a}	0.27	<0.0001	<0.0001	<0.0001	<0.0001
4-h	59.55 ^{d}	60.77 ^{c}	61.25 ^{c}	62.66 ^{b}	63.88 ^{a}	0.31	<0.0001	<0.0001	<0.0001	0.005
Mean	57.69 ^{e}	58.66 ^{d}	58.90 ^{c}	59.68 ^{b}	60.46 ^{a}	0.19	<0.0001	<0.0001	<0.0001	<0.0001
					Propionic acid (%molar)					
0-h	29.70	29.71	29.72	29.73	29.74	0.01	0.25	0.26	0.55	0.87
2-h	31.03 ^{e}	33.04 ^{d}	35.32 ^{c}	36.49 ^{b}	37.38 ^{a}	0.48	0.01	0.01	0.01	0.01
4-h	32.44 ^{e}	33.33 ^{d}	36.12 ^{c}	37.18 ^{b}	39.08 ^{a}	0.5	0.01	0.01	0.01	0.003
Mean	31.06 ^{e}	32.02 ^{d}	33.72 ^{c}	34.47 ^{b}	35.40 ^{a}	0.32	0.01	0.01	0.01	0.01

Table 9. *Cont.*

Items	Control	Normal Neem Foliage		Purple Neem Foliage		SEM	*p*-Value			
		3%	6%	3%	6%		Control vs. Treatment	Cultivars	Levels	C × L
Butyric acid (%molar)										
0-h	14.21	14.24	14.19	14.20	14.22	0.01	0.78	0.79	0.20	0.01
2-h	15.04 ^{e}	17.67 ^{d}	18.82 ^{c}	19.83 ^{b}	20.64 ^{a}	0.4	0.01	0.01	0.01	0.004
4-h	17.19 ^{e}	18.94 ^{d}	20.32 ^{c}	21.66 ^{b}	22.59 ^{a}	0.39	0.01	0.01	0.01	0.01
Mean	14.48 ^{e}	16.95 ^{d}	17.78 ^{c}	18.57 ^{b}	19.15 ^{a}	0.26	0.01	0.01	0.01	0.0002
Acetic acid: Propionic (mmol/L)										
0-h	1.84	1.85	1.86	1.87	1.88	0.01	0.07	0.12	0.56	0.93
2-h	1.95 ^{e}	2.28 ^{d}	2.70 ^{c}	2.84 ^{b}	2.92 ^{a}	0.07	0.01	0.01	0.01	0.01
4-h	2.06 ^{e}	2.54 ^{d}	2.76 ^{c}	2.99 ^{b}	3.13 ^{a}	0.08	0.01	0.01	0.01	0.04
Mean	1.95 ^{e}	2.22 ^{d}	2.44 ^{c}	2.57 ^{b}	2.65 ^{a}	0.05	0.01	0.01	0.01	0.01
Total VFA (mmol/L)										
0-h	54.63	54.64	54.65	54.69	54.73	0.01	0.13	0.04	0.39	0.64
2-h	56.41 ^{e}	58.42 ^{d}	63.19 ^{c}	67.59 ^{b}	75.82 ^{a}	1.42	0.01	0.01	0.01	0.01
4-h	54.46 ^{e}	61.36 ^{d}	67.83 ^{c}	74.43 ^{b}	82.20 ^{a}	1.98	0.01	0.01	0.01	0.01
Mean	55.17 ^{e}	58.14 ^{d}	61.89 ^{c}	65.57 ^{b}	70.92 ^{a}	1.13	0.01	0.01	0.01	0.01

SEM, standard error of the mean; a, b, c, d, e in the same row there is a statistically significant difference $p < 0.05$.

Table 10. Effect of purple neem foliage rich in anthocyanins on rumen microbial population in growing goats.

Items	Control	Normal Neem Foliage		Purple Neem Foliage		SEM	*p*-Value			
		3%	6%	3%	6%		Control vs. Treatment	Cultivars	Levels	C × L
Total bacteria (lg10 copies/mL)										
0-h	5.55	5.54	5.56	5.62	5.51	0.05	0.94	0.88	0.72	0.61
2-h	5.36 ^{e}	6.34 ^{d}	7.46 ^{c}	8.58 ^{b}	10.59 ^{a}	0.38	0.01	0.01	0.01	0.03
4-h	5.38 ^{e}	6.41 ^{d}	7.58 ^{c}	8.55 ^{b}	9.59 ^{a}	0.32	0.01	0.01	0.0002	0.78
Mean	5.43 ^{e}	6.10 ^{d}	6.87 ^{c}	7.58 ^{b}	8.56 ^{a}	0.23	0.01	0.01	0.01	0.54
Butyrivibrio fibrisolven (lg10 copies/mL)										
0-h	7.58	7.57	7.55	7.56	7.59	0.03	0.9	0.85	1.00	0.75
2-h	4.92 ^{e}	6.66 ^{d}	7.59 ^{c}	8.14 ^{b}	8.75 ^{a}	0.28	0.01	0.01	0.0005	0.39
4-h	5.15 ^{d}	6.43 ^{c}	7.55 ^{b}	8.07 ^{ab}	8.64 ^{a}	0.27	0.01	0.01	0.0018	0.26
Mean	5.88 ^{d}	6.89 ^{c}	7.56 ^{b}	7.92 ^{ab}	8.32 ^{a}	0.18	0.01	0.01	0.0008	0.33
Fibrobacter succinogenes (lg10 copies/mL)										
0-h	3.54	3.58	3.59	3.58	3.67	0.06	0.68	0.76	0.72	0.79
2-h	5.03 ^{d}	5.14 ^{d}	6.04 ^{c}	7.09 ^{b}	9.25 ^{a}	0.33	0.01	0.01	0.01	0.01
4-h	4.83 ^{d}	5.03 ^{d}	5.99 ^{c}	7.03 ^{b}	9.02 ^{a}	0.32	0.01	0.01	0.01	0.03
Mean	4.47 ^{d}	4.58 ^{d}	5.21 ^{c}	5.90 ^{b}	7.31 ^{a}	0.22	0.01	0.01	0.01	0.01
Ruminococcus albus (lg10 copies/mL)										
0-h	3.55	3.63	3.60	3.66	3.70	0.05	0.49	0.62	0.96	0.75
2-h	5.01 ^{e}	6.09 ^{d}	6.94 ^{d}	7.95 ^{b}	9.06 ^{a}	0.30	0.01	0.01	0.0007	0.60
4-h	4.95 ^{e}	6.05 ^{d}	6.74 ^{c}	7.55 ^{b}	8.78 ^{a}	0.28	0.01	0.01	0.0003	0.23
Mean	4.51 ^{e}	5.26 ^{d}	5.76 ^{c}	6.38 ^{b}	7.18 ^{a}	0.20	0.01	0.01	0.0005	0.36

Table 10. *Cont.*

Items	Control	Normal Neem Foliage		Purple Neem Foliage		SEM	*p*-Value			
		3%	6%	3%	6%		Control vs. Treatment	Cultivars	Levels	C × L
Ruminococcus flavefacises (lg10 copies/mL)										
0-h	3.47	3.57	3.60	3.61	3.63	0.05	0.36	0.76	0.86	0.99
2-h	5.00 ^{e}	6.03 ^{d}	7.03 ^{c}	8.11 ^{b}	9.47 ^{a}	0.33	0.01	0.01	0.01	0.43
4-h	4.95 ^{e}	5.91 ^{d}	6.99 ^{c}	7.99 ^{b}	9.36 ^{a}	0.33	0.01	0.01	0.01	0.51
Mean	4.47 ^{e}	5.17 ^{d}	5.88 ^{c}	6.57 ^{b}	7.49 ^{a}	0.22	0.01	0.01	0.01	0.50
Streptococcus bovis (lg10 copies/mL)										
0-h	3.30	3.49	3.60	3.63	3.70	0.05	0.02	0.29	0.41	0.84
2-h	4.04 ^{d}	5.03 ^{c}	5.18 ^{c}	6.53 ^{b}	9.03 ^{a}	0.36	0.01	0.01	0.01	0.01
4-h	3.91 ^{d}	4.73 ^{c}	5.03 ^{c}	6.31 ^{b}	8.95 ^{a}	0.37	0.01	0.01	0.01	0.01
Mean	3.75 ^{d}	4.42 ^{c}	4.60 ^{c}	5.49 ^{b}	7.23 ^{a}	0.25	0.01	0.01	0.01	0.01
Protozoa (lg10 copies/mL)										
0-h	4.59	4.50	4.70	4.59	4.39	0.05	0.7	0.27	0.98	0.06
2-h	8.61 ^{a}	7.16 ^{b}	6.70 ^{b}	5.51 ^{c}	3.97 ^{d}	0.36	0.01	0.01	0.02	0.19
4-h	8.55 ^{a}	7.12 ^{b}	6.19 ^{c}	5.15 ^{d}	3.17 ^{e}	0.39	0.01	0.01	0.01	0.08
Mean	7.25 ^{a}	6.26 ^{b}	5.86 ^{b}	5.08 ^{c}	3.85 ^{d}	0.25	0.01	0.01	0.001	0.07
Methanogen (lg10 copies/mL)										
0-h	1.70	1.55	1.58	1.54	1.53	0.06	0.40	0.84	0.93	0.88
2-h	8.24 ^{a}	6.15 ^{b}	5.30 ^{c}	2.91 ^{d}	2.00 ^{e}	0.47	0.01	0.01	0.0009	0.90
4-h	7.74 ^{a}	6.12 ^{b}	4.78 ^{c}	2.59 ^{d}	1.97 ^{e}	0.45	0.01	0.01	0.01	0.08
Mean	5.89 ^{a}	4.61 ^{b}	3.89 ^{c}	2.34 ^{d}	1.83 ^{e}	0.31	0.01	0.01	0.001	0.53

SEM, standard error of the mean; a, b, c, d, e: in the same row there is a statistically significant difference ($p < 0.05$).

Table 11. Effect of purple neem foliage rich in anthocyanins on antioxidant activity in plasma of growing goat.

Items	Control	Normal Neem Foliage		Purple Neem Foliage		SEM	*p*-Value			
		3%	6%	3%	6%		Control vs. Treatment	Cultivars	Levels	C × L
Total antioxidant (nmol/uL)										
0-h	0.85	0.83	0.81	0.85	0.86	0.01	0.75	0.28	0.98	0.64
2-h	0.87 ^{c}	0.71 ^{d}	1.05 ^{b}	1.08 ^{b}	1.27 ^{a}	0.04	0.01	0.01	0.01	0.026
4-h	0.81 ^{b}	0.84 ^{b}	0.86 ^{b}	0.88 ^{b}	0.99 ^{a}	0.02	0.006	0.0012	0.009	0.08
Mean	0.84 ^{c}	0.79 ^{d}	0.91 ^{b}	0.93 ^{b}	1.04 ^{a}	0.02	0.01	0.01	0.01	0.76
SOD (inhibition rate %)										
0-h	86.50	87.72	88.27	88.70	89.30	1.38	0.60	0.77	0.86	0.99
2-h	91.95 ^{d}	92.19 ^{d}	93.25 ^{c}	94.06 ^{b}	95.40 ^{a}	0.26	0.01	0.01	0.01	0.33
4-h	89.74 ^{d}	90.29 ^{c}	90.49 ^{c}	90.90 ^{b}	91.42 ^{a}	0.12	0.01	0.01	0.0003	0.06
Mean	89.40	90.06	90.67	91.22	92.04	0.48	0.20	0.25	0.51	0.92
GPX (Units/mL)										
0-h	68.45	68.20	68.53	68.57	68.49	0.05	0.98	0.16	0.28	0.08
2-h	83.85 ^{d}	84.02 ^{c}	84.11 ^{b}	84.16 ^{b}	84.28 ^{a}	0.03	0.01	0.01	0.01	0.66
4-h	75.94 ^{d}	76.03 ^{cd}	76.11 ^{bc}	76.18 ^{b}	76.28 ^{a}	0.03	0.01	0.01	0.001	0.57
Mean	76.08 ^{b}	76.08 ^{b}	76.25 ^{a}	76.30 ^{a}	76.35 ^{a}	0.03	0.0003	0.0002	0.01	0.09

Table 11. *Cont.*

Items	Control	Normal Neem Foliage		Purple Neem Foliage		SEM	*p*-Value			
		3%	6%	3%	6%		Control vs. Treatment	Cultivars	Levels	C × L
MDA (µg/mL)										
0-h	21.23	21.91	21.60	21.39	21.35	0.1	0.18	0.09	0.43	0.55
2-h	35.40 [a]	29.48 [b]	26.77 [c]	26.75 [c]	22.12 [d]	0.91	0.01	0.01	0.01	0.07
4-h	30.91 [a]	25.00 [b]	22.29 [c]	22.27 [c]	21.23 [c]	0.75	0.01	0.002	0.002	0.12
Mean	29.18 [a]	25.46 [b]	23.56 [c]	23.47 [c]	21.57 [d]	0.55	0.01	0.01	0.01	1.00
DPPH scavenging activity (%)										
0-h	28.50	27.35	28.80	27.36	27.13	0.31	0.28	0.24	0.38	0.23
2-h	21.35 [d]	26.14 [d]	37.54 [c]	66.71 [b]	78.06 [a]	4.64	0.01	0.01	0.01	0.99
4-h	20.97 [d]	30.00 [c]	36.04 [bc]	41.04 [b]	49.69 [a]	2.09	0.01	0.01	0.0002	0.42
Mean	23.61 [e]	27.83 [d]	34.13 [c]	45.04 [b]	51.62 [a]	2.19	0.01	0.01	0.01	0.89
CAT (nmol/min/mL)										
0-h	8.74	8.86	8.93	8.88	8.96	0.03	0.02	0.68	0.22	0.95
2-h	14.22 [c]	14.32 [c]	14.69 [b]	14.83 [b]	15.03 [a]	0.06	0.01	0.01	0.01	0.02
4-h	13.56 [d]	13.63 [cd]	13.70 [c]	13.83 [b]	14.16 [a]	0.04	0.01	0.01	0.01	0.01
Mean	12.17 [d]	12.27 [c]	12.44 [b]	12.51 [b]	12.72 [a]	0.04	0.01	0.01	0.01	0.68

Superoxide dismutase, (SOD); glutathione peroxidase, (GPX); Malondialdehyde, (MDA); 2,2-diphenyl-1-picrylhydrazyl, (DPPH); catalase, (CAT); a, b, c, d, e in the same row there is a statistically significant difference ($p < 0.05$); SEM, standard error of the mean.

4. Discussion

4.1. Feed Intake, Nutrient Intake and Growth Performance

In this study, we found that although purple neem foliage displayed a high level of anthocyanin, anthocyanins are phenolic compounds that contribute the characteristic of bitter flavor to plants. Our current study revealed that goats that consumed a 6% purple neem foliage diet had higher levels of feed intake (gDM/d, %BW and g/kgBW$^{0.75}$) and nutrient intake, which might be attributed to the high amount of purple neem foliage in the diet because it contains anthocyanins, and they affect palatability, which can improve animal production [21]. This indicates that giving anthocyanins to goats did not reduce their palatability. Although anthocyanins had the capacity to affect palatability, they also had the ability to boost antioxidant activity without reducing DMI. According to [22], the animal's feed intake is highly proportional to the rate of DM removal from reticulorumen during the digestive process.

In this study, the richness of anthocyanins from purple neem foliage improved the apparent digestibility of DM, OM, CP, EE, NDF and ADF. This might be because anthocyanin's sugar structure is involved in digestion and metabolism [23]. The feeding experiment demonstrated that goats consuming a 6% purple neem foliage diet had higher apparent digestibility for all nutrients. Furthermore, it is widely recognized that the nutritional value of feed is mainly determined by its digestion properties and nutrient makeup, particularly for the CP and fiber fractions. According to [24–27], goats fed neem (*Azadirachta indica*) and Leucaena (*Leucaena leucocephala*) fodders, cassava leaf pellets and pods of Pterocarpus lucens or Senegal displayed increased total DM intake, digestibility, animal performance as a result of better ruminal fermentation and microbiological production. Furthermore, anthocyanins may be associated with the nutrients to be digested, limiting the digestive antibacterial properties of enzymes [28].

The growth performance of goats, final weight, weight change and ADG were relatively high in goats fed a 6% purple neem foliage diet. The significant difference in the final weight, weight change and ADG of the goats in the treatment of 6% purple neem foliage could be due to the relatively good quality of the basal (purple neem foliage). Because of

the comparatively high intake DM of purple neem foliage, the total intake per day of CP content in purple neem foliage had a relatively high final weight, weight change and ADG of goats consuming a 6% purple neem foliage diet. Although anthocyanins had the capacity to affect palatability, they also had the ability to boost antioxidant activity without reducing DMI. According to [29,30], supplementation with mixes and solitary multifunctional trees, rather than standard supplements such as wheat bran, increases animal performance in terms of live weight gain [31,32]. The addition of Leucaena (*Leucaena leucocephala*) and Madras thorn (*Pithecellobium dulce*) foliage to rice straw suggests that protein foliage from locally-produced shrubs and trees can replace commercial feed. We concluded that the feeding experiment demonstrated the goats' growth performance throughout the experimental period, indicating that the purple neem foliage diet with nutritional composition and high anthocyanin composition is more effective at improving growth performance.

4.2. Nitrogen Utilization

In terms of nitrogen utilization in this study, goats receiving normal neem foliage and purple neem foliage had a significant difference in the interaction between cultivars and levels. The goats receiving a 6% purple neem foliage diet increased their level of nitrogen intake, N urine, N digestion, N digestion (%) and N retention (%) compared to the other treatment groups. The high CP content and anthocyanin concentration of purple neem foliage may have the ability to promote ruminal fermentation and microbial protein synthesis. As a result, anthocyanins appear to have little effect on digestion, while the nutritional balance remains constant. Moreover, anthocyanins can attach to dietary proteins, reducing rumen fermentation and improving nitrogen utilization [33–35]. We established that sheep fed polyphenol-rich plants have higher nitrogen utilization and CP digestibility [36–38]. As predicted, feeding purple corn pigment increased CP digestibility, which could be attributed to the high anthocyanin concentration of purple corn pigment. Our outcomes are consistent with those of [39].

4.3. Rumen Fermentation Parameters

The ruminal pH, ammonia nitrogen and blood urea nitrogen levels after feeding did not significantly differ among treatments. The ruminal pH was decreased after feeding at 2 and 4 h. The decreased ruminal pH is relative to the accumulation of VFA [40]. In this study, it was found that the pH value was in the appropriate range. The rumen pH had average values ranging from 6.75 to 7.00, which is optimal for microbial digestion in the rumen [41]. This finding suggested that microbial decomposition of grain might lower the pH value in the rumen.

The NH_3-N had significant differences among all the treatments. In addition, the ruminal concentration of NH_3-N is generally affected by the CP content in the diet [42]. In the current study, the goats receiving a 6% purple neem foliage diet had high NH_3-N at 2 and 4 h after morning feeding. A high-level CP of anthocyanin in purple neem foliage did not affect the ruminal pH value, but there was a high level of NH_3-N concentration. This result is in accordance with [43], which demonstrated that in lactating dairy cows, receiving higher-level CP of anthocyanin-rich corn silages did not affect the ruminal fluid pH value, but they showed a higher level of NH_3-N concentration compared to the control silage group. The ruminal concentration of ammonia is generally affected by the CP content in a diet [44]. Furthermore, because urea is generated in the liver from ammonia absorbed from the digestive organs, blood urea nitrogen content is positively related to ruminal fluid ammonia concentration [45]. In this study, the average ruminal NH_3-N value for goats in the different treatment groups was 11.11–17.20 mg/dL and was within the range of optimal rumen NH_3–N concentration for microbial growth. An average range value for optimal NH_3–N concentration of 15 to 30 mg/dL is recommended by [46,47]. It can be suggested that the 6% purple neem foliage was likely affected by the high level of CP, which is a supplemental source of nitrogen to increase NH_3-N.

The BUN concentration was no different among the experiments at 0 h and there was a significantly different effect of the goats fed a 6% purple neem foliage diet, which had a higher BUN than other treatments at 2 and 4 h after feeding. In this study, the BUN concentration was 6.16–12.36 mg/dL. These results show that the BUN concentration for all treatments was within or above the optimal level of 10–20 mg/dL [48]. The increase in ruminal ammonia and BUN concentration in goats fed 6% purple neem foliage was probably caused by a higher CP content of purple neem foliage or by a higher concentration of ruminal ammonia. It can be suggested that purple neem foliage can affect the protein content.

There was no significant difference in the current research of volatile fatty acid among the five treatment groups at 0 h, and there was a significant difference in acetic acid, propionic acid, butyric acid, acetic acid/propionic acid ratio and total VFA at 2 and 4 h after feeding. In this study, the goats receiving 6% purple neem foliage had a higher acetic acid, propionic acid, butyric acid, acetic acid/propionic acid ratio and total VFA in rumen fluid at 2 and 4 h post feeding compared with other treatments. The major sources of energy for ruminant metabolism are volatile fatty acids. Anthocyanins could increase the formation of volatile fatty acid by regulating intestinal flora [49]. Volatile fatty acid, which is formed via organic matter fermentation in the rumen, can have a significant impact on ruminant production and product composition [50]. Several variables determine the relative quantities of the VFA generated, considering substrate composition, substrate availability, depolymerization rate and the presence of microbial species [51].

By raising the amount of propionic acid, anthocyanins may be able to influence glucose metabolism and offer more energy to ruminants [52]. The acetic acid concentration was higher for the goats fed 6% purple neem foliage when compared with normal neem foliage at 2 and 4 h post feeding. This is likely due to the high digestibility of NDF and ADF content. Our prior research suggested that anthocyanins might influence glucose metabolism to offer additional energy to ruminants by increasing acetic acid production. Furthermore, the acetic acid content in rumen digesta transferred from buffalo to cattle changed, whereas propionic acid and butyric acid remained similar, indicating an active function of rumen microorganisms and continued fiber fermentation by cellulolytic bacteria [53]. The formation of acetic acid is always accompanied by H_2 and CO_2 production. In the current study, the goats fed a 6% purple neem foliage diet had significantly higher levels of propionic acid, butyric acid, a ratio of AA to PA and total VFA at 2 and 4 h after feeding than goats fed standard neem foliage. Owing to the high fiber content of the purple neem foliage diet treatment, it would be difficult to digest in the rumen. According to one study, this might be because, while anthocyanin-rich purple corn silage had a higher level of anthocyanin, it did not appear to be degraded in the rumen [54]. Furthermore, the type of VFA generated in the rumen is determined by the fermented substrate, the rumen environment and the microbial population [55].

4.4. Microbial Population

Anthocyanins have been shown to influence gut bacteria. Furthermore, the fermentation substrate, rumen environment and microbial population are all important factors in determining the kind of rumen microbial population [56]. As a result, this disparity might be attributed to fermentation products, anthocyanin sources and animal physiological phases. In this study, there was a substantial change in the microbial community in growing goats at days 2 and 4 following feeding. According to our findings, the goats given a 6% purple neem foliage diet had higher levels of total bacteria, *Butyrivibrio fibrisolvens*, *Fibrobacter succinogenes*, *Ruminococcus albus*, *Ruminococcus flavefaciens* and *Streptococcus bovis*. The microorganisms were predicted to boost anthocyanin bioavailability in the gastrointestinal system, hence increasing the gut antioxidant capacity [57]. Furthermore, anthocyanins have potent antioxidant properties that can protect the organism from peroxidation damage, hence boosting rumen microbiota. As a result, anthocyanins can lower heat stress and modify the rumen microbiota, perhaps promoting the expansion of beneficial anaerobic bacteria.

As a result, we discovered that our study was compatible with the study of [58], which showed that feeding anthocyanin might boost the number of different bacteria and found that anthocyanin-rich berry seed residues might increase the total bacteria in ruminal fluid in a batch culture system. Furthermore, [59] found that dairy cows fed anthocyanin-rich plants had a higher relative abundance of ruminal fluid, and [60] *Piper betle* L. powder supplementation enhanced the presence of ruminal *Butyrivibrio fibrisolvens* in dairy goats.

As a gut modulator, anthocyanins may promote the development of good anaerobic bacteria while suppressing harmful bacteria [61,62]. Furthermore, owing to intracellular interactions, anthocyanin has antibacterial activity and inhibits the development of a range of pathogens [60]. In the current investigation, there was a significant difference between the treatments for protozoa and methanogen at 2 and 4 h after feeding. Data show that goats given a 6% purple neem foliage diet reduced protozoa and methanogen levels as compared to other treatments, suggesting that the high anthocyanin content suppressed microbes in the rumen. According to [61], phenolic-rich plants demonstrated antibacterial effects in feedlot cattle; according to [62], piper oil combined with sunflower oil reduced methane mitigation agents without negatively affecting rumen fermentation; according to [63], anthocyanin from purple maize affects rumen microbiota by modulating the relative abundance of rumen microorganisms, increasing the variety of rumen microbes; and [64] revealed the effectiveness of Siamese neem leaves in inhibiting ruminal CH_4 emission.

4.5. Antioxidant Activity in Plasma

Anthocyanins are a source of secondary metabolites of plants that are powerful natural antioxidants and free radical (FR) scavengers, have a variety of key physiological functions for consumers and have vast development and application potential, according to the literature [65]. Previous research has demonstrated that anthocyanins are absorbed into the bloodstream in their entirety [66]. As a result, anthocyanins can boost the activity of antioxidant enzymes including superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and catalase (CAT) to further limit malondialdehyde formation (MDA). In our study, there was a significant difference in total antioxidant, SOD, GPX, DPPH and CAT levels at 2 and 4 h after feeding, indicating that goats given a 6% purple neem foliage diet increased total antioxidant, SOD, GPX, DPPH and CAT levels in plasma. The goats fed a 6% purple neem foliage diet dramatically increased plasma SOD activity, possibly indicating that anthocyanins were absorbed into the blood, supplying electrons to O_2 and therefore alleviating the OS condition. It is worth noting that the activity of total antioxidant, GPX and CAT in plasma increased in the goats fed a 6% purple neem foliage diet, maybe because they improved SOD activity in plasma, alleviating the stress on the antioxidant defense system. Furthermore, antho-cyanin-rich foods may increase messenger RNA production, influencing SOD activity in the blood [66]. The current findings are consistent with the findings of [66], who observed a considerable increase in SOD in the plasma of lactating dairy cows given anthocyanin-rich purple corn silage [67]. Furthermore, our findings are consistent with the findings of [68], who found that anthocyanin-rich purple corn stover silage can boost plasma antioxidant capacity in nursing dairy goats. Because anthocyanins have considerable antioxidant activity, studies have demonstrated that anthocyanins are absorbed intact into the blood of goats given a 6% purple neem foliage diet and dramatically increase plasma DPPH. DPPH is a kind of FR that is reduced in an aqueous solution containing an antioxidant [67]. As a result, anthocyanins in plasma can donate electrons to DPPH, boosting the degree of DPPH-scavenging activity. Anthocyanin-rich grape juices, as effectively highlighted by [69], can boost blood antioxidant capacity in humans. In this study, MDA concentrations in plasma were considerably lower after the goats were given a 6% purple neem foliage diet compared to other treatments. Because FRs' high reactivity has an effect on antioxidant enzymes, lipid peroxidation occurs [70]. Antioxidant enzymes reduce lipid hydroperoxide and H_2O_2 concentrations, making them efficient in preventing lipid peroxidation and maintaining cell membrane integrity and function, resulting in the lowest MDA concentration. Previous research [70,71] found that

it serves to minimize oxidative stress caused by MDA byproducts formed from DNA and lipid oxidation, and this stress has mostly been utilized to monitor the oxidation state.

5. Conclusions

This study found that goats fed a 6% purple neem foliage diet improved their growth performance, changed the type of rumen fermentation and enhanced their VFA content. Furthermore, the goats fed anthocyanin-rich 6% purple neem foliage increased rumen fermentation parameters via modulating microbial population abundance and antioxidant activity in growing goat plasma. Future research is required to validate the current findings and examine the impact of anthocyanin-rich purple neem leaves on carcass characteristics such as antioxidant content, fatty acid profile and meat texture.

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