CHAPTER IV
EFFECTS OF HEAT TREATMENT ON RUMEN DEGRADABILITY AND PROTEIN INTESTINAL DIGESTIBILITY OF BLACK SOLDIER FLY (*Hermetia illucens* L.)

4.1 Abstract

The black soldier fly larvae (BSF) were used as a substitute for soybean meal due to their high crude protein content. This experiment aims to assess the impact of heat treatment on the rumen degradability of BSF and protein digestion in the small intestine using the *in situ* nylon bag method and the three-step *in vitro* method. This study comprises a total of 8 groups (n= 6). The negative control group included only full-fat soybeans (FFS) and BSF (FF group and BS group). The positive control groups consisted of a 95% BSF or 95% FFS mixed with 5% cassava (FFC and BSC groups). The treatment groups involved adding 75% water to the positive control mixture, followed by vigorous kneading to achieve a uniform mixing. The resulting mixture was then pressed to a thickness of approximately 5 cm, placed in an oven, and dried for 120 min at temperatures of 120°C and 140°C (12FFC, 14FFC, 12BSC, and 14BSC groups). Nylon bags were incubated in the rumen for 0, 2, 4, 8, 12, 24, and 48 h, and the small intestine protein digestion rate was analyzed at 16 h. Compared to the BS group, heat-treated BSF showed increased ($P < 0.05$) rumen DM degradability and effective degradability. The 14BSC group increased ($P < 0.05$) rumen CP degradability and degradation kinetic parameters, while the 12BSC group decreased ($P < 0.05$) these parameters. The CP degradability of BSF was significantly higher ($P < 0.05$) than that of full-fat soybeans. The Idg and IDCP of heat-treated full-fat soybeans were significantly higher ($P < 0.05$) than those of other treatment groups. At the same time, heat treatment was beneficial for increasing ($P < 0.05$) the Idg and IDCP of BSF, and the 14BSC treatment effect was significantly better ($P < 0.05$) than that of the 12BSC group. Therefore, based on the results of this experiment, it was recommended to supplement BSF with cassava and subject them to heat treatment at 140°C.

Keywords: black soldier fly larvae, degradation kinetic, full-fat soybeans, nylon bag, three-step *in vitro*

4.2 Introduction

Global warming, the Russia-Ukraine war, the Israeli-Palestinian conflict, and the trade war between China and the United States have led to a surge in global food prices. Additionally, the urgent search for sustainable protein sources for livestock feed to address the challenge of feeding, approximately 9.5 billion people globally by 2050 has led humans to explore the potential of the BSF, as one of the most promising insect species for industrial protein production to replace protein feeds such as soybean and fish meal (Traksele et al., 2021). This is because they are capable of efficiently upgrading organic waste into high-value protein sources, thereby enhancing the productivity and efficiency of the food chain (Campbell et al., 2020b). The evidence of the high content of protein, fat, and minerals in BSF has been previously reported in our earlier studies (Lu Taethaisong Meethip Surakhunthod Sinpru Sroichak Archa Thongpea Paengkoum andPurba, 2022). Nylon bag technique is simple to operate, cost-effective, and yields accurate results (DONG et al., 2017), making it crucial for assessing the degradation rate and quantity of nutrients in the rumen of ruminant animals (DONG et al., 2017; Madsen et al., 1997).

Nitrogen-containing compounds entering the small intestine of ruminants include feed proteins that escape rumen degradation, microbial proteins synthesized in the reticulorumen, and endogenous proteins (Antoniewicz et al., 1992). The rumen-undegradable protein (RUP) entering the small intestine depends on the degradability and passage rate of feed proteins in the reticulorumen (Mupangwa et al., 2003). Because indigestible feed in the rumen is closely related to microbial biomass and endogenous secretions, it is challenging to accurately assess the true small intestinal digestion rate of nutrients in ruminants (Mantovani et al., 2019). One method to overcome these challenges is the use of the nylon bag technique.

Enhancing the passage rate of RUP and reducing the rumen-degradable protein (RDP) is beneficial for improving the production performance of animals (Abdelrahman et al., 2022; Bachmann et al., 2020). Methods to improve RUP mainly include formaldehyde treatment, tannin treatment, heat treatment, and physical coating (Atole

and Bestil, 2014; Belverdy et al., 2021). Due to the toxic side effects of formaldehyde and the anti-nutritional properties of tannins, their use is restricted. The physical coating method is currently less utilized and requires further validation. In comparison, heat treatment is a relatively ideal approach as it is neither toxic nor exhibits anti-nutritional properties. The structures of proteins and starch undergo reorganization in the presence of water. This is due to the interaction between water molecules and protein and starch molecules (Scott and Awika, 2023). Hydrophobic side chains tend to be buried within the protein, forming a hydrophobic core that stabilizes the protein structure. On the other hand, hydrophilic side chains are more exposed to the solvent (Wang et al., 2021). Under high-temperature conditions, hydroxy compounds and amino groups undergo the Maillard reaction, and the resulting complex can reduce degradation in the rumen (Huang et al., 2023).

Many studies have reported that heat treatment of feed could increase RUP. (Linda Karlsson et al., 2012) reported that, after heat treatment of hempseed cake, both RUP and the intestinal digestion rate of RUP showed a linear increase with the temperature rise. The research discovered by (Aufrère et al., 2001), (A Subuh et al., 1996), (Ljøkjel et al., 2003a), and (Huang et al., 2015) indicates that high-temperature treatment could reduce the degradation of crude protein in the rumen without lowering rumen digestibility. Building upon the aforementioned studies, we employed, for the first time, the *in situ* nylon bag method and an improved three-step *in vitro* method to assess the impact of different temperature treatments on the ruminal degradation and intestinal protein digestibility of BSF.

4.3 Materials and methods

4.3.1 Ethics approval and consent to participate

This experiment was approved by the Animal Welfare Committee of Suranaree University of Technology (korat, Thailand) (SUT-IACUC-023/2021).

4.3.2 Experimental design

This experiment will adopt a single-factor completely randomized experimental design. This study comprises 8 treatments, with 6 replications per treatment, each replication consisting of 1 sample. The supplement level of cassava was determined regarding (Manzocchi et al., 2023) the quantitative relationship

between CP rumen degradability and intestinal digestibility after high-pressure treatment of reducing sugars and proteins. The treatment group mixes 95% BSF or 95% full-fat soybeans (FFS) with 5% cassava. Subsequently, 75% water was added, and the mixture was vigorously kneaded to achieve uniform blending. The resulting mixture is then pressed to a thickness of approximately 5 cm, and will be placed in an oven for drying, with a time setting of 120 min and temperatures of 120℃ and 140℃, respectively (Ljøkjel et al., 2003b). There are 8 groups, with FF and BS as the negative control groups, FFC and BSC as the positive control groups, and 12FFC to 14BSC as the treatment groups. Group details are provided in Table 4.1.

Table 4.1 Design factor level.

Items	FF	BS	FFC	BSC	12FFC	14FFC	12BSC	14BSC
Feed	FFS	BSF	FFS95%+ Cassava5%	BSF95%+ Cassava5%	FFS 95%+ Cassava5%	FFS 95%+ Cassava5%	BSF95%+ Cassava5%	BSF95%+ Cassava5%
Time					120 min	120 min	120 min	120 min
Temperature					120℃	140℃	120℃	140℃

BSF= black soldier fly, FFS= Full-fat soybeans.

4.3.3 Animals and diets

Three Nubian rams (29.1 ± 2.16 kg) each were equipped with permanent cannulas in the rumen and proximal duodenum. They were housed individually in pens (3×2.5 m2), and each ram was fed twice a day at 08:00 and 17:00. The diet was formulated to feed 3% of the body weight, and goats had free access to fresh water and mineral salt blocks. The BSF larvae were purchased from a farmer in Henan Province, China. The basic daily feed for BSF consisted of kitchen waste. At 12 days old, the larvae were immersed in water at 90℃ for 90 seconds, following a ratio of BSF to water was 1:8. Subsequently, the larvae were immersed in cold water and drained, then dried in a 65℃ oven for 48 h. The formulated diet, by the nutritional requirements for goats (NRC 2007), is detailed in Table 4.2, specifying its composition and nutritional levels.

Table 4.2 Feed ingredients and nutrient components.

Ingredient	**Contents (dry matter basis, %)**
Corn meal	4.00
Soybean meal	5.00
Soybean hull	5.00
Rice bran	4.80
Molasses	0.60
Calcium Phosphorus	0.20
NaCl	0.20
Premix [1]	0.20
Silage corn	80.00
Chemical composition, % of DM	
DM	34.80
CP	13.48
EE	2.26
Ash	10.82
NDF	59.17
ADF	34.28
Ca	0.53
P	0.44

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.

ADF = acid detergent fiber, CP = crude protein, DM = dry matter, EE = ether extract. NDF = Neutral detergent fiber.

4.3.4 Rumen degradability

The nylon bag method, as determined by (DONG et al., 2017), was employed for the rumen culture to assess the degradation kinetics of DM and CP. In short, approximately 3 g of sample (on an air-dry matter basis) were weighed and placed into nylon bags (4.5 × 10 cm^2; 40 μm pore size) labeled with numerical codes. Considering that goats are small ruminants, to facilitate the normal degradation of

nutrients in the nylon bags in this experiment, the nylon bags were placed into the rumen twice in chronological order. The bags were then incubated in the rumen for 0, 2, 4, 8, 12, 24, and 48 h before being immediately removed. Subsequently, all bags were placed in a bucket filled with cold tap water to halt microbial fermentation. After manual cleaning in cold tap water, the bags were dried in an oven at 65°C for 48 h. Weigh the extracted material using an analytical balance. Subsequently, grind the residual material from the nylon bag, pass it through a 1 mm sieve, and prepare it for measurement. The 0 h (2 bags for each sample) incubation samples were not incubated in the rumen, but they were washed as described above. Residues from the bags were pooled within the incubation time and treatment.

4.3.5 *In vitro* three-step procedure

Drawing inspiration from the approach of (Boucher et al., 2009), improvements were made to the *in vitro* three-step technique. In summary, at the 16th hour, the nylon bag (5 × 12 cm^2; 40 μm pore size) inside the rumen was retrieved, suspended in a 0.1% concentration solution of methylcellulose, and incubated at 37°C for 30 min. Subsequently, it was removed and stored in a -20°C freezer. Before gastric protease treatment, the nylon bag was thawed, and subjected to three 5-minute wash cycles in a washing machine to eliminate bacteria from rumen residues. Subsequently, the bag was dried in a 65°C oven and sieved through a 1mm mesh.

The P-7000 gastric protease and P-7545 pancreatic protease from Sigma Corporation in the United States were employed. One gram of undegraded rumen feed residue is weighed and placed into a nylon bag (5 × 12 cm^2; 40 μm pore size). The sample is then immersed in a solution consisting of 10 mL of pH 1.9 and 0.1 N HCl, with 1 g/L gastric protease (Sigma P-7000, Sigma), followed by vortexing and incubation for 1 h at 39°C in a shaking water bath. After incubation, 0.5 mL of 1 N NaOH solution and 13.5 mL of pancreatic enzyme solution (0.5 M KH2PO4 buffer, standardized at pH 7.75, containing 3 g/L pancreatic enzyme, Sigma P-7545, Sigma) were added. The sample was vortexed and incubated for 24 h at 39°C in a shaking water bath, with vortexing every 4 h. After the 24-h incubation, the bags were rinsed with tap water and then dried at 65°C until a constant weight was achieved (approximately 48 h). Weighing was carried out using an analytical balance, and the data were recorded. The collected

samples in the sample bags were subjected to laboratory analysis to determine the CP content in the samples.

4.3.6 Conventional nutrients

All feed samples were dried at 65℃ in a vacuum oven for 72 h, ground, and sieved through a 1-mm sieve for further analysis. Following the standard procedures outlined by AOAC (2012) 934.01, 927.02, and 976.05, dry matter (DM), crude ash, and Kjeldahl nitrogen (N) analyses were conducted on the original feed samples, rumen undegraded residues, and gastric protease/pancreatic enzyme-digested residues. In which the conversion factor of protein was 6.25 that of N.Neutral detergent fiber (NDF) and acid detergent fiber (ADF) analyses were performed using the method of (Van Soest et al., 1991) without the inclusion of sodium sulfite.

4.3.7 Minerals analysis

Mineral content is determined following the method outlined by (Elsje Pieterse et al., 2019). In brief, 5 mL of 6 mol L-1 hydrochloric acid is added to 0.5 g of the sample. The mixture was placed in an oven at 50℃ 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.

4.3.8 Fatty acid (FA) analysis

FA was extracted using a chloroform-methanol solution following the method outlined by (X Tian et al., 2020). In brief, utilizing n-hexane as the internal standard, a gas chromatograph-mass spectrometer (GC-MS; Thermo Fisher Scientific) was employed under the following conditions: Thermo TG-FAME capillary column (50 m × 0.25 mm × 0.20 μm), 1 μL injection volume, 8:1 split ratio; inlet temperature 250℃, ionization temperature 230℃, transmission line temperature 250℃, quadrupole temperature 150℃. Helium served as the carrier gas with a flow rate of 0.63 mL/min, and the ionization energy was set at 70 eV.

4.3.9 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.

4.3.10 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 in 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).

4.3.11 Kinetic modeling and statistical analysis

The results of DM and CP disappearance from nylon bags were fitted into the following exponential equation of (Ørskov and McDonald, 1979) using non-linear regression (SAS 2003):

$$\mathbf{P} = \boldsymbol{a} + \boldsymbol{b}\,(\mathbf{1} - \boldsymbol{e}^{-\boldsymbol{ct}})$$

Where P is the disappearance of nutrients during time t, a is the soluble nutrient fraction rapidly washed out of the bags and assumed to be completely degradable, b is the proportion of insoluble nutrients potentially degradable by microorganisms, e is the natural logarithm, c is the degradation rate of fraction b per hour (i.e. k) and t is a time of incubation.

The effective degradability (ED) of DM and CP *in situ* of each feed sample for each of the three lambs was calculated as follows:

$$\mathbf{ED} = \boldsymbol{a} + \boldsymbol{b} \times \boldsymbol{c}/(\boldsymbol{k} + \boldsymbol{c})$$

where ED denotes the effective degradation rate (%); k signifies the ruminal outflow velocity of the feed. For reference, (Castro et al., 2007) recommend using k = 0.08%/h.

The effective degradability of CP is also referred to as the rumen-degradable protein (RDP) content of CP in the feed. The calculation is as follows:

$$\mathbf{RDP(g/kg)} = \mathbf{CP}(\%) \times \mathbf{ED/10}$$

$$\mathbf{RUP(g/kg)} = \mathbf{CP}(\%) \times \mathbf{10} - \mathbf{RDP}$$

The content of rumen undegradable protein (RUP), and the digestible crude protein (IDCP) in the small intestine are as follows:

$$\mathbf{Idg}(\%) = \mathbf{100} \times (\mathbf{CP_{16h}} - \mathbf{CP_N})/\mathbf{CP_{16h}}$$

$$\mathbf{IDCP\ (g/kg) = RDP \times 0.85 \times 0.775 + RUP \times Idg}$$

where, CP16 h: protein in rumen-degraded residues of 16 h (g/kg), CPN: protein in residues after small intestinal digestion (g/kg), the rumen-degraded protein coefficient of MCP is 0.85, and the coefficient of small intestinal digestibility of MCP is 0.775 (NRC, 2001).

Data for a, b, and c values and ED and results from the three-step *in vitro* procedure were analyzed using the GLM option of the software program of SAS (1987) according to the following model:

$$\mathbf{Yij = m + dij + eij}$$

where Yij= the criteria under study, m = overall mean, dij = feed source effect, and eij = residual error.

Data were analyzed by ANOVA with SAS 9.1.3. The statistically significant differences were determined by Duncan's multiple-range tests. Data were presented as the MEAN and SEM. The significance level was indicated at $P < 0.05$.

4.4 Results

4.4.1 Proximate composition of FF and BSF

The proximate composition of FF and BSF is shown in Table 4.3. The CP and P content of FF were significantly higher ($P < 0.05$) than that of BSF, while other nutritional components and mineral content were significantly higher ($P < 0.05$) in BSF than in FF.

Table 4.3 The proximate composition of FF and BSF (% of DM).

Items	FF	BSF	SEM	p-Value
DM	90.92[b]	97.36[a]	1.43	< 0.01
CP	37.21[a]	34.13[b]	0.69	< 0.01
EE	21.63[b]	37.92[a]	4.86	< 0.01
Ash	10.82[b]	12.42[a]	2.40	< 0.01
Chitin	-	7.01	-	-
Ca, g/kg	20.97[b]	31.00[a]	2.22	< 0.01
Mg, g/kg	18.62[b]	23.04[a]	1.11	< 0.01
Fe, mg/kg	62.37[b]	158.00[a]	21.36	< 0.01
P, %	0.72[a]	0.65[b]	0.15	< 0.01
Cu, mg/kg	0.21[b]	5.80[a]	1.26	< 0.01
Se, mg/kg	0.01[b]	0.26[a]	0.06	< 0.01

DM = dry matter, CP = crude protein, EE = ether extract, FF = Full-fat soybeans. Values represent the means of twelve replicates (n = 3), mean ± sem.

In the table, when the P-value is less than 0.05, it indicates a significant difference, annotated with different letter labels.

4.4.2 Amino acid content of FF and BSF

The amino acid composition of FF and BSF is shown in Table 4.4. In indispensable amino acids, the levels of arginine, histidine, isoleucine, leucine, lysine, and threonine in FF were significantly higher ($P < 0.05$) than those in BSF, whereas methionine and phenylalanine in BSF were significantly higher ($P < 0.05$) than in FF. There was no statistically significant difference ($P > 0.05$) in valine between FF and BSF. Overall, the content of indispensable amino acids in FF was higher than that in BSF.

Table 4.4 Amino acid content of FF and BSF (g/100g).

Items	FF	BSF	SEM	p-Value
Indispensable amino acids				
Arginine	2.51[a]	1.38[b]	0.25	< 0.01
Histidine	0.37[a]	0.22[b]	0.33	< 0.01
Isoleucine	1.02[a]	0.81[b]	0.49	< 0.01
Leucine	2.68[a]	1.82[b]	0.19	< 0.01
Lysine	2.02[a]	1.33[b]	0.15	< 0.01
Methionine	0.03[b]	0.10[a]	0.15	< 0.01
Phenylalanine	1.88[b]	3.01[a]	0.25	< 0.01
Threonine	1.32[a]	1.02[b]	0.68	< 0.01
Valine	1.09	1.14	0.18	0.20
Dispensable amino acids				
Alanine	1.68[b]	2.01[a]	0.07	< 0.01
Aspartic acid	4.43[a]	2.65[b]	0.40	< 0.01
Glycine	1.61	1.72	0.03	0.12
Glutamic acid	7.24[a]	3.59[b]	0.82	< 0.01
Proline	1.69[a]	1.52[b]	0.04	0.01
Serine	2.05[a]	1.18[b]	0.20	< 0.01
Tyrosine	1.08[b]	1.43[a]	0.08	< 0.01

BSF = black soldier fly, FF = Full-fat soybeans.

In the table, when the P-value is less than 0.05, it indicates a significant difference, annotated with different letter labels.

4.4.3 Fatty acid content FF and BSF

The fatty acid composition of FF and BSF is shown in Table 4.5. Overall, BSF exhibited a richer fatty acid composition. FF lacked fatty acids ranging from C6:0 to C12:0, while BSF was abundant in C12:0, C14:0, and C16:0. The content of C18:1 n-9t in BSF was significantly higher ($P < 0.05$) than in FF, whereas FF had higher ($P < 0.05$) levels of C18:2 n-6c and C18:3 n-3 compared to BSF. However, BSF had higher ($P < 0.05$) levels of C18:3 n-6. Saturated fatty acid was significantly higher in BSF compared to FF,

while unsaturated fatty acid (UFA) was significantly lower ($P < 0.05$). The content of n-3 PUFA and n-6 PUFA was significantly higher ($P < 0.05$) in FF than in BSF. There was no statistically significant difference ($P < 0.05$) in the n-3/n-6 ratio between FF and BSF.

Table 4.5 Fatty acid content of FF and BSF (g/100g).

Items	FF	BSF	SEM	p-Value
C6:0	-	0.11	-	-
C8:0	-	0.03	-	
C10:0	-	0.67	-	-
C12:0	-	18.56	-	-
C14:0	0.08^{b}	3.51^{a}	0.77	< 0.01
C14:1	-	0.08	-	-
C15:0	-	0.14	-	-
C16:0	10.10^{b}	18.57^{a}	1.90	< 0.01
C16:1	0.08^{b}	1.76^{a}	0.37	< 0.01
C17:0	0.08^{b}	0.46^{a}	0.08	< 0.01
C17:1	0.04^{b}	0.16^{a}	0.03	< 0.01
C18:0	3.46	3.64	0.06	0.15
C18:1 n-9t	0.25^{b}	0.39^{a}	0.03	< 0.01
C18:1 n-9c	26.25	26.12	0.06	0.42
C18:2 n-6t	-	0.08		
C18:2 n-6c	47.84^{a}	19.42^{b}	6.35	< 0.01
C20:0	4.45^{a}	2.43^{b}	0.45	< 0.01
C18:3 n-6	0.02^{b}	0.14^{a}	0.03	< 0.01
C20:1 n-9	0.21	0.22	0.004	0.52
C18:3 n-3	6.04^{a}	2.35^{b}	0.82	< 0.01
C21:0	0.52^{a}	0.14^{b}	0.09	< 0.01
C20:2	0.41^{a}	0.04^{b}	0.08	< 0.01
C22:0	0.03^{b}	0.12^{a}	0.02	< 0.01
C20:3 n-6	-	0.02	-	-
C20:4 n-6	-	0.20	-	-
C23:0	-	0.03	-	-

Table 4.5 (Continue).

Items	FF	BSF	SEM	p-Value
C22:2	0.15[a]	0.02[b]	0.03	< 0.01
C24:0	0.03[b]	0.18[a]	0.03	< 0.01
C20:5 n-3	-	0.36	-	-
C24:1 n-9	-	0.04	-	-
SFA	18.76[b]	48.60[a]	6.67	< 0.01
UFA	81.33[a]	51.40[b]	6.69	< 0.01
n-3 PUFA	6.08[a]	2.86[b]	0.75	< 0.01
n-6 PUFA	47.86[a]	19.87[b]	6.26	< 0.01
n-3/ n-6	0.13	0.14	0.02	0.39

BSF = black soldier fly, FF = Full-fat soybeans, PUFA = polyunsaturated fatty acid, UFA = unsaturated fatty acid, SFA = saturated fatty acid.

In the table, when the P-value is less than 0.05, it indicates a significant difference, annotated with different letter labels.

4.4.4 Effect of different treatments on DM degradability

The ruminal degradability of DM for each treatment is presented in Table 4.6. Throughout the experimental period, the ruminal degradation rate of DM in the 14BSC group consistently remained the highest ($P < 0.05$), while that of the BS group consistently remained the lowest ($P < 0.05$), there was no difference ($P > 0.05$) between the BSC group and the 12BSC and 14BSC groups between 2 h and 24 h. Heat treatment did not affect the group containing full-fat soybeans ($P > 0.05$). The BSF without heat treatment (BS and BSC groups) showed a significant reduction ($P < 0.05$) at 48 h.

There were no significant differences ($P > 0.05$) in a* and c* among all treatments; however, the b* in the FF group was significantly higher ($P < 0.05$) than in all treatments containing BSF. The a + b* in the FF group was significantly higher ($P < 0.05$) than in the BS, BSC, and 12BSC groups. There was only the BS group had a significantly lower ($P < 0.05$) effective degradability (ED) compared to all other groups.

Table 4.6 Effect of different treatments on DM degradability, %.

Item	FF	BS	FFC	BSC	12FFC	14FFC	12BSC	14BSC	SEM	p-Value
2	17.50^{ab}	15.15^{b}	17.65^{ab}	19.15^{ab}	20.25^{ab}	19.65^{ab}	21.10^{a}	21.85^{a}	0.63	0.04
4	22.75^{ab}	18.15^{b}	22.30^{ab}	23.20^{ab}	24.95^{a}	24.35^{a}	25.25^{a}	26.35^{a}	0.69	0.04
8	31.70^{a}	26.10^{b}	30.30^{ab}	30.20^{ab}	33.00^{a}	32.40^{a}	32.40^{a}	34.15^{a}	0.68	0.03
12	39.10^{a}	31.75^{b}	36.95^{a}	35.85^{ab}	39.65^{a}	39.05^{a}	38.20^{a}	40.50^{a}	0.75	0.04
24	54.05^{a}	43.30^{b}	50.35^{ab}	47.50^{ab}	53.10^{a}	52.55^{a}	50.15^{ab}	53.45^{a}	0.95	0.02
48	64.60^{a}	53.15^{c}	61.75^{ab}	57.40^{bc}	64.55^{a}	64.05^{a}	60.25^{ab}	64.50^{a}	1.08	0.02
a*	11.75	11.80	12.45	14.65	15.00	14.45	16.55	16.90	0.62	0.25
b*	60.60^{a}	47.35^{bc}	54.20^{abc}	47.10^{c}	54.50^{ab}	54.55^{ab}	48.10^{bc}	52.40^{bc}	1.21	0.02
c*	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.00	1.00
a+ b*	72.35^{a}	59.15^{d}	66.65^{abc}	61.75^{cd}	69.50^{ab}	69.00^{ab}	64.65^{bcd}	69.30ab	1.15	0.02
ED	63.50^{a}	62.40^{b}	63.60^{a}	63.45^{a}	63.75^{a}	63.65^{a}	63.60^{a}	63.65^{a}	0.11	< 0.01

a* is the soluble nutrient fraction rapidly washed out of the bags and assumed to be completely degradable, b* is the proportion of insoluble nutrients potentially degradable by microorganisms, c* is the degradation rate of fraction b* per hour, a+ b* is potential degradability. ED = effective degradability. FF=full-fat soybeans, FFC=full-fat soybeans+cassava, 12FFC=120℃ processed full-fat soybeans+cassava, 14FFC=140℃ processed full-fat soybeans+cassava. BS=black soldier fly, BSC=black soldier fly+cassava, 12BSC=120℃ treatment of black soldier fly+cassava, 14BSC=140℃ treatment of black soldier fly+cassava.

In the table, when the P-value is less than 0.05, it indicates a significant difference, annotated with different letter labels.

4.4.5 Effect of different treatments on CP degradability

The ruminal degradation rates of CP for each treatment are presented in Table 4.7. The ruminal CP degradation rate was highest ($P < 0.05$) in the 14BSC group, followed by BSC, BS, and the 12BSC group ($P < 0.05$). There was no statistical difference ($P > 0.05$) between the 12FFC and 14FFC groups. The ruminal protein degradation rate was lowest ($P < 0.05$) in the FF and FFC groups, with no statistical difference ($P > 0.05$) between these two groups.

For degradation kinetic parameters, the order of values from high to low was as follows: 14BSC, BSC, 12BSC, BS, FFC, 14FFC, 12FFC, and FF ($P < 0.05$). All a* values containing BSF were higher ($P < 0.05$) than those containing full-fat soybeans,

and there was no statistical difference (P > 0.05) among the soybean groups. The order of b* values from high to low was as follows: 14BSC, FF, BS, BSC, 12BSC, 12FFC, 14FFC, and FFC (P < 0.05). The a+ b* was highest (P < 0.05) in 14BSC, and lowest (P < 0.05) in FFC, 12FFC, and 14FFC, there was no difference (P > 0.05) between FF, BS, FFC, and 12BSC. The effective degradability (ED) in 14BSC was significantly higher (P < 0.05) than in all groups, and BS and BSC were significantly higher (P < 0.05) than in FF, FFC, 12FFC, 14FFC, and 12BSC.

Table 4.7 Effect of different treatments on CP degradability, %.

Item	FF	BS	FFC	BSC	12FFC	14FFC	12BSC	14BSC	SEM	p-Value
2	0.30^{e}	12.35^{bc}	1.95^{de}	16.45^{b}	7.05^{cd}	7.15^{cd}	8.20^{c}	27.50^{a}	2.09	< 0.01
4	3.80^{d}	15.80^{bc}	4.45^{d}	19.35^{b}	9.85^{cd}	9.90^{cd}	10.95^{cd}	32.25^{a}	2.21	< 0.01
8	10.05^{de}	21.80^{b}	8.65^{e}	24.35^{b}	14.70^{cd}	14.65^{cd}	15.80^{c}	40.45^{a}	5.65	< 0.01
12	15.25^{cd}	26.70^{b}	12.15^{d}	28.40^{b}	18.65^{c}	18.50^{c}	19.75^{c}	47.25^{a}	2.64	< 0.01
24	25.80^{cd}	36.60^{b}	19.20^{d}	36.75^{b}	26.70^{c}	26.45^{c}	27.85^{c}	60.95^{a}	3.06	< 0.01
48	34.70^{c}	45.05^{b}	25.25^{d}	43.80^{b}	34.00^{c}	33.20^{cd}	34.65^{c}	72.60^{a}	3.44	< 0.01
a*	3.95^{d}	8.55^{cd}	7.15^{cd}	13.25^{b}	3.95^{d}	4.10^{d}	9.15^{c}	22.20^{a}	1.48	< 0.01
b*	42.50^{b}	40.20^{bc}	28.60^{d}	33.60^{bcd}	32.50^{cd}	32.00^{cd}	32.55^{cd}	55.40^{a}	2.14	< 0.01
c*	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.00	1.00
a+ b*	46.45^{b}	48.75^{b}	35.75^{c}	46.85^{b}	36.45^{c}	36.10^{c}	41.70^{bc}	77.60^{a}	3.29	< 0.01
ED	22.70^{c}	26.90^{b}	21.30^{c}	27.60^{b}	23.10^{c}	22.75^{c}	23.40^{c}	43.50^{a}	1.71	< 0.01

a* is the soluble nutrient fraction rapidly washed out of the bags and assumed to be completely degradable, b* is the proportion of insoluble nutrients potentially degradable by microorganisms, c* is the degradation rate of fraction b* per hour, a+ b* is potential degradability. ED = effective degradability. FF=full-fat soybeans, FFC=full-fat soybeans+cassava, 12FFC=120°C processed full-fat soybeans+cassava, 14FFC=140°C processed full-fat soybeans+cassava. BS=black soldier fly, BSC=black soldier fly+cassava, 12BSC=120°C treatment of black soldier fly+cassava, 14BSC=140°C treatment of black soldier fly+cassava.

In the table, when the P-value is less than 0.05, it indicates a significant difference, annotated with different letter labels.

4.4.6 Effects of different temperature treatments on Idg and IDCP of RUP

The effects of different temperature treatments on Idg and IDCP of RUP are presented in Table 4.8. The Idg of full-fat soybeans after heat treatment (in the

12FFC and 14FFC groups) was significantly higher (P < 0.05) than in other groups. There was no significant difference (P > 0.05) in Idg among FF, FFC, and the 14BSC group. The Idg of BSF without heat treatment (in the BS and BSC groups) was the lowest (P < 0.05). The IDCP of full-fat soybeans after heat treatment (in the 12FFC and 14FFC groups) was significantly higher (P < 0.05) than in other groups, followed by the FF (P < 0.05) and FFC groups (P < 0.05). All IDCP values containing BSF were significantly lower (P < 0.05) than those containing full-fat soybeans, in the following order from high to low: 14BSC (P < 0.05), 12BSC (P < 0.05), BSC (P < 0.05), and BS groups (P < 0.05).

Table 4.8 Effects of different temperature treatments on Idg and IDCP of RUP.

Item	FF	BS	FFC	BSC	12FFC	14FFC	12BSC	14BSC	SEM	p-Value
Idg, %	70.29^{b}	47.83^{e}	71.15^{b}	58.14^{d}	78.82^{a}	77.27^{a}	61.76^{c}	67.75^{b}	2.50	< 0.01
IDCP, g/kg	348.87^{b}	187.52^{g}	337.01^{c}	219.67^{f}	383.93^{a}	380.73^{a}	235.19^{e}	258.70^{d}	18.25	< 0.01

Idg = small intestinal digestibility of protein, IDCP = small intestine digests crude protein. FF=full-fat soybeans, FFC=full-fat soybeans+cassava, 12FFC=120℃ processed full-fat soybeans+cassava, 14FFC=140℃ processed full-fat soybeans+cassava. BS=black soldier fly, BSC=black soldier fly+cassava, 12BSC=120℃ treatment of black soldier fly+cassava, 14BSC=140℃ treatment of black soldier fly+cassava.

In the table, when the P-value is less than 0.05, it indicates a significant difference, annotated with different letter labels.

4.5 Discussion

4.5.1 Effect of different treatments on DM degradability

The degradation rate of DM in the rumen of BSF supplemented with cassava and heat-treated was higher than that of untreated BSF, while the DM degradation rate of full-fat soybeans falls between these two, furthermore, there was no difference between heat-treated and untreated full-fat soybeans. There are considerable differences in the research findings regarding the effect of heat treatment on the degradation rate of DM in the rumen. A study reported by (Molosse et al., 2023) demonstrated that different times and temperatures of treatment reduced the degradation rate of DM in the rumen of cottonseed meal. However, previous research

has found that the degradation rate of DM in green tea waste treated at 121°C increased. Additionally, the concentrations of volatile fatty acid (VFA), acetate-to-propionate ratio, ammonia nitrogen (NH3-N) concentration, and cumulative gas production during *in vitro* fermentation significantly increased (Chowdhury et al., 2022). Interestingly, a study reported by (Campbell et al., 2020a) found that there were no changes in the *in vitro* digestibility rates of monogastric and ruminant animals after BSF were treated at 90°C. The factors leading to these results may be related to starch content because the degradation rate of DM in the cassava-containing BSF group in this study did not also significantly differ from that of the heat-treated group. There was only the effective degradability (ED) of the BS group was significantly lower than that of the other groups. This may be related to the presence of chitin, which has been shown to have anti-nutritional properties (Austin et al., 1981), heat treatment and the presence of starch may slightly alter its anti-nutritional properties. Heat treatment did not affect the rapidly washable fraction (a*) but reduced the potentially degradable fraction (b*). This finding contrasts with the results of (Lund et al., 2008) who suggested that heat treatment increased the potentially degradable fraction, allowing rumen microbes to enter and degrade. The decrease in the potentially degradable fraction in this study may be related to the protective layer formed by the Maillard reaction between starch and protein (Starowicz and Zieliński, 2019). In summary, heat treatment of BSF was advantageous for increasing the degradation rate of DM, did not affect the rapidly washable fraction (a*), and reduced the potentially degradable fraction (b*).

4.5.2 Effect of different treatments on CP degradability

Heat treatment can protect feed proteins from microbial degradation in the rumen, thereby increasing the amount of digestible amino acids available in the small intestine. The results of this study revealed that the degradation rate and degradation parameters of proteins in the 14BSC group were the highest, while those of the FF control group, which underwent no treatment, were the lowest. Among the treatments containing BSF, the 12BSC group showed the lowest degradation rate. This indicates that the protective effect of the 12BSC group was slightly better. A study reported by (L. Karlsson et al., 2012) found that the rumen undegradable protein (RUP) of hempseed cake treated at 110°C, 120°C, and 130°C increased linearly with

temperature. (Rigon et al., 2023) also found that the rapidly washable fraction (a*) of CP in heat-treated peanut meal was higher, similar to the 14BSC group, with a decrease in effective degradability (ED) and an increase in RUP, which is similar to the 12BSF group. A result of (Stern et al., 1985), concluded that heat treatment led to a decrease in the apparent degradation rate of proteins. The above research findings are consistent with those of the 12BSC group. However, it is disappointing that the results of the 14BSC group did not meet expectations. At present, we are unable to explain the reason for this outcome. The CP degradation rate of BSF was higher than that of full-fat soybeans, the factors leading to this result may be related to the protein source. Animal-derived proteins have a higher nutritional quality, implying that their digestibility was significantly greater than that of plant-derived proteins (Day et al., 2022). Furthermore, the degradation rate of soybean protein in this study appears to be quite low, possibly due to the high-fat content in both soybeans and BSF. The elevated fat concentration reduces the pH of the rumen, inhibiting the microbial activity associated with the degradation of plant-based protein sources (Dijkstra et al., 2012). In summary, the treatment of BSF at 120℃ increased the RUP, while treatment at 140℃ enhanced the degradation kinetic parameters.

4.5.3 Effects of different temperature treatments on Idg and IDCP of RUP

The small intestinal protein digestibility of full-fat soybeans after heat treatment was significantly higher than that of all other treatments, and the small intestinal protein digestibility of full-fat soybeans was higher than that of treatments containing BSF. The factors that led to these results may be related to anti-nutritional factors. In whole-fat soybeans, the main anti-nutritional factors are pancreatic protease inhibitors and soy agglutinins, which are more easily destroyed at high temperatures (Yang et al., 2014). In contrast, the anti-nutritional factor in BSF is mainly chitin (Ghimire, 2021). Chitin is less damaged under heat treatment compared to anti-nutritional factors in soybeans (Prandi et al., 2021; Ravi et al., 2020). Therefore, the digestibility of soybean proteins in the small intestine was higher. However, heat treatment also benefits the digestion of BSF proteins in the small intestine, as the digestibility of untreated BSF small intestine proteins was the lowest. In summary, the treatment of BSF at 140℃ was conducive to enhancing the digestibility of its proteins in the small intestine.

4.6 Conclusions

Compared to the BS group, heat-treated BSF showed increased rumen DM degradability and effective degradability. The 14BSC group increased rumen CP degradability and degradation kinetic parameters, while the 12BSC group decreased these parameters. The CP degradability of BSF was significantly higher than that of full-fat soybeans. The Idg and IDCP of heat-treated full-fat soybeans were significantly higher than those of other treatment groups. At the same time, heat treatment was beneficial to increasing the Idg and IDCP of BSF, and the 14BSC treatment effect was significantly better than that of the 12BSC group. Therefore, based on the results of this experiment, it is recommended to supplement BSF with cassava and subject them to heat treatment at 140°C.

4.7 References

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