CHAPTER III

EFFECTS OF DIFFERENT LEVELS OF BLACK SOLDIER FLY LARVAE (H*ermetia illucens* L.) ON GROWTH PERFORMANCE, DIGESTIBILITY, SERUM ANTIOXIDANTS, AND MICROORGANISMS OF GOATS

3.1 Abstract

This experiment aimed to investigate the potential impact of supplementing different levels of BSF on growth performance, serum antioxidants, and rumen microbiota of goats. Twenty-four native Anglo-Thai male goats (18.43 ± 0.76 kg), were distributed across four dietary treatments with 6 repetitions in each group. The control treatment (BSF0) did not include BSF, while the other treatments (BSF5, BSF10, and BSF15) contained 5%, 10%, and 15% of BSF, respectively. BSF supplementation did not affect ($P > 0.05$) growth performance. With increasing supplementation levels, the digestibility of dry matter (DM) decreased linearly and quadratically ($P < 0.05$), while organic matter (OM) decreased linearly, quadratically, and cubically ($P < 0.05$). Crude protein (CP) decreased linearly, and neutral detergent fiber (NDF) and acid detergent fiber (ADF) decreased linearly and quadratically ($P < 0.05$). Serum MDA levels showed a linear ($P < 0.05$) response at 0 h, while SOD and DPPH exhibited linear responses ($P < 0.05$) at 4 h. BSF supplementation did not affect ($P > 0.05$) rumen pH. In the BSF15 group, NH3-N decreased quadratically and cubically ($P < 0.05$) at 0 h, and linearly ($P < 0.05$) at 2 h and 4 h. Acetic acid decreased linearly ($P < 0.05$) at 2 and 4 h in the BSF15 group, while propionic acid decreased cubically and linearly ($P < 0.05$) at 0 h and 4 h, respectively. However, the proportion of butyric acid significantly increased ($P < 0.05$). Total VFAs were significantly highest ($P < 0.05$) in the BSF5 group, equal in BSF0 and BSF10, and significantly lowest ($P < 0.05$) in the BSF15 group. The supplementation of BSF did not affect Chao, Shannon, and Simpson ($P > 0.05$). The most abundant phylum was *Bacillota*, *Bacteroidota,* and *Candidatus Saccharibacteria*, the most abundant genera were *Xylanibacter*, *Saccharibacteria*, *Butyrivibrio,* and *Ruminococcus*, and with no statistical difference among the four treatments ($P > 0.05$). In summary, supplementing with BSF did

not affect the growth performance and rumen microbiota of goats. It was noteworthy that BSF5 and BSF10 were beneficial, as they increased antioxidant levels and the proportion of short-chain fatty acids. In contrast, BSF15 results in decreased digestibility, antioxidant levels, and VFA parameters. Therefore, we recommend limiting the addition of BSF in goat diets to no more than 10%.

Keywords: Antioxidant; Black soldier fly larvae; Crude protein; Growth performance; Rumen microorganisms

3.2 Introduction

The International Feed Industry Federation (IFIF) report states that by 2050, the world's population will surpass 10 billion (Statistics). At that time, the continuously growing population and the protein consumed by animals will be twice the current levels, with insufficient arable land to meet this demand (Abril et al., 2022). Additionally, protein, being the most expensive and constrained component in feed formulations, faces direct or indirect impacts on the global feed industry due to production factors, human-animal competition, and geopolitical events such as the Russia-Ukraine conflict, U.S.-China trade war, COVID-19, leading to a rise in prices of traditional protein feeds (Goh and Chou, 2022; Zhang and Zhang, 2019). To fulfill the protein requirements of humans and animals, the production systems of the global livestock industry in the future will be compelled to explore new sources of high-quality and sustainable protein feeds as raw materials. Against this backdrop, humans were continually experimenting with animal protein feeds in livestock, and black soldier fly larvae (*Hermetia illucens* L. Diptera: Stratiomyidae), mealworm larvae (*Tenebrio molitor* L.), and crickets (*Orthoptera: Gryllidae*) were currently the hotspots of research.

BSF originates from the sparsely wooded grasslands of South America and is widely distributed in temperate, subtropical, and tropical regions. It thrives in a temperature range of 25°C-30°C, lacking cold resistance and unable to survive in Northwestern Europe and climate zones with temperatures below 5°C (Spranghers Noyez et al., 2017). BSF primarily feeds on organic waste, including plant residues, animal dung, waste, food scraps, agricultural by-products, or straws (Nana et al., 2018). Adult BSF only consumes water, does not approach humans, does not bite or sting, and does not transmit any

specific diseases (Park, 2016; Sheppard et al., 2002). BSF exhibits better feed conversion rates compared to crickets and mealworms, with its survival rate and nitrogen-phosphorus composition showing minimal changes with dietary variations (AA Shah et al., 2022). BSF can convert organic waste into amino acids, peptides, proteins, oil, chitin, and vitamins (Ebeneezar et al., 2021). Its protein content ranges from 35% to 65%, comparable to soybean meal (approximately 46%-49%) and slightly lower than fishmeal (approximately 68%). The fat content ranges from 29% to 63%, making it a valuable source of protein and energy for livestock (Lu Taethaisong Meethip Surakhunthod Sinpru Sroichak Archa Thongpea Paengkoum andPurba, 2022). Moreover, BSF contains approximately 4%-7% chitin and is rich in lauric acid (C12:0) (40%-58%) (Ravi et al., 2021). The former plays a crucial role in immunity, antioxidation, and maintaining the rumen environment's stability, while the latter contributes significantly to reducing methane emissions from ruminant animals (Ngo and Kim, 2014a; Patra, 2013).

Chitin was a homopolymer of *N-acetyl*-d-glucosamine (GlcNAc), (1–4)-linked 2-acetamido-2-deoxy-β-d-glucan, known for its resistance to easy degradation, digestion, and absorption (Ngo and Kim, 2014a). Chitin and its derivatives possess significant biological properties and exhibit a broad spectrum of potential applications, including enzyme inhibition, immunostimulation, anticoagulation, antibacterial, anti-hyperlipidemic, and wound healing activities (Elieh Ali Komi et al., 2018; Synowiecki and Al-Khateeb, 2003). The mode of action of chitosan in the rumen depends on pH. Chitosan polymers exhibit their main antibacterial effect by influencing cell permeability through polycationic chitosan ($R\text{-}NH_3+$). When the rumen pH falls below 6.3, electronegative charges on the microbial surface come into play, fostering the hydrolysis of peptidoglycan in the microbial wall and leading to cell lysis (AM Shah et al., 2022). Primarily, it reduces the abundance of *Fibrobacteroidetes* and *Firmicutes* while increasing the abundance of *Proteobacteria* and *Bacteroidetes* (Uyanga et al., 2023).

Currently, numerous studies focus on BSF in fish, poultry, and pigs. Substituting 30% of fish meal with BSF, as reported by (Hender et al., 2021a), had no impact on growth performance, feed utilization, and total fatty acid composition of the meat; however, it increased the expression of immune-related genes. (Agbohessou et al., 2021) discovered that a complete replacement of fish meal with BSF had no significant effect on the growth parameters and innate immune status of Nile tilapia; however, it improved the systemic

biochemical quality. Research on laying hens fed 5% or 10% whole-fat BSF revealed no significant differences between the treated hens and those fed a diet based on corn kernels, soybean meal, and soybean oil (Kawasaki et al., 2019). Nevertheless, when feeding 15% BSF, the experimental results were controversial. Some studies indicated that supplementing 15% BSF negatively affects the growth performance and digestibility of poultry (Biasato Ferrocino Dabbou et al., 2020; Sihem Dabbou et al., 2018b), However, other studies have found that it can still improve the growth performance of animals (Schiavone et al., 2019; Tahamtani et al., 2021), with similar results observed in pigs (Biasato Ferrocino Colombino et al., 2020; Sogari et al., 2019a). In conclusion, BSF can serve as a substitute for soybean meal as a protein source within 15% of the animal diet. However, currently, there is a lack of relevant studies on the application of BSF in goats. Therefore, the objective of this experiment was to investigate whether supplementation with varying levels of BSF would affect goat growth performance, antioxidant levels, and ruminal fermentation parameters and microorganisms.

3.3 Materials and methods

3.3.1 Animal ethics statement

This study was approved by the Animal Welfare Department of Suranaree University of Technology, number: SUT-IACUC-023/2021.

3.3.2 Experimental design, animal diets, and management

Twenty-four native Anglo-Thai male goats (18.43 ± 0.76 kg, Mean ± SD), were distributed across four dietary treatments, with six replicates per treatment and one goat per replicate. The distribution was done using a completely randomized design (CRD). The control treatment (BSF0) did not include BSF, while the other treatments (BSF5, BSF10, and BSF15) contained 5%, 10%, and 15% of BSF, respectively. Throughout the 14-day adjustment period and the subsequent 75-day experimental period, the goats were fed a diet comprising 3% of their body weight in dry matter (BW DM/day). The diet consisted of silage corn and concentrate feed with 14% crude protein, provided at a ratio of 40:60 during the morning (AM 08:00) and evening (PM 16:00) each day. The goats were individually housed in rearing pens, with each pen equipped with mineral blocks. Clean water was freely provided to all animals. The experimental diet composition and chemical composition utilized in the treatment are shown in Table 3.1.

Table 3.1 Ingredients and chemical composition of experimental diets used in treatments (DM basis, %).

Items	Treatments			
	BSF0	**BSF5**	**BSF10**	**BSF15**
Ingredient				
Corn	20.00	18.00	15.00	12.00
Soybean meal	25.00	21.00	17.00	13.00
Rice bran	27.99	27.45	27.52	27.49
Cassava paul	22.01	24.05	26.48	29.51
BSF	-	5.00	10.00	15.00
Soybean oil	2.00	1.50	1.00	-
Limestone	1.00	1.00	1.00	1.00
NaCl	1.00	1.00	1.00	1.00
Premix[1)]	1.00	1.00	1.00	1.00
Chemical composition, % of DM[2)]				
DM	87.8	88.7	88.8	89.0
Ash	5.0	5.4	5.4	5.5
CP	14.3	14.2	14.1	14.0
EE	4.0	4.5	5.5	5.5
NDF	44.92	41.79	42.54	43.54
ADF	24.40	23.84	23.89	23.26
Fatty acid composition, %				
C6:0	0.06	0.01	-	-
C8:0	0.04	0.02	-	-
C10:0	-	0.22	0.26	0.51
C12:0	0.09	5.87	6.99	13.79
C14:0	0.31	1.22	1.39	2.50

Table 3.1 (Continue).

Items	Treatments			
	BSF0	BSF5	BSF10	BSF15
C14:1	0.04	0.06	0.07	0.09
C16:0	21.12	18.83	19.12	19.19
C16:1	0.18	0.58	0.64	1.20
C18:0	4.12	3.62	3.53	3.22
C18:1 n-9c	37.45	32.46	32.05	29.71
C18:2 n-6c	31.06	32.18	31.24	26.03
C18:3 n-3	2.46	2.83	2.67	2.12
C18:3 n-6	0.07	0.08	0.07	0.06
C20:0	0.80	0.56	0.56	0.43
C20:1 n-9	0.31	0.26	0.26	0.24
C20:2	0.27	0.14	0.13	0.08
C20:3 n-3	0.06	0.06	-	-
C20:4 n-6	-	0.06	0.08	0.17
C20:5 n-3	-	0.05	0.06	0.08
C21:0	0.05	0.03	0.03	0.01
C22:0	0.58	0.39	0.37	0.21
C22:1 n-9	0.02	0.05	0.02	0.03
C24:0	0.91	0.51	0.50	0.35
SFA	28.07	31.27	32.75	40.22
UFA	71.93	68.73	67.25	59.78
n-3 PUFA	2.53	2.89	2.73	2.21
n-6 PUFA	31.13	32.32	31.39	25.25
n-3 PUFA/ n-6 PUFA	0.08	0.09	0.09	0.08

1) 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.

2) Nutritional levels were actual measured values.

ADF = acid detergent fiber, CP = crude protein, DM = dry matter, EE = ether extract, NDF = neutral detergent fiber, PUFA = polyunsaturated fatty acids, UFA = unsaturated fatty acids, SFA = saturated fatty acid. BSF0 = No BSF, BSF5 = 5%BSF, BSF10 = 10%BSF, BSF15 = 15%BSF

"-" not detected.

3.3.3 Growth Performance

During the entire study period, the daily dry matter intake (DMI) was calculated. On the 1st and 75th days of the experiment, the fasting morning weights of each goat were measured. According to (X Tian et al., 2020), growth performance was calculated using the following formulas: Average daily gain (ADG) (g/d) = total weight gain (kg) / 75 / 1000; $\%\mathrm{BW(body\ weight)} = \left(\frac{\mathrm{DMI}}{\mathrm{BW}}\right) * 100.$

3.3.4 Chemical composition and apparent digestibility

Approximately 100 g of basic diet were collected weekly and mixed at the end of the feeding trial. Additionally, 500 g of feed were collected, dried at 65°C in a vacuum oven for 72 h, ground, and sieved through a 1 mm screen for further analysis. During the 75-day experimental period, voluntary feed intake was measured daily. In the last 7 days of the experiment, all fecal samples were collected from each goat, dried at 65°C in an oven for 72 h, ground, and sieved through a 1mm screen. Subsequently, analyses were conducted for DM, ash, EE, and CP (AOAC, 1995), as well as NDF and ADF (Van Soest et al., 1991). The goats were weighed before morning feeding, and the average daily weight gain was calculated for the first and last days of the experiment. Each sample was run in triplicate. Apparent nutrient digestibility is determined using the acid-insoluble ash (AIA) method (Bovera et al., 2012) with the following formula: Apparent nutrient digestibility (%) = 100% – (AIA in diet/AIA in feces × Nutrient in feces/Nutrient in diet) × 100.

3.3.5 Chitin analysis

The chitin content of the BSF meal was analyzed following the method outlined by (Liu et al., 2012) with minor modifications. In brief, an aliquot of the prepupae meal (90-100 mg) was enclosed in an ANKOM filter bag (ANKOM Technology, Macedon, NY, USA) shaped to fit a 15 mL screw cap centrifuge tube. This aliquot underwent demineralization for 30 min in 5 mL of 1 M HCl at 100°C. The demineralization process was followed by five washing steps in ASTM Type I water, ensuring neutrality. Subsequently, a deproteinization step was carried out in 5 mL of 1 M NaOH at 80°C for 24 h. Finally, the sample was washed five times in ASTM Type I water until neutrality was achieved. After drying at 105°C in an air-forced oven for 2 h, the chitin content (CT, g/kg DM) was calculated using the following formula:

$$CT = 1000 \times \frac{Fw - (Bw \times C)}{Sw}$$

Where Fw = weight after demineralization, deproteinization, and drying (g), Bw = weight of the modified ANKOM extraction bag (g), C = dimensionless factor taking into account the MEAN weight loss of extraction bags (0.999, n = 6) treated according to the same procedure used for the samples, and Sw = exact amount of sample processed (g).

3.3.6 Minerals analysis

Mineral content was determined following the method outlined by (Pieterse et al., 2019). In brief, 5 mL of 6 mol L-1 hydrochloric acid was added to 0.5 g of the sample. The mixture was placed in an oven at 50°C for 30 min, removed, and then 35 mL of distilled water was added. The solution was filtered and adjusted to a final volume of 50 mL. Mineral concentrations were determined using an iCAP 6000 series inductively coupled plasma (ICP) spectrophotometer (Thermo Electron Corporation, Strada Rivoltana, 20090 Rodana, Milan, Italy), which was equipped with a vertical quartz torch and a Cetac ASX-520 autosampler. Mineral concentrations were calculated using TEVA analyst software.

3.3.7 Fatty acid (FA) analysis

The analysis of FA was conducted according to the method by (X Tian et al., 2020), FA from both BSF and FFS were extracted using a chloroform-methanol solution. The procedure was as follows: approximately 50 mg of the sample was mixed with 3 mL of chloroform-methanol solution (2:1) and agitated in a tissue lyser at 60 Hz for 15 min. The extract was collected and 0.6 mL of physiological saline was added, then centrifuged at 4000 × g for 10 min to obtain a lipid extract. 1 mL of lipid extract was combined to 0.2 mL of 5.00 mg/mL glycerol undecanoic acid triglyceride (C36H68O6, CAS: 13552-80-2) as an internal standard, and all of the samples were esterified with 0.2 mL methanol. All samples were esterified by 8 mL of 2% sodium hydroxide-methanol solution. Then 1 mL of n-heptane was added and centrifuged at 10,000 × g for 5 min. The supernatant was gathered and 100 mg of powdered anhydrous sodium sulfate was added. The extract was filtered through a 13 mm 0.45 μm nylon syringe filter and analyzed for individual fatty acids by gas chromatography (GC-MS; Thermo Fisher

Scientific). Thermo TG-FAME capillary column (50 m × 0.25 mm × 0.20 μm), 1 μL injection volume, 8:1 split ratio; inlet temperature 250°C, ionization temperature 230°C, transmission line temperature 250°C, quadrupole temperature 150°C. Helium served as the carrier gas with a flow rate of 0.63 mL/min, and the ionization energy was set at 70 eV.

3.3.8 Amino acid analysis

The preprocessing of BSF amino acid analysis followed the method outlined by (Tian Li Luo Wang Xiao et al., 2022). The UPLC conditions were as follows: individual amino acids (AAs) were separated on an ACQUITY UPLC BEH C18 column (2.1 × 100 mm × 1.7 μm, Waters, Milford, USA) with a column temperature of 40°C; the injection volume was 5 μL. The mobile phase consisted of A = 10% methanol (containing 0.1% formic acid) and B = 50% methanol (containing 0.1% formic acid). The gradient elution conditions were as follows: 0-6.5 min, 10-30% B; 6.5-7 min, 30-100% B; 7-8 min, 100% B; 8-8.5 min, 10-100% B; 8.5-12.5 min, 10% B. The flow rate was as follows: 0-8.5 min, 0.3 mL/min; 8.5-12.5 min, 0.3-0.4 mL/min. The mass spectrometry (MS) conditions were as follows: electrospray ionization source, positive ion ionization mode; ion power temperature was 500°C, ion source voltage was 5,500 V; collision gas pressure of 6 psi, curtain gas pressure of 30 psi; nebulization gas pressure and aux gas pressure were both 50 psi; and multiple-reaction monitoring scan mode.

3.3.9 Antioxidant analysis

On the last day of the experiment, blood was collected on an empty stomach in the morning and 2 and 4 h after feeding, allowed to stand for 30 min and then centrifuged at 3500 × g for 15 min at 4°C. The supernatant was then extracted and stored at -80°C for analysis of serum antioxidant parameters. Sigma antioxidant kits were utilized, and analytical procedures were conducted following the provided instructions. The kit numbers for the assays were as follows: Glutathione peroxidase (GSH-Px) - MAK437, Catalase (CAT) - MAK381, Total antioxidant capacity (T-AOC) - MAK187. Lipid peroxidation (MDA) was measured at 532 nm using kit number MAK085. 2,2-Diphenyl-1-trinitrophenylhydrazine (DPPH) was measured using kit number MAK085. Superoxide dismutase (SOD) was assessed using kit number CS0009. The BUN assay was conducted with kit number MAS008.

3.3.10 Rumen fermentation parameters

At the same time as the blood collection, rumen fluid was sampled using a gastric tube connected to a vacuum pump. Rumen fluid samples will be promptly measured for pH using a portable pH meter (Mettler Five Easy Plus Series, Columbus, OH, USA) and then filtered through four layers of cheesecloth. 5 mL of rumen fluid with 1 mL of 15% metaphosphoric acid was mixed, stored at −20°C, and analyzed volatile fatty acids (VFA) following the method described by (Suong et al., 2022). In brief, the concentration of VFA in the filtrate was determined using gas chromatography (Agilent 6890 GC, Agilent Technologies, Santa Clara, CA, USA) with a silica capillary column (30 m × 250 μm × 0.25 μm). The initial temperature was 40°C for 2 min, followed by an increase 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 boiling chamber temperature was 250°C, and the carrier gas, helium (99.99%), had a pressure of 31.391 psi. The carrier gas flow rate was 3.0 mL/min, and the solvent delay time was 3 min. The technique of Nur et al.(Nur Atikah et al., 2018) was used to detect ammonia nitrogen (NH3-N).

3.3.11 DNA extraction and PCR amplification

The instructions of the kit were followed, MagPure Soil DNA LQ Kit (Magan) was used to extract genomic DNA from the samples. The DNA concentration and purity were evaluated using NanoDrop 2000 (Thermo Fisher Scientific, USA) and agarose gel electrophoresis, and the extracted DNA was stored at -20°C. The extracted genomic DNA was then used as a template for bacterial 16S rRNA gene PCR amplification. Universal primers 343F (5'-TACGRAGGCAGCAG-3') and 798R (5'-AGGGTATCTAATCCT-3') were used to target the V3-V4 variable region of the bacterial 16S rRNA gene (Nossa et al., 2010), for diversity analysis. PCR products were analyzed by agarose gel electrophoresis, sequenced on the Illumina NovaSeq 6000 platform, generating paired-end reads of 250 bp. Library construction, sequencing, and data analysis were performed by Shenzhen Huada Gene Co., Ltd. After data collection, Cutadapt software was used to trim primer sequences from the raw data sequences. The default parameters of QIIME 2 (2020.11) (Bolyen et al., 2019) were used, and DADA2 was employed to perform quality filtering, denoising, merging, and removal of chimeric sequences on qualified paired-end raw data, resulting in representative data sequences and an ASV abundance table. After representative sequences were selected for each ASV using the QIIME 2 software

package, all representative sequences were aligned and annotated against the Silva database (version 138). Alpha and Beta diversity analyses were performed using the QIIME 2 software. Alpha diversity of samples was assessed using metrics such as the Chao1 index and Shannon index. Unweighted UniFrac principal coordinates analysis (PCoA) was conducted using an unweighted UniFrac distance matrix computed by R to assess the beta diversity of samples. Differential analysis was performed using ANOVA statistical methods based on the R package.

3.3.12 Statistical analysis

Data were subjected to one-way ANOVA analysis using SPSS statistical software (Version 27.0 for Windows; SPSS, Chicago, IL, USA). Statistically significant differences were determined using Tukey's multiple-range tests. The data were presented as the mean and standard error of the mean (SEM). The significance level was set at $P < 0.05$.

3.4 Results

3.4.1 The proximate composition of BSF

The proximate composition of BSF is shown in Table 3.2. The composition included a dry matter (DM) content of 97.351%, crude protein (CP) content of 40.81%, and ether extract (EE) content of 32.90%. The minerals with the highest content were ranked in the following order: Fe (150.00 mg/kg), Mg (1.80 g/kg), and Ca (26.00 g/kg). The chitin content was 77.83 g/kg.

3.4.2 Amino acid content of BSF

The amino acid contents are presented in Table 3.3. BSF exhibited a high content of indispensable amino acids. Phenylalanine constituted 3%, followed by leucine at 1.81%, lysine at 1.42%, and arginine at 1.37%. Both threonine and Valine exceeded 1% in content.

Table 3.2 The proximate composition of BSF.

Items	Contents, (DM basis, %)
DM	97.35
CP	40.81
EE	32.90
Ash	8.29
Ca	26.00 g/kg
Mg	1.80 g/kg
Fe	150.00 mg/kg
P	5.70 mg/kg
u	5.80 mg/kg
Se	0.26 mg/kg
Chitin	77.83 g/kg

CF = crude fiber, CP = crude protein, DM = dry matter, EE = ether extract.

Table 3.3 Amino acid content of BSF.

Items	Contents, %
Indispensable amino acids	
Arginine	1.37
Histidine	0.22
Isoleucine	0.71
Leucine	1.81
Lysine	1.42
Methionine	0.10
Phenylalanine	3.00
Threonine	1.00
Valine	1.10
Dispensable amino acids	
Alanine	2.01
Aspartic acid	2.67
Glycine	1.53
Glutamic acid	3.91
Proline	1.52
Serine	1.33
Tyrosine	1.42

3.4.3 Fatty acids content of BSF

The contents of fatty acid is shown in Table 3.4. The saturated fatty acid with the highest content was C12:0 (20.02%), followed by C16:0 (18.25%), C14:0 (4.13%), and C18:0 (3.38%). The contents of C18:1, C18:2, and C18:3 were 26.36%, 20.76%, and 3.05%, respectively. The contents of SFA and UFA were 46.62% and 53.38%, respectively. Additionally, the contents of n-3PUFA, and n-6PUFA were 3.25% and 21.31%, respectively, with a n-3/n-6 ratio of 0.15.

Table 3.4 Fatty acid content of BSF.

Items	Contents, %
C10:0	0.70
C12:0	20.02
C14:0	4.13
C14:1	0.14
C16:0	18.25
C16:1	2.18
C18:0	3.38
C18:1n-9c	26.36
C18:2n-6c	20.76
C18:3n-3	2.94
C18:3n-6	0.11
C20:0	0.07
C20:1n-9	0.11
C20:2	0.04
C20:4n-6	0.43
C20:5n3	0.30
C21:0	0.06
SFA	46.62
UFA	53.38
n-3 PUFA	3.25
n-6 PUFA	21.31
n-3/ n-6	0.15

PUFA = polyunsaturated fatty acid, UFA = unsaturated fatty acid, SFA = saturated fatty

3.4.4 Effects of BSF on dry matter intake and growth performance and apparent digestibility in goats

The effects of BSF on feed intake, growth performance, and apparent digestibility of goats are shown in Table 3.5. BSF showed no significant effect ($P > 0.05$) on final weight, ADG, DMI, and % BW. However, BSF supplementation exhibited a tendency to increase ($P > 0.05$) both ADG and DMI. Digestion of DM and NDF decreased linearly and quadratically ($P < 0.05$) as BSF was supplemented. Digestion of OM decreased linearly, quadratically, and cubically ($P < 0.05$). Digestion of CP decreased linearly ($P < 0.05$), and ADF decreased quadratically ($P < 0.05$). Supplementation of BSF did not affect digestion of EE ($P > 0.05$).

Table 3.5 Effects of BSF on growth performance and apparent digestibility in goats.

Item	BSF0	BSF5	BSF10	BSF15	SEM	p- Value			
						Treatment	L	Q	C
Growth performance									
Initial weight, kg	18.55	18.64	18.02	18.45	0.16	0.607	0.547	0.621	0.262
Final weight, kg	27.61	28.27	27.39	27.50	0.38	0.858	0.739	0.740	0.493
ADG, g	120.80	128.33	124.93	120.67	4.68	0.934	0.933	0.564	0.825
DMI, g/d	748.95	778.98	775.45	764.24	8.24	0.617	0.585	0.241	0.738
% BW	3.24	3.33	3.42	3.33	0.03	0.249	0.181	0.141	0.450
Apparent digestibility, %									
DM	65.74 a	66.15 a	64.50 b	62.22 c	0.24	< 0.001	< 0.001	< 0.001	0.049
OM	68.06 a	67.71 b	66.19 c	64.69 d	0.23	< 0.001	< 0.001	< 0.001	0.001
EE	92.20	92.67	93.34	92.49	0.24	0.377	0.517	0.183	0.390
CP	73.94 ab	74.98 a	71.61 bc	69.97 c	0.53	< 0.001	< 0.001	0.063	0.055
NDF	56.31 ab	57.16 a	56.10 ab	53.55 b	0.49	0.019	0.011	0.028	0.878
ADF	40.79 b	47.72 a	45.78 a	41.92 b	0.85	< 0.001	0.516	< 0.001	0.104

ADFD = acid detergent fiber digestibility, ADG = average weight gain, BW = body weight, CPD = crude protein digestibility, DMI = dry matter intake. OMD = Organic matter digestibility, DMD = dry matter digestibility, EED = Ether extract digestibility, ADFD = acid detergent fiber digestibility, NDFD = neutral detergent fiber digestibility. BSF0= No BSF, BSF5= 5%BSF, BSF10= 10%BSF,BSF15= 15%BSF, L = linearly, Q = quadratically, C = cubically. The significance level was indicated at $P < 0.05$, with different shoulder letters.

3.4.5 The effect of supplementing BSF on blood urea nitrogen (BUN) and antioxidant capacity

The impact of BSF on blood urea nitrogen (BUN) and antioxidant capacity is presented in Table 3.6. At 0 h, SOD exhibited a cubic response ($P < 0.05$), while MDA showed a linear response ($P < 0.05$), with no impact ($P > 0.05$) on the antioxidant parameters between groups. At 2 h, BSF had no effect ($P > 0.05$) on the antioxidant parameters. At 4 h, both SOD and DPPH exhibited linear responses ($P < 0.05$), with no significant differences ($P > 0.05$) in other parameters.

Table 3.6 The effect of supplementing BSF on BUN and antioxidant capacity.

Item	BSF0	BSF5	BSF10	BSF15	SEM	p- Value			
						Treatment	L	Q	C
0 h									
BUN, mg/ dL	12.14	13.97	15.60	14.40	0.85	0.577	0.290	0.383	0.727
SOD, U/ mL	69.76 b	75.89 a	75.08 a	72.99 ab	0.88	0.048	0.633	0.208	0.014
CAT, U/ mL	17.05	16.83	17.59	17.63	0.31	0.766	0.405	0.843	0.569
GSH-Px, U/ mL	51.56	52.20	52.58	52.20	0.15	0.149	0.090	0.092	0.696
DPPH, %	89.05	90.87	90.27	90.46	0.41	0.483	0.345	0.343	0.399
MDA, nmol/ mL	0.97	0.96	0.88	0.89	0.07	0.123	0.038	0.681	0.232
T-AOC, nmol/ uL	8.52	9.76	10.88	8.70	0.64	0.568	0.774	0.197	0.588
2 h									
BUN, mg/ dL	12.52	14.35	15.95	14.77	0.77	0.521	0.258	0.349	0.715
SOD, U/ mL	65.09	67.93	68.17	69.27	0.90	0.453	0.136	0.640	0.678
CAT, U/ mL	16.17	16.23	14.78	14.14	0.42	0.186	0.048	0.663	0.521
GSH-Px, U/ mL	52.28	51.35	52.32	50.82	0.35	0.380	0.295	0.695	0.184
DPPH, %	85.94	86.16	87.12	86.26	0.86	0.975	0.821	0.776	0.764
MDA, nmol/ mL	1.05	1.23	1.18	1.18	0.08	0.904	0.677	0.619	0.709
T-AOC, nmol/ uL	6.04	5.59	6.69	6.36	0.37	0.768	0.552	0.936	0.398
4 h									
BUN, mg/ dL	13.56	15.03	16.57	15.27	0.82	0.691	0.404	0.428	0.704
SOD, U/ mL	68.80	72.13	74.36	73.53	0.84	0.108	0.029	0.197	0.782
CAT, U/ mL	20.48	21.29	21.75	21.92	0.31	0.386	0.097	0.606	0.980
GSH-Px, U/ mL	52.07	54.23	54.17	53.94	0.39	0.191	0.117	0.130	0.553
DPPH, %	86.47	89.22	88.91	89.38	0.46	0.092	0.039	0.195	0.320
MDA, nmol/ mL	0.92	1.29	1.24	0.97	0.08	0.264	0.896	0.056	0.755
T-AOC, nmol/ uL	8.63	9.38	10.61	9.96	0.34	0.246	0.095	0.306	0.436

BSF0= No BSF, BSF5= 5%BSF, BSF10= 10%BSF,BSF15= 15%BSF.

L = linearly, Q = quadratically, C = cubically. The significance level was indicated at $P < 0.05$, with different shoulder letters.

3.4.6 Effect of BSF supplementation on rumen pH and NH_3-N

The effects of BSF on rumen pH and NH_3-N are presented in Table 3.7. Supplementation of BSF had no significant effect ($P > 0.05$) on rumen pH. With BSF supplementation, NH_3-N decreased quadratically and cubically ($P < 0.05$) at 0 h, and linearly ($P < 0.05$) at 2 h and 4 h.

Table 3.7 Effect of BSF supplementation on rumen pH and NH_3-N.

Item	BSF0	BSF5	BSF10	BSF15	SEM	p- Value			
						Treatment	L	Q	C
pH									
0 h	7.05	7.01	7.15	7.07	0.04	0.793	0.654	0.860	0.378
2 h	6.75	6.60	6.55	6.63	0.06	0.718	0.467	0.351	0.968
4 h	6.95	7.00	6.98	6.99	0.03	0.964	0.742	0.802	0.754
NH_3-N mg/dl									
0 h	9.74^{b}	12.61^{a}	14.64^{a}	9.71^{b}	0.43	< 0.001	0.424	< 0.001	0.021
2 h	17.75^{a}	15.63^{ab}	15.60^{ab}	14.21^{b}	0.39	0.014	0.002	0.612	0.274
4 h	16.08^{a}	13.84^{ab}	12.80^{b}	11.72^{b}	0.45	0.003	< 0.001	0.457	0.720

BSF0= No BSF, BSF5= 5%BSF, BSF10= 10%BSF, BSF15= 15%BSF.

L = linearly, Q = quadratically, C = cubically. The significance level was indicated at $P < 0.05$, with different shoulder letters.

3.4.7 Effect of BSF supplementation on ruminal VFA

The effect of BSF supplementation on ruminal VFA is depicted in Table 3.8. Acetic acid showed a quadratic decreased ($P < 0.05$) at 0 h and 2 h, with no significant difference ($P > 0.05$) at 4 h. Propionic acid showed a cubic decreased ($P < 0.05$) at 0 h, a quadratic increased ($P < 0.05$) at 2 h and a linear decreased ($P < 0.05$) at 4 h. Butyric acid increased linearly and quadratically ($P < 0.05$) at 0 h and 2 h, and quadratically ($P < 0.05$) at 4 h. Total VFA decreased quadratically ($P < 0.05$) at 0 h, linearly, quadratically and cubically ($P < 0.05$) at 2 h, and linearly and quadratically ($P < 0.05$) at 4 h.

Table 3.8 Effect of BSF supplementation on ruminal VFA.

Item	BSF0	BSF5	BSF10	BSF15	SEM	p- Value			
						Treatment	L	Q	C
Acetic acid (% mol)									
0 h	59.00^{ab}	59.68^{a}	61.96^{a}	55.11^{b}	0.67	0.004	0.076	0.003	0.052
2 h	66.10^{ab}	68.27^{a}	67.65^{a}	64.67^{b}	0.37	0.001	0.080	< 0.001	0.879
4 h	66.02	67.65	68.62	67.39	0.45	0.236	0.209	0.120	0.705
Propionic acid (% mol)									
0 h	19.26^{ab}	21.32^{a}	18.94^{b}	20.01^{ab}	0.32	0.026	0.970	0.415	0.007
2 h	21.06	19.69	19.49	20.61	0.28	0.140	0.519	0.029	0.955
4 h	20.10	19.23	17.98	17.60	0.36	0.058	0.006	0.716	0.686
Butyric acid (% mol)									
0 h	21.74^{ab}	19.00^{b}	19.10^{ab}	24.87^{a}	0.58	< 0.001	0.018	< 0.001	0.478
2 h	12.84^{b}	12.04^{b}	12.86^{b}	14.72^{a}	0.24	< 0.001	< 0.001	0.001	0.738
4 h	13.88^{ab}	13.12^{b}	13.40^{ab}	15.01^{a}	0.25	0.033	0.077	0.014	0.882
A: P									
0 h	3.09	2.83	3.23	2.84	0.08	0.050	0.423	0.366	0.009
2 h	3.15	3.49	3.51	3.16	0.62	0.147	0.950	0.019	0.909
4 h	3.31	3.59	3.89	3.87	0.09	0.079	0.016	0.416	0.667
Total VFA (mM)									
0 h	52.35^{ab}	61.29^{a}	61.42^{a}	45.49^{b}	1.95	0.006	0.188	0.001	0.639
2 h	97.72^{b}	108.95^{a}	92.57^{b}	76.35^{c}	2.36	< 0.001	< 0.001	< 0.001	0.031
4 h	86.02^{ab}	94.00^{a}	88.79^{a}	73.75^{b}	2.02	0.002	0.008	0.002	0.826

BSF0= No BSF, BSF5= 5%BSF, BSF10= 10%BSF, BSF15= 15%BSF.

L = linearly, Q = quadratically, C = cubically. The significance level was indicated at $P < 0.05$, with different shoulder letters.

3.4.8 Effects of BSF on rumen microbial community dynamics and species diversity

High-quality filtering and chimeric sequence removal resulted in 961,392 sequences, with an average coverage of 48,070 sequences per sample. Across all samples, a total of 8042 OTUs were calculated (Figure 3.1A). The number of OTUs common to the 4 treatments was 503, and the number of OTUs unique to BSF0, BSF5, BSF10, and BSF15 treatments were 646, 654, 720, and 704, respectively. Principal coordinates analysis of dissimilarity matrices showed that rumen bacterial communities in the four groups clustered together based on their ratio treatment and were separated (Figure 1B). The results of the analysis of similarities support the tendency of differences

in the community structure of the four groups (P = 0.180). From the coverage index, the coverage of each sample was very close to 1.0, reflecting that the sample quality of all samples was sufficient (Table 3.9). There was no statistically significant difference (P > 0.05) in species diversity among the 4 treatments.

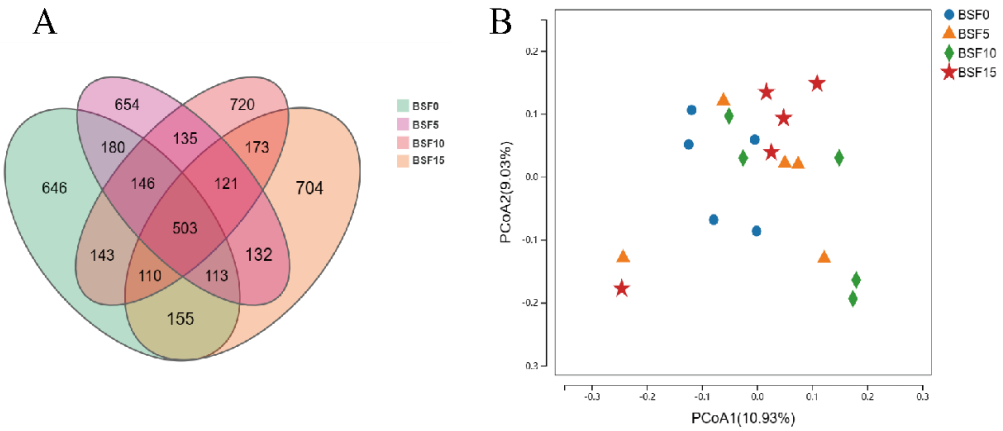

Figure 3.1 Effects of BSF supplementation on rumen microorganisms. **A**, A Venn diagram of operational taxonomic units (OTUs). **B**, points of different colors or shapes represent sample groups. The scales of the horizontal and vertical axes were the projected coordinates of the sample points on the two-dimensional plane respectively. BSF0= No BSF, BSF5= 5%BSF, BSF10= 10%BSF, BSF15= 15%BSF.

Table 3.9 Operational taxonomic unit count and diversity were estimated from sequencing analysis based on the 16S rRNA gene libraries.

Item	BSF0	BSF5	BSF10	BSF15	SEM	p- Value			
						Treatment	L	Q	C
Chao	648.10	621.41	626.00	619.00	16.12	0.930	0.601	0.780	0.786
Shannon	4.91	4.96	4.91	4.72	0.12	0.912	0.593	0.642	0.960
Simpson	0.04	0.03	0.03	0.06	0.01	0.554	0.435	0.236	0.935
Ace	648.25	621.50	626.09	619.06	16.11	0.929	0.600	0.780	0.785
Coverage	1.00	1.00	1.00	1.00	0.00	0.230	0.075	0.393	0.543

BSF0= No BSF, BSF5= 5%BSF, BSF10= 10%BSF, BSF15= 15%BSF.

L = linearly, Q = quadratically, C = cubically. The significance level was indicated at P < 0.05, with different shoulder letters.

3.4.9 Comparison of bacterial community composition among treatments

The bacterial abundance is depicted in Figure 3.2A and B. At the phylum level, the most abundant species were *Bacillota* (BSF0: 56.51%, BSF5: 48.92%, BSF10: 53.03%, BSF15: 61.73%), *Bacteroidota* (BSF0: 28.48%, BSF5: 29.83%, BSF10: 30.47%, BSF15: 15.60%), and *Candidatus Saccharibacteria* (BSF0: 4.48%, BSF5: 9.16%, BSF10: 3.01%, BSF15: 3.86%), with *Verrucomicrobiota* and *Pseudomonadota* both exceeding 1%. The most abundant genera were *Xylanibacter* (BSF0: 7.24%, BSF5: 7.73%, BSF10: 6.09%, BSF15: 2.73%), *Saccharibacteria* (BSF0: 4.48%, BSF5: 9.16%, BSF10: 3.01%, BSF15: 3.81%), *Butyrivibrio* (BSF0: 2.27%, BSF5: 2.00%, BSF10: 2.47%, BSF15: 1.27%), and *Ruminococcus* (BSF0: 1.61%, BSF5: 1.04%, BSF10: 1.30%, BSF15: 0.73%). At the phylum and genus levels (Table 3.10 and Table 3.11), among the top 10 species, only the genus *Cyanobacteriota* showed significant differences ($P < 0.01$), while other bacterial groups exhibited no statistically significant differences. To provide clarity and visualization, a heatmap depicted the top 13 phyla and 52 genera (Figures 3.2C and D). At the genus and phylum levels, there was no correlation between increased BSF and the relatedness of the bacterial flora. The LEfSe algorithm was used to identify ASV biomarkers (Figure 3.3). Compared to the BSF0 group, the relative abundance of *Mycobacteriales, Staphylococcus, Staphylococcaceae, Caryophanales*, *Corynebacterium,* and *Atopobiaceae* increased in the BSF15 group. The relative abundance of *Massiliimalia* increased in the BSF10 group, and the relative abundance of *Negativicutes* increased in the BSF5 group.

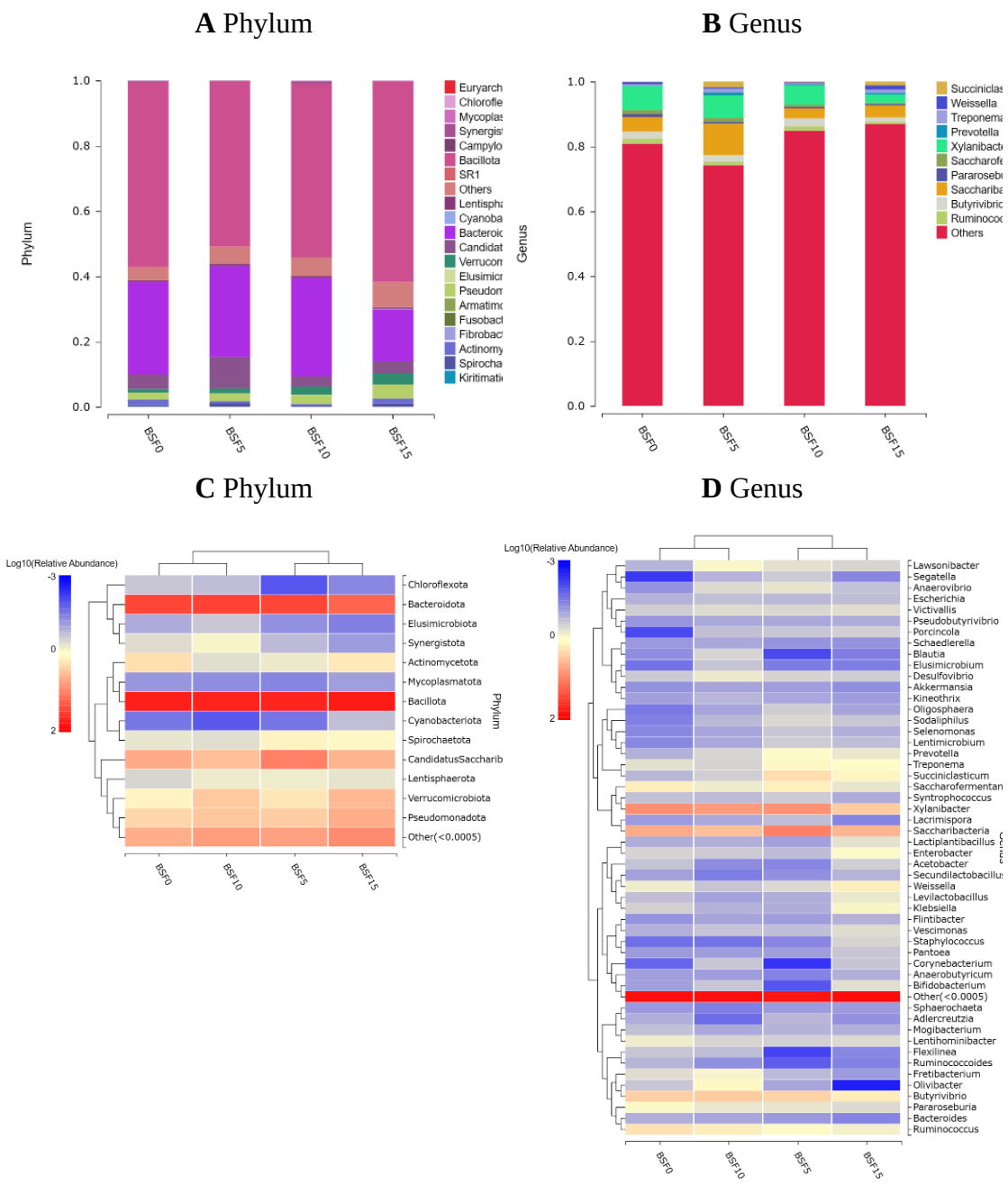

Figure 3.2 Effects of BSF supplementation on rumen microorganisms. **A** and **B**, rumen microbial composition at phylum and genus levels, species that were not annotated at this taxonomic level and whose abundance was less than 0.5% in the sample were merged into others. **C** and **D** The heatmap showing the composition of the phylum and genus level microbiota combined with the results from the cluster analysis. BSF0= No BSF, BSF5= 5%BSF, BSF10= 10%BSF, BSF15= 15%BSF.

Table 3.10 Effects of BSF supplementation on rumen microorganisms (phylum-level, %).

Item	BSF0	BSF5	BSF10	BSF15	SEM	p- Value			
						Treatment	L	Q	C
Mycoplasmatota	0.06	0.04	0.05	0.08	0.01	0.841	0.686	0.430	0.944
Cyanobacteriota	0.03[b]	0.02[b]	0.01[b]	0.18[a]	0.01	0.001	0.002	0.006	0.139
Elusimicrobiota	0.11	0.05	0.24	0.03	0.01	0.129	0.903	0.239	0.039
Synergistota	0.41	0.17	0.83	0.07	0.04	0.351	0.800	0.427	0.115
Lentisphaerota	0.34	0.69	0.61	0.52	0.03	0.853	0.720	0.468	0.761
Spirochaetota	0.53	1.21	0.43	1.06	0.05	0.492	0.674	0.953	0.114
Actinomycetota	1.72	0.58	0.43	1.41	0.05	0.067	0.523	0.011	0.930
Verrucomicrobiota	1.16	1.48	2.53	3.37	0.09	0.189	0.037	0.734	0.785
Pseudomonadota	2.02	2.40	2.62	4.32	0.10	0.245	0.070	0.437	0.665
CandidatusSacchar -ibacteria	4.48	9.16	3.01	3.86	0.23	0.122	0.345	0.313	0.045
Bacteroidota	28.48	29.83	30.47	15.60	0.72	0.323	0.195	0.216	0.606
Bacillota	56.51	48.92	53.03	61.73	0.68	0.535	0.489	0.211	0.802
Others	4.13	5.41	5.51	7.64	0.19	0.592	0.205	0.818	0.695

Species that were not annotated at this taxonomic level and whose abundance was less than 0.5% in the sample were merged into Others. BSF0= No BSF, BSF5= 5%BSF, BSF10= 10%BSF, BSF15= 15%BSF.

L = linearly, Q = quadratically, C = cubically. The significance level was indicated at $P < 0.05$, with different shoulder letters.

Table 3.11 Effects of BSF supplementation on rumen microorganisms (genus-level, %).

Item	BSF0	BSF5	BSF10	BSF15	SEM	p- Value			
						Treatment	L	Q	C
Prevotella	0.12	1.00	0.34	0.56	0.20	0.476	0.725	0.418	0.199
Pararoseburia	0.92	0.51	0.55	0.38	0.11	0.325	0.108	0.567	0.490
Weissella	0.65	0.39	0.22	1.32	0.22	0.323	0.356	0.134	0.554
Treponema	0.46	1.11	0.31	0.98	0.20	0.450	0.673	0.986	0.126
Succiniclasticum	0.15	0.02	0.29	1.13	0.28	0.098	0.556	0.408	0.023
Saccharofermentans	1.32	1.29	0.64	0.54	0.27	0.213	0.054	0.907	0.419
Ruminococcus	1.61	1.04	1.30	0.73	0.28	0.745	0.375	0.996	0.531
Butyrivibrio	2.27	2.00	2.47	1.27	0.24	0.311	0.238	0.330	0.259
Saccharibacteria	4.48	9.16	3.01	3.86	1.01	0.122	0.345	0.313	0.045
Xylanibacter	7.24	7.73	6.09	2.37	1.57	0.654	0.282	0.528	0.995
Others	80.79ab	73.92[b]	84.78[a]	86.86[a]	1.62	0.011	0.020	0.095	0.032

Species that were not annotated at this taxonomic level and whose abundance was less than 0.5% in the sample were merged into Others. BSF0= No BSF, BSF5= 5%BSF, BSF10= 10%BSF, BSF15= 15%BSF., L = linearly, Q = quadratically, C = cubically. The significance level was indicated at $P < 0.05$, with different shoulder letters.

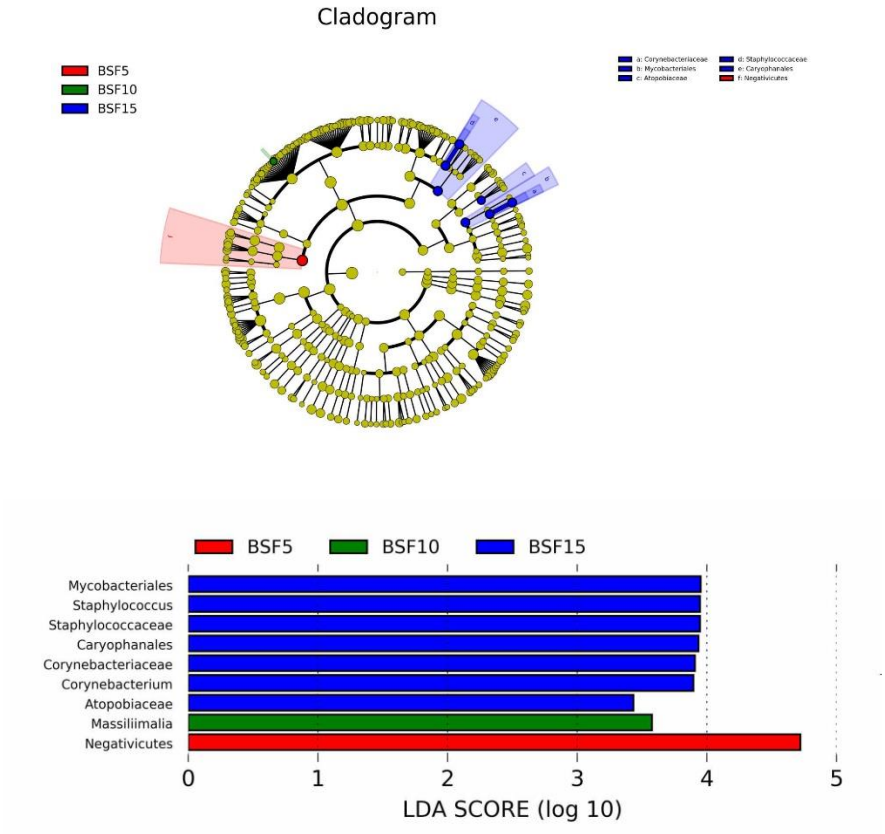

Figure 3.3 LEfSe analysis (BSF0= No BSF, BSF5= 5%BSF, BSF10= 10%BSF, BSF15= 15%BSF).

3.5 Discussion

3.5.1 Effects of BSF on dry matter intake and growth performance and apparent digestibility in goats

The intake of animals is influenced by the composition, availability, palatability, and feedback mechanisms of the diet (Nur Atikah et al., 2018). In this study, the inclusion of BSF did not alter the DMI and growth performance of goats. This is consistent with the findings of (Bellezza Oddon et al., 2021), who supplementing 5% BSF or *Tenebrio molitor* did not significantly affect the growth performance parameters of broilers overall. However, (de Souza Vilela Andronicos et al., 2021) obtained different results when supplementing 5%, 10%, 15%, and 20% BSF in broilers, body weight increased linearly with the increase in BSF supplementation. The research from (Ipema et al., 2021) on piglets also concluded that supplying BSF did not affect piglet growth, feed efficiency, energy efficiency, or fecal consistency. (Biasato Renna et al., 2019) reported that overall, BSF did not affect the growth performance of pigs. The results above indicate that BSF at least maintained the growth performance of animals. Apparent digestibility reflects the degree of absorption and utilization of nutrients in the diet. In this study, BSF5 was beneficial in increasing the digestibility of nutrients, but as the supplementation of BSF increased, the digestibility decreased. An *in vitro* study indicated that BSF reduced the digestibility of DM and OM. However, another *in vitro* fermentation study, reported by (Kahraman et al., 2023c), found that supplementing with 20% and 40% BSF increased the digestibility rates of DM and NDF at 24 h and 48 h. This differs from the findings of our experiment. Furthermore, supplementing 20% BSF in the diet of beagle dogs reduced the digestibility of CP and OM, while 8% BF did not affect the apparent nutrient digestibility (S. Jian et al., 2022). In contrast, some studies have also found that feeding 5% or 20% BSF decreased the digestibility of CP in both beagle dogs and cats (Do et al., 2022; Kröger et al., 2020). Interestingly, in studies conducted on pigs, BSF showed no effect on apparent digestibility rates (Biasato Renna et al., 2019). In poultry, apart from a reduction in the digestibility rate of EE, there were no significant differences in the apparent digestibility rates of DM, CP, starch, and energy (Cullere et al., 2016). The studies above indicate that the digestion of BSF in animals varies significantly, which may be related to factors such as the growth cycle of BSF, temperature, substrate, and environmental conditions (Seyedalmoosavi et al., 2022). In

this study, the reduced digestibility rates observed with BSF10 and BSF15 may be associated with the content of chitin and C12:0. C12:0 inhibits rumen fermentation, leading to a decrease in the digestion of nutrients (Hristov et al., 2009). Chitin is a linear polymer of β-(1-4) N-acetyl-D-glucosamine units with high molecular weight, poor water solubility, strong protein binding activity, minimal chitin-degrading enzyme activity in rumen microorganisms, and exhibits anti-nutritional effects, negatively impacting protein digestibility rates (Longvah et al., 2011).

3.5.2 The effect of supplementing BSF on blood urea nitrogen (BUN) and antioxidant capacity

Oxidative stress can result in the activation of enzymes within the organism and oxidative damage to cellular systems. Free radicals attack large molecules such as DNA, proteins, and lipids, leading to disruptions in bodily functions (Ngo and Kim, 2014b). Due to the presence of chitin and C12:0, BSF exhibits antioxidant properties (Quintieri et al., 2023). In this study, there were no differences observed in the analyzed antioxidant parameters among treatment levels. However, SOD at 0 h exhibited a cubic response, while MDA showed a linear response. By the 4-h mark, both SOD and DPPH showed a linear increase. This indicates that BSF does not impair the antioxidant system of goats. (Caimi et al., 2020) reported that feeding 25% and 50% BSF increased the activities of SOD, CAT, and GSH-Px in the fish liver while reduced the MDA levels. There was a study by (S. Dabbou et al., 2018; Gariglio et al., 2019), who indicated that 5%, 10%, and 15% BSF supplementation enhanced the GSH-Px and T-AOC levels in broiler chickens while also lowering the levels of MDA. In summary, BSF can exert antioxidant effects in goats similar to its effects in other animals.

3.5.3 Effect of BSF supplementation on rumen pH and NH_3-N

Rumen pH is an important indicator of rumen nutrient metabolism and digestive environment homeostasis, usually varying between 5.0-7.5 (Dijkstra et al., 2020). In this study, the pH values at all periods were within the normal range without significant differences, indicating that BSF supplementation may not affect the rumen environment. NH_3-N was an intermediate product of rumen microorganisms decomposing nitrogenous substances. It comes from the degradation of feed protein and was used to synthesize microbial protein (MCP). Its optimal concentration range NH_3-N was 2.37-27.3 mg/dl, which was the most important nitrogen source for ruminants (Hervás et al., 2022). Our results showed that at 0 h, BSF5 and BSF10 were significantly higher than BSF0 and

BSF15, but at 2 h and 4 h, BSF treatment was significantly lower than BSF0. It is known that soybean meal contains a high level of rapidly degradable protein fractions, leading to an increase in ammonia production in the rumen at 2 h and 4 h of feeding in this study (Maxin et al., 2013). An *in vitro* study showed that the level of NH_3-N decreased with the decrease in soybean meal levels in the substrate (Jeong et al., 2015). In contrast, the reduction in NH_3-N concentration in the BSF15 group at 0 h may be due to the increase in C12:0 and C14:0 in BSF, which inhibits the activity of protein microbes. A previous *in vitro* study found that BSF at 20% and 40% in the TMR diet reduced NH3-N concentration (Kahraman et al., 2023c), and (A. Jayanegara et al., 2017) also observed a decrease in NH3-N by adding 50% BSF. In summary, low levels of BSF favor ammonia nitrogen production, while high levels inhibit rumen ammonia nitrogen concentration.

3.5.4 Effect of BSF supplementation on ruminal VFA

VFA was the major product of ruminal fermentation and were positively correlated with the digestibility of the substrate, accounting for approximately 40% to 70% of digestible energy intake (Cabezas-Garcia et al., 2017). In this study, acetic acid, propionic acid, and total VFA were generally highest in the BSF5 and BSF10 groups, while lowest in the BSF15 group. The A/P ratio did not show significant differences. This may be related to the levels of saturated fatty acid and chitin because a small amount of chitin can change the rumen fermentation pattern and increase propionic acid concentration (de Paiva et al., 2016; Dias et al., 2017; Goiri et al., 2010; Vendramini et al., 2016). As the proportion of BSF increased, the concentration of chitin also increases, but the level of chitinase in the rumen was very low, resulting in a decreased in the concentration of VFA (Tabata et al., 2018). This had been confirmed by Renna et al. (Renna et al., 2022). In addition, the role of fatty acid in reducing VFA had been confirmed (Hristov et al., 2011; Vargas et al., 2020), it could be due to the partial exchange of easily fermentable carbohydrates by lipids in the diet. The decreased in VFA concentration is consistent with the reduced digestibility of OM and NDF. However, an unexpected increase in the proportion of butyrate in the BSF15 group was observed. This explanation can be attributed to the decrease in acetic acid and propionic acid concentrations, as the increase in butyric acid concentration inhibits the production of acetic acid and propionic acid (Górka et al., 2018). In summary, low levels of BSF promote the production of VFA, while high levels of BSF inhibit VFA production.

3.5.5 Effects of BSF on rumen microbial community dynamics and species diversity

Ruminal microbiota plays a key role in intestinal ecology. The dominant microbial community in the rumen of ruminants was not static and changes with variations in feed type (McCann et al., 2016; Yadav and Jha, 2019). In this study, the supplementation of BSF did not significantly differ in rumen bacterial diversity indices. This indicates that adding BSF to the daily diet did not affect the diversity of the goat rumen bacterial community, consistent with research findings on BSF in other animals (Dabbou Lauwaerts Ferrocino Biasato Sirri Zampiga Bergagna Pagliasso Gariglio andColombino, 2021; Shiyan Jian et al., 2022). The predominant bacterial phyla in this experiment were *Pseudomonadota, CandidatusSaccharibacteria, Bacteroidota,* and *Bacillota*. Although their differences were not significant, there was a decreasing trend in *Bacteroidota* associated with protein hydrolysis (Gary D Wu et al., 2011), this also explains the observed decrease in protein apparent digestibility in this study. *Xylanibacter*, *Saccharibacteria*, *Butyrivibrio,* and *Ruminococcus* were dominant bacterial genera among the four groups. Although the differences between groups were not statistically significant, with the supplementation of BSF, there ass a decreasing trend in its abundance, attributable to the antibacterial properties of BSF fatty acid (C12:0) (Spranghers et al., 2018). *Butyrivibrio* was mainly responsible for the production of butyric acid, and *Ruminococcus* was mainly involved in the degradation of cellulose to produce acetic acid and propionic acid (Liang et al., 2021), this also explains the reason for the decrease in VFA concentration observed in this study. The results of other studies were completely different from this experiment. A study found that BSF reduced the abundance of beneficial bacteria and increased the abundance of harmful bacteria (Shiyan Jian et al., 2022). (Biasato Ferrocino Dabbou et al., 2020) conducted a study on the use of BSF in broiler chickens, revealing that 15% BSF reduced α-diversity, increased β-diversity, and different BSF levels had distinct effects on the characteristics of the intestinal microbial community. Meanwhile, (Dabbou et al., 2020) discovered that BSF had a positive impact on the cecal microbiota of rabbits. The reason for this difference may be that the rumen microbiota of ruminant animals is far more complex than that of monogastric animals. In summary, in this experiment, the supplementation of BSF did not result in statistically significant differences in rumen microbial diversity and

community composition. However, there was a trend of decreasing abundance in dominant bacterial groups.

3.6 Conclusions

BSF supplementation did not affect growth performance, feed intake, and rumen pH. With the supplementation of BSF, the digestibility of nutrients decreased. BSF5 and BSF10 increased the production of ruminal acetic acid, propionic acid, and total VFA, while BSF15 increased the content of butyric acid. On the other hand, BSF supplementation increased resistance to oxidation levels. The most abundant genera observed were *Xylanibacter, Saccharibacteria, Butyrivibrio*, and *Ruminococcus*, while the most abundant phyla were *Bacillota, Bacteroidota*, and *Candidatus Saccharibacteria*. However, there was no statistical difference observed among the four treatments. Therefore, we recommend supplementing 5%-10% BSF in goat diets.

3.7 References

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