

Article

Mulberry Silage as Alternative to Soybean Meal Protein in Ruminant Diet: Effect on Growth Performance, Digestion, Antioxidant Capacity, and Carcass Characteristics of Goats

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Simple Summary

Using 45 Xiangdong black goat kids split into three groups, the study examined substituting mulberry silage for soybean meal protein in goat diets at 50% and 100% substitution levels. The findings indicate that whereas full replacement (MS-100) decreased the digestibility of organic matter and fiber, mulberry silage enhanced feed intake of dry matter, protein, fiber, and organic matter. Health indicators improved; MS-100 goats had higher levels of antioxidant capacity, albumin, calcium, and plasma protein, but they also had higher levels of oxidative stress markers. While MS-50 maximized the amino acid content of meat, MS-100 improved growth performance with higher body weight, daily gain, and carcass weight. All things considered, mulberry silage shows promise as a substitute for soybean meal, promoting growth, health, and meat quality in goats that were either equivalent or better.



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Abstract

Current research is still striving to maximize the expansion of protein feed sources in order to reduce the production costs of the livestock industry. In this context, the current study aimed to evaluate the impacts of replacing soybean meal protein in diets with mulberry silage (at 50 and 100% substitution levels) on the growth, health status, and carcass characteristics of growing goat kids. Forty-five Xiangdong black goat kids weighing 18.2 ± 1.6 kg (approximately 6 months old) were divided into three experimental groups and fed one of the following diets: a control diet consisting of a 50:50 concentrate-to-roughage ratio on a dry matter basis (control), a diet replacing 50% of soybean meal protein with mulberry silage (MS-50), or a diet replacing 100% of soybean meal protein with mulberry silage (MS-100). The results show that replacing soybean meal protein with mulberry silage significantly increased ($p < 0.05$) the intakes of dry matter (DMI), crude protein (CPI), neutral detergent fiber (NDFI), and organic matter (OMI). However, no

significant ($p > 0.05$) differences were observed in the digestibility of dry matter (DM), crude protein (CP), ether extract (EE), or neutral detergent fiber (NDF) among the groups, whereas the digestibility of OM and acid detergent fiber (ADF) was significantly reduced ($p < 0.05$) in the MS-100 group. Additionally, the MS-100 group exhibited significantly higher ($p < 0.05$) plasma total protein, albumin, and calcium levels. Total antioxidant capacity (TAC) was significantly enhanced in both the MS-50 and MS-100 groups compared to the control, although the MS-100 showed a significant increase ($p < 0.05$) in malondialdehyde (MDA) levels related to the other groups. In terms of growth performance, the MS-100 group improved ($p < 0.05$) final body weight, average daily gain, and carcass weights. Meanwhile, the MS-50 group recorded the highest contents ($p < 0.05$) of the following amino acids: aspartic acid, threonine, serine, glutamic acid, alanine, lysine, and proline. Overall, the results of the present study indicate that replacing soybean meal protein with mulberry silage generally produced comparable impacts, with additional positive effects on growth performance, meat quality, and health status of goats.

Keywords: mulberry silage; soybean meal protein; goat kids; growth performance; antioxidant capacity; carcass characteristics

1. Introduction

In China and other developing nations, there is a rising need for animal products, which in turn is boosting the demand for sufficient and cost-effective animal feed, especially for protein feed, which is a vital part of animal diets and is essential for growth, reproduction, and general health [1,2]. China demonstrates a substantial dependence on imported protein feed ingredients, notably represented by soybeans. According to data from the General Administration of Customs of China, the import volume in 2025 reached 111.8 million tons [3], constituting over 83% of the nation's annual total soybean consumption. Fluctuations in international soybean prices exert the most direct and profound impact on the production costs of livestock. Consequently, the development of non-grain protein feed resources to substitute soybean meal is critically imperative for reducing costs and enhancing efficiency in the animal husbandry sector.

Forage mulberry (*Morus alba* L.) is characterized by a well-developed root system and strong stress resistance, leading to its widespread cultivation across China. Concurrently, its herbaceous cultivation characteristics, tolerance to frequent cutting, high biomass yield, and balanced nutritional profile hold significant importance for enhancing protein feed supply and promoting the reduction of grain-based feed in grass-fed animal husbandry.

Forage mulberry is known for its high nutritional value, containing noticeable amounts of crude protein (CP), essential amino acids, vitamins, and minerals [4,5]. The leaves are particularly rich in protein (15–25% of dry matter (DM)) and contain bioactive compounds such as flavonoids, phenolic acids, and alkaloids, which contribute to the plant's antioxidant properties [6–8]. Furthermore, mulberry leaves are elevated in amino acids especially essential amino acids such as lysine, methionine, and tryptophan, which are vital for various physiological functions and overall animal health [5,9–12]. Also, mulberry leaves are rich in vitamins A, C, and E, as well as minerals like calcium, phosphorus, and potassium, which play crucial roles in maintaining metabolic processes and overall health [4,6,13,14].

As a high-quality protein feed resource, forage mulberry has demonstrated significant potential in animal production. Studies indicate that mulberry leaves can serve as an effective protein source for herbivores [7,15,16], and their incorporation into lamb diets reduces the reliance on conventional protein supplements [17]. Notably, the digestible energy and

CP content of mulberry leaves are comparable to those of alfalfa hay, underscoring their nutritional competitiveness [18]. In terms of processing technology, silage fermentation offers distinct advantages over dry processing for preserving mulberry leaves. Unlike the production of dried leaves, which is highly dependent on favorable weather conditions, silage fermentation is less constrained by ambient climate fluctuations. During this process, lactic acid bacteria metabolize carbohydrates in the leaves to produce organic acids (e.g., lactic, acetic, propionic, and butyric acids), which act as natural preservatives and enhance the storage stability of the feed.

The ensiling fermentation process effectively degrades the fiber fraction, which can improve nutrient availability, digestibility, and palatability of mulberry leaves. These enhancements contribute to increased voluntary feed intake [19,20]. For instance, studies have demonstrated that the incorporation of mulberry silage into diets promotes higher DM consumption, as observed in goats supplemented with mulberry leaf meal, where DMI rose correspondingly with inclusion levels. [17,18]. Additionally, the inclusion of mulberry silage in ruminant diets has been associated with improved nutrient and fiber digestibility [19]. Furthermore, bioactive compounds present in forage mulberry, such as flavonoids and phenolic acid, enhance antioxidant defenses in animals. Research indicates that diets containing mulberry silage improve plasma protein profiles and mineral status [8,20,21].

Although previous studies have confirmed the potential of mulberry leaves as feed ingredients, systematic research on the substitution of soybean meal with whole-plant mulberry silage in the diets of finishing goat remains limited. In particular, the mechanisms underlying the effects of different substitution ratios on physiological functions and meat quality are still unclear. Therefore, this study used local black goats as subjects and formulated dietary regimens with varying replacement levels of whole-plant mulberry silage for soybean meal. It aimed to systematically evaluate the effects on growth performance, health indicators, and carcass characteristics, in order to provide a theoretical basis for the rational application of mulberry feed in goat meat production.

2. Materials and Methods

2.1. Experimental Protocol

The experiment was carried out on the experimental station and laboratories of the Institute of Subtropical Agriculture, Chinese Academy of Sciences. The protocol of the study was approved by the animal care committee of the Institute of Subtropical Agriculture, Chinese Academy of Sciences, Changsha, Hunan Province, China (permission No. CAS202200045).

2.2. Mulberry Silage Making

Whole mulberry plants were chopped, wilted for 4 h, and treated with an enzyme bacteria complex (*Lactobacillus plantarum* 4×10^8 CFU/kg, α -galactosidase 40 U/kg, acid cellulase 20 U/kg, and *Trichoderma reesei* xylanase 40 U/kg) on a fresh weight basis. The amounts of bacteria and enzymes added were modified according to the Hu et al. [18] and Yang et al. [22] scheme. The material was ensiled in plastic bags for two months before being fed to the goats. The quality of the whole mulberry plant silage was as follows: pH 4.04, acetate 3.08 g/kg, propionate 0.677 g/kg, lactic acid 25.1 g/kg, ammonia nitrogen 0.694 g/kg. The chemical composition based on DM was: CP 155.0 g/kg, neutral detergent fiber (NDF) 379.2 g/kg, acid detergent fiber (ADF) 227.1 g/kg, organic matter (OM) 900.2 g/kg, and ether extract (EE) 35.1 g/kg.

2.3. Animals and Diets

Forty-five healthy Xiangdong black wether goats (6 months old; body weight 18.2 ± 1.6 kg) were selected and randomly divided into three groups (control, MS-50, and MS-100), with 15 goats per group. Each goat was housed in an individual cage. The control group received a basal diet, while the experimental groups (MS-50 and MS-100) had 50% and 100% of soybean meal protein replaced with mulberry silage, respectively. The diets were formulated according to the goat feeding standard (NY/T816–2004, Ministry of Agriculture, China) [23]. The diet compositions and nutritional levels are shown in Table 1.

Table 1. Chemical composition of the experimental diets.

Items	Diet Composition (g/kg DM)		
	Control	MS-50	MS-100
Corn	258.6	244.8	194.8
Wheat bran	44.0	128.9	251.9
Mulberry silage	0.00	268.0	500.0
Soybean meal	147.0	73.5	0.00
Rice Straw	500.0	232.0	0.00
CaCO ₃	7.0	3.5	1.3
CaHCO ₃	18.5	24.2	26.9
NaCl	5.0	5.0	5.0
Premix	20.0	20.0	20.0
Nutritional value			
ME (MJ/kg)	8.68	8.75	8.70
CP (g/kg DM)	115.4	115.8	116.3
NDF (g/kg DM)	415.8	380.4	356.5
ADF (g/kg DM)	267.5	253.7	243.9
Ca (g/kg DM)	10.0	14.0	17.2
P (g/kg DM)	6.4	9.0	11.0

Premix contains: 35 mg of iron, 15 mg of copper, 90 mg of manganese, 70 mg of zinc, 0.05 mg of selenium, 1 mg of iodine, and 0.15 mg of cobalt per kg of dry matter. ME: metabolic energy; CP: crude protein; NDF: neutral detergent fiber; ADF: acid detergent fiber. Ca: calcium; P: phosphorus. MS-50: 50% of soybean meal protein replaced with mulberry silage; MS-100: 100% of soybean meal protein replaced with mulberry silage.

Prior to the experiment, the goat house was thoroughly cleaned and disinfected. All goats were vaccinated against foot-and-mouth disease and goat pox, and were dewormed and tested for brucellosis. During the pre-trial period, the goats were initially fed a diet provided by Liu'an Agriculture Co., Ltd. (Liu'an, Anhui, China). The diet was gradually transitioned to the experimental diets by replacing 15% of the previous feed daily until the transition was complete. The experimental procedures were conducted over a total period of 90 days, which included a 10-day pre-trial adaptation phase and a subsequent 100-day formal experimental period. Following an initial weighing, all goats were housed in individual pens. The animals were fed twice per day at 09:00 and 16:00 and were provided with ad libitum access to feed and water throughout the study to ensure voluntary intake.

2.4. Intake, Nutrient Digestibility, and Chemical Analyses

2.4.1. Feed Intake Recording and Body Weight

Body weight was measured on the first and last day of the formal experiment. Daily feed offered and residues were recorded for each goat to calculate dry matter intake (DMI). Weekly feed samples were collected and composited for post-trial nutritional analysis.

2.4.2. Digestibility

In the last week (the 75th to 80th day) of the formal period, fecal samples were collected by the full fecal collection method. Fecal samples were collected once a day for 6 consecutive days. After weighing the fecal samples of each goat, 10% of the samples were taken, and 10% tartaric acid was added at a ratio of 5% of the fecal weight. The samples were mixed evenly to prepare air-dried samples for testing. To calculate the coefficients of digestion, the procedure of Ferret et al. [24] was used.

2.4.3. Blood Collection and Treatment

On the last day of the formal period, 15 mL of blood was collected from the jugular vein before morning feeding, anti-coagulated (EDTA), and centrifuged at 3000 rpm for 20 min to obtain plasma, which was divided into four 2 mL centrifuge tubes and stored at -20°C to be used for the detection of biochemical items and antioxidant indicators.

2.4.4. Slaughter and Muscle Sample

Ten animals were randomly selected from each group for slaughter sampling. The test goats were fasted for 12 h before slaughter, bled to death, and right *Longissimus dorsi* muscle (LDM) samples were collected [25].

2.4.5. Feed and Feces Samples Analysis

Feed, orts, and fecal samples were analyzed for ash after heating samples in a muffle furnace at 550°C for 12 h (method ID 942.05). N was measured using the Kjeldahl method (method ID 954.01), and ether extract (EE) using diethyl ether in a Soxhlet extractor (method ID 920.39), according to AOAC [26] official methods. Neutral detergent fiber (NDF) was determined by the procedure of Van Soest et al. [27] without alpha amylase but with sodium sulfite. Acid detergent fiber (ADF; method ID 973.18) was analyzed according to AOAC [26] (method ID 973.18).

2.4.6. Analysis of Blood Plasma Samples

Blood plasma samples were analyzed for different blood biochemical parameters. The plasma total protein (TP), albumin (ALB), alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), gamma glutamyl transpeptidase (GGT), triglyceride (TG), total cholesterol (CHOL), low-density lipoprotein cholesterol (LDL-c), high-density lipoprotein cholesterol (HDL-c), glucose (GLU), amylase (amy), lactate dehydrogenase (LDH), blood urea nitrogen (BUN), calcium (Ca) and phosphorus (P) concentrations were measured on a Mindray BS-230 automatic chemistry analyzer (Shenzhen, China).

Plasma levels of total antioxidant capacity (TAC), malondialdehyde (MDA), superoxide dismutase (SOD), and glutathione peroxidase (GSH-Px), key parameters of antioxidant status and oxidative stress, were quantified using commercial ELISA kits according to the manufacturers' instructions, following the methodological details outlined by Khattab et al. [28].

2.5. Carcass Characteristics and Measurements

After slaughter, the external offal (hide, head and four feet) was removed. The dressed carcasses were then eviscerated and split into two halves along the vertebral column. Dressing percentage was calculated as: $(\text{carcass weight}/\text{live body weight}) \times 100$. A fresh sample of the LDM at the 9th rib was dissected. The height and width of its cross-section were measured using a digital vernier calipers, while the area of the eye muscle (cm^2) was calculated by the height $\times$ width $\times 0.7$.

The chemical composition of the kids' meat samples was analyzed with a Food ScanTM meat analyzer (Foss Analytical A/S, Model 78810, FOSS Company, Hilleroed, Denmark,

DK-3400 Hilleroed, FOSS Company, Hilleroed, Denmark). Color parameters were determined with a chroma meter (Konica Minolta, model CR 410, Chiyoda-ku, Konica Minolta Sensing Ltd., Tokyo, 100-0005, Japan) and expressed in the CIE L^* , a^* , and b^* color system (CIE, 1986). Three spectral readings were obtained from different locations on each LDM sample to record lightness (L^*), redness (a^*) and yellowness (b^*). Hue angle was used to evaluate the transition of myoglobin pigment from red to brown, while chroma (repression color saturation) indicated color intensity, calculated according to the method described by Majdoub-Mathlouthi et al. [29].

Amino acids in the LDM muscle were analyzed following the official method 985.28 of AOAC [26]. An ion-exchange amino acid analyzer (Hitachi L-8900, Hitachi high-technologies Corporation, Tokyo, Japan) was employed for quantification. For sample preparation, approximately 0.1 g of ground muscle tissue was hydrolyzed with 10 mL of 6 mol/L HCl at 110 °C for 24 h. After hydrolysis, the solution was quantitatively transferred to a 100 mL volumetric flask and made up to the mark with distilled water. A 1 mL aliquot of the clarified supernatant was passed through a 0.45 µm syringe filter. The resulting filtrate was subsequently diluted tenfold for instrumental analysis.

2.6. Statistical Analysis

With the exception of eye muscle data, the data generated were analyzed using the PROC MIXED procedure of SAS (version 9.4; SAS Inc., SAS Campus Drive Cary, NC 27513-2414, USA). The model was:

$$Y_{ijk} = \mu + T_i + L_j(T_i) + E_{ijk}$$

where Y_{ijk} = observation of the j th kid given i th treatment, T_i = treatments effect, $L_j(T_i)$ = kid within treatments, and E_{ijk} = experimental error. For eye muscle area, the carcass weight was included in the model as a covariance factor based on the above model. The differences between the means were determined using the Duncan multiple comparison test, the results were expressed as mean and SEM, and those where $p \leq 0.05$ were considered significant.

3. Results

3.1. Growth Performance

Growth performance parameters and carcass characteristics are presented in Table 2. Compared with other experiment groups, MS-100 significantly improved final body weight ($p = 0.002$), total weight gain ($p < 0.001$), average daily gain ($p < 0.001$), and feed conversion ratio (expressed as DM feed intake per daily gain; $p = 0.005$).

Table 2. Effect of replacing soybean meal in growing goat kids' diets with mulberry silage on growth performance and feed conversion.

Items	Experimental Groups			SEM	p-Value
	Control	MS-50	MS-100		
Initial body weight (kg)	18.3	17.9	18.5	0.43	0.643
Final body weight (kg)	22.9 ^b	23.5 ^b	25.3 ^a	0.57	0.012
Total weight gain (kg)	4.62 ^b	5.53 ^b	6.82 ^a	0.321	<0.001
Average daily gain (g/d)	46.2 ^b	55.3 ^b	68.2 ^a	3.21	<0.001
Feed conversion (feed intake/daily gain)	16.7 ^a	16.2 ^a	13.3 ^b	0.47	0.005

MS-50: 50% of soybean meal protein replaced with mulberry silage; MS-100: 100% of soybean meal protein replaced with mulberry silage; SEM: standard error of mean. a, & b: means within a row with different superscripts differ ($p < 0.05$).

3.2. Feed Intake and Nutrient Digestibility

Records of nutrient feed intake and digestibility showed significant differences between experimental groups (Table 3). The results show that replacing soybean meal protein with mulberry silage (MS-50 and MS-100) significantly improved intakes of DMI ($p < 0.001$), crude protein (CPI) ($p < 0.001$), neutral detergent fiber (NDFI) ($p = 0.029$), and organic matter (OMI) ($p < 0.001$). Compared with the control, DMI increased by 12.6% and 17.6% for MS-50 and MS-100, respectively. Similarly, CPI values improved by 17.8% and 18.6% for MS-50 and MS-100, respectively, compared to the control.

Table 3. Effect of replacing soybean meal in growing goat kids' diets with mulberry silage on nutrient intake and digestibility.

Items	Experimental Groups			SEM	<i>p</i> -Value
	Control	MS-50	MS-100		
Nutrient intake (g/d)					
DMI	657 ^b	740 ^a	773 ^a	10.4	<0.001
CPI	82.9 ^b	97.7 ^a	98.3 ^a	1.30	<0.001
EEI	17.3 ^c	33.9 ^a	32.7 ^b	1.15	<0.001
NDFI	287 ^c	312 ^a	313 ^a	4.7	0.029
ADFI	152	162	153	2.8	0.305
OMI	572 ^c	658 ^b	695 ^a	10.1	<0.001
Nutrient digestibility (%)					
DMD	74.4	71.5	72.6	0.93	0.097
CPD	78.8	80.1	77.7	0.91	0.187
EED	89.2	89.7	84.6	2.43	0.274
NDFD	65.7	64.7	64.8	1.32	0.850
ADFD	67.3 ^a	63.6 ^{ab}	60.9 ^b	1.85	0.047
OMD	78.9 ^a	76.1 ^b	75.4 ^b	0.77	0.006

a–c: means within a row with different superscripts differ ($p < 0.05$). DMI: dry matter intake; CPI: crude protein intake; EI: ether extract intake; NDFI: neutral detergent fiber intake; ADFI: acid detergent fiber intake; OMI: organic matter intake; DMD: dry matter digestibility; CPD: crude protein digestibility; EED: ether extract digestibility; NDFD: neutral detergent fiber digestibility; ADFD: acid detergent fiber digestibility; OMD: organic matter digestibility; MS-50: 50% of soybean meal protein replaced with mulberry silage; MS-100: 100% of soybean meal protein replaced with mulberry silage; SEM: standard error of mean.

Digestibility coefficients of dry matter (DMD), crude protein (CPD), ether extract (EED), and neutral detergent fiber (NDFD) showed no significant differences between the experimental groups ($p > 0.05$). The MS-100 group showed significantly lower organic matter (OMD) ($p = 0.006$) and acid detergent fiber (ADFD) ($p = 0.047$) compared with the control. Meanwhile, MS-50 also showed significantly reduced OMD values ($p = 0.006$) compared to the control.

3.3. Carcass Traits, Viscera and Meat Characteristics

Carcass and meat quality parameters are summarized in Table 4. In general, the replacement of soybean meal with mulberry silage significantly increased certain parameters. Specifically, the MS-100 group showed a significant increase in carcass weight ($p = 0.001$), dressing ($p = 0.022$), eye muscle area ($p = 0.047$), and pH of LDM ($p = 0.005$) after 24 h compared to the control group. The MS-50 group also resulted in a significant enhancement of carcass weight ($p = 0.002$) and dressing ($p = 0.002$) relative to the control.

Table 4. Effect of replacing soybean meal in growing goat kids' diets with mulberry silage on carcass traits and meat quality.

Items	Experimental Groups			SEM	<i>p</i> -Value
	Control	MS-50	MS-100		
Carcass weight (kg)	10.4 ^c	11.5 ^b	12.5 ^a	0.33	0.001
Dressing (%)	45.7 ^b	47.2 ^a	47.9 ^a	0.66	0.022
Eye muscle area (cm ²)	683 ^b	795 ^{ab}	847 ^a	49.8	0.047
Drip loss (%)	1.65	1.99	1.49	0.139	0.353
Cooking loss (%)	32.9	32.3	28.5	0.94	0.116
Shear force (N)	41.7	42.8	42.7	1.19	0.918
Dorsal muscle moisture (%)	75.0	75.5	75.4	0.31	0.425
Dorsal muscle protein (%)	78.2	79.0	78.6	1.30	0.913
Dorsal muscle fat (%)	12.3	11.3	11.1	0.68	0.413
Ash of dorsal muscle (%)	4.40	4.70	4.70	0.209	0.516
LDM pH 0 h	6.84	6.77	6.72	0.054	0.230
LDM pH 24 h	5.67 ^b	5.65 ^b	6.21 ^a	0.095	0.005
Meat color <i>L</i> * 0 h	34.8	34.1	33.7	0.68	0.798
Meat color <i>a</i> * 0 h	15.8	15.4	15.3	0.34	0.816
Meat color <i>b</i> * 0 h	1.40	1.32	1.79	0.200	0.610
Meat color <i>L</i> * 24 h	37.3	33.6	36.0	1.07	0.384
Meat color <i>a</i> * 24 h	16.3	16.3	16.6	0.35	0.904
Meat color <i>b</i> * 24 h	4.14	3.16	3.38	0.245	0.276

a–c: means within a row with different superscripts differ ($p < 0.05$). LDM: *Longissimus dorsi* muscle; MS-50: 50% of soybean meal protein replaced with mulberry silage; MS-100: 100% of soybean meal protein replaced with mulberry silage; SEM: standard error of mean.

In contrast, no significant differences ($p > 0.05$) were observed among the experimental groups for the remaining metrics. These included meat quality traits (cooking loss, shear force, drip loss, dorsal muscle moisture, dorsal muscle protein, dorsal muscle fat, ash content of dorsal muscle, pH value of the LDM at 0 h, and meat color values *L**, *a**, and *b** at 0 and 24 h).

3.4. Blood Plasma Biochemical Kinetics

Plasma biochemical parameter values were within the normal ranges for healthy animals [30]. Data for plasma biochemical kinetics are presented in Table 5. The MS-100 group showed a significant increase in total protein ($p = 0.004$), albumin ($p < 0.001$), and calcium concentration ($p = 0.004$) as compared with the control group. On the other hand, the MS-50 group did not differ significantly from the control group ($p > 0.05$).

Table 5. Effect of replacing soybean meal in growing goat kids' diets with mulberry silage on different blood plasma kinetics.

Items	Experimental Groups			SEM	<i>p</i> -Value
	Control	MS-50	MS-100		
Total protein (g/L)	72.6 ^b	74.5 ^b	81.1 ^a	1.19	0.004
Albumin (g/L)	32.6 ^b	35.6 ^b	40.4 ^a	0.87	<0.001
Glucose (mmol/L)	4.49	4.08	4.94	0.186	0.170
Triglycerides (mmol/L)	0.204	0.246	0.255	0.0101	0.075
Cholesterol (mmol/L)	2.69	2.97	2.62	0.100	0.331

Table 5. Cont.

Items	Experimental Groups			SEM	p-Value
	Control	MS-50	MS-100		
Low-density lipoprotein cholesterol (mmol/L)	0.993	0.972	0.752	0.0612	0.204
High-density lipoprotein cholesterol (mmol/L)	1.64	2.00	1.84	0.066	0.081
Alanine transaminase (u/L)	41.0	37.7	39.0	2.10	0.823
Aspartate transaminase (u/L)	173	159	172	11.4	0.869
Blood urea nitrogen (mmol/L)	6.47	5.83	6.59	0.305	0.566
Lactate dehydrogenase (u/L)	794	879	9160	78.2	0.819
Alkaline phosphatase (u/L)	397	556	1006	118.9	0.091
Gamma glutamyl transpeptidase (u/L)	51.2	45.1	51.3	1.99	0.359
Amylase (u/L)	39.2	39.1	45.2	4.08	0.795
Calcium (mmol/L)	2.13 ^b	2.28 ^{ab}	2.44 ^a	0.040	0.004
Phosphorus (mmol/L)	2.96	3.05	3.22	0.127	0.705

a, b: means within a row with different superscripts differ ($p < 0.05$). MS-50: 50% of soybean meal protein replaced with mulberry silage; MS-100: 100% of soybean meal protein replaced with mulberry silage; SEM: standard error of mean.

3.5. Plasma Antioxidant Parameters

Replacing soybean meal with mulberry silage improved TAC (Figure 1). However, MDA values were significantly ($p < 0.05$) increased in the MS-100 group when compared to the other groups. Additionally, no significant differences were noted between the experimental groups for SOD and GSH-Px (Figure 1).

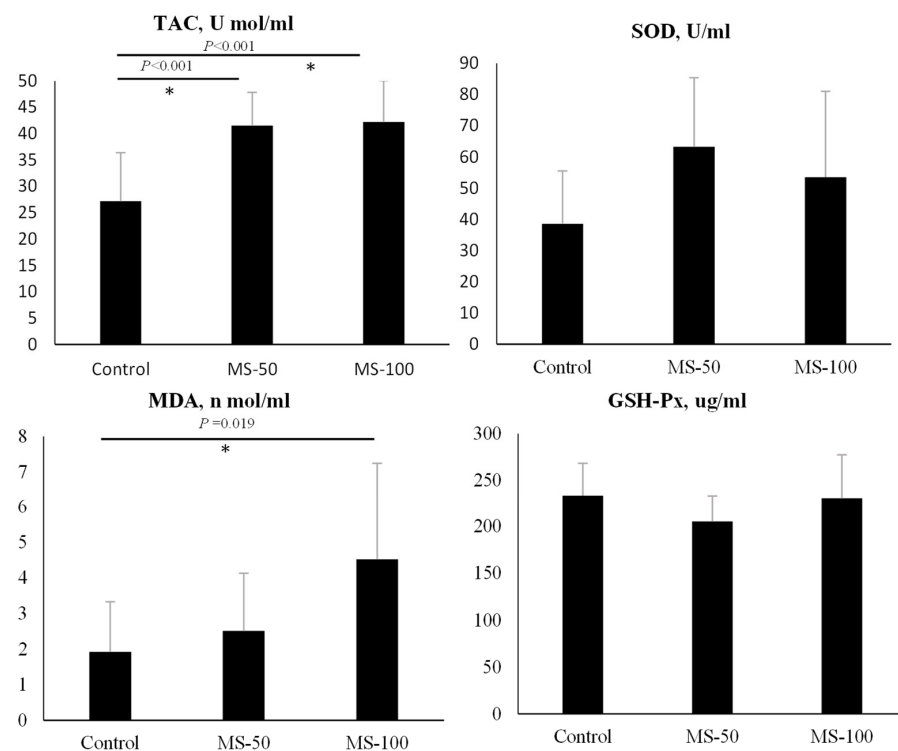


Figure 1. Effect of replacing soybean meal in growing kids' diets with mulberry silage on antioxidant parameters. * means $p < 0.05$. TAC: total antioxidant capacity; SOD: superoxide dismutase; MDA: malondialdehyde; GSH-Px: glutathione peroxidase; MS-50: 50% of soybean meal protein replaced with mulberry silage; MS-100: 100% of soybean meal protein replaced with mulberry silage.

3.6. Amino Acid Profile

The amino acid profile of carcass meat in the different experimental groups is presented in Table 6. The MS-50 group exhibited the highest contents ($p < 0.05$) of aspartate, threonine, serine, glutamate, alanine, lysine, proline, total amino acids (TAA), non-essential amino acids (NEAA), flavor amino acids (DAA), and functional amino acids (FAA). In contrast, the control group showed the lowest values of these amino acids but the highest ratio of EAA to NEAA. On the other hand, no significant differences ($p > 0.05$) were observed among the groups for valine, methionine, isoleucine, leucine, tryptophan, phenylalanine, histidine, arginine, essential amino acids (EAA), limited amino acids (LAA), and branched-chain amino acids (BCAA).

Table 6. Effect of replacing soybean meal in growing goat kids' diets with mulberry silage on amino acid profile (mg/g) of carcass meat.

Items	Experimental Groups			SEM	p-Value
	Control	MS-50	MS-100		
Aspartate (Asp)	7.37 ^b	7.88 ^a	7.58 ^{ab}	0.082	0.029
Threonine (Thr)	3.73 ^b	3.99 ^a	3.84 ^{ab}	0.042	0.028
Serine (Ser)	2.79 ^b	3.12 ^a	2.98 ^a	0.040	<0.001
Glutamate (Glu)	12.0 ^b	13.0 ^a	12.3 ^{ab}	0.15	0.021
Glycine (Gly)	3.53 ^b	3.80 ^a	3.94 ^a	0.058	0.007
Alanine (Ala)	4.71 ^b	5.20 ^a	5.14 ^a	0.060	<0.001
Valine (Val)	3.98	4.17	4.03	0.042	0.137
Methionine (Met)	2.21	2.35	2.25	0.029	0.118
Isoleucine (Iso)	3.80	3.95	3.76	0.042	0.142
Leucine (Leu)	6.61	6.88	6.50	0.075	0.115
Tyrosine (Tyr)	1.63	2.72	2.53	0.035	0.083
Phenylalanine (Phe)	3.32	3.49	3.39	0.038	0.197
Lysine (Lys)	7.42 ^b	7.90 ^a	7.57 ^{ab}	0.082	0.045
Histidine (His)	2.93	5.35	5.03	0.058	0.139
Arginine (Arg)	5.14	3.81	3.72	0.059	0.065
Proline (Pro)	3.30 ^b	7.88 ^a	7.58 ^a	0.082	<0.001
TAA	75.5 ^b	80.6 ^a	77.5 ^{ab}	1.31	0.028
EAA	33.7	35.4	33.9	0.62	0.106
NEAA	38.8 ^b	42.1 ^a	40.7 ^{ab}	0.66	0.005
LAA	9.63	10.25	9.83	0.178	0.057
BCAA	14.4	15.0	14.3	0.26	0.134
DAA	35.4 ^b	37.9 ^a	36.5 ^{ab}	0.61	0.021
FAA	23.7 ^b	25.2 ^a	23.8 ^{ab}	0.45	0.049
EAA:NEAA	0.868 ^a	0.841 ^b	0.833 ^b	0.0054	<0.001

a, b: means within a row with different superscripts differ ($p < 0.05$). TAA: total amino acids; EAA: essential amino acids (sum of Lys, Try, Phe, Met, Thr, Ile, Leu, and Val); NEAA: non-essential amino acid (sum of Asp, Ser, Glu, Gly, Ala, Tyr, and Pro); LAA: limited amino acids (sum of Lys and Met); BCAA: branched-chain amino acids (sum of Val, Ile, and Leu); FAA: functional amino acids (sum of Glu, Leu, and Arg); DAA: flavor amino acids (sum of Asp, Glu, Gly, Ala, Arg, and Tyr); EAA:NEAA: ratio of essential amino acids to non-essential amino acids. MS-50: 50% of soybean meal protein replaced with mulberry silage; MS-100: 100% of soybean meal protein replaced with mulberry silage; SEM: standard error of mean.

4. Discussion

Replacing soybean meal protein with mulberry silage (MS-50 and MS-100) increased DMI (by 12.6 and 17.6%, respectively), OMI (by 15 and 21.5%, respectively), CPI (by 17.8

and 18.6%, respectively), NDFI (by 8.6 and 8.9%, respectively), and EEI (by 95.6 and 88.7%, respectively). These increases can be largely attributed to the higher palatability of mulberry silage compared to soybean meal, which promotes greater consumption in goats [29,30]. The enhanced palatability is primarily due to the high nutrient content and excellent digestibility of mulberry silage [31–33]. Additionally, the ensiling process enhances the flavor and reduces potential anti-nutritional factors, thereby increasing the accessibility and digestibility of the nutrients [34]. These findings are consistent with previous experiments in which goats fed diets containing mulberry leaves also demonstrated increased feed intakes [16,21,35].

A noticeable improvement in final body weight, total weight gain, and feed conversion ratio was recorded for the total replacement of soybean meal with mulberry silage (MS-100), which reflects the cumulative effect of improved feed intake, nutrient utilization, and absorption in this treatment group. The richness of mulberry leaves or mulberry silage in essential nutrients, including proteins, vitamins, and minerals supports better growth and development in animals, leading to increased muscle mass development [28,34]. Additionally, the fermentation process involved in making mulberry silage breaks down complex carbohydrates and fibers, making the feed more digestible and its nutrients more easily absorbed by the animals [28]. Furthermore, mulberry silage is highly palatable, which encourages goats to consume more feed and contributes to better growth performance [36]. The bioactive compounds in mulberry leaves, such as flavonoids and phenolic acids, possess antioxidant properties that help reduce oxidative stress and improve overall health [37]. Previous studies have also observed that goats fed mulberry silage exhibit improved feed intake, total weight gain, average daily gain, and reduced feed-to-gain ratio (an indicator of improved feed conversion efficiency) [37].

Although replacing soybean meal with mulberry silage (MS-50 and MS-100) resulted in a noticeable reduction in OM (3.5 and 4.5%) and ADF (5.5% and 9.5%) digestibility, the overall protein/dry matter digestibility remained stable. This decrease is contrary to the results of previous studies [38,39]. This might be because mold, which was observed in some silage bags, influenced the digestibility of OM and ADF.

Furthermore, the lack of significant differences in the digestibility of DM, CP, EE, and NDF between the mulberry silage groups and the control group, despite the dietary change, might be explained by the concurrent increase in DMI observed in the MS-50 and MS-100 groups. Although a general increase in DMI can sometimes lead to a decrease in apparent nutrient digestibility due to faster passage rate, the similar digestibility coefficients in this case indicate that the goats effectively utilized the nutrients from the mulberry silage-based diets. This compensatory effect suggests that the higher intake provided more total digestible nutrients.

Liu et al. [40] demonstrated that supplementing lamb diets with mulberry leaves improved the rumen microbial environment and increased feed intake. Consistent with this, the present study observed that dietary inclusion of mulberry silage significantly increased the DMI and ADG in black goats, aligning with the findings reported by Jia et al. [34]. This improvement in growth performance can be attributed to the high palatability of mulberry silage, which promotes higher voluntary feed consumption, thereby supporting enhanced nutrient utilization and weight gain [31]. The data of the feeding trials and the nutrient digestion tests fully confirm the nutritional value of silage made from whole mulberry plants and suggest that, when aiming for higher levels of animal production performance, a solution can be adopted where forage mulberry completely replaces soybean meal.

All measured blood metabolite parameters in the experimental goats fell within the established reference ranges for healthy animals [30]. Of particular interest were the changes in plasma total protein and antioxidant capacity. Plasma proteins, particularly

albumin, play a significant role in the body's antioxidant defense system. For instance, albumin not only serves as a carrier protein but also exhibits antioxidant properties by scavenging free radicals and reactive oxygen species. Elevated levels of plasma total protein, including albumin, can thereby enhance the body's overall antioxidant capacity, providing better protection against oxidative stress and cellular damage [41].

In the present study, the increase in total protein and albumin levels in goats fed the MS-100 diet implies enhanced protein synthesis and an improvement in overall antioxidant capacity compared to the control group. This elevation in plasma protein and albumin concentrations might be due to the higher intake of protein and feed in mulberry silage, which provides abundant substrates for the synthesis of plasma proteins, including albumin [32]. Additionally, the ensiling process may improve nutrient absorption by reducing anti-nutritional factors, thereby enhancing the digestibility and absorption of nutrient, which could also contribute to increased protein synthesis [32]. The concentration of plasma total protein and albumin reflects the state of protein metabolism in the body. Thus, the observed increase suggests a strengthening of protein anabolism in goats fed this mulberry silage-based diet.

Replacing soybean meal with mulberry silage improved total antioxidant capacity, attributed to the presence of bioactive compounds in mulberry leaves, such as flavonoids, phenolic acids, and alkaloids. These compounds possess strong antioxidant properties that help neutralize free radicals and reduce oxidative stress [42]. Additionally, the fermentation process involved in making mulberry silage can enhance the antioxidant activity of the feed, and leading to improved total antioxidant capacity in the animal [42].

Malondialdehyde is a marker of oxidative stress and cell membrane damage, higher MDA levels indicate that animals may experience greater oxidative damage. In previous studies, plasma MDA levels in sheep were higher than those in the present study [41,42]. Ding et al. [43] reported that MDA levels in plasma ranged from 28.64 to 51.38 nmol/mL in Hu sheep during early and late pregnancy, lactation, and non-pregnant periods when supplemented with N-Carbamylglutamic at doses from 0 to 2.0 g/d. Gao et al. [44] noted that MDA levels decreased from 21.09 to 10.33 $\mu\text{mol/L}$ when capsaicin was fed to Hu sheep. In a study of normal human serum in Guiyang, MDA concentrations were 4.21 ± 0.57 and 3.99 ± 0.47 nmol/mL in males and females, respectively [45].

Although MDA levels were higher in the MS-100 group compared to the control and MS-50 groups, they remained within the normal range. This suggests that goats fed MS-100 diets did not necessarily experience oxidative damage. We observed some mold spots in certain mulberry silage feed bags, which may explain why increased mulberry silage inclusion led to elevated plasma MDA levels in goats. This discovery further highlights the necessity of conducting a thorough assessment of the quality of whole-plant mulberry silage, especially when used at high doses, to determine the risks posed to animal health.

Previous research indicates that replacing soybean meal with mulberry silage maintains a balanced protein content in the diet, which is crucial for supporting muscle and organ development [34]. Adequate protein intake provides the essential substrates required for the growth of lean muscle mass and the maintenance of healthy organs [34]. Interestingly, in the present study, it was the MS-50 group, rather than the MS-100 group, that exhibited higher contents of TAA, NEAA, DAA, and FAA compared to the soybean meal group. These findings are consistent with Long et al. [31], who reported elevated levels of TAA, NEAA, and DAA in goats fed a diet containing fresh mulberry leaves (40% DM diet) compared to a control group. Furthermore, Wang et al. [42], observed that as the inclusion rate of mulberry silage increased from 5% to 15% in the diet of Hu lambs, the content of EAA decreased, while certain flavor-enhancing amino acids (such as Asp, Gly, and Tyr) increased. The deposition of amino acids in muscles is closely related to the content of

absorbable amino acids in the intestines. The MS-50 group exhibited higher levels of the aforementioned functional and flavor-related amino acids compared to the MS-100 and control groups, which might be attributed to its ability to provide more of these amino acids in the digestive tract. When the amount of mulberry silage is further increased, the content of these amino acids in the intestines may decrease. However, since this study did not analyze the content of these amino acids in the feed and digestive tract, further research is needed to confirm this hypothesis. Collectively, these alterations in the muscle amino acid profile suggest that incorporating mulberry silage into the diet has the potential to improve meat quality and flavor. However, as comprehensive studies on the impact of whole-plant mulberry silage specifically on the meat quality of goats are still limited, it remains to be further explored how much whole-plant mulberry silage should be fed to achieve the best improvement in meat quality.

5. Conclusions

Using mulberry silage as an alternative protein source for Xiangdong black goat kids' diets had similar effects to using soybean meal. MS-100 enhanced different nutrients' feed intakes, daily gain, body weight and final body weight, carcass weight and feed conversion. At the same time, MS-50 improved the meat quality (such as flavor and functional amino acid component). It could be concluded that mulberry silage is an effective protein alternative feed for soybean meal in growing goat kids' diets. The amount of whole-plant mulberry silage in the diet should be adjusted based on specific production objectives.

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