APPLICATION RESEARCH OF BLACK SOLDIER FLIES (*HERMETIA ILLUCENS* L.) AS PROTEIN SOURCE IN GOATS

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การวิจัยการประยุกต์ใช้หนอนแมลงวัน (*Hermetia illucens* L.) เป็นแหล่งโปรตีนในแพะ

นายเชียงหยง หลู

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาปรัชญาดุษฎีบัณฑิต
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เชียงหยง หลู : การวิจัยการประยุกต์ใช้หนอนแมลงวัน (*Hermetia illucens* L.) เป็นแหล่งโปรตีนในแพะ (APPLICATION RESEARCH OF BLACK SOLDIER FLIES (*HERMETIA ILLUCENS* L.) AS PROTEIN SOURCE IN GOATS) อาจารย์ที่ปรึกษา : รองศาสตราจารย์ ดร. ปราโมทย์ แพงคำ, 172 หน้า.

คำสำคัญ: หนอนแมลงวัน/แพะ/การเจริญเติบโต/จุลินทรีย์ในรูเมน/การต้านอนุมูลอิสระ/การแสดงออกของยีน

เป้าหมายของการวิจัยนี้คือการประเมินผลของการเสริมหนอนแมลงวัน ในระดับต่างๆ (Black Soldier Fly: BSF) ต่อประสิทธิภาพในการเจริญเติบโต การต้านอนุมูลอิสระ และจุลินทรีย์ในกระเพาะรูเมนของแพะ รวมถึงผลของการให้อาหารด้วยหนอนแมลงวันที่ได้รับการทำความร้อน ต่อประสิทธิภาพในการเจริญเติบโต จุลินทรีย์ในกระเพาะอาหาร ประสิทธิภาพซาก คุณภาพเนื้อ เสริมภูมิคุ้มกัน การต้านอนุมูลอิสระ และการแสดงออกของยีนที่เกี่ยวข้องในแพะ การวิจัยนี้ประกอบด้วยสามส่วน

ในการทดลองที่ 1 แพะตัวผู้พื้นเมืองแองโกล-ไทยจำนวน 24 ตัว (น้ำหนักตัว 18.43± 0.76 กก. ค่าเฉลี่ย± SD) แบ่งออกเป็น 4 ทรีทเมนต์ แต่ละทรีทเมนต์มี 6 ซ้ำ ได้แก่ กลุ่มควบคุม (BSF0) กลุ่มที่เสริมด้วยหนอนแมลงวันที่ (BSF5, BSF10 และ BSF15) มี 5%, 10% และ 15% ของ BSF ตามลำดับ การเสริมด้วยหนอนแมลงวันไม่ส่งผลต่อการเจริญเติบโต ($P > 0.05$) การเสริมหนอนแมลงวันในระดับที่สูงทำส่งผลให้ลดการย่อยได้ของโภชนะ ($P < 0.05$) ระดับ MDA ในซีรั่มแสดงการตอบสนองเชิงเส้น ($P < 0.05$) ที่ 0 ชั่วโมง ในขณะที่ SOD และ DPPH แสดงการตอบสนองเชิงเส้น ($P < 0.05$) ที่ 4 ชั่วโมง การเสริมด้วย BSF0, BSF5 และ BSF10 มีกรดไขมันระเหยได้รวมสูงที่สุดอย่างมีนัยสำคัญ ($P < 0.05$) และในกลุ่มที่เสริมด้วย BSF15 มีกรดไขมันระเหยได้ทั้งหมดต่ำที่สุดอย่างมีนัยสำคัญทางสถิติ ($P < 0.05$) ไฟลัมที่มีมากที่สุดคือ *Bacillota* และ *Bacteroidota* และจำพวกที่มีมากที่สุดคือ *Xylanibacter*, *Saccharibacteria*, *Butyrivibrio* และ *Ruminococcus*

ในการทดลองที่ 2 กลุ่มควบคุมเชิงลบประกอบด้วยถั่วเหลืองไขมันเต็ม (FFS) และ BSF (กลุ่ม FF และกลุ่ม BS) กลุ่มควบคุมเชิงบวกประกอบด้วย BSF 95% หรือ FFS 95% ผสมกับมันสำปะหลัง 5% (กลุ่ม FFC และ BSC) กลุ่มทดลองที่ได้รับการเติมน้ำ 75% ลงในส่วนผสมควบคุมเชิงบวก ตามด้วยการนวดอย่างแรงเพื่อให้ได้การผสมที่สม่ำเสมอ และการทำให้แห้งเป็นเวลา 120 นาทีที่อุณหภูมิ 120 °C และ 140 °C (กลุ่ม 12FFC, 14FFC, 12BSC และ 14BSC) เมื่อเปรียบเทียบกับกลุ่ม BS BSF ที่ได้รับการบำบัดด้วยความร้อนมีความสามารถในการย่อยสลาย DM ในกระเพาะรูเมนเพิ่มขึ้น ($P < 0.05$) และความสามารถในการย่อยสลายที่มีประสิทธิผล กลุ่ม 14BSC เพิ่มความสามารถในการย่อยโปรตีนในกระเพาะรูเมนและพารามิเตอร์จลนศาสตร์ของการย่อยสลาย ($P < 0.05$) ในขณะที่กลุ่ม

12BSC ลด ($P < 0.05$) พารามิเตอร์เหล่านี้ ความสามารถในการย่อยโปรตีน ของ BSF สูงกว่าถั่วเหลืองไขมันเต็ม ($P < 0.05$) อย่างมีนัยสำคัญ การอบด้วยความร้อนมีประโยชน์ในการเพิ่ม Idg และ IDCP ($P < 0.05$) ของ BSF และผลการรักษา 14BSC ก็ดีกว่ากลุ่ม 12BSC อย่างมีนัยสำคัญ ($P < 0.05$)

ในการทดลองที่ 3 แพะจำนวน 30 ตัว (น้ำหนัก 20.30 ± 1.09 ค่าเฉลี่ย ± SD) ถูกแบ่งออกเป็นสามกลุ่มแบบสุ่ม: กลุ่มควบคุม (FFS) เสริมด้วยถั่วเหลืองที่มีไขมันเต็ม 10%, กลุ่มที่ 1 (BSF) เสริมด้วย BSF ที่ไม่ผ่านการบำบัด 10% และการบำบัด 2 (HTBSF) เสริมด้วย BSF ที่ได้รับความร้อน 10% ผลการวิจัยพบว่า ไม่มีความแตกต่างอย่างมีนัยสำคัญในด้านประสิทธิภาพการเจริญเติบโตระหว่างทั้งสามกลุ่ม ($P > 0.05$) ระดับซีรัมของ IgG, IgM, IL-6, IL-8 และ IL-10 ในกลุ่ม FFS และ HTBSF สูงกว่าระดับในกลุ่ม BSF อย่างมีนัยสำคัญ ($P <0.05$) ระดับซีรั่มของ CAT, GSH-Px และ T-AOC และระดับสารต้านอนุมูลอิสระของกล้ามเนื้อในกลุ่ม FFS สูงกว่าระดับในกลุ่ม BSF และ HTBSF อย่างมีนัยสำคัญ ($P <0.05$) ในระดับไฟลัม ไฟลัมที่โดดเด่น ได้แก่ *Bacteroidetes, Firmicutes*, *Proteobacteria*, *Spirochaetes*, and *Fibrobacteres*. ในระดับสกุล สกุลที่โดดเด่นถูกระบุเป็น *Prevotella*, Rikenellaceae_RC9_gut_group, Bacteroidales_RF16_group และ Prevotellaceae_UCG_001 อัตราการฆ่า น้ำหนักซาก และคุณภาพเนื้อสัตว์โดยรวมลดลงในกลุ่ม BSF และ HTBSF ($P < 0.05$) ระดับการแสดงออก mRNA ของ CAT, SOD, GPX-1, GPX-4, IL-6, IL-8, TNF-α และ IL-1β นั้นสูงขึ้นอย่างมีนัยสำคัญในกลุ่ม HTBSF มากกว่าในกลุ่ม FFS ($P <0.05$) .

จากที่กล่าวมาข้างต้น ภายใต้เงื่อนไขของการศึกษานี้ ระดับการเสริม BSF ในอาหารแพะไม่ควรเกิน 10% และการให้อาหารหลังจากที่ได้ผ่านการอบที่ 140°C จะให้ผลลัพธ์ที่ดีกว่า

มหาวิทยาลัยเทคโนโลยีสุรนารี

สาขาวิชาเทคโนโลยีและนวัตกรรมทางสัตว์ ลายมือชื่อนักศึกษา__________
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SHENGYONG LU : APPLICATION RESEARCH OF BLACK SOLDIER FLIES (*HERMETIA ILLUCENS* L.) AS PROTEIN SOURCE IN GOATS. THESIS ADVISOR: ASSOC. PROF. PRAMOTE PAENGKOUM, PH. D., 172 PP.

Keyword: ANTIOXIDANT/BLACK SOLDIER FLY/GOAT/GROWTH PERFORMANCE/MEAT QUALITY

The purpose of this study was to evaluate the effects of different levels of black soldier fly (BSF) supplementation on the growth performance, antioxidant activity, and rumen microbiota of goats, as well as the effects of heat-treated BSF on the growth performance, rumen microbiota, slaughter performance, meat quality, immunity, and antioxidant activity, and related gene expression in goats. This study consists of three parts.

In Experiment I, twenty-four native Anglo-Thai male goats, (body weight 18.43± 0.76 kg, Mean± SD), were distributed across four dietary treatments with 6 repetitions in each group. The control treatment (BSF0) did not include BSF, while the treatments (BSF5, BSF10, and BSF15) contained 5%, 10%, and 15% of BSF, respectively. BSF supplementation did not affect ($P > 0.05$) growth performance. High levels of BSF reduced the digestibility of nutrients ($P < 0.05$). 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. Total VFAs were significantly higher ($P < 0.05$) in the BSF5 group, equal in BSF0 and BSF10, and significantly lower ($P < 0.05$) in the BSF15 group. The most abundant phylum was *Bacillota* and *Bacteroidota*, and the most abundant genera were *Xylanibacter*, *Saccharibacteria*, *Butyrivibrio,* and *Ruminococcus*.

In Experiment II, the negative control group includes only full-fat soybeans (FFS) and BSF (FF group and BS group). The positive control groups consist of a 95% BSF or 95% FFS mixed with 5% cassava (FFC and BSC groups). The treatment groups involve adding 75% water to the positive control mixture, followed by vigorous kneading to achieve uniform mixing, and drying for 120 minutes at temperatures of 120℃ and 140℃ (12FFC, 14FFC, 12BSC, and 14BSC groups). 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. Heat treatment was beneficial to 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.

In Experiment III, thirty goats (weighing 20.30 ± 1.09, Mean ± SD) were randomly divided into three groups: the control group (FFS) supplemented with 10% full-fat soybean, treatment 1 (BSF) supplemented with 10% untreated BSF, and treatment 2 (HTBSF) supplemented with 10% heat-treated BSF. The results showed that there were no significant differences in growth performance among the three groups (P > 0.05). The serum levels of IgG, IgM, IL-6, IL-8, and IL-10 in the FFS and HTBSF groups were significantly higher than those in the BSF group (P < 0.05). The serum levels of CAT, GSH-Px, and T-AOC and muscle antioxidant levels in the FFS group were significantly higher than those in the BSF and HTBSF groups (P < 0.05). At the phylum level, the dominant phyla were *Bacteroidetes, Firmicutes, Proteobacteria, Spirochaetes,* and *Fibrobacteres*. At the genus level, dominant genera were identified as *Prevotella, Rikenellaceae_RC9_gut_group, F082, Bacteroidales_RF16_group,* and *Prevotellaceae_UCG_001*. The slaughter rate, carcass weight, and overall meat quality decreased in the BSF and HTBSF groups (P < 0.05). The mRNA expression levels of CAT, SOD, GPX-1, GPX-4, IL-6, IL-8, TNF-α, and IL-1β were significantly higher in the HTBSF group than in the FFS group (P < 0.05).

Based on the above, under the conditions of this study, the supplementation level of BSF in goat diets should not exceed 10%, and feeding them after treatment at 140°C yields better results.

School of Animal Technology and Innovation

Academic Year 2023

Student's Signature__________

Advisor's Signature__________

Co-advisor's Signature__________

มหาวิทยาลัยเทคโนโลยีสุรนารี

ACKNOWLEDGEMENT

I have walked a long and arduous journey to present this doctoral thesis.

I was born in a remote mountain village in Guanling County, Guizhou Province. In my childhood, there was no electricity, roads, or shops in the village. We lived in poverty, and the farthest place I had been to was the town 20 km away from my home. When I was in fourth grade, a teacher named Ms. Liu Feixia came to our village from the town. She told us in class that the world outside was wonderful and encouraged us to study hard and break out of the mountains. It was the first time in my life that the seed of a dream sprouted. Before that, I did farm work every day after school, such as mowing grass and herding cattle. In sixth grade, I transferred to a school in the town, beginning my hellish life experiences.

Because I had to walk for an hour along a mountain road to catch the bus to the neighboring village, I used to wake up at 4:30 in the morning, wash up, and rush to the bus stop before six o'clock. I remember many times when it was pouring rain, and I was worried about getting my shoes dirty, so I tied the laces around my neck and arrived, and washed my feet clean before putting on my shoes at the bus stop. At 4 a.m., I walked alone through dense forests and graveyards. Birds whose names I couldn't call out cried like ghosts, making my hair stand on end with fear, and sweat soaked my back. I walked this path from sixth grade to my undergraduate graduation.

During my time in the town, I didn't feel as excited as I had imagined. I am from an ethnic minority and couldn't speak the local dialect of Guizhou. It took me about five years to become fluent in it. Later, when I went to university, I couldn't speak Mandarin either. It took me about six years to become fluent in Mandarin. Now, in Thailand, I've spent three years and still can't speak English. When I learn a new language, many people mock my accent and non-standard pronunciation. I often feel inferior and hesitant to communicate with others. Therefore, some classmates thought I was mute during school. Coming from a rural area with poor academic performance and simple, rustic clothing, I faced discrimination from many

people, including classmates, teachers, and members of society. This situation persisted until I pursued my graduate studies. Of course, I also made many friends during my academic journey at various stages.

I lived with my grandmother since I was young. She expressed her desire to witness my wedding and carried a lot of firewood back home, saying it could be used to make a fire for my wedding. However, when I was in my third year of graduate school, she suddenly passed away, leaving me with deep pain. I felt she wasn't prepared to leave, and I couldn't accept it. I often dream of her, as if she were still alive, speaking to me. During my first year of doctoral studies, my father also passed away. Since then, memories and emotions of that small mountain village have abruptly fragmented, and even the cherry blossoms blooming at the doorstep feel somewhat melancholic and mournful.

Thanks for this challenging journey. Including it in the acknowledgments of my thesis serves as a constant reminder to keep striving and never forget my original intentions, especially when facing difficulties.

I express gratitude to my mother (Ms. Wumei Xu), who supported me through all my journeys with her blood and tears. I am thankful to Mr. Jianhong Cao for his tireless efforts and silent support.

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LIST OF ABBREVIATIONS

Abbreviation		Meaning
AA	=	Amino acids
ADF	=	Acid detergent fiber
ADFD	=	Acid detergent fiber digestibility
ADG	=	Average daily weight gain
BSF	=	Black soldier fly
BUN	=	Blood urea nitrogen
BW	=	Body weight
BWC	=	Body weight change
C	=	Cubically
CAT	=	Catalase
CP	=	Crude protein
CPD	=	Crude protein digestibility
DM	=	Dry matter
DMD	=	Dry matter digestion
DMI	=	Dry matter intake
DPPH	=	1,1-diphenyl-2-picrylhydrazyl
ED	=	Effective degradability
EE	=	Ether extract
FA	=	Fatty acid
FFS	=	Full-fat soybeans
GE	=	Gross energy
GSH-Px	=	Glutathione peroxidase
HTBSF	=	Treated black soldier fly
L	=	Linearly
MDA	=	Malondialdehyde
N	=	Nitrogen
NDF	=	Neutral detergent fiber
NFC	=	Non-fibrous carbohydrates

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LIST OF ABBREVIATIONS (Continued)

OM	=	Organic matter
PUFA	=	Polyunsaturated fatty acids
Q	=	Quadratically
RDP	=	Rumen degradable protein
RUP	=	Rumen undegradable protein
SFA	=	Saturated fatty acid
SOD	=	Superoxide dismutase
T-AOC	=	Total antioxidant
UAA	=	Umami amino acids
UFA	=	Unsaturated fatty acids
VFAs	=	Volatile fatty acids

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CHAPTER I

INTRODUCTION

1.1 Introduction

The International Feed Industry Federation (IFIF) reports that the world population will exceed 10 billion by 2050 (Statistics). By then, the increased population will consume twice as much animal protein as it does today; between 2010 and 2050, consumption of pork and poultry is expected to grow by 105% and 173%, respectively (McLeod, 2011; Tiengtam et al., 2017), which will create enormous challenges in the production of protein feed ingredients, estimated as being more than 1.3 billion tons of dry matter (Paengkoum et al., 2017). Soy grains and some oilseed cakes (e.g., soybean meal, rapeseed meal, and cottonseed meal), and some algal biomass are currently the main sources of protein for ruminants and monogastric animals (Kaewwongsa et al., 2011). Global livestock feed production was estimated to be 1.104 billion tons in 2018 alone, with a total value of more than USD 400 billion (Parisi et al., 2020). Protein is the most costly and restrictive ingredient in feed formulations, and the price of traditional sources of protein has risen significantly due to yield factors and competition between humans and animals (Parisi et al., 2020; Van Huis and Oonincx, 2017). To meet the demand for highly nutritious animal food, the future will drive animal production systems to find new sources of high-quality and sustainable protein-fed raw materials. Economic and environmental concerns must be considered in this context, while competition with plant-based human and animal food chains is reduced (Allegretti et al., 2018; Dicke, 2018). Therefore, urea can be added as a protein source in ruminant diets to improve their growth performance and milk production performance (Paengkoum et al., 2006; Vorlaphim et al., 2021; Wanapat et al., 2000). However, in monogastric species, ammonia concentrations in the gastrointestinal tract and the environment can cause damage to the gastrointestinal mucosa, resulting in impaired nutrient absorption, energy inefficiency, and reduced growth performance (Patra and Aschenbach, 2018). Thence, people have turned their attention to insects with high protein content.

As protein raw materials, insects such as BSF, mealworm larvae (*Tenebrio molitor L.*), and crickets (*Orthoptera: Gryllidae*) are the focus of emerging research fronts and are already used as alternative nutrient sources for poultry and swine feed because they contain nearly 100 percent of the edible portion of protein (Gasco et al., 2019; Oonincx and De Boer, 2012). The feed conversion rate of BSF is better than that of mealworms and crickets, and its survival rate and nitrogen and phosphorus composition do not change greatly with a change in diet (Oonincx et al., 2015). BSF are characterized by a high food conversion rate, short reproductive cycle, and high content of fat, protein, minerals, and vitamins (El-Hack et al., 2020; Spranghers Ottoboni et al., 2017). They are also highly sustainable as they can be raised on a large scale in organic streams, and at a much lower environmental cost than traditional protein sources (Meneguz Schiavone et al., 2018). Multiple studies have shown that BSF can be used as a food or feed source, ultimately helping to solve the global food problem. However, consumers prefer to use BSF in animal feed rather than directly for human consumption, because people have a certain degree of psychological aversion to eating insects (Bukkens, 1997; Nyakeri et al., 2017; Tabata et al., 2017b). Diets supplemented with BSF appear to improve growth performance and digestibility in pigs and poultry compared to other protein feeds (Veldkamp and Bosch, 2015; Zuki et al., 2021). (Schiavone et al., 2018) used whole-fat BSF to replace 50% or 100% soybean oil, and the results showed that the growth performance, blood biochemical immune parameters, and intestinal health of broilers were not impaired. BSF can also reduce the quality and nutrient content of pig manure with an efficiency similar to that of poultry manure, which is beneficial for improving farm hygiene (Nguyen et al., 2015; Zhou et al., 2013), reducing pest numbers, and reducing nutrient pollution in runoff (Mallin and Cahoon, 2003). An *in vitro* study found that BSF reduced CH4 emissions (A Jayanegara et al., 2017). Another *in vitro* study was also found by (Kahraman et al., 2023a) that BSF improved the digestibility of DM and NDF in dairy cows. However, the current research on BSF in goats has not been found. Therefore, the purpose of this study is to:

1) Evaluate the effects of supplementing different levels of BSF on the growth performance, digestibility, antioxidant, and rumen microorganisms of goats.

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2) Evaluate the Effects of heat treatment on rumen degradability and protein intestinal digestibility of BSF.

3) Evaluate the effects of heat-treated BSF on goat growth performance, digestion, meat quality, rumen microorganisms, blood immunity, and antioxidant and related gene expression.

1.2 Research purposes

1.2.1 To Investigate the effects of different levels of BSF on the growth performance, blood antioxidant capacity, and rumen microbiota of goats.

1.2.2 To Investigate the effects of heat-treated BSF as a bypass protein on rumen degradation and small intestine digestibility rate in goats.

1.2.3 To Investigate the effects of heat-treated BSF as a bypass protein on the growth performance, rumen microbiota, and meat quality of goats.

1.3 Research hypothesis

1.3.1 Different levels of BSF can promote or have no effects negative on growth performance blood antioxidant capacity, and rumen microbiota of goats.

1.3.2 Heat treatment of BSF can increase bypass protein, and improve goat growth performance, rumen microorganisms, and meat quality.

1.4 Scope and limitations of the study

1.4.1 The experiment was conducted on Xishui Fuxing Animal Husbandry Co., Ltd. (China) and SUT's farm.

1.4.2 Nubian goats and Qianbei goats were used in this study.

1.4.3 The BSF from Henan Province, China, was used in this study.

1.5 Expected results

1.5.1 BSF enhances the growth performance, blood antioxidant capacity, and rumen microbiota abundance of goats, and 15% of BSF can still be supplemented in the daily diet of goats.

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1.5.2 Heat-treated BSF increases rumen bypass protein, promotes the growth performance of goats, enhances rumen microbiota abundance, and improves meat quality, contributing to a new processing method for the application of BSF in ruminant animals.

1.6 References

Allegretti, G., Talamini, E., Schmidt, V., Bogorni, P. C., & Ortega, E. (2018). Insect as feed: An emergy assessment of insect meal as a sustainable protein source for the Brazilian poultry industry. *Journal of Cleaner Production*, *171*, 403-412.

Bukkens, S. G. (1997). The nutritional value of edible insects. *Ecology of Food and Nutrition*, *36*(2-4), 287-319.

Dicke, M. (2018). Insects as feed and the Sustainable Development Goals. *Journal of Insects as Food and Feed*, *4*(3), 147-156.

El-Hack, A., Mohamed, E., Shafi, M. E., Alghamdi, W. Y., Abdelnour, S. A., Shehata, A. M., Noreldin, A. E., Ashour, E. A., Swelum, A. A., & Al-Sagan, A. A. (2020). Black soldier fly (*Hermetia illucens*) meal as a promising feed ingredient for poultry: A comprehensive review. *Agriculture*, *10*(8), 339.

Gasco, L., Dabbou, S., Trocino, A., Xiccato, G., Capucchio, M. T., Biasato, I., Dezzutto, D., Birolo, M., Meneguz, M., & Schiavone, A. (2019). Effect of dietary supplementation with insect fats on growth performance, digestive efficiency and health of rabbits. *Journal of animal science and biotechnology*, *10*(1), 1-9.

Jayanegara, A., Yantina, N., Novandri, B., Laconi, E., & Ridla, M. (2017). Evaluation of some insects as potential feed ingredients for ruminants: chemical composition, *in vitro* rumen fermentation and methane emissions. *Journal of the Indonesian Tropical Animal Agriculture*, *42*(4).

Kaewwongsa, W., Traiyakun, S., Yuangklang, C., Wachirapakorn, C., & Paengkoum, P. (2011). Protein enrichment of cassava pulp fermentation by *Saccharomyces cerevisiae*. *Journal of Animal and Veterinary Advances*, *10*(18), 2434-2440.

Kahraman, O., Gülşen, N., İnal, F., Alataş, M. S., İnanç, Z. S., Ahmed, İ., Şişman, D., & Küçük, A. E. (2023). Comparative Analysis of In Vitro Fermentation Parameters in Total Mixed Rations of Dairy Cows with Varied Levels of Defatted Black Soldier Fly Larvae (*Hermetia illucens*) as a Substitute for Soybean Meal.

Fermentation, *9*(7), 652. https://www.mdpi.com/2311-5637/9/7/652

Mallin, M. A., & Cahoon, L. B. (2003). Industrialized animal production-a major source of nutrient and microbial pollution to aquatic ecosystems. *Population and Environment*, *24*(5), 369-385.

McLeod, A. (2011). *World livestock 2011-livestock in food security*. Food and agriculture organization of the united nations (FAO).

Meneguz, M., Schiavone, A., Gai, F., Dama, A., Lussiana, C., Renna, M., & Gasco, L. (2018). Effect of rearing substrate on growth performance, waste reduction efficiency and chemical composition of black soldier fly (*Hermetia illucens*) larvae. *Journal of the Science of Food and Agriculture*, *98*(15), 5776-5784.

Nguyen, T. T., Tomberlin, J. K., & Vanlaerhoven, S. (2015). Ability of black soldier fly (Diptera: Stratiomyidae) larvae to recycle food waste. *Environmental entomology*, *44*(2), 406-410.

Nyakeri, E., Ogola, H., Ayieko, M., & Amimo, F. (2017). An open system for farming black soldier fly larvae as a source of proteins for smallscale poultry and fish production. *Journal of Insects as Food and Feed*, *3*(1), 51-56.

Oonincx, D. G., & De Boer, I. J. (2012). Environmental impact of the production of mealworms as a protein source for humans–a life cycle assessment. *PloS one*, *7*(12), e51145.

Oonincx, D. G., Van Broekhoven, S., Van Huis, A., & van Loon, J. J. (2015). Feed conversion, survival and development, and composition of four insect species on diets composed of food by-products. *PloS one*, *10*(12), e0144601.

Paengkoum, P., Liang, J., Jelan, Z., & Basery, M. (2006a). Utilization of steam-treated oil palm fronds in growing goats: 1. supplementation with dietary urea. *Asian-australasian journal of animal sciences*, *19*(9), 1305-1313.

Paengkoum, P., Liang, J., Jelan, Z., & Basery, M. (2006b). Utilization of steam-treated oil palm fronds in growing saanen goats: II. Supplementation with energy and urea. *Asian-australasian journal of animal sciences*, *19*(11), 1623-1631.

Paengkoum, P., Thongpea, S., & Paengkoum, S. (2017). Utilization of concentrate supplements containing varying levels of cassava leaf pellet by growing goats fed a basal diet of pangola hay. *Indian Journal of Animal Research*, *51*(6).

Parisi, G., Tulli, F., Fortina, R., Marino, R., Bani, P., Dalle Zotte, A., De Angelis, A.,

Piccolo, G., Pinotti, L., & Schiavone, A. (2020). Protein hunger of the feed sector: The alternatives offered by the plant world. *Italian Journal of Animal Science*, *19*(1), 1204-1225.

Patra, A. K., & Aschenbach, J. R. (2018). Ureases in the gastrointestinal tracts of ruminant and monogastric animals and their implication in urea-N/ammonia metabolism: A review. *Journal of Advanced Research*, *13*, 39-50.

Purba, R., Yuangklang, C., Paengkoum, S., & Paengkoum, P. (2020). Milk fatty acid composition, rumen microbial population and animal performance in response to diets rich in linoleic acid supplemented with Piper betle leaves in Saanen goats. *Animal Production Science*.

Purba, R. A. P., Paengkoum, S., Yuangklang, C., & Paengkoum, P. (2020). Flavonoids and their aromatic derivatives in Piper betle powder promote in vitro methane mitigation in a variety of diets. *Ciência e Agrotecnologia*, *44*.

Schiavone, A., Dabbou, S., De Marco, M., Cullere, M., Biasato, I., Biasibetti, E., Capucchio, M., Bergagna, S., Dezzutto, D., & Meneguz, M. (2018). Black soldier fly larva fat inclusion in finisher broiler chicken diet as an alternative fat source. *Animal*, *12*(10), 2032-2039.

Schiavone, A., De Marco, M., Martínez, S., Dabbou, S., Renna, M., Madrid, J., Hernandez, F., Rotolo, L., Costa, P., & Gai, F. (2017). Nutritional value of a partially defatted and a highly defatted black soldier fly larvae (*Hermetia illucens* L.) meal for broiler chickens: apparent nutrient digestibility, apparent metabolizable energy and apparent ileal amino acid digestibility. *Journal of animal science and biotechnology*, *8*(1), 1-9.

Spranghers, T., Ottoboni, M., Klootwijk, C., Ovyn, A., Deboosere, S., De Meulenaer, B., Michiels, J., Eeckhout, M., De Clercq, P., & De Smet, S. (2017). Nutritional composition of black soldier fly (*Hermetia illucens*) prepupae reared on different organic waste substrates. *Journal of the Science of Food and Agriculture*, *97*(8), 2594-2600.

Statistics, F. G. F. International Feed Industry Federation; 2021. In.

Tabata, E., Kashimura, A., Wakita, S., Ohno, M., Sakaguchi, M., Sugahara, Y., Kino, Y., Matoska, V., Bauer, P. O., & Oyama, F. (2017). Gastric and intestinal proteases resistance of chicken acidic chitinase nominates chitin-containing organisms

for alternative whole edible diets for poultry. *Scientific reports*, *7*(1), 1-11.

Tian, X., Lu, Q., Paengkoum, P., & Paengkoum, S. (2020). Effect of purple corn pigment on change of anthocyanin composition and unsaturated fatty acids during milk storage. *Journal of dairy science*, *103*(9), 7808-7812.

Tiengtam, N., Paengkoum, P., Sirivoharn, S., Phonsiri, K., & Boonanuntanasarn, S. (2017). The effects of dietary inulin and Jerusalem artichoke (*Helianthus tuberosus*) tuber on the growth performance, haematological, blood chemical and immune parameters of Nile tilapia (*Oreochromis niloticus*) fingerlings. *Aquaculture Research*, *48*(10), 5280-5288.

Van Huis, A., & Oonincx, D. G. (2017). The environmental sustainability of insects as food and feed. A review. *Agronomy for Sustainable Development*, *37*(5), 1-14.

Veldkamp, T., & Bosch, G. (2015). Insects: a protein-rich feed ingredient in pig and poultry diets. *Animal Frontiers*, *5*(2), 45-50.

Vorlaphim, T., Paengkoum, P., Purba, R. A. P., Yuangklang, C., Paengkoum, S., & Schonewille, J. T. (2021). Treatment of rice stubble with Pleurotus ostreatus and urea improves the growth performance in slow-growing goats. *Animals*, *11*(4), 1053.

Wanapat, M., Chumpawadee, S., & Paengkoum, P. (2000). Utilization of urea-treated rice straw and whole sugar cane crop as roughage sources for dairy cattle during the dry season. *Asian-australasian journal of animal sciences*, *13*(4), 474-477.

Zhou, F., Tomberlin, J. K., Zheng, L., Yu, Z., & Zhang, J. (2013). Developmental and waste reduction plasticity of three black soldier fly strains (Diptera: Stratiomyidae) raised on different livestock manures. *Journal of medical entomology*, *50*(6), 1224-1230.

Zuki, N. M., Ibrahim, N., Mat, K., Rusli, N., Harun, H., Mahmud, M., Samat, N., & Al-Amsyar, S. (2021). Hybrid treatment of black soldier fly larvae (*Hermetia illucens*) as a sustainable and efficient protein source in poultry diets. IOP Conference Series: Earth and Environmental Science; IOP Publishing.

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CHAPTER II

LITERATURE REVIEW

2.1 Black soldier fly (BSF (*Hermetia illucens* L.; Diptera: Stratiomyidae))

BSF is a saprophytic insect that primarily feeds on organic wastes such as plant residues, animal manure, and waste, food waste, agricultural byproducts, or straw (Banks et al., 2014; Lalander et al., 2013; Nguyen et al., 2015; Zheng et al., 2012). BSF is an excellent candidate for human and animal protein sources, and the utilization of organic waste can help to reduce pollution (Erickson et al., 2004; Paengkoum, 2010; Purba et al., 2021). In the process of degrading waste, BSF converts organic waste into amino acids, peptides, proteins, oils, chitin, and vitamins, thereby controlling certain harmful bacteria (such as Salmonella and Escherichia coli) and pests, and are also used in medicine and chemical and various animal feeds (mainly pets, pigs and poultry) (Erickson et al., 2004; Liu et al., 2008).

BSF originated in the South American savannah and is widely distributed in temperate, subtropical, and tropical regions, with an optimum temperature range of 25°C to 30°C (James, 1935). Due to their lack of resistance to cold, they cannot survive in northwestern Europe and regions with temperatures below 5°C (Spranghers Noyez et al., 2017). BSF is one of five genera in the subfamily Hermetiinae of the order Diptera (Woodley, 2011). The other four genera are Patagiomyia, Chaetosargus, Notohermetia, and Chaetohermetia; *Hermetia illucens* is the most widespread of all species (Singh and Kumari, 2019). It is a large, slender black species with three segments-head, thorax, and bell-with brownish wings and tentacles projecting from the head (Üstüner et al., 2003). There are five segments on the abdomen with white spots. Males are longer than females but have smaller end genitals and wings. Females have body lengths between 12 and 20 mm and wings between 8 and 14.8 mm (Üstüner et al., 2003). Their life cycle has five stages: egg, larva, pupa, pre- and adult. The larval and pupal stages are the most nutrient-rich and largely depend on the quality of food, with about 18–33% fat and 32–53% protein (Chippindale et al.,

2004; St-Hilaire et al., 2007; Yu et al., 2009). BSF has a lifespan of approximately 20-22 days, with a pupa for the first 6–8 days and an adult metamorphosis for the last 14 days. Adult worms have no mouth, digestive system, or stinger, pose no threat to other organisms (Park, 2016), and have no affinity for the human body and fresh food. Therefore, they also do not serve as vectors for disease transmission (Sheppard et al., 2002).

The bioconversion rate is one of the important indicators of waste efficiency used to treat BSF (Gold et al., 2020; Lalander et al., 2019), and the biotransformation rate depends on many factors, such as the concentration of digestible nutrients, protein, fat, fiber, pH, feeding rate (Banks et al., 2014), density and water content of substrates, etc. (Banks et al., 2014; Dortmans et al., 2017). The ideal moisture content is between 60% and 80%, with a lower limit of about 40% (Bortolini et al., 2020). The management requirements of livestock manure around the world are getting higher and higher (Paengkoum et al., 2019). Supplementing BSF in livestock feed can reduce the excretion of manure by 60% (Siddiqui et al., 2022). Moreover, the larvae can also decompose more than 50% of chicken manure, and convert it into high-quality amino acids, protein, and fat for animal feed, reducing the cost of breeding (Newton et al., 2005). At the same time, BSF nutrients are also rich in minerals and chitin and have antioxidant and immune-boosting properties.

2.2 Nutritional Value of BSF

2.2.1 Regular Nutrition Facts

In animal feed, there are currently two types of BSF: defatted and full fat, with the primary difference being in fat and saturated fatty acid content. Table 1 shows its nutritional value. The average crude protein content of BSF was 414.7 g/kg, ranging from 216 g/kg (Yildirim-Aksoy et al., 2020) to 655 g/kg (Schiavone Cullere et al., 2017), which was lower than a conventional soybean meal (CSBM) (494.4 g/kg) and fish meal (675.3 g/kg) (Council, 2012). The protein content of full-fat BSF is relatively similar, but the protein content after defatting is very different (from 216 g/kg to 655 g/kg), which may be related to the method of defatting, such as the irreversible damage to the protein caused by high temperatures. In full-fat BSF, the fat content

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ranged from 294 g/kg (Onsongo et al., 2018) to 515.3 g/kg (Tyshko et al., 2021), with an average of 353.2 g/kg; these were both higher than CSBM (14 g/kg) and fish meal (103.6 g/kg) (Council, 2012). The average fat level after defatting (69.2 g/kg) was also higher than CSBM. BSF contained ash at an average of 82.4 g/kg, ranging from 27 g/kg (Spranghers Noyez et al., 2017) to 132 g/kg (Onsongo et al., 2018), higher than CSBM (71.9 g/kg) and lower than a fish meal (171.5 g/kg) (Council, 2012). The average level of crude fiber was 95.4 g/kg, ranging from 41 g/kg (Spranghers Noyez et al., 2017) to 213 g/kg (Onsongo et al., 2018), which was lower than CSBM (74.3 g/kg), but higher than a fish meal (2.6 g/kg) (Council, 2012). The average content of chitin was 61.7 g/kg, ranging from 38.7 to 72.1 g/kg. The active ingredient of chitin is chitosan, which is another important polysaccharide in addition to cellulose. Chitin (linear polymer of -(1-4)N-acetyl-d-glucosamine units) and cellulose (linear polymer of -(1-4)d-glucopyranose units) have similar molecular structures (Finke, 2007). Chitin is considered an indigestible fiber, but it can improve the immune function of animals (Li et al., 2013; Swiatkiewicz et al., 2015). The variation of crude fiber content in BSF may be related to developmental stages, and the closer to metamorphic adults, the higher the fiber content (H Wang et al., 2020). Therefore, different research results produced different levels of chitin content.

Table 2.1 Regular nutrition facts of BSF (g kg-1 dry matter basis).

	BSF									CSBM	FM
Type	FF	DF	DF	FF	FF	FF	FF	FF	DF		
Crude protein	431.0	655.0	216.0	411.0	439.0	350.0	401.0	275.4	554.2	494.4	675.3
Crude fat	386.0	46.0	63.0	301.0	294.0	298.0	325.0	515.3	98.5	14.0	103.6
Crude fiber	41.0		70.0		213.0	79.0			74.0	74.3	2.6
Ash	27.0	93.0	93.0	93.0	132.0	53.0	104.0	65.9	81.0	71.9	171.5
Chitin	67.0	69.0						38.7	72.1		

CSBM = conventional soybean meal, FM = fish meal, FF = full-fat, DF = defatted.

2.2.2 Amino Acid Profile

Both defatted and full-fat BSF has a rich amino acid profile and are thus considered a more sustainable protein source than CSBM or fish meal (Crosbie et al., 2020). The amino acid profile of BSF is shown in Table 2.2. The most abundant essential amino acids were leucine (average 44.6 g/kg, from 27.8 g/kg to 78.3 g/kg),

lysine (average 38.8 g/kg, from 23.0 g/kg to 68.2 g/kg), and valine (average 40.1 g/kg, ranging from 28.2 g/kg to 67.9 g/kg). These three amino acid contents are higher than those of soybean meal, and even the valine content is higher than that of fish meal (Council, 2012). The least abundant essential amino acids are methionine and tryptophan, which are comparable to soybean meal and are much lower than fish meal (Council, 2012). The content of histidine ranged from 9.8 g/kg to 48 g/kg, and the content of isoleucine ranged from 17.7 g/kg to 48 g/kg, which was slightly higher than soybean meal and fish meal (Council, 2012). The content of phenylalanine ranged from 16.4 g/kg to 77.6 g/kg, and the content of threonine ranged from 16.2 g/kg to 45 g/kg, which is basically the same as soybean meal and fish meal. Arginine and histidine are lower than soybean meal and fish meal (Council, 2012).

Table 2.2 Amino acid composition of BSF (g kg−1 dry matter basis).

	BSF							CSBM	FM
Indispensable amino acids									
Type	FF	DF	FF	FF	FF	FF	FF		
Arginine	19.9	20.7	21.1	54.7	62.0	21.9	18.7	35.7	41.0
Histidine	13.8	16.3	13.5	32.5	48.0	9.8	13.7	14.2	15.4
Isoleucine	19.1	24.0	17.7	47.3	48.0	19.1	20.6	22.1	27.3
Leucine	30.6	36.7	27.8	78.3	77.0	32.1	29.4	38.6	47.7
Lysine	23.0	25.2	28.1	68.2	74.0	27.2	25.9	31.1	48.7
Methionine	7.1	8.56	8.0	21.2	6.0	6.0	7.1	.6.8	18.5
Phenylalanine	16.4	21.8	16.4	77.6	62.0	18.3	18.7	25.5	26.4
Threonine	16.2	21.8	16.3	44.3	45.0	26.5	16.7	19.8	27.5
Tryptophan	5.4					5.6	6.3	6.6	6.7
Valine	28.2	34.5	25.0	67.9	67.0	28.7	28.8	21.7	32.7
Dispensable amino acids									
Alanine	27.8	43.7	25.6	82.1	62.0		26.6	21.6	41.9
Aspartic acid	36.9	48.8	38.7	73.0	103.0		35.6	55.0	57.7
Cysteine	2.2	0.2	3.5	7.6	5.0	4.2	3.2	7.7	6.5
Glycine	25.2	30.3	24.6	61.5	54.0	26.8	24.8	21.3	50.3
Glutamic acid	45.8	63.7	46.1	131.0	102.0		38.4	88.6	84.1
Proline	25.1	32.7	23.6	66.8	62.0		23.1	27.4	30.8
Serine	15.9	26.8	17.6	48.8	41.0	19.2	15.2	24.1	25.9
Tyrosine		34.1		67.1	60.0	26.5	26.9	15.5	20.1

CSBM = conventional soybean meal, DF = defatted, FM = fish meal, FF = full fat.

2.2.3 Fatty Acid Profile

The fatty acid content of BSF is shown in Table 2.3. The most abundant saturated fatty acids (SFA) are lauric acid (C12:0), which ranges from 75 to 575. An amount of 6 g/kg, myristic acid (C14:0), which ranges from 23 to 98.7 g/kg, palmitic acid (C16:0), which ranges from 10.3 to 192.0 g/kg, and stearic acid (C18:0), which ranges from 9.8 to 69.0 g/kg. The highest content of monounsaturated fatty acids is oleic acid (C18:1 c9), which ranges from 79.7 to 266.0 g/kg, palmitoleic acid (C16:1), which ranges from 10.3 to 192.0 g/kg, linoleic acid (C18:2n6), which ranges from 38.0 to 314.0 g/kg, and linolenic acid (C18:3n3), which ranges from 9.8 to 36.0 g/kg. SFA content ranges from 362.0 to 782.9 g/kg, MUFA ranges from 85.5 to 287.0 g/kg, n-6 PUFA ranges from 80.0 to 314.0 g/kg and n-3 PUFA ranges from 9.8 to 36.0 g/kg. The MUFA/SFA ratio ranges from 15.3% to 79.3% and the n-3 PUFA/n-6 PUFA ratio ranges from 8.5% to 17.9%.

Table 2.3 Fatty acid composition of BSF (g kg-1 dry matter basis).

Type	FF	FF	FF	FF	FF	FF
C10:0	20.3		8.6	14.3		8.6
C12:0	575.6	75.0	459.7	526	468.6	407.9
C14:0	71.4	23.0	87	85.4	98.7	65.6
C15:0			1.5		143.8	1.3
C16:0	10.3	192.0	122.1	109	143.8	162.7
C18:0	9.8	69.0	25.3	15.3	17.9	14.3
SFA	782.9	362.0	707.2	750.0	742.4	664.2
C16:1	33.4	8.0	19.1	19.8	27.8	23.6
c9C18:1	79.7	266.0	112.4	61.6	77.3	182.4
c11C18:1	1.2			2.4		
MUFA	119.9	287.0	134.1	85.5	115.8	218.8
C18:2n-6	78.3	314.0	38.0	116.0	127.7	100.7
n-6 PUFA	80.0	314.0	142.2	119.0	106.0	100.9
C18:3n-3	11.0	36.0	16.5	10.1	9.8	16.0
C18:4n-3	0.5					
C20:5n-3	2.3					0.2
C22:6n-3	0.1					
n-3 PUFA	14.3	36.0	16.5	10.1	9.8	16.2
MUFA /SFA, %	15.3	79.3	18.9	11.4	15.6	32.9
n-3 PUFA/ n-6 PUFA, %	17.9	11.5	11.6	8.5	9.0	16.1

DF = defatted, FF = full fat, MUFA = monounsaturated fatty acid, PUFA = polyunsaturated fatty acids, SFA = saturated fatty acid.

2.2.4 Minerals Composition

Table 2.4 shows the mineral content of BSF. BSF is rich in minerals; calcium (Ca) is the most abundant and ranges from 1.2 g/kg to 35.7 g/kg. Copper (Cu) ranges from 0.1 g/kg to 15.0 g/kg. Iron (Fe) ranges from 0.1 g/kg to 191.0 g/kg. Magnesium (Mg) ranges from 1.0 g/kg to 3.5 g/kg. Manganese (Mn) ranges from 0.2 g/kg to 166.0 g/kg. Phosphorus (P) ranges from 1.0 g/kg to 10.3 g/kg. Potassium (K) ranges from 1.7 g/kg to 15.4 g/kg. Sodium (Na) ranges from 0.7 g/kg to 15.6 g/kg. Zinc (Zn) ranges from 0.7 g/kg to 103.0 g/kg. However, in addition to the accumulation of the above minerals, some toxic and harmful elements (such as Ba, Hg, and Mo) will also bioaccumulate in BSF (Bulak et al., 2020), which will pose a challenge to the safety of feed and food production (Petlum et al., 2019).

Table 2.4 Mineral compositions of BSF (g kg-1dry matter basis).

Type	FF	DF	FF	FF	FF
Calcium (Ca)	1.2	13.0	1.9.0	34.6	35.7
Copper (Cu)	0.1	15.0	0.6	10.7	0.7
Iron (Fe)	0.1	125.0	2.1	191.0	14.0
Magnesium (Mg)	2.1	3.0	1.0	3.5	3.4
Manganese (Mn)	0.2	45.0	0.3	166.0	33.5
Phosphorus (P)	4.1	8.0	1.0	10.3	7.0
Potassium (K)	6.0	11.0	1.7	15.4	9.2
Sodium (Na)	0.7	5.0	3.3	1.7	15.6
Zinc (Zn)	0.7	90.0	0.9	103.0	9.0

DF = defatted, FF = full fat.

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2.2.5 Different Factors of Nutritional Value of BSF

The content of minerals and other nutrients in BSF significantly varies across different studies, and the reasons may be as follows:

First, it may be that the growth stages of BSF are different. On the 4–14th days, the crude fat content of larvae increased rapidly, and the highest level reached 28.4%, while crude protein showed a continuous downward trend at the same developmental stage. With the development of pupa, crude fat dropped

sharply to 24.2%. The maximum crude protein in adulthood is 57.6% and the fat level is 21.6% (Liu et al., 2017).

Second, in relation to the nutritional structure ingested by BSF, the content of fat and ash fed from vegetable waste, chicken feed, and kitchen waste varies greatly (Spranghers Ottoboni et al., 2017; Tschirner and Simon, 2015). In addition, BSF on cow dung grows at a much slower rate of individual size than on poultry feed (Diener et al., 2009; Myers et al., 2014).

Third, it may be related to the processing method. Different killing methods (such as blanching, drying, freezing, high hydrostatic pressure grinding, and asphyxiation) also had an effect on pH, ash, fat content, and oxidative capacity (Larouche et al., 2019; Purba and Paengkoum, 2019). The temperature and method during storage also affect the nutritional quality of BSF (Saucier et al., 2022). Different extraction methods also have different nutrient content; for example, the best separation of protein is through alkali extraction (Caligiani et al., 2018). When the processing temperature is 25°C, the shelf life of BSF can reach seven months (Kamau et al., 2018).

Fourth, it is related to various factors such as temperature, humidity, sunlight, moisture content, pH, etc. Humidity and temperature will obviously affect the incubation, development, and lifespan of BSF (Chia et al., 2018; Tomberlin et al., 2009). Temperatures between 26°C and 40°C and relative humidity between 40%–70% are the ideal living conditions for BSF (Holmes, 2010; Sheppard et al., 2002). Sunlight also affects the nutrient composition of BSF, with black soldier flies developing best in the wavelength range between 450 and 700 nm (Park, 2016; Zhang et al., 2010). When the water content in the feed matrix is 60%–80%, the survival rate and growth rate of BSF are the highest (Banks et al., 2014; Cheng et al., 2017). The growth of black soldier flies is better under alkaline conditions than under acidic conditions, and a suitable pH value is between 6– 9 (Ma et al., 2018; Meneguz Gasco et al., 2018).

2.3 Antioxidant mechanism of BSF

The BSF has been confirmed to possess antioxidant and immunomodulatory functions, primarily through three mechanisms: chitin, minerals (such as Se and Mg), and lauric acid (C12:0). Chitin or chitosan (β-(1⟶4)-*N-acetyl*-d-glucosamine) is the

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second most abundant natural polymer after cellulose and was first discovered in 1884 (Figure 2.1). Under alkaline conditions, chitin structures are prone to alterations, and when these alterations reach 50%, it is referred to as chitosan (Younes and Rinaudo, 2015). Chitin exists in an orderly crystalline microfibril form and is a structural component of the exoskeleton of arthropods or the cell walls of fungi and yeast (Rinaudo, 2013). In general, chitin exhibits its functionality only when the pH is below 6.5 (Synowiecki and Al-Khateeb, 2003). Chitosan and its derivatives can act as antioxidants by scavenging reactive oxygen species (such as hydroxyl, superoxide, and alkyl radicals), or by serving as hydrogen donors to prevent oxidation cascades, and they exhibit highly stable DPPH radicals (Park et al., 2003). Although the exact mechanism of free radical scavenging activity is not fully understood, it is attributed to the reaction of amino and hydroxyl groups (attached to the C-2, C-3, and C-6 positions of the pyranose ring) with unstable radicals, thereby promoting the formation of stable macromolecular radicals (Je et al., 2004).

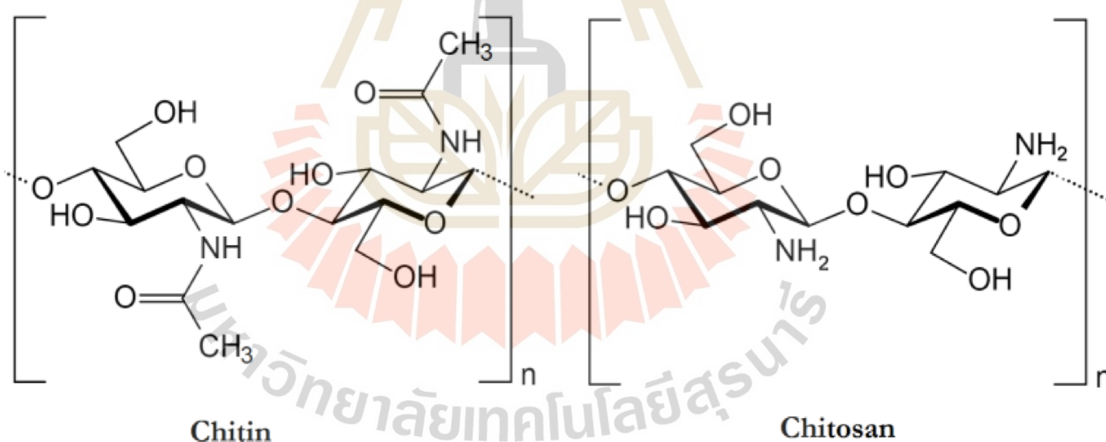

Figure 2.1 The structure of chitin.

Selenium primarily exerts its antioxidant function in the form of amino acids (selenomethionine and selenocysteine) and enzymes (such as GPX) (Kryukov et al., 2003). Additionally, selenium can regulate the expression of TOAX, GPX, CAT, SOD, and GSH genes to alleviate oxidative stress (Chan et al., 2016). The antioxidant properties of selenium proteins are summarized in Table 2.5.

Table 2.5 The antioxidant properties of selenium proteins (Xiao et al., 2021).

Selenoproteins	**Function**
Selenophosphate synthetase 2	Selenophosphate synthetase 2 plays a crucial role in the biosynthesis of all selenoproteins, including itself.
Selenoprotein W	Antioxidant effects are important for muscle growth.
Selenoprotein T	Deficiency leads to early embryonic lethality.
Selenoprotein O	Mitochondrial protein consisted of a cytosine-nucleotide-nucleotide-uridine motif suggestive of the redox role.
Selenoprotein H	Responsible for Nuclear localization, which is associated with redox sensing and transcription.
GPX 1	Cellular reduction of H2O2.
GPX 2	Reduction of peroxide in the gut.
GPX 3	Reduction of peroxide in the blood.
GPX 4	Causes the Reduction of hydrogen peroxide radicals and facilitates lipid peroxides to water and lipid alcohols and the cellular ferroptosis induced by iron.

GPX 1 = glutathione peroxidase 1, GPX 2 = glutathione peroxidase 2, GPX 3 = glutathione peroxidase 3, GPX 4 = glutathione peroxidase 4.

BSF is rich in C12:0, which is a medium-chain saturated fatty acid that has been proven to possess antioxidant properties. Research revealed (Nevin and Rajamohan, 2006) that mice fed a diet containing C12:0 showed increased activity of enzymatic antioxidants such as catalase and superoxide dismutase in serum. In addition to enhancing antioxidant enzyme activity, C12:0 also elevated the levels of intracellular reduced glutathione in cell cultures and animal models, actively participating in phase II detoxification systems (Illam et al., 2017).

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2.4 Effect mechanism of medium-chain fatty acids (MCFA) on rumen

CH_4 emissions decrease with the supplementation of dietary MCFA. This can be attributed to the direct inhibition of methane production by MCFA, as well as the reduction in the quantity and activity of rumen protozoa and/or archaea populations (Patra et al., 2017). Consequently, the digestion rates of rumen microbial populations, OM, and NDF associated with fiber substrates also decrease (Kim et al., 2014). Because fatty acids can adsorb onto microbes or feed particles in the rumen, their small molecules readily dissolve in the lipid layer of cell membranes, effectively causing physical damage to cell membranes, disrupting energy metabolism and nutrient transport, leading to the death of cellulolytic bacteria and ciliated protozoa (Kang et al., 2016; Patra, 2009). MCFA reduces the number of rumen microbes, and the intensity of this antimicrobial effect is proportional to the concentration of MCFA (Debruyne et al., 2018). This leads to a decrease in the growth of protozoa, methanogens, and total archaea species. Consequently, the remaining H_2 can be optimized through non-inhibited microbial populations, forming C_3 through interspecies H_2 transfer, reducing the proportion of C2/C3 and CH_4 synthesis (Patra, 2016; Wang et al., 2017).

2.5 References

Banks, I. J., Gibson, W. T., & Cameron, M. M. (2014). Growth rates of black soldier fly larvae fed on fresh human faeces and their implication for improving sanitation. *Tropical medicine & international health*, *19*(1), 14-22.

Bortolini, S., Macavei, L. I., Saadoun, J. H., Foca, G., Ulrici, A., Bernini, F., Malferrari, D., Setti, L., Ronga, D., & Maistrello, L. (2020). *Hermetia illucens* (L.) larvae as chicken manure management tool for circular economy. *Journal of Cleaner Production*, *262*, 121289.

Bulak, P., Wiącek, D., & Bieganowski, A. (2020). Hermetia illucens exhibits bioaccumulative potential for 15 different elements-Implications for feed and food production. *The Science of the Total Environment*, *723*, 138125-138125.

Caligiani, A., Marseglia, A., Leni, G., Baldassarre, S., Maistrello, L., Dossena, A., & Sforza, S. (2018). Composition of black soldier fly prepupae and systematic

approaches for extraction and fractionation of proteins, lipids and chitin. *Food research international*, *105*, 812-820.

Chan, J. M., Darke, A. K., Penney, K. L., Tangen, C. M., Goodman, P. J., Lee, G.-S. M., Sun, T., Peisch, S., Tinianow, A. M., & Rae, J. M. (2016). Selenium-or vitamin E–related gene variants, interaction with supplementation, and risk of high-grade prostate cancer in SELECT. *Cancer Epidemiology, Biomarkers & Prevention*, *25*(7), 1050-1058.

Cheng, J. Y., Chiu, S. L., & Lo, I. M. (2017). Effects of moisture content of food waste on residue separation, larval growth and larval survival in black soldier fly bioconversion. *Waste management*, *67*, 315-323.

Chia, S. Y., Tanga, C. M., Khamis, F. M., Mohamed, S. A., Salifu, D., Sevgan, S., Fiaboe, K. K., Niassy, S., van Loon, J. J., & Dicke, M. (2018). Threshold temperatures and thermal requirements of black soldier fly *Hermetia illucens*: Implications for mass production. *PloS one*, *13*(11), e0206097.

Chippindale, A. K., Leroi, A. M., Kim, S. B., & Rose, M. R. (2004). Phenotypic plasticity and selection in Drosophila life-history evolution. I. Nutrition and the cost of reproduction. In *Methuselah flies: A case study in the evolution of aging* (pp. 122-144). World Scientific.

Council, N. R. (2012). Nutrient requirements of swine. *Natl. Acad. Press, Washington, DC*.

Crosbie, M., Zhu, C., Shoveller, A. K., & Huber, L.-A. (2020). Standardized ileal digestible amino acids and net energy contents in full fat and defatted black soldier fly larvae meals (*Hermetia illucens*) fed to growing pigs. *Translational Animal Science*, *4*(3), txaa104.

Daszkiewicz, T., Murawska, D., Kubiak, D., & Han, J. (2022). Chemical composition and fatty acid profile of the pectoralis major muscle in broiler chickens fed diets with full-fat black soldier fly (*Hermetia illucens*) larvae meal. *Animals*, *12*(4), 464.

de Souza Vilela, J., Alvarenga, T. I., Andrew, N. R., McPhee, M., Kolakshyapati, M., Hopkins, D. L., & Ruhnke, I. (2021). Technological quality, amino acid and fatty acid profile of broiler meat enhanced by dietary inclusion of black soldier fly larvae. *Foods*, *10*(2), 297.

Debruyne, S., Ruiz-González, A., Artiles-Ortega, E., Ampe, B., Van Den Broeck, W., De Keyser, E., Vandaele, L., Goossens, K., & Fievez, V. (2018). Supplementing goat kids with coconut medium chain fatty acids in early life influences growth and rumen papillae development until 4 months after supplementation but effects on in vitro methane emissions and the rumen microbiota are transient. *Journal of Animal Science*, *96*(5), 1978-1995.

Diener, S., Zurbrügg, C., & Tockner, K. (2009). Conversion of organic material by black soldier fly larvae: establishing optimal feeding rates. *Waste Management & Research*, *27*(6), 603-610.

Dortmans, B., Diener, S., Bart, V., & Zurbrügg, C. (2017). *Black soldier fly biowaste processing: a step-by-step guide*. eawag.

Erickson, M. C., Islam, M., Sheppard, C., Liao, J., & Doyle, M. P. (2004). Reduction of Escherichia coli O157: H7 and Salmonella enterica serovar Enteritidis in chicken manure by larvae of the black soldier fly. *Journal of food protection*, *67*(4), 685-690.

Ewald, N., Vidakovic, A., Langeland, M., Kiessling, A., Sampels, S., & Lalander, C. (2020). Fatty acid composition of black soldier fly larvae (*Hermetia illucens*)–Possibilities and limitations for modification through diet. *Waste Management*, *102*, 40-47.

Finke, M. D. (2007). Estimate of chitin in raw whole insects. *Zoo biology: published in affiliation with the American zoo and aquarium association*, *26*(2), 105-115.

Gold, M., Cassar, C. M., Zurbrügg, C., Kreuzer, M., Boulos, S., Diener, S., & Mathys, A. (2020). Biowaste treatment with black soldier fly larvae: Increasing performance through the formulation of biowastes based on protein and carbohydrates. *Waste Management*, *102*, 319-329.

Holmes, L. (2010). Role of abiotic factors on the development and life history of the black soldier fly, *Hermetia illucens* (L.)(Diptera: Stratiomyidae).

Illam, S. P., Narayanankutty, A., & Raghavamenon, A. C. (2017). Polyphenols of virgin coconut oil prevent pro-oxidant mediated cell death. *Toxicology mechanisms and methods*, *27*(6), 442-450.

James, M. T. (1935). The genus Hermetia in the United States (Diptera: Stratiomyidae). *Bull. Brooklyn Entomol. Soc*, *30*(4), 165-170.

มหาวิทยาลัยเทคโนโลยีสุรนารี

Je, J.-Y., Park, P.-J., & Kim, S.-K. (2004). Free radical scavenging properties of hetero-chitooligosaccharides using an ESR spectroscopy. *Food and Chemical Toxicology*, *42*(3), 381-387.

Kamau, E., Mutungi, C., Kinyuru, J., Imathiu, S., Tanga, C., Affognon, H., Ekesi, S., Nakimbugwe, D., & Fiaboe, K. (2018). Moisture adsorption properties and shelf-life estimation of dried and pulverised edible house cricket Acheta domesticus (L.) and black soldier fly larvae *Hermetia illucens* (L.). *Food research international*, *106*, 420-427.

Kang, S., Wanapat, M., Phesatcha, K., Norrapoke, T., Foiklang, S., Ampapon, T., & Phesatcha, B. (2016). Using krabok (*Irvingia malayana*) seed oil and Flemingia macrophylla leaf meal as a rumen enhancer in an in vitro gas production system. *Animal Production Science*, *57*(2), 327-333.

Kim, D., Mizinga, K., Kube, J., Friesen, K., McLeod, K., & Harmon, D. (2014). Influence of monensin and lauric acid distillate or palm oil on in vitro fermentation kinetics and metabolites produced using forage and high concentrate substrates. *Animal Feed Science and Technology*, *189*, 19-29.

Kryukov, G. V., Castellano, S., Novoselov, S. V., Lobanov, A. V., Zehtab, O., Guigó, R., & Gladyshev, V. N. (2003). Characterization of mammalian selenoproteomes. *Science*, *300*(5624), 1439-1443.

Lalander, C., Diener, S., Magri, M. E., Zurbrügg, C., Lindström, A., & Vinnerås, B. (2013). Faecal sludge management with the larvae of the black soldier fly (Hermetia illucens)—From a hygiene aspect. *Science of the Total Environment*, *458*, 312-318.

Lalander, C., Diener, S., Zurbrügg, C., & Vinnerås, B. (2019). Effects of feedstock on larval development and process efficiency in waste treatment with black soldier fly (*Hermetia illucens*). *Journal of Cleaner Production*, *208*, 211-219.

Larouche, J., Deschamps, M.-H., Saucier, L., Lebeuf, Y., Doyen, A., & Vandenberg, G. W. (2019). Effects of killing methods on lipid oxidation, colour and microbial load of black soldier fly (*Hermetia illucens*) larvae. *Animals*, *9*(4), 182.

Li, J., Shi, B., Yan, S., Jin, L., Guo, Y., Xu, Y., Li, T., & Guo, X. (2013). Effects of dietary supplementation of chitosan on humoral and cellular immune function in weaned piglets. *Animal Feed Science and Technology*, *186*(3-4), 204-208.

มหาวิทยาลัยเทคโนโลยีสุรนารี

Liu, Q., Tomberlin, J. K., Brady, J. A., Sanford, M. R., & Yu, Z. (2008). Black soldier fly (Diptera: Stratiomyidae) larvae reduce Escherichia coli in dairy manure. *Environmental entomology*, *37*(6), 1525-1530.

Liu, X., Chen, X., Wang, H., Yang, Q., ur Rehman, K., Li, W., Cai, M., Li, Q., Mazza, L., & Zhang, J. (2017). Dynamic changes of nutrient composition throughout the entire life cycle of black soldier fly. *PloS one*, *12*(8), e0182601.

Ma, J., Lei, Y., Rehman, K. u., Yu, Z., Zhang, J., Li, W., Li, Q., Tomberlin, J. K., & Zheng, L. (2018). Dynamic effects of initial pH of substrate on biological growth and metamorphosis of black soldier fly (Diptera: Stratiomyidae). *Environmental entomology*, *47*(1), 159-165.

Meneguz, M., Gasco, L., & Tomberlin, J. K. (2018). Impact of pH and feeding system on black soldier fly (*Hermetia illucens*, L; Diptera: Stratiomyidae) larval development. *PloS one*, *13*(8), e0202591.

Müller, A., Wolf, D., & Gutzeit, H. O. (2017). The black soldier fly, Hermetia illucens–a promising source for sustainable production of proteins, lipids and bioactive substances. *Zeitschrift für Naturforschung C*, *72*(9-10), 351-363.

Myers, H. M., Tomberlin, J. K., Lambert, B. D., & Kattes, D. (2014). Development of black soldier fly (Diptera: Stratiomyidae) larvae fed dairy manure. *Environmental entomology*, *37*(1), 11-15.

Nayohan, S., Susanto, I., Permata, D., Pangesti, R. T., Rahmadani, M., & Jayanegara, A. (2022). Effect of dietary inclusion of black soldier fly larvae (*Hermetia illucens*) on broiler performance: A meta-analysis. E3S Web of Conferences,

Nevin, K., & Rajamohan, T. (2006). Virgin coconut oil supplemented diet increases the antioxidant status in rats. *Food Chemistry*, *99*(2), 260-266.

Newton, G., Sheppard, D., Watson, D., Burtle, G., Dove, C., Tomberlin, J., & Thelen, E. (2005). The black soldier fly, *Hermetia illucens*, as a manure management/resource recovery tool. Symposium on the state of the science of Animal Manure and Waste Management,

Nguyen, T. T., Tomberlin, J. K., & Vanlaerhoven, S. (2015). Ability of black soldier fly (Diptera: Stratiomyidae) larvae to recycle food waste. *Environmental entomology*, *44*(2), 406-410.

Onsongo, V., Osuga, I., Gachuiri, C., Wachira, A., Miano, D., Tanga, C., Ekesi, S., Nakimbugwe, D., & Fiaboe, K. (2018). Insects for income generation through animal feed: effect of dietary replacement of soybean and fish meal with black soldier fly meal on broiler growth and economic performance. *Journal of economic entomology*, *111*(4), 1966-1973.

Paengkoum, P. (2010). Effects of neem (*Azadirachta indica*) and leucaena (*Leucaena leucocephala*) fodders on digestibility, rumen fermentation and nitrogen balance of goats fed corn silage. *Journal of Animal and Veterinary Advances*, *9*(5), 883-886.

Paengkoum, P., Chen, S., & Paengkoum, S. (2019). Effects of crude protein and undegradable intake protein on growth performance, nutrient utilization, and rumen fermentation in growing Thai-indigenous beef cattle. *Tropical animal health and production*, *51*(5), 1151-1159.

Park, H. H. (2016). Black soldier fly larvae manual.

Park, P.-J., Je, J.-Y., & Kim, S.-K. (2003). Free radical scavenging activity of chitooligosaccharides by electron spin resonance spectrometry. *Journal of agricultural and food chemistry*, *51*(16), 4624-4627.

Patra, A. (2016). Recent advances in measurement and dietary mitigation of enteric methane emissions in ruminants. Front Vet Sci 2016; 3: 39. In.

Patra, A., Park, T., Kim, M., & Yu, Z. (2017). Rumen methanogens and mitigation of methane emission by anti-methanogenic compounds and substances. *Journal of Animal Science and Biotechnology*, *8*, 1-18.

Patra, A. K. (2009). A meta-analysis on effects of supplementing low-quality roughages with foliages from browses and tree fodders on intake and growth in sheep. *Livestock Science*, *121*(2-3), 239-249.

Petlum, A., Paengkoum, P., Liang, J., Vasupen, K., & Paengkoum, S. (2019). Molecular weight of condensed tannins of some tropical feed-leaves and their effect on in vitro gas and methane production. *Animal Production Science*, *59*(12), 2154-2160.

Purba, R. A. P., & Paengkoum, P. (2019). Bioanalytical HPLC method of Piper betle L. for quantifying phenolic compound, water-soluble vitamin, and essential oil

in five different solvent extracts. *Journal of Applied Pharmaceutical Science*, *9*(5), 033-039.

Purba, R. A. P., Yuangklang, C., & Paengkoum, P. (2020). Enhanced conjugated linoleic acid and biogas production after ruminal fermentation with Piper betle L. supplementation. *Ciência Rural*, *50*.

Purba, R. A. P., Yuangklang, C., Paengkoum, S., & Paengkoum, P. (2021). Piper oil decreases in vitro methane production with shifting ruminal fermentation in a variety of diets. *Int. J. Agric. Biol*, *25*, 231-240.

Rabani, V., Cheatsazan, H., & Davani, S. (2019). Proteomics and lipidomics of black soldier fly (Diptera: Stratiomyidae) and blow fly (Diptera: Calliphoridae) larvae. *Journal of Insect Science*, *19*(3), 29.

Rawski, M., Mazurkiewicz, J., Kierończyk, B., & Józefiak, D. (2020). Black soldier fly full-fat larvae meal as an alternative to fish meal and fish oil in Siberian sturgeon nutrition: The effects on physical properties of the feed, animal growth performance, and feed acceptance and utilization. *Animals*, *10*(11), 2119.

Rinaudo, M. (2013). Materials based on chitin and chitosan. *Bio‐Based Plastics: Materials and Applications*, 63-87.

Romano, N., Fischer, H., Kumar, V., Francis, S. A., & Sinha, A. K. (2022). Productivity, conversion ability, and biochemical composition of black soldier fly (*Hermetia illucens*) larvae fed with sweet potato, spent coffee or dough. *International Journal of Tropical Insect Science*, *42*(1), 183-190.

Saucier, L., M'ballou, C., Ratti, C., Deschamps, M.-H., Lebeuf, Y., & Vandenberg, G. (2022). Comparison of black soldier fly larvae pre-treatments and drying techniques on the microbial load and physico-chemical characteristics. *Journal of Insects as Food and Feed*, *8*(1), 45-64.

Schiavone, A., Cullere, M., De Marco, M., Meneguz, M., Biasato, I., Bergagna, S., Dezzutto, D., Gai, F., Dabbou, S., & Gasco, L. (2017). Partial or total replacement of soybean oil by black soldier fly larvae (*Hermetia illucens* L.) fat in broiler diets: Effect on growth performances, feed-choice, blood traits, carcass characteristics and meat quality. *Italian Journal of Animal Science*, *16*(1), 93-100.

มหาวิทยาลัยเทคโนโลยีสุรนารี

Schiavone, A., De Marco, M., Martínez, S., Dabbou, S., Renna, M., Madrid, J., Hernandez, F., Rotolo, L., Costa, P., & Gai, F. (2017). Nutritional value of a partially defatted and a highly defatted black soldier fly larvae (*Hermetia illucens* L.) meal for broiler chickens: apparent nutrient digestibility, apparent metabolizable energy and apparent ileal amino acid digestibility. *Journal of animal science and biotechnology*, *8*(1), 1-9.

Shah, A. A., Totakul, P., Matra, M., Cherdthong, A., Hanboonsong, Y., & Wanapat, M. (2022). Nutritional composition of various insects and potential uses as alternative protein sources in animal diets. *Animal Bioscience*, *35*(2), 317.

Sheppard, D. C., Tomberlin, J. K., Joyce, J. A., Kiser, B. C., & Sumner, S. M. (2002). Rearing methods for the black soldier fly (Diptera: Stratiomyidae). *Journal of medical entomology*, *39*(4), 695-698.

Shumo, M., Khamis, F. M., Tanga, C. M., Fiaboe, K. K., Subramanian, S., Ekesi, S., Van Huis, A., & Borgemeister, C. (2019). Influence of temperature on selected life-history traits of black soldier fly (*Hermetia illucens*) reared on two common urban organic waste streams in Kenya. *Animals*, *9*(3), 79.

Shumo, M., Osuga, I. M., Khamis, F. M., Tanga, C. M., Fiaboe, K. K., Subramanian, S., Ekesi, S., van Huis, A., & Borgemeister, C. (2019). The nutritive value of black soldier fly larvae reared on common organic waste streams in Kenya. *Scientific reports*, *9*(1), 1-13.

Siddiqui, S. A., Ristow, B., Rahayu, T., Putra, N. S., Yuwono, N. W., Mategeko, B., Smetana, S., Saki, M., Nawaz, A., & Nagdalian, A. (2022). Black soldier fly larvae (BSFL) and their affinity for organic waste processing. *Waste Management*, *140*, 1-13.

Singh, A., & Kumari, K. (2019). An inclusive approach for organic waste treatment and valorisation using Black Soldier Fly larvae: A review. *Journal of Environmental Management*, *251*, 109569.

Smets, R., Verbinnen, B., Van De Voorde, I., Aerts, G., Claes, J., & Van Der Borght, M. (2020). Sequential extraction and characterisation of lipids, proteins, and chitin from black soldier fly (*Hermetia illucens*) larvae, prepupae, and pupae. *Waste and Biomass Valorization*, *11*(12), 6455-6466.

Spranghers, T., Noyez, A., Schildermans, K., & De Clercq, P. (2017). Cold hardiness of the black soldier fly (Diptera: Stratiomyidae). *Journal of economic entomology*, *110*(4), 1501-1507.

Spranghers, T., Ottoboni, M., Klootwijk, C., Ovyn, A., Deboosere, S., De Meulenaer, B., Michiels, J., Eeckhout, M., De Clercq, P., & De Smet, S. (2017). Nutritional composition of black soldier fly (*Hermetia illucens*) prepupae reared on different organic waste substrates. *Journal of the Science of Food and Agriculture*, *97*(8), 2594-2600.

St-Hilaire, S., Sheppard, C., Tomberlin, J. K., Irving, S., Newton, L., McGuire, M. A., Mosley, E. E., Hardy, R. W., & Sealey, W. (2007). Fly prepupae as a feedstuff for rainbow trout, Oncorhynchus mykiss. *Journal of the world aquaculture society*, *38*(1), 59-67.

Swiatkiewicz, S., Swiatkiewicz, M., Arczewska-Wlosek, A., & Jozefiak, D. (2015). Chitosan and its oligosaccharide derivatives (chito-oligosaccharides) as feed supplements in poultry and swine nutrition. *Journal of animal physiology and animal nutrition*, *99*(1), 1-12.

Synowiecki, J., & Al-Khateeb, N. A. (2003). Production, properties, and some new applications of chitin and its derivatives.

Tomberlin, J. K., Adler, P. H., & Myers, H. M. (2009). Development of the black soldier fly (Diptera: Stratiomyidae) in relation to temperature. *Environmental entomology*, *38*(3), 930-934.

Tschirner, M., & Simon, A. (2015). Influence of different growing substrates and processing on the nutrient composition of black soldier fly larvae destined for animal feed. *Journal of Insects as Food and Feed*, *1*(4), 249-259.

Tyshko, N. V., Zhminchenko, V. M., Nikitin, N. S., Trebukh, M. D., Shestakova, S. I., Pashorina, V. A., & Sadykova, E. O. (2021). The comprehensive studies of *Hermetia illucens* larvae protein's biological value. *Problems of nutritions*, *90*, 49-58.

Üstüner, T., Hasbenlí, A., & Rozkošný, R. (2003). The first record of Hermetia illucens (Linnaeus, 1758)(Diptera, Stratiomyidae) from the Near East. *Studia dipterologica*(1).

Wang, H., ur Rehman, K., Feng, W., Yang, D., ur Rehman, R., Cai, M., Zhang, J., Yu, Z., & Zheng, L. (2020). Physicochemical structure of chitin in the developing stages

of black soldier fly. *International journal of biological macromolecules*, *149*, 901-907.

Wang, S., Kreuzer, M., Braun, U., & Schwarm, A. (2017). Effect of unconventional oilseeds (safflower, poppy, hemp, camelina) on in vitro ruminal methane production and fermentation. *Journal of the Science of Food and Agriculture*, *97*(11), 3864-3870.

Woodley, N. E. (2011). A world catalog of the Stratiomyidae (Insecta: Diptera): a supplement with revisionary notes and errata. *Myia*, *12*, 379-415.

Xiao, J., Khan, M. Z., Ma, Y., Alugongo, G. M., Ma, J., Chen, T., Khan, A., & Cao, Z. (2021). The Antioxidant Properties of Selenium and Vitamin E; Their Role in Periparturient Dairy Cattle Health Regulation. *Antioxidants*, *10*(10).

Yildirim‐Aksoy, M., Eljack, R., & Beck, B. H. (2020). Nutritional value of frass from black soldier fly larvae, *Hermetia illucens*, in a channel catfish, Ictalurus punctatus, diet. *Aquaculture nutrition*, *26*(3), 812-819.

Younes, I., & Rinaudo, M. (2015). Chitin and Chitosan Preparation from Marine Sources. Structure, Properties and Applications. *Marine Drugs*, *13*(3), 1133-1174.

Yu, G., Chen, Y., Yu, Z., & Cheng, P. (2009). Research progress on the larvae and prepupae of black soldier fly *Hermetia illucens* used as animal feedstuff. *Chinese bulletin of entomology*, *46*(1), 41-45.

Zhang, J., Huang, L., He, J., Tomberlin, J. K., Li, J., Lei, C., Sun, M., Liu, Z., & Yu, Z. (2010). An artificial light source influences mating and oviposition of black soldier flies, *Hermetia illucens*. *Journal of insect science*, *10*(1).

Zheng, L., Li, Q., Zhang, J., & Yu, Z. (2012). Double the biodiesel yield: Rearing black soldier fly larvae, *Hermetia illucens*, on solid residual fraction of restaurant waste after grease extraction for biodiesel production. *Renewable energy*, *41*, 75-79.

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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

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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.

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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:

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

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 ($C_{36}H_{68}O_6$, 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

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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.

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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.

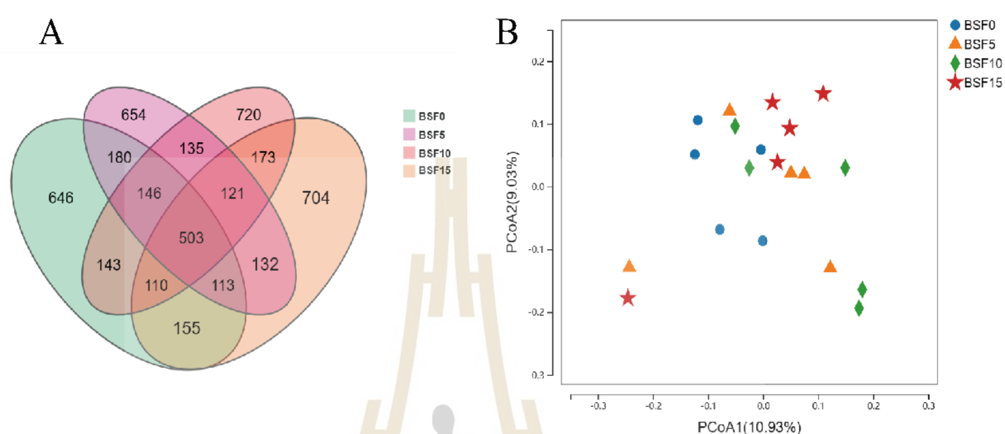

Figure 3.1 Effects of BSF supplementation on rumen microorganisms. **A**, A Venn diagram of operational taxonomic units (OTUs). **B**, points of different colors or shapes represent sample groups. The scales of the horizontal and vertical axes were the projected coordinates of the sample points on the 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.

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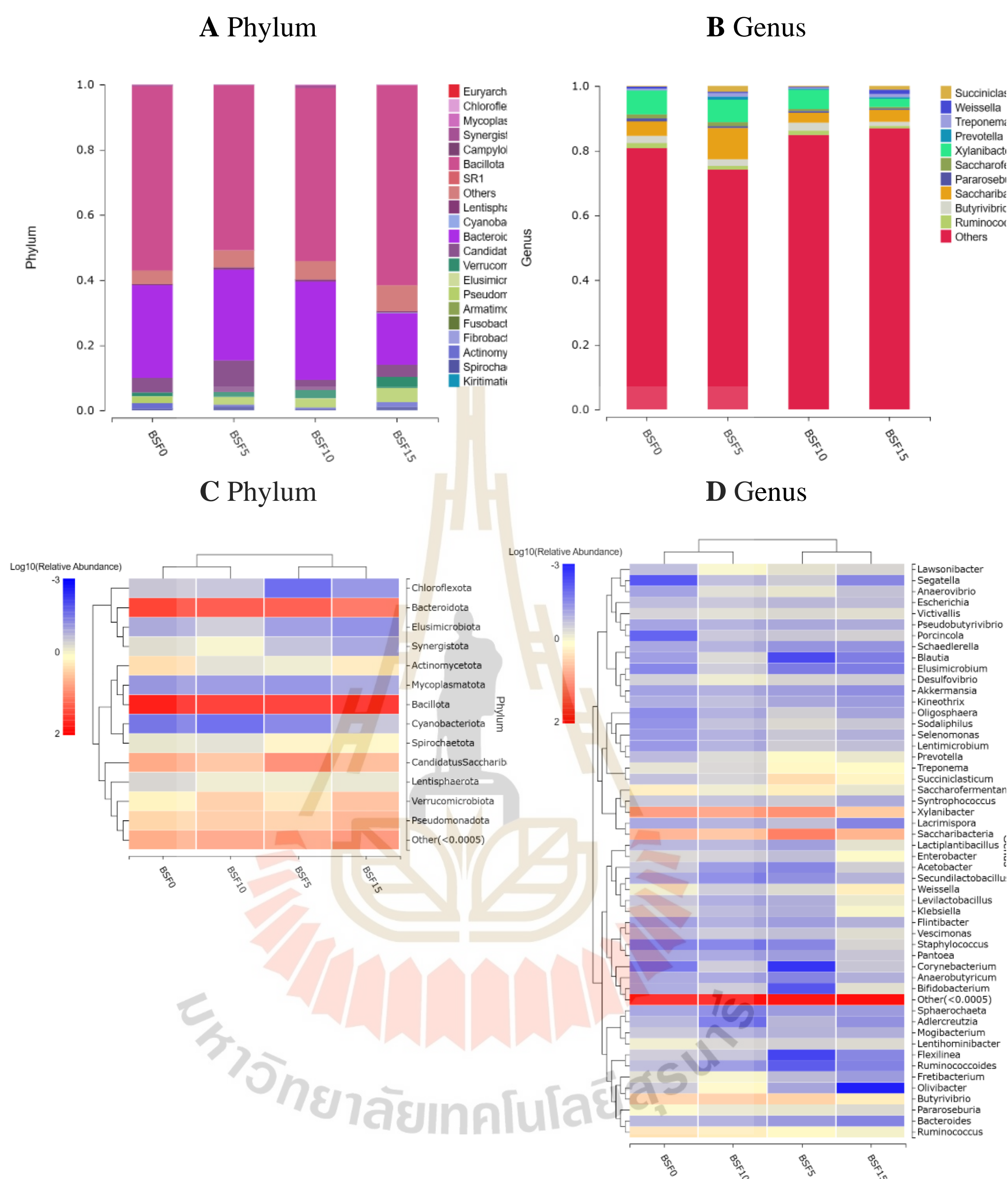

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Figure 3.2 Effects of BSF supplementation on rumen microorganisms. **A** and **B**, rumen microbial composition at phylum and genus levels, species that were not annotated at this taxonomic level and whose abundance was less than 0.5% in the sample 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.

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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.

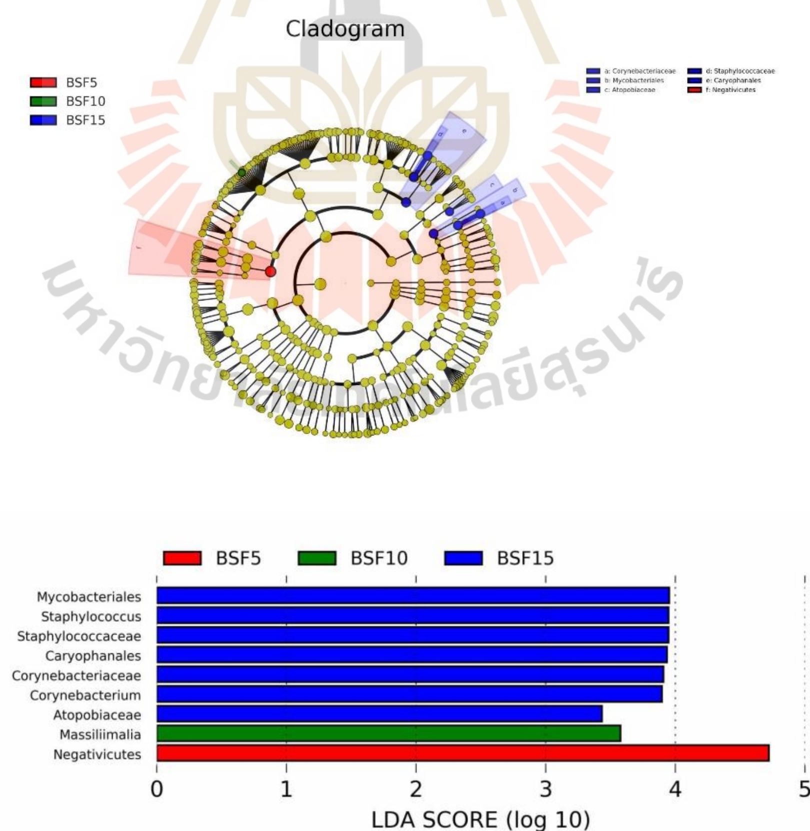

Figure 3.3 LEfSe analysis (BSF0= No BSF, BSF5= 5%BSF, BSF10= 10%BSF, BSF15= 15%BSF).

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

Abril, S., Pinzón, M., Hernández-Carrión, M., & Sánchez-Camargo, A. d. P. (2022). Edible insects in latin america: a sustainable alternative for our food security. *Frontiers in nutrition*, *9*, 904812.

Agbohessou, P. S., Mandiki, S. N., Gougbédji, A., Megido, R. C., Hossain, M. S., De Jaeger, P., Larondelle, Y., Francis, F., Lalèyè, P. A., & Kestemont, P. (2021). Total replacement of fish meal by enriched-fatty acid *Hermetia illucens* meal did not substantially affect growth parameters or innate immune status and improved whole body biochemical quality of Nile tilapia juveniles. *Aquaculture nutrition*, *27*(3), 880-896.

Bellezza Oddon, S., Biasato, I., Imarisio, A., Pipan, M., Dekleva, D., Colombino, E., Capucchio, M. T., Meneguz, M., Bergagna, S., Barbero, R., Gariglio, M., Dabbou, S., Fiorilla, E., Gasco, L., & Schiavone, A. (2021). Black soldier fly and yellow mealworm live larvae for broiler chickens: Effects on bird performance and health status. *J Anim Physiol Anim Nutr (Berl)*, *105 Suppl 1*(Suppl 1), 10-18. https://doi.org/10.1111/jpn.13567.

Biasato, I., Ferrocino, I., Colombino, E., Gai, F., Schiavone, A., Cocolin, L., Vincenti, V., Capucchio, M. T., & Gasco, L. (2020). Effects of dietary *Hermetia illucens* meal inclusion on cecal microbiota and small intestinal mucin dynamics and infiltration with immune cells of weaned piglets. *Journal of animal science and biotechnology*, *11*(1), 1-11.

Biasato, I., Ferrocino, I., Dabbou, S., Evangelista, R., Gai, F., Gasco, L., Cocolin, L., Capucchio, M. T., & Schiavone, A. (2020). Black soldier fly and gut health in broiler chickens: insights into the relationship between cecal microbiota and intestinal mucin composition. *Journal of animal science and biotechnology*, *11*, 1-12.

Biasato, I., Renna, M., Gai, F., Dabbou, S., Meneguz, M., Perona, G., Martinez, S., Lajusticia, A. C. B., Bergagna, S., Sardi, L., Capucchio, M. T., Bressan, E., Dama, A., Schiavone, A., & Gasco, L. (2019). Partially defatted black soldier fly larva meal inclusion in piglet diets: effects on the growth performance, nutrient digestibility, blood profile, gut morphology and histological features. *J Anim Sci Biotechnol*, *10*, 12. https://doi.org/10.1186/s40104-019-0325-x.

Bolyen, E., Rideout, J. R., Dillon, M. R., Bokulich, N. A., Abnet, C. C., Al-Ghalith, G. A., Alexander, H., Alm, E. J., Arumugam, M., Asnicar, F., Bai, Y., Bisanz, J. E., Bittinger, K., Brejnrod, A., Brislawn, C. J., Brown, C. T., Callahan, B. J., Caraballo-Rodríguez, A. M., Chase, J., . . . Caporaso, J. G. (2019). Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nature Biotechnology*, *37*(8), 852-857. https://doi.org/10.1038/s41587-019-0209-9.

Bovera, F., Lestingi, A., Iannaccone, F., Tateo, A., & Nizza, A. (2012). Use of dietary mannanoligosaccharides during rabbit fattening period: Effects on growth performance, feed nutrient digestibility, carcass traits, and meat quality. *Journal of Animal Science*, *90*(11), 3858-3866. https://doi.org/10.2527/jas.2011-4119.

Cabezas-Garcia, E., Krizsan, S., Shingfield, K. J., & Huhtanen, P. (2017). Between-cow variation in digestion and rumen fermentation variables associated with methane production. *Journal of Dairy Science*, *100*(6), 4409-4424.

Caimi, C., Gasco, L., Biasato, I., Malfatto, V., Varello, K., Prearo, M., Pastorino, P., Bona, M. C., Francese, D. R., Schiavone, A., Elia, A. C., Dorr, A. J. M., & Gai, F. (2020). Could Dietary Black Soldier Fly Meal Inclusion Affect the Liver and Intestinal Histological

มหาวิทยาลัยเทคโนโลยีสุรนารี

Traits and the Oxidative Stress Biomarkers of Siberian Sturgeon (Acipenser baerii) Juveniles? *Animals (Basel)*, *10*(1). https://doi.org/10.3390/ ani10010155.

Cullere, M., Tasoniero, G., Giaccone, V., Miotti-Scapin, R., Claeys, E., De Smet, S., & Dalle Zotte, A. (2016). Black soldier fly as dietary protein source for broiler quails: apparent digestibility, excreta microbial load, feed choice, performance, carcass and meat traits. *animal*, *10*(12), 1923-1930. https://doi.org/10.1017/ S1751731116001270.

Dabbou, S., Ferrocino, I., Gasco, L., Schiavone, A., Trocino, A., Xiccato, G., Barroeta, A. C., Maione, S., Soglia, D., & Biasato, I. (2020). Antimicrobial effects of black soldier fly and yellow mealworm fats and their impact on gut microbiota of growing rabbits. *Animals*, *10*(8), 1292.

Dabbou, S., Gai, F., Biasato, I., Capucchio, M. T., Biasibetti, E., Dezzutto, D., Meneguz, M., Placha, I., Gasco, L., & Schiavone, A. (2018). Black soldier fly defatted meal as a dietary protein source for broiler chickens: Effects on growth performance, blood traits, gut morphology and histological features. *J Anim Sci Biotechnol*, *9*, 49. https://doi.org/10.1186/s40104-018-0266-9.

Dabbou, S., Gai, F., Biasato, I., Capucchio, M. T., Biasibetti, E., Dezzutto, D., Meneguz, M., Plachà, I., Gasco, L., & Schiavone, A. (2018). Black soldier fly defatted meal as a dietary protein source for broiler chickens: Effects on growth performance, blood traits, gut morphology and histological features. *Journal of animal science and biotechnology*, *9*, 1-10.

Dabbou, S., Lauwaerts, A., Ferrocino, I., Biasato, I., Sirri, F., Zampiga, M., Bergagna, S., Pagliasso, G., Gariglio, M., & Colombino, E. (2021). Modified black soldier fly larva fat in broiler diet: Effects on performance, carcass traits, blood parameters, histomorphological features and gut microbiota. *Animals*, *11*(6), 1837.

de Paiva, P. G., de Jesus, E. F., Del Valle, T. A., de Almeida, G. F., Costa, A. G. B. V. B., Consentini, C. E. C., Zanferari, F., Takiya, C. S., da Silva Bueno, I. C., & Rennó, F. P. (2016). Effects of chitosan on ruminal fermentation, nutrient digestibility, and milk yield and composition of dairy cows. *Animal Production Science*, *57*(2), 301-307.

de Souza Vilela, J., Andronicos, N. M., Kolakshyapati, M., Hilliar, M., Sibanda, T. Z., Andrew, N. R., Swick, R. A., Wilkinson, S., & Ruhnke, I. (2021). Black soldier fly larvae in broiler diets improve broiler performance and modulate the immune system.

Animal Nutrition, *7*(3), 695-706. https://doi.org/https://doi.org/ 10.1016/j.aninu. 2020.08.014.

Dias, A., Goes, R., Gandra, J., Takiya, C., Branco, A., Jacaúna, A., Oliveira, R., Souza, C., & Vaz, M. (2017). Increasing doses of chitosan to grazing beef steers: Nutrient intake and digestibility, ruminal fermentation, and nitrogen utilization. *Animal Feed Science and Technology*, *225*, 73-80.

Dijkstra, J., Van Gastelen, S., Dieho, K., Nichols, K., & Bannink, A. (2020). Rumen sensors: Data and interpretation for key rumen metabolic processes. *Animal*, *14*(S1), s176-s186.

Do, S., Koutsos, E. A., McComb, A., Phungviwatnikul, T., de Godoy, M. R. C., & Swanson, K. S. (2022). Palatability and apparent total tract macronutrient digestibility of retorted black soldier fly larvae-containing diets and their effects on the fecal characteristics of cats consuming them. *Journal of Animal Science*, *100*(4), skac068. https://doi.org/10.1093/jas/skac068.

Ebeneezar, S., Tejpal, C., Jeena, N., Summaya, R., Chandrasekar, S., Sayooj, P., & Vijayagopal, P. (2021). Nutritional evaluation, bioconversion performance and phylogenetic assessment of black soldier fly (*Hermetia illucens*, Linn. 1758) larvae valorized from food waste. *Environmental Technology & Innovation*, *23*, 101783.

Elieh Ali Komi, D., Sharma, L., & Dela Cruz, C. S. (2018). Chitin and its effects on inflammatory and immune responses. *Clinical reviews in allergy & immunology*, *54*, 213-223.

Ebeneezar, S., Tejpal, C., Jeena, N., Summaya, R., Chandrasekar, S., Sayooj, P., & Vijayagopal, P. (2021). Nutritional evaluation, bioconversion performance and phylogenetic assessment of black soldier fly (*Hermetia illucens*, Linn. 1758) larvae valorized from food waste. *Environmental Technology & Innovation*, *23*, 101783.

Elieh Ali Komi, D., Sharma, L., & Dela Cruz, C. S. (2018). Chitin and its effects on inflammatory and immune responses. *Clinical reviews in allergy & immunology*, *54*, 213-223.

Gariglio, M., Dabbou, S., Crispo, M., Biasato, I., Gai, F., Gasco, L., Piacente, F., Odetti, P., Bergagna, S., Placha, I., Valle, E., Colombino, E., Capucchio, M. T., & Schiavone, A. (2019). Effects of the Dietary Inclusion of Partially Defatted Black Soldier Fly

(*Hermetia illucens*) Meal on the Blood Chemistry and Tissue (Spleen, Liver, Thymus, and Bursa of Fabricius) Histology of Muscovy Ducks (Cairina moschata domestica). *Animals (Basel)*, *9*(6). https://doi.org/10.3390/ani9060307.

Goh, P. G., & Chou, M. C.-f. (2022). *Global Supply Chains in a Glocal World: The Impact of COVID-19 and Digitalisation*. World Scientific.

Goiri, I., Oregui, L., & Garcia-Rodriguez, A. (2010). Use of chitosans to modulate ruminal fermentation of a 50: 50 forage-to-concentrate diet in sheep. *Journal of animal science*, *88*(2), 749-755.

Górka, P., Kowalski, Z. M., Zabielski, R., & Guilloteau, P. (2018). Invited review: Use of butyrate to promote gastrointestinal tract development in calves. *Journal of Dairy Science*, *101*(6), 4785-4800. https://doi.org/https://doi.org/10.3168/jds.2017-14086.

Hender, A., Siddik, M. A., Howieson, J., & Fotedar, R. (2021). Black soldier fly, *Hermetia illucens* as an alternative to fishmeal protein and fish oil: impact on growth, immune response, mucosal barrier status, and flesh quality of juvenile barramundi, Lates calcarifer (Bloch, 1790). *Biology*, *10*(6), 505.

Hervás, G., Boussalia, Y., Labbouz, Y., Della Badia, A., Toral, P. G., & Frutos, P. (2022). Insect oils and chitosan in sheep feeding: Effects on in vitro ruminal biohydrogenation and fermentation. *Animal Feed Science and Technology*, *285*, 115222.

Hristov, A., Lee, C., Cassidy, T., Long, M., Heyler, K., Corl, B., & Forster, R. (2011). Effects of lauric and myristic acids on ruminal fermentation, production, and milk fatty acid composition in lactating dairy cows. *Journal of Dairy Science*, *94*(1), 382-395.

Hristov, A. N., Vander Pol, M., Agle, M., Zaman, S., Schneider, C., Ndegwa, P., Vaddella, V. K., Johnson, K., Shingfield, K. J., & Karnati, S. K. R. (2009). Effect of lauric acid and coconut oil on ruminal fermentation, digestion, ammonia losses from manure, and milk fatty acid composition in lactating cows. *Journal of Dairy Science*, *92*(11), 5561-5582. https://doi.org/https://doi.org/10.3168/jds.2009-2383 .

Ipema, A. F., Bokkers, E. A. M., Gerrits, W. J. J., Kemp, B., & Bolhuis, J. E. (2021). Providing live black soldier fly larvae (*Hermetia illucens*) improves welfare while maintaining performance of piglets post-weaning. *Scientific Reports*, *11*(1), 7371. https://doi.org/10.1038/s41598-021-86765-3.

มหาวิทยาลัยเทคโนโลยีสุรนารี

Jayanegara, A., Novandri, B., Yantina, N., & Ridla, M. (2017). Use of black soldier fly larvae (*Hermetia illucens*) to substitute soybean meal in ruminant diet: An in vitro rumen fermentation study. *Vet World*, *10*(12), 1439-1446. https://doi.org/10.14202/vetworld.2017.1439-1446.

Jeong, C. D., Mamuad, L. L., Kim, S. H., Choi, Y. J., Soriano, A. P., Cho, K. K., Jeon, C. O., Lee, S. S., & Lee, S. S. (2015). Effect of Soybean Meal and Soluble Starch on Biogenic Amine Production and Microbial Diversity Using In vitro Rumen Fermentation. *Asian-Australas J Anim Sci*, *28*(1), 50-57. https://doi.org/10.5713/ajas.14.0555.

Jian, S., Zhang, L., Ding, N., Yang, K., Xin, Z., Hu, M., Zhou, Z., Zhao, Z., Deng, B., & Deng, J. (2022). Effects of black soldier fly larvae as protein or fat sources on apparent nutrient digestibility, fecal microbiota, and metabolic profiles in beagle dogs. *Frontiers in Microbiology*, *13*, 1044986.

Jian, S., Zhang, L., Ding, N., Yang, K., Xin, Z., Hu, M., Zhou, Z., Zhao, Z., Deng, B., & Deng, J. (2022). Effects of black soldier fly larvae as protein or fat sources on apparent nutrient digestibility, fecal microbiota, and metabolic profiles in beagle dogs. *Front Microbiol*, *13*, 1044986. https://doi.org/10.3389/fmicb.2022.1044986.

Kahraman, O., Gülşen, N., İnal, F., Alataş, M. S., İnanç, Z. S., Ahmed, İ., Şişman, D., & Küçük, A. E. (2023). Comparative Analysis of In Vitro Fermentation Parameters in Total Mixed Rations of Dairy Cows with Varied Levels of Defatted Black Soldier Fly Larvae (*Hermetia illucens*) as a Substitute for Soybean Meal. *Fermentation*, *9*(7). https://doi.org/10.3390/fermentation9070652.

Kawasaki, K., Hashimoto, Y., Hori, A., Kawasaki, T., Hirayasu, H., Iwase, S.-i., Hashizume, A., Ido, A., Miura, C., & Miura, T. (2019). Evaluation of black soldier fly (*Hermetia illucens*) larvae and pre-pupae raised on household organic waste, as potential ingredients for poultry feed. *Animals*, *9*(3), 98.

Kröger, S., Heide, C., & Zentek, J. (2020). Evaluation of an extruded diet for adult dogs containing larvae meal from the Black soldier fly (*Hermetia illucens*). *Animal Feed Science and Technology*, *270*, 114699. https://doi.org/https://doi.org/10.1016/j.anifeedsci.2020.114699.

Liang, J., Fang, W., Wang, Q., Zubair, M., Zhang, G., Ma, W., Cai, Y., & Zhang, P. (2021). Metagenomic analysis of community, enzymes and metabolic pathways during

มหาวิทยาลัยเทคโนโลยีสุรนารี

corn straw fermentation with rumen microorganisms for volatile fatty acid production. *Bioresource technology*, *342*, 126004.

Liu, S., Sun, J., Yu, L., Zhang, C., Bi, J., Zhu, F., Qu, M., Jiang, C., & Yang, Q. (2012). Extraction and characterization of chitin from the beetle Holotrichia parallela Motschulsky. *Molecules*, *17*(4), 4604-4611.

Longvah, T., Mangthya, K., & Ramulu, P. (2011). Nutrient composition and protein quality evaluation of eri silkworm (Samia ricinii) prepupae and pupae. *Food Chemistry*, *128*(2), 400-403. https://doi.org/https://doi.org/10.1016/j.foodchem.2011.03.041.

Lu, S., Taethaisong, N., Meethip, W., Surakhunthod, J., Sinpru, B., Sroichak, T., Archa, P., Thongpea, S., Paengkoum, S., & Purba, R. A. P. (2022). Nutritional composition of black soldier fly larvae (*Hermetia illucens* L.) and its potential uses as alternative protein sources in animal diets: A review. *Insects*, *13*(9), 831.

Maxin, G., Ouellet, D. R., & Lapierre, H. (2013). Ruminal degradability of dry matter, crude protein, and amino acids in soybean meal, canola meal, corn, and wheat dried distillers grains. *Journal of Dairy Science*, *96*(8), 5151-5160. https://doi.org/ https://doi.org/10.3168/jds.2012-6392.

McCann, J. C., Luan, S., Cardoso, F. C., Derakhshani, H., Khafipour, E., & Loor, J. J. (2016). Induction of subacute ruminal acidosis affects the ruminal microbiome and epithelium. *Frontiers in Microbiology*, *7*, 701.

Nana, P., Kimpara, J. M., Tiambo, C. K., Tiogue, C. T., Youmbi, J., Choundong, B., & Fonkou, T. (2018). Black soldier flies (*Hermetia illucens* L.) as recyclers of organic waste and possible livestock feed. *International Journal of Biological and Chemical Sciences*, *12*(5), 2004-2015.

Ngo, D.-H., & Kim, S.-K. (2014a). Antioxidant effects of chitin, chitosan, and their derivatives. *Advances in food and nutrition research*, *73*, 15-31.

Ngo, D.-H., & Kim, S.-K. (2014b). Chapter Two - Antioxidant Effects of Chitin, Chitosan, and Their Derivatives. In S.-K. Kim (Ed.), *Advances in Food and Nutrition Research* (Vol. 73, pp. 15-31). Academic Press. https://doi.org/https://doi.org/ 10.1016/B978-0-12-800268-1.00002-0.

Nossa, C. W., Oberdorf, W. E., Yang, L., Aas, J. A., Paster, B. J., Desantis, T. Z., Brodie, E. L., Malamud, D., Poles, M. A., & Pei, Z. (2010). Design of 16S rRNA gene primers for

มหาวิทยาลัยเทคโนโลยีสุรนารี

454 pyrosequencing of the human foregut microbiome. *World J Gastroenterol*, *16*(33), 4135-4144. https://doi.org/10.3748/wjg.v16.i33.4135.

Nur Atikah, I., Alimon, A. R., Yaakub, H., Abdullah, N., Jahromi, M. F., Ivan, M., & Samsudin, A. A. (2018). Profiling of rumen fermentation, microbial population and digestibility in goats fed with dietary oils containing different fatty acids. *BMC Vet Res*, *14*(1), 344. https://doi.org/10.1186/s12917-018-1672-0.

Park, H. H. (2016). Black soldier fly larvae manual.

Patra, A. K. (2013). The effect of dietary fats on methane emissions, and its other effects on digestibility, rumen fermentation and lactation performance in cattle: A meta-analysis. *Livestock science*, *155*(2-3), 244-254.

Quintieri, L., Nitride, C., De Angelis, E., Lamonaca, A., Pilolli, R., Russo, F., & Monaci, L. (2023). Alternative Protein Sources and Novel Foods: Benefits, Food Applications and Safety Issues. *Nutrients*, *15*(6). https://doi.org/10.3390/ nu15061509.

Ravi, H. K., Guidou, C., Costil, J., Trespeuch, C., Chemat, F., & Vian, M. A. (2021). Novel insights on the sustainable wet mode fractionation of black soldier fly larvae (*Hermetia illucens*) into lipids, proteins and chitin. *Processes*, *9*(11), 1888.

Renna, M., Coppa, M., Lussiana, C., Le Morvan, A., Gasco, L., & Maxin, G. (2022). Full-fat insect meals in ruminant nutrition: in vitro rumen fermentation characteristics and lipid biohydrogenation. *Journal of animal science and biotechnology*, *13*(1), 138.

Schiavone, A., Dabbou, S., Petracci, M., Zampiga, M., Sirri, F., Biasato, I., Gai, F., & Gasco, L. (2019). Black soldier fly defatted meal as a dietary protein source for broiler chickens: Effects on carcass traits, breast meat quality and safety. *Animal*, *13*(10), 2397-2405.

Seyedalmoosavi, M. M., Mielenz, M., Veldkamp, T., Das, G., & Metges, C. C. (2022). Growth efficiency, intestinal biology, and nutrient utilization and requirements of black soldier fly (*Hermetia illucens*) larvae compared to monogastric livestock species: a review. *J Anim Sci Biotechnol*, *13*(1), 31. https://doi.org/ 10.1186/s40104-022-00682-7.

Shah, A. A., Totakul, P., Matra, M., Cherdthong, A., Hanboonsong, Y., & Wanapat, M. (2022). Nutritional composition of various insects and potential uses as alternative protein sources in animal diets. *Animal Bioscience*, *35*(2), 317.

มหาวิทยาลัยเทคโนโลยีสุรนารี

Shah, A. M., Qazi, I. H., Matra, M., & Wanapat, M. (2022). Role of Chitin and Chitosan in Ruminant Diets and Their Impact on Digestibility, Microbiota and Performance of Ruminants. *Fermentation*, *8*(10), 549.

Sheppard, D. C., Tomberlin, J. K., Joyce, J. A., Kiser, B. C., & Sumner, S. M. (2002). Rearing methods for the black soldier fly (Diptera: Stratiomyidae). *Journal of medical entomology*, *39*(4), 695-698.

Sogari, G., Amato, M., Biasato, I., Chiesa, S., & Gasco, L. (2019). The potential role of insects as feed: A multi-perspective review. *Animals*, *9*(4), 119.

Spranghers, T., Michiels, J., Vrancx, J., Ovyn, A., Eeckhout, M., De Clercq, P., & De Smet, S. (2018). Gut antimicrobial effects and nutritional value of black soldier fly (*Hermetia illucens* L.) prepupae for weaned piglets. *Animal Feed Science and Technology*, *235*, 33-42.

Spranghers, T., Noyez, A., Schildermans, K., & De Clercq, P. (2017). Cold hardiness of the black soldier fly (Diptera: Stratiomyidae). *Journal of economic entomology*, *110*(4), 1501-1507.

Statistics, F. G. F. International Feed Industry Federation; 2021. In.

Suong, N. T. M., Paengkoum, S., Schonewille, J. T., Purba, R. A. P., & Paengkoum, P. (2022). Growth performance, blood biochemical indices, rumen bacterial community, and carcass characteristics in goats fed anthocyanin-rich black cane silage. *Frontiers in Veterinary Science*, *9*, 880838.

Synowiecki, J., & Al-Khateeb, N. A. (2003). Production, properties, and some new applications of chitin and its derivatives.

Tabata, E., Kashimura, A., Kikuchi, A., Masuda, H., Miyahara, R., Hiruma, Y., Wakita, S., Ohno, M., Sakaguchi, M., & Sugahara, Y. (2018). Chitin digestibility is dependent on feeding behaviors, which determine acidic chitinase mRNA levels in mammalian and poultry stomachs. *Scientific Reports*, *8*(1), 1461.

Tahamtani, F. M., Ivarsson, E., Wiklicky, V., Lalander, C., Wall, H., Rodenburg, T. B., Tuyttens, F. A., & Hernandez, C. E. (2021). Feeding live Black Soldier Fly larvae (*Hermetia illucens*) to laying hens: effects on feed consumption, hen health, hen behavior, and egg quality. *Poultry Science*, *100*(10), 101400

Tian, X., Li, J.-X., Luo, Q.-Y., Wang, X., Xiao, M.-M., Zhou, D., Lu, Q., & Chen, X. (2022). Effect of supplementation with selenium-yeast on muscle antioxidant activity,

มหาวิทยาลัยเทคโนโลยีสุรนารี

meat quality, fatty acids and amino acids in goats. *Frontiers in veterinary science*, 1683.

Tian, X., Lu, Q., Paengkoum, P., & Paengkoum, S. (2020). Effect of purple corn pigment on change of anthocyanin composition and unsaturated fatty acids during milk storage. *Journal of dairy science*, *103*(9), 7808-7812.

Uyanga, V. A., Ejeromedoghene, O., Lambo, M. T., Alowakennu, M., Alli, Y. A., Ere-Richard, A. A., Min, L., Zhao, J., Wang, X., & Jiao, H. (2023). Chitosan and chitosan‐based composites as beneficial compounds for animal health: Impact on gastrointestinal functions and biocarrier application. *Journal of Functional Foods*, *104*, 105520.

Vargas, J. E., Andrés, S., López-Ferreras, L., Snelling, T. J., Yáñez-Ruíz, D. R., García-Estrada, C., & López, S. (2020). Dietary supplemental plant oils reduce methanogenesis from anaerobic microbial fermentation in the rumen. *Scientific Reports*, *10*(1), 1613.

Vendramini, T. H. A., Takiya, C. S., Silva, T., Zanferari, F., Rentas, M. F., Bertoni, J., Consentini, C. E. C., Gardinal, R., Acedo, T. S., & Rennó, F. P. (2016). Effects of a blend of essential oils, chitosan or monensin on nutrient intake and digestibility of lactating dairy cows. *Animal Feed Science and Technology*, *214*, 12-21.

Wu, G. D., Chen, J., Hoffmann, C., Bittinger, K., Chen, Y.-Y., Keilbaugh, S. A., Bewtra, M., Knights, D., Walters, W. A., & Knight, R. (2011). Linking long-term dietary patterns with gut microbial enterotypes. *Science*, *334*(6052), 105-108.

Yadav, S., & Jha, R. (2019). Strategies to modulate the intestinal microbiota and their effects on nutrient utilization, performance, and health of poultry. *Journal of animal science and biotechnology*, *10*(1), 1-11.

Zhang, H., & Zhang, H. (2019). Food Power in the Context of Sino-American Rivalry. *Securing the 'Rice Bowl' China and Global Food Security*, 207-233.

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.

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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.

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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°C 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°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.

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°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.

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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:

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

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

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

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

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

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.

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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.

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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°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.

4.4.5 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,

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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°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.

4.5 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

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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°C increased the RUP, while treatment at 140°C 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°C 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

Abdelrahman, M., Wang, W., Lv, H., Di, Z., An, Z., Lijun, W., Shaukat, A., Bo, W., Guangsheng, Z., & Liguo, Y. (2022). Evaluating the Effect of Forage Rape (*Brassica Napus*) Ensiling Kinetics on Degradability and Milk Performance as Non-Conventional Forage for Dairy Buffalo. *Frontiers in Veterinary Science*, *9*, 926906.

Antoniewicz, A., Van Vuuren, A., Van der Koelen, C., & Kosmala, I. (1992). Intestinal digestibility of rumen undegraded protein of formaldehyde-treated feedstuffs measured by mobile bag and in vitro technique. *Animal Feed Science and Technology*, *39*(1-2), 111-124.

Atole, A. F. F., & Bestil, L. C. (2014). Extrapolating Bypass Protein Potential of Treated Soybean Meal by Degradation in Rumen-Fistulated Brahman Cattle. *Annals of Tropical Research*, *36*(1), 50-62.

Aufrère, J., Graviou, D., Melcion, J.-P., & Demarquilly, C. (2001). Degradation in the rumen of lupin (*Lupinus albus* L.) and pea (*Pisum sativum* L.) seed proteins: Effect of heat treatment. *Animal Feed Science and Technology*, *92*(3-4), 215-236.

Bachmann, M., Kuhnitzsch, C., Thierbach, A., Michel, S., Bochnia, M., Greef, J., Martens, S., Steinhöfel, O., & Zeyner, A. (2020). Effects of toasting temperature and duration on in vitro ruminal gas production kinetics and post-ruminal crude

protein from field pea (*Pisum sativum*) legume grain silages. *Livestock science*, *233*, 103944.

Belverdy, M. S., Khadem, A. A., Alamouti, A. A., Khani, J., Calsamiglia, S., & Jacobs, J. (2021). Use of fat-coated or heat-treated soybean meal for partial replacement of solvent-extracted soybean meal in the diets of early lactation dairy cows. *Animal Production Science*.

Boucher, S., Calsamiglia, S., Parsons, C., Stern, M., Moreno, M. R., Vázquez-Añón, M., & Schwab, C. G. (2009). In vitro digestibility of individual amino acids in rumen-undegraded protein: The modified three-step procedure and the immobilized digestive enzyme assay. *Journal of Dairy Science*, *92*(8), 3939-3950.

Campbell, M., Ortuño, J., Stratakos, A. C., Linton, M., Corcionivoschi, N., Elliott, T., Koidis, A., & Theodoridou, K. (2020a). Impact of Thermal and High-Pressure Treatments on the Microbiological Quality and In Vitro Digestibility of Black Soldier Fly (*Hermetia illucens*) Larvae. *Animals*, *10*(4).

Campbell, M., Ortuño, J., Stratakos, A. C., Linton, M., Corcionivoschi, N., Elliott, T., Koidis, A., & Theodoridou, K. (2020b). Impact of thermal and high-pressure treatments on the microbiological quality and in vitro digestibility of black soldier fly (*Hermetia illucens*) larvae. *Animals*, *10*(4), 682.

Castro, S. B., Phillip, L., Lapierre, H., Jardon, P., & Berthiaume, R. (2007). Ruminal degradability and intestinal digestibility of protein and amino acids in treated soybean meal products. *Journal of Dairy Science*, *90*(2), 810-822.

Day, L., Cakebread, J. A., & Loveday, S. M. (2022). Food proteins from animals and plants: Differences in the nutritional and functional properties. *Trends in Food Science & Technology*, *119*, 428-442.

DONG, S.-z., Azarfar, A., Yang, Z., LI, S.-l., WANG, Y.-j., & CAO, Z.-j. (2017). Effects of sequence of nylon bags rumen incubation on kinetics of degradation in some commonly used feedstuffs in dairy rations. *Journal of Integrative Agriculture*, *16*(1), 162-168.

Ghimire, S. (2021). *The effect of black soldier fly (Hermetia illucens) larvae fractions in diets for Atlantic salmon (Salmo salar) on extruder parameters, pellet quality, growth performance and nutrient utilization* Norwegian University of Life Sciences, Ås].

Huang, X., Khan, N. A., Zhang, X., & Yu, P. (2015). Effects of canola meal pellet conditioning temperature and time on ruminal and intestinal digestion, hourly effective degradation ratio, and potential nitrogen to energy synchronization in dairy cows. *Journal of Dairy Science*, *98*(12), 8836-8845.

Huang, X., Wang, P., Xue, W., Cheng, J., Yang, F., Yu, D., & Shi, Y. (2023). Preparation of meaty flavor additive from soybean meal through the Maillard reaction. *Food Chemistry: X*, *19*, 100780.

Karlsson, L., Ruiz-Moreno, M., Stern, M., & Martinsson, K. (2012). Effects of temperature during moist heat treatment on ruminal degradability and intestinal digestibility of protein and amino acids in hempseed cake. *Asian-Australasian journal of animal sciences*, *25*(11), 1559.

Ljøkjel, K., Harstad, O. M., Prestløkken, E., & Skrede, A. (2003a). In situ digestibility of protein in barley grain (Hordeum vulgare) and peas (*Pisum sativum* L.) in dairy cows: influence of heat treatment and glucose addition. *Animal Feed Science and Technology*, *107*(1-4), 87-104.

Ljøkjel, K., Harstad, O. M., Prestløkken, E., & Skrede, A. (2003b). In situ digestibility of starch in barley grain (*Hordeum vulgare*) and peas (*Pisum sativum* L.) in dairy cows: influence of heat treatment and glucose addition. *Animal Feed Science and Technology*, *107*(1), 105-116. https://doi.org/https://doi.org/10.1016/S0377-8401(03)00122-6

Lu, S., Taethaisong, N., Meethip, W., Surakhunthod, J., Sinpru, B., Sroichak, T., Archa, P., Thongpea, S., Paengkoum, S., & Purba, R. A. P. (2022). Nutritional composition of black soldier fly larvae (*Hermetia illucens* L.) and its potential uses as alternative protein sources in animal diets: A review. *Insects*, *13*(9), 831.

Madsen, J., Hvelplund, T., & Weisbjerg, M. (1997). Appropriate methods for the evaluation of tropical feeds for ruminants. *Animal Feed Science and Technology*, *69*(1-3), 53-66.

Mantovani, H. C., Huws, S. A., Creevey, C. J., Oyama, L. B., Mizrahi, I., Denman, S. E., Popova, M., Muñoz-Tamayo, R., Forano, E., & Waters, S. M. (2019). Addressing global ruminant agricultural challenges through understanding the rumen microbiome: past, present, and future.

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Manzocchi, E., Ferlay, A., Mendowski, S., Chesneau, G., Chapoutot, P., Lemosquet, S., Cantalapiedra-Hijar, G., & Nozière, P. (2023). Extrusion of lupines with or without addition of reducing sugars: Effects on the formation of Maillard reaction compounds, partition of nitrogen and Nε-carboxymethyl-lysine, and performance of dairy cows. *Journal of Dairy Science*, *106*(11), 7675-7697.

Mupangwa, J., Ngongoni, N., Topps, J., Acamovic, T., & Hamudikuwanda, H. (2003). Rumen degradability and post-ruminal digestion of dry matter, nitrogen and amino acids in three tropical forage legumes estimated by the mobile nylon bag technique. *Livestock Production Science*, *79*(1), 37-46.

Ørskov, E.-R., & McDonald, I. (1979). The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. *The Journal of Agricultural Science*, *92*(2), 499-503.

Pieterse, E., Erasmus, S. W., Uushona, T., & Hoffman, L. C. (2019). Black soldier fly (*Hermetia illucens*) pre-pupae meal as a dietary protein source for broiler production ensures a tasty chicken with standard meat quality for every pot. *Journal of the Science of Food and Agriculture*, *99*(2), 893-903.

Prandi, B., Zurlini, C., Maria, C. I., Cutroneo, S., Di Massimo, M., Bondi, M., Brutti, A., Sforza, S., & Tedeschi, T. (2021). Targeting the nutritional value of proteins from legumes by-products through mild extraction technologies. *Frontiers in nutrition*, *8*, 695793.

Ravi, H. K., Degrou, A., Costil, J., Trespeuch, C., Chemat, F., & Vian, M. A. (2020). Effect of devitalization techniques on the lipid, protein, antioxidant, and chitin fractions of black soldier fly (*Hermetia illucens*) larvae. *European Food Research and Technology*, *246*, 2549-2568.

Scott, G., & Awika, J. M. (2023). Effect of protein–starch interactions on starch retrogradation and implications for food product quality. *Comprehensive Reviews in Food Science and Food Safety*, *22*(3), 2081-2111.

Stern, M., Santos, K., & Satter, L. (1985). Protein degradation in rumen and amino acid absorption in small intestine of lactating dairy cattle fed heat-treated whole soybeans. *Journal of Dairy Science*, *68*(1), 45-56.

Subuh, A., Rowan, T., & Lawrence, T. (1996). Effect of heat or formaldehyde treatment on the rumen degradability and intestinal tract apparent digestibility of protein

in soya-bean meal and in rapeseed meals of different glucosinolate content. *Animal Feed Science and Technology*, *57*(3), 257-265.

Tian, X., Li, J.-X., Luo, Q.-Y., Wang, X., Xiao, M.-M., Zhou, D., Lu, Q., & Chen, X. (2022). Effect of supplementation with selenium-yeast on muscle antioxidant activity, meat quality, fatty acids and amino acids in goats. *Frontiers in veterinary science*, 1683.

Tian, X., Lu, Q., Paengkoum, P., & Paengkoum, S. (2020). Effect of purple corn pigment on change of anthocyanin composition and unsaturated fatty acids during milk storage. *Journal of dairy science*, *103*(9), 7808-7812.

Traksele, L., Speiciene, V., Smicius, R., Alencikiene, G., Salaseviciene, A., Garmiene, G., Zigmantaite, V., Grigaleviciute, R., & Kucinskas, A. (2021). Investigation of in vitro and in vivo digestibility of black soldier fly (*Hermetia illucens* L.) larvae protein. *Journal of Functional Foods*, *79*, 104402. https://doi.org/https://doi.org/10.1016/j.jff.2021.104402.

Van Soest, P. v., Robertson, J. B., & Lewis, B. A. (1991). Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *Journal of Dairy Science*, *74*(10), 3583-3597.

Wang, J., Zhao, S., Min, G., Qiao, D., Zhang, B., Niu, M., Jia, C., Xu, Y., & Lin, Q. (2021). Starch-protein interplay varies the multi-scale structures of starch undergoing thermal processing. *International Journal of Biological Macromolecules*, *175*, 179-187.

Yang, H. W., Hsu, C. K., & Yang, Y. F. (2014). Effect of thermal treatments on anti-nutritional factors and antioxidant capabilities in yellow soybeans and green-cotyledon small black soybeans. *Journal of the Science of Food and Agriculture*, *94*(9), 1794-1801

CHAPTER V

EFFECTS OF HEAT TREAT BLACK SOLDIER FLY LARVAE (*Hermetia illucens* L.) ON GROWTH PERFORMANCE, SERUM ANTIOXIDANT AND IMMUNITY, RUMEN MICROORGANISMS, MEAT QUALITY, AND RELATED GENE EXPRESSION OF GOAT

5.1 Abstract

The purpose of this study was to evaluate the effects of heat treatment on black soldier fly (BSF) on serum antioxidants and immunity, rumen microorganisms, meat quality, and related gene expression in goats. Thirty goats (20.30 ± 1.09 kg) were randomly divided into three groups: the control group (FFS) supplemented with 10% full-fat soybean, treatment 1 (BSF) supplemented with 10% untreated BSF, and treatment 2 (HTBSF) supplemented with 10% heat-treated BSF. The results showed that there were no significant differences in growth performance among the three groups (P > 0.05). The NDF digestibility and NH3-N concentration of the HTBSF group were significantly higher than those of the FFS and BSF groups (P < 0.05). Overall, there were no significant differences in VFA among the three groups (P > 0.05). At 0 h, the serum levels of IgG, IgM, IL-6, IL-8, and IL-10 in the FFS and HTBSF groups were significantly higher than those in the BSF group (P < 0.05). However, at 4 h, the levels in the BSF group were significantly higher than those in the FFS and HTBSF groups (P < 0.05). The serum levels of CAT, GSH-Px, and T-AOC and muscle antioxidant levels in the FFS group were significantly higher than those in the BSF and HTBSF groups (P < 0.05). At the phylum level, the dominant phyla were *Bacteroidetes, Firmicutes, Proteobacteria, Spirochaetes,* and *Fibrobacteres*. The abundance of the phylum *Bacteroidetes* in the HTBSF group was significantly higher than that in the FFS and BSF groups (P < 0.05), while the abundance of the phylum *Firmicutes* was significantly lower than that in these two groups (P < 0.05). At the genus level, dominant genera were identified as *Prevotella, Rikenellaceae_RC9_gut_group, F082, Bacteroidales_ RF16_group,* and *Prevotellaceae_UCG_001*. Both the BSF and HTBSF groups increased

the abundance of *Prevotella*. There were no statistical differences in moisture, DM, CP, ash, AA, and minerals content of muscles among the three groups ($P > 0.05$). The slaughter rate, carcass weight, and overall meat quality decreased in the BSF and HTBSF groups ($P < 0.05$). The individual unsaturated fatty acid and total unsaturated fatty acid in the FFS and HTBSF groups were higher than those in the BSF group ($P < 0.05$). The mRNA expression levels of CAT, SOD, GPX-1, GPX-4, IL-6, IL-8, TNF-α, and IL-1β were significantly higher in the HTBSF group than in the FFS group ($P < 0.05$). The mRNA expression levels of SOD, GPX-4, IL-6, IL-8, and TNF-α were significantly higher in the BSF group than in the FFS group ($P < 0.05$). Additionally, the mRNA expression levels of SOD, GPX-4, IL-8, and TNF-α were significantly higher in the HTBSF group than in the BSF group ($P < 0.05$). The BSF and HTBSF groups had a negative impact on serum and muscle antioxidant levels and meat quality. However, the HTBSF group increased the abundance of the phylum *Bacteroidetes* and the content of unsaturated fatty acid in muscles, as well as enhancing the expression levels of antioxidant and immune mRNA.

Keywords: Antioxidant; black soldier fly; goats; mRNA expression; rumen microorganisms

5.2 Introduction

Due to the projected 76% increase in global demand for meat (Henchion et al., 2021), it is imperative to explore new methods for augmenting protein production while minimizing the utilization of natural resources to ensure food security within the carrying capacity of Earth (Mok et al., 2020; Sekaran et al., 2021). Hence, there is a particular need to identify additional protein-rich feed ingredients for livestock with less environmental impact (Taelman et al., 2015). In this context, insects have been explicitly recommended as sustainable protein sources for both human consumption and livestock feed (Churchward-Venne et al., 2017; Sogari et al., 2019b). However, in the livestock breeding industry, the use of insect powder is more readily accepted by consumers than direct human consumption, as in some societies, there is a certain level of aversion towards consuming insect powder (Tabata et al., 2017a). Currently,

insects such as BSF, crickets (Orthoptera: *Gryllidae*), and mealworm larvae (*Tenebrio molitor* L.) are being utilized as protein sources in animal feed (Lu Taethaisong Meethip Surakhunthod Sinpru Sroichak Archa Thongpea Paengkoum Purba et al., 2022). These three insects are rich in protein and essential amino acids (AA), minerals, fats, and a significant amount of saturated fatty acid (FA). Among other insect species, BSF stands out due to its excellent protein conversion rate (Wang and Shelomi, 2017). However, a high content of saturated fatty acid (SFA) may have adverse effects on the rumen fermentation, and meat quality of goats (Goetsch et al., 2011). Although the FA composition of ruminant tissues is less influenced by dietary lipid components compared to non-ruminant animals, differences in dietary FA composition can still lead to variations in tissue FA composition (Rhee et al., 2000). This difference primarily arises from the hydrogenation of dietary lipids by rumen microorganisms (Buccioni et al., 2012). However, n-3 and n-6 FA from feed can be incorporated into the adipose tissues and muscles of ruminant animals (Enser et al., 2003). Moreover, the levels of n-3 polyunsaturated fatty acid (PUFA) in ruminant tissues can be increased by feeding a diet rich in dietary lipids, which can be protected from rumen biohydrogenation through chemical or physical processing (Enser et al., 2003). Previous research on rapeseed indicated that heat treatment reduced the biohydrogenation of unsaturated fatty acid (UFA) in the rumen, which subsequently increased the proportion of undegraded protein in the rumen (AMH Subuh et al., 1996). In a study by (Enser et al., 2003), feeding a protected lipid supplement is composed of soybean, flaxseed, and sunflower seeds. It resulted in a slight increase in the concentration of 18:3 n-3 in muscle phospholipids, rising from 12.7 mg/100 g to 16.0 mg/100 g, but there was no occurrence of chain elongation or desaturation of long-chain n-3 PUFA. Simultaneously, feeding heat-treated protein supplements can also enhance the tenderness of meat and intramuscular fat (Hao et al., 2019). Hemp seed cake was treated by (L. Karlsson et al., 2012) at temperatures of 110°C, 120°C, and 130°C. Compared to the control group, the RUP increased from 259 g/kg to 629 g/kg after treatment at 130°C, while the small intestine digestibility rate also increased from 176 g/kg RUP to 730 g/kg RUP. The research conducted by (Krizsan et al., 2017) indicated that heat-treated rapeseed meal promotes the production of rumen volatile fatty acid (VFA) and NH_3-N in cows, and increases the intake of dry matter (DM), neutral detergent

fiber (NDF), and organic matter (OM). We hypothesized that heat-treated BSF could yield similar results. This experiment represents the final stage of a series of three trials. Earlier, we supplemented goat diets with varying levels of BSF and investigated the effects of different temperatures on BSF rumen degradation and protein digestion in the small intestine. From these preliminary trials, we determined suitable supplementation levels and treatment temperatures. Based on this, we conducted the current experiment.

Guizhou Province is located in the eastern part of the Yunnan-Guizhou Plateau in southwestern China, there is very lack of protein feed sources. Therefore, looking for new protein feed sources was very important. The cultivation of BSF does not require extensive land, significant labor costs, or time investments, making it particularly suitable for development in countries and regions with limited arable land. The Qianbei Ma Goat (the goats raised in the north of Guizhou Province) is known for its excellent meat quality, with mild odor and tender texture (Tian Li et al., 2022a). Currently, it is not clear whether BSF has any negative impact on the meat quality of the goat. Therefore, this study investigated the growth performance, serum antioxidant and immunity, rumen microorganisms, meat quality, and related gene expression of goats. At the same time, it is also the first time to evaluate the impact of BSF on ruminant meat quality, providing data support for the application of BSF in goats.

5.3 Materials and methods

Ethical permission for this study was obtained from the Experimental Animal Ethics Committee of Guizhou University (protocol number EAE-GZU-2021-E024).

5.3.1 Black soldier fly

Black soldier fly purchased from farmers in Henan Province, China, was ground and sieved through a 1 mm sieve to prepare the experimental powder. The chemical composition and minerals of BSF are listed in Table 5.1. The amino acid and fatty acid levels of full-fat soybean and black soldier fly are shown in Table 5.2 and Table 5.3 respectively.

Table 5.1 The proximate composition of black soldier fly (DM basis, %).

Items	Contents
DM	97.28 ± 0.22
OM	87.74 ± 0.99
CP	35.85 ± 0.32
EE	37.65 ± 0.81
Ca, g/kg	45.40 ± 0.78
Cu, mg/kg	13.20 ± 0.45
Fe, mg/kg	829.00 ± 40.55
K, g/kg	14.53 ± 0.48
Mg, g/kg	3.84 ± 0.28
Mn, mg/kg	121.00 ± 6.98
Na, g/kg	3.09 ± 0.11
P, g/kg	8.44 ± 0.38
Se, mg/kg	0.50 ± 0.01
Zn, mg/kg	79.53 ± 0.77

DM = dry matter, CP = crude protein, EE = ether extract. Values represent the means of twelve replicates (n = 3), mean ± se.

Table 5.2 The amino acid content of full-fat soybeans and black soldier fly (g/100g).

Items	Full-fat soybeans	Black soldier fly
Indispensable amino acids		
Arginine	2.54 ± 0.01	1.37 ± 0.00
Histidine	0.37 ± 0.00	0.21 ± 0.00
Isoleucine	1.03 ±0.00	0.71 ± 0.00
Leucine	2.68 ±0.02	1.80 ± 0.01
Lysine	2.04 ±0.02	1.42 ± 0.00
Methionine	0.03 ± 0.00	0.10 ± 0.00
Phenylalanine	1.85 ±0.01	2.99 ± 0.00
Threonine	1.34 ±0.01	1.00 ± 0.00
Valine	1.07 ±0.01	1.09 ± 0.00
Dispensable amino acids		
Alanine	1.67 ±0.02	2.00 ± 0.01
Aspartic acid	4.47 ±0.06	2.65 ± 0.01
Glycine	1.61 ±0.00	1.52 ± 0.01
Glutamic acid	7.24 ±0.03	3.89 ± 0.01
Proline	1.70 ± 0.03	1.49 ± 0.03
Serine	2.05 ±0.01	1.33 ± 0.00
Tyrosine	1.10 ±0.01	1.39 ± 0.02

Values represent the means of twelve replicates (n = 3), mean ± se.

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Table 5.3 The fatty acid content of full-fat soybeans and black soldier fly (g/100g).

Items	Full-fat soybeans	Black soldier fly
C6:0	-	0.11 ± 0.00
C8:0	-	0.03 ± 0.00
C10:0	-	0.74 ± 0.00
C12:0	-	18.99 ± 0.03
C14:0	0.07 ± 0.00	3.66 ± 0.01
C14:1	-	0.07 ± 0.00
C15:0	-	0.13 ± 0.00
C16:0	10.08 ± 0.00	18.00 ± 0.01
C16:1	0.08 ± 0.00	1.90 ±0.00
C17:0	0.08 ± 0.00	0.42 ± 0.00
C17:1	0.04 ± 0.00	0.15 ± 0.00
C18:0	3.45 ± 0.00	3.88 ± 0.01
C18:1n9t	0.24 ± 0.00	0.39 ± 0.00
C18:1n9c	26.24 ± 0.00	26.80 ± 0.03
C18:2n6t	-	0.09 ± 0.00
C18:2n6c	47.83 ± 0.00	18.95 ± 0.03
C20:0	4.44 ± 0.00	2.03 ± 0.01
C18:3n6	0.02 ± 0.00	0.12 ± 0.00
C20:1n9	0.20 ± 0.00	0.20 ± 0.00
C18:3n3	6.03 ± 0.00	2.15 ± 0.00
C21:0	0.52 ± 0.00	0.13 ± 0.00
C20:2	0.41 ± 0.00	0.06 ± 0.00
C22:0	0.03 ± 0.00	0.15 ± 0.00
C20:3n6	-	0.02 ± 0.00
C20:3n3	0.05 ± 0.00	-
C20:4n6	-	0.20 ± 0.00
C23:0	-	0.02 ± 0.00
C22:2	0.15 ± 0.00	0.02 ± 0.00
C24:0	0.03 ± 0.00	0.20 ± 0.00
C20:5n3	0.00 ± 0.00	0.38 ± 0.00
C24:1n9	-	0.19 ± 0.16
SFA	18.71 ± 0.02	48.48 ± 0.05
UFA	81.29 ± 0.04	51.52 ± 0.03
n-3 PUFA	6.07 ± 0.01	2.52 ± 0.01
n-6 PUFA	47.85 ± 0.03	19.38 ± 0.02
n-3/ n-6	0.13 ± 0.00	0.13 ± 0.01

PUFA = polyunsaturated fatty acid, UFA = unsaturated fatty acid, SFA = saturated fatty acid. Values represent the means of twelve replicates (n = 3), mean ± se.

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5.3.2 Animals, Diets, and Experimental Design

This study was conducted at Fuxing Husbandry Co., Ltd., Guizhou, China (106.198244 E, 28.26403 N). After acclimatizing animals for 14 days, we tested them for the following 60 days. Thirty Qianbei Ma goats (20.30 ± 1.09 kg) were randomly divided into three groups, and all of goats were fed individually. The control group (FFS) was supplemented with 10% full-fat soybean, treatment 1 (BSF) was supplemented with 10% untreated BSF, and treatment 2 (HTBSF) was supplemented with 10% heat-treated BSF. Before formulating the feed, cassava was mixed with either FFS or BSF in a 95:5 ratio. For the heat-treated group, an additional 75% water was added. The mixture was vigorously kneaded, then firmly pressed evenly into a heated square pan (100 cm × 55 cm × 6.5 cm) to a thickness of about 5 cm. It was then treated in an oven at 140°C for 120 min. The nutritional requirements for goats should be formulated according to NRC2004, as detailed in Table 5.4. Equal amounts of feed were offered daily at 09: 00 and 17: 00 for ad libitum water and intake, and 10% refusals on an as-fed basis. Amino acid (AA) and fatty acid (FA) were detailed respectively in Table 5.5 and Table 5.6.

Table 5.4 Concentrate supplement formula and chemical composition (DM basis, %).

Item	FFS	BSF	HTBSF
Corn	64.0	64.0	64.0
Soybean meal	10.0	10.0	10.0
FFS	10.0	-	-
BSF	-	10.0	-
HTBSF	-	-	10.0
Wheat bran	10.0	10.0	10.0
Ca (H2PO4)2	1.0	1.0	1.0
NaCl	1.0	1.0	1.0
Premix[1]	4.0	4.0	4.0
Total	100.0	100.0	100.0
Chemical composition, %			
DM	94.79	95.19	95.19
OM	92.60	91	90.46

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Table 5.4 (Continue).

Item	FFS	BSF	HTBSF
CP	16.10	16.19	16.09
EE	5.50	5.50	5.50
Ca	0.92	0.95	0.95
P	0.36	0.37	0.37
NDF	18.27	19.57	21.35
ADF	6.63	7.25	7.41
GE (KJ/g)	16.96	16.53	16.63

[1] The vitamin-mineral premix was purchased from the Feed Division of Beijing Sanyuan Seed Industry Technology Co., Ltd. (Beijing, China), containing the following per kg: 100000 IU of VA, 400 IU of VE, 11% of Ca, 1500 mg/kg of Mg, 50 mg/kg of Cu, 1500 mg/kg of Zn, 1100 mg/kg of Mn, 20 mg/kg of P, 5 mg/kg of Se, 8 mg/kg of Co.

ADF = acid detergent fiber, BSF = black soldier fly, CP = crude protein, DM = dry matter, EE = ether extract, NDF = Neutral detergent fiber, BSF = black soldier fly, FFS = full-fat soybeans, HTBSF = heat-treated black soldier fly.

Table 5.5 AA composition of concentrate.

Item (mg/100 g)	FFS	BSF	HTBSF
Indispensable amino acids			
Arginine	0.44 ± 0.01	0.64 ± 0.01	0.91 ± 0.01
Histidine	-	-	-
Isoleucine	0.10 ± 0.00	0.20 ± 0.00	0.31 ± 0.00
Leucine	0.89 ± 0.00	1.10 ± 0.01	1.21 ± 0.00
Lysine	-	0.11 ± 0.01	0.47 ± 0.01
Methionine	-	-	-
Phenylalanine	0.13 ± 0.04	0.45 ± 0.01	0.63 ± 0.00
Threonine	0.21 ± 0.00	0.32 ± 0.01	0.47 ± 0.00
Valine	0.13 ± 0.00	0.25 ± 0.00	0.39 ± 0.00

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Table 5.5 (Continue).

Item (mg/100 g)	FFS	BSF	HTBSF
Dispensable amino acids			
Alanine	0.65 ± 0.01	0.81 ± 0.01	0.87 ± 0.00
Aspartic acid	0.74 ± 0.00	1.08 ± 0.01	1.54 ± 0.00
Glycine	0.39 ± 0.00	0.56 ± 0.00	0.70 ± 0.00
Glutamic acid	2.05 ± 0.01	2.62 ± 0.01	3.00 ± 0.01
Proline	0.72 ± 0.01	0.79 ± 0.04	0.91 ± 0.00
Serine	0.40 ± 0.00	0.57 ± 0.00	0.75 ± 0.00
Tyrosine	-	0.07 ± 0.00	0.25 ± 0.00

Values represent the means of twelve replicates (n = 3), mean ± se. BSF = black soldier fly, FFS = full-fat soybeans, HTBSF = heat-treated black soldier fly.

Table 5.6 Fatty acid composition of concentrate.

Item (mg/100 g)	FFS	BSF	HTBSF
C6:0	-	0.23 ± 0.00	0.22 ± 0.00
C8:0	-	-	0.08 ± 0.00
C10:0	-	0.74 ± 0.01	0.68 ± 0.00
C12:0	-	16.32 ± 0.01	15.51 ± 0.01
C14:0	0.08 ± 0.00	2.57 ± 0.01	2.45 ± 0.01
C14:1	-	0.05 ± 0.00	-
C15:0	-	0.09 ± 0.00	0.09 ± 0.00
C16:0	13.93 ± 0.01	16.82 ± 0.01	17.41 ± 0.01
C16:1	0.14 ± 0.00	1.17 ± 0.00	1.03 ± 0.00
C17:0	0.09 ± 0.00	0.23 ± 0.00	0.23 ± 0.00
C18:0	2.93 ± 0.01	2.80 ± 0.01	2.99 ± 0.01
C18:1n9t	0.18 ± 0.00	0.18 ± 0.00	0.19 ± 0.00
C18:1n9c	30.71 ± 0.02	27.31 ± 0.01	29.10 ± 0.01
C18:2n6c	47.21 ± 0.02	28.84 ± 0.02	27.46 ± 0.01
C20:0	0.60 ± 0.00	0.34 ± 0.00	0.40 ± 0.00

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Table 5.6 (Continue).

Item (mg/100 g)	FFS	BSF	HTBSF
C18:3n6	0.29 ± 0.00	0.18 ± 0.00	0.20 ± 0.00
C20:1n9	-	-	0.31 ± 0.00
C18:3n3	2.87 ± 0.00	1.50 ± 0.00	1.07 ± 0.00
C21:0	0.21 ± 0.00	-	-
C20:2	0.31 ± 0.00	0.11 ± 0.00	0.13 ± 0.00
C20:4n6	-	0.07 ± 0.00	-
C22:2	0.22 ± 0.00	0.11 ± 0.00	0.13 ± 0.00
C24:0	0.12 ± 0.00	0.18 ± 0.00	0.17 ± 0.00
C20:5n3	0.10 ± 0.00	0.17 ± 0.00	0.15 ± 0.00
SFA	17.96 ± 0.01	40.32 ± 0.02	40.22 ± 0.02
UFA	82.04 ± 0.03	59.68 ± 0.02	59.78 ± 0.02
PUFA/SFA	2.84 ± 0.01	0.72 ± 0.00	0.72 ± 0.00
n-3 PUFA	2.97 ± 0.01	1.66 ± 0.00	1.22 ± 0.00
n-6 PUFA	47.51 ± 0.03	29.09 ± 0.01	27.66 ± 0.01
n-3/ n-6	0.06 ± 0.00	0.06 ± 0.00	0.04 ± 0.00

PUFA = polyunsaturated fatty acid, SFA = saturated fatty acid, UFA = unsaturated fatty acid. Values represent the means of twelve replicates (n = 3), mean ± se. BSF = black soldier fly, FFS = full-fat soybeans, HTBSF = heat-treated black soldier fly.

5.3.3 Chemical Composition

200 g of feed was collected every week, dried in a 65°C oven for 72 h, combined at the end of the experiment, ground, and passed through a 1mm sieve. During the last 7 days of the experiment, feces were collected, dried in a 65°C oven for 72 h ground, passed through a 1mm sieve, and stored at 4°C until further analysis. Additionally, the *longissimus thoracis and lumborum* (LTL) was collected and lyophilized. BSF and diet were analyzed for DM, CP, EE, NDF, ADF, and ash according to the methods of (AOAC, 2005). Mineral content in BSF was analyzed according to the methods of (AOAC, 2005). Gross energy (GE) was determined using an adiabatic cartridge calorimeter (WGR-WR3, Changsha Benyi Instrument Co., Ltd., Changsha, China). Each sample was assayed in triplicate.

5.3.4 Growth Performance

During the entire study period, the daily dry matter intake (DMI) was calculated. On the 1st and 60th days of the experiment, the fasting morning weights of each goat were measured. According to (Tian Li et al., 2022b), growth performance was calculated using the following formulas: Body weight change (BWC) (kg) = Final weight (kg) - Initial weight (kg); Average daily gain (ADG) (g/d) = total weight gain (kg)/60/1000; Feed conversion ratio (FCR) = DMI (g/d)/ADG (g/d).

5.3.5 Serum sample collection and analysis

On the 60th day of the experiment, blood samples were collected in the morning after fasting and 4 h post-feeding. The samples were 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 and immune-inflammatory parameters. Serum total antioxidant capacity (T-AOC), blood urea nitrogen (BUN), 1,1-diphenyl-2-picrylhydrazyl (DPPH), glutathione peroxidase (GSH-Px), malondialdehyde (MDA), superoxide dismutase (SOD), and catalase (CAT) levels were determined according to the manufacturer's protocol using commercial assay kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). Enzyme-linked immunosorbent assay (ELISA) kits (MEIMIAN, Jiangsu Meimian Industrial Co., Ltd., Jiangsu) were used to detect serum immunoglobulin A (IgA), IgG, and IgM, tumor necrosis factor-alpha (TNF-α), interleukin 6 (IL-6), IL-8, IL-10, and IL-1β.

5.3.6 Rumen fermentation parameters

Ruminal fluid will be sampled on the last day of the experiment using a stomach tube connected to a vacuum pump at 0 h and 4 h post-feeding. Rumen fluid samples will be promptly measured for pH using a portable pH meter (Mettler Five Easy Plus Series, Columbus, OH, USA) and then mixture was then filtered through four layers of coarse cotton cloth and divided into three portions. One portion involved mixing 5 mL of rumen fluid with 1 mL of 15% metaphosphoric acid were mixed, store at −20°C, and analyze volatile fatty acid (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 second portion was analyzed for ammonia nitrogen (NH_3-N) using a Kjeldahl nitrogen analyzer following the methodology described by (Nur Atikah et al., 2018). While the third portion was used for 16S rRNA analysis. All samples were stored at -80°C.

5.3.7 DNA extraction and PCR amplification

The MagPure Soil DNA LQ Kit (Magan) was used to extract genomic DNA from samples, following the kit instructions. DNA content and purity were determined using NanoDrop 2000 (Thermo Fisher Scientific, USA) and agarose gel electrophoresis before being kept at -20°C. The isolated genomic DNA served as a template for PCR amplification of bacterial 16S rRNA genes. To specifically target the V3-V4 variable region of the 16S rRNA gene, universal primers 343F (5'-TACGRAGGCAGCAG-3') and 798R (5'-AGGGTATCTAATCCT-3') were employed (Nossa et al., 2010), followed by bacterial primer diversity analysis.

5.3.8 Bioinformatics analysis

Shanghai OE Biomedical Technology Co., Ltd. completed the library's creation, sequencing, and data analysis. Following data collection, DADA2 was used to quality filter, merge, and delete chimeric sequences on qualifying paired-end raw data to create representative Data sequences and ASV abundance tables, which were used as the default parameters of QIIME 2 (2020.11) (Bolyen et al., 2019). After identifying representative sequences for each ASV with the QIIME 2 software suite, all representative sequences were aligned and annotated against the silva database (version 138). The q2-feature-classifier software was used with its default defaults to accomplish species alignment annotation. QIIME 2 software was used to perform an alpha and beta diversity study. Sample alpha diversity was examined using measures such as the Chao1 index (Chao and Bunge, 2002) and the Shannon index (Hill et al., 2003). To measure sample beta diversity, unweighted UniFrac principal coordinate analysis (PCoA) was used with the unweighted UniFrac distance matrix produced in R. The ANOVA statistical approach, which is included in the R package, was used to analyze differences. Difference study of species abundance distributions with LEfSe.

5.3.9 Slaughtering procedure, carcass dissection, and meat sampling

Slaughter and dissection procedures were conducted according to the Livestock and Poultry Slaughter Operation Regulations (Goat) (NY/T 3469-2019). Six randomly selected goats were slaughtered in each group after a 12 fasting period and weighed before slaughter. The LTL muscle was separated from each carcass for meat quality testing. The dressing percentage (%) was calculated as (carcass weight/live weight before slaughter) × 100. The pH of the LTL muscle was measured at 45 miand 24 h post-mortem (maintained at 4°C) using a pH meter (Matthäus, Eckelsheim, Germany) that had been calibrated with standard pH 4.0 and 7.0 buffers. Meat color attributes, including lightness (L*), redness (a*), and yellowness (b*), were assessed using a portable meat colorimeter (NR110, Qinyou Science and Technology Development Co., Ltd., Chongqing, China). The colorimeter was pre-calibrated with a manual white calibration plate provided by the manufacturer, with an illuminance level of D65, an 8-degree standard viewer, and an aperture size of 4.0 mm for the closed candle. To determine water loss, fresh meat samples were cut and weighed after 45 min from slaughter (W3). They were then covered with 17 layers of filter paper and compressed using a meat press (Tenovo Meat-1; Tenovo International Co., Limited Beijing, China). The samples were then reweighed (W4), water loss (%) = [(W3 - W4) / W3] × 100%. Cooking loss, 50 g of the sample (W5) was weighed after removal of tendons, fascia or fat. The sample was then boiled in water for 30 min, cooled for 30 min and reweighed (W6), cooking loss (%) = [(W5 - W6) / W5] × 100%. Water holding capacity (WHC), samples 2.5 cm in diameter and 1 cm thick were cut and weighed (W7). The samples were then pressed under a 35 kg weight for 5 min and reweighed (W8), WHC (%) = [(W7 - W8) / W7] × 100. Longitudinal sections of 1×1×3 cm parallel to the muscle fibres was cut and the shear force (in Newtons) was measured with a digital flexometer (Harbin Xeriki Co., Ltd., Harbin, China). Each sample was tested in triplicate.

5.3.10 Amino acid (AA) analysis

The preprocessing of BSF AA analysis followed the method outlined by (Tian et al., 2021). The UHPLC conditions were as follows: individual AA were isolated on an ACQUITY UPLC BEH C18 column (2.1 × 100 mm × 1.7 µm, Waters, Milford, USA) at 40°C with an injection volume of 5 µL. The mobile phases were A = 10% methanol (containing 0.1% formic acid) & B = 50% of methanol (with 0.1% formic acid). B = 50%

of methanol (with 0.1% formic acid). The elution conditions were as follows: 0 to 6.5 min, 10 to 30% B; 6.5 to 7 min, 30 to 100% B; 7 to 8 min, 100% B; 8 to 8.5 min, 10 to 100% B; 8.5 to 12.5 min, 10% B: 0 to 8.5 min, 0.3 ml/min; 8.5 to 12.5 min, 0.3 to 0.4 ml/min. The mass spectral conditions were as follows: the electrospray ion source, positive mode of ions ionisation; the ion source temperature was 500℃; the source voltage was 5,500 V; the gas pressure for the impact gas was 6 psi, the gas pressure for the curtain gas was 30 psi; the gas pressure for the atomisation gas and the pressure for the auxiliary gas was 50 psi; and the scan mode for multi-reaction monitoring was used.

5.3.11 Fatty acid (FA) profiles analysis

The analysis of FAs was conducted according to method by (XZ Tian et al., 2020), FAs from both BSF and FFS were extracted using a chloroform-methanol solution. The procedure was as follows: approximately 50 mg of 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 ($C_{36}H_{68}O_6$, 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). Instrumental conditions: capillary column: polydicyanopropylsiloxane strong polar stationary phase, column length 100 m, inner diameter 0.25 mm, film thickness 0.2 μm; injector temperature: 270℃; detector temperature: 280℃; program temperature: the initial temperature of 100℃, continued for 13min; 100℃ - 180℃, the heating rate of 10℃/min, keep 6min; 180℃ - 200℃, heating rate 1℃/min, keep 20 min; 200℃ - 230℃, the heating rate of 4℃/min, keep 10.5 min. 200℃, heating rate 1℃/min, keep 20 min; 200℃-230℃, heating rate 4℃/min, keep 10.5 min. Carrier gas: nitrogen. Shunt ratio: 100:1. Sample volume: 1.0 μL. The assay conditions should meet the theoretical plate number (n) of at least 2000 /m and the separation degree (R) of at least 1.25.

5.3.12 Gene expression

Approximately 10 grams of the longest loin muscle from each goat were immediately collected and preserved in liquid nitrogen for further analysis using RT-PCR. Total RNA was extracted from meat using (Cat. No. G3013; Wuhan Servicebio Technology Co., Ltd., Wuhan, China) according to the manufacturer's specifications. The yield of RNA was determined using a NanoDrop 2000 spectrophotometer (Thermo Scientific, USA), and the integrity was evaluated using agarose gel electrophoresis stained with ethidium bromide.

5.3.13 Real-time quantitative (RT-PCR)

The quantification was carried out through a two-step reaction process: reverse transcription (RT) and PCR. Each RT reaction included 0.5 μg of RNA, 2 μL of 5× TransScript All-in-one SuperMix for qPCR, and 0.5 μL of gDNA Remover, with a total volume of 10 μL. The reaction was conducted in a GeneAmp® PCR System 9700 (Applied Biosystems, USA) at 42°C for 15 min, followed by 85°C for 5 seconds. Subsequently, the 10 μL RT reaction mixture was diluted tenfold with nuclease-free water and stored at -20°C. Real-time PCR was performed using a LightCycler® 480 II real-time PCR instrument (Roche, Switzerland), with PCR reaction mixture consisting of 1 μL of cDNA, 5 μL of 2×PerfectStartTM Green qPCR SuperMix, 0.2 μL of forward primer, 0.2 μL of reverse primer, and 3.6 μL of nuclease-free H2O. The reaction was incubated at 94°C for 30 seconds, followed by 45 cycles of 94°C for 5 seconds and 60°C for 30 seconds in a 384-well optical plate (Roche, Switzerland). Each sample was analyzed in triplicate. The expression levels of mRNAs were normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and the primer sequences are derived from NCBI (https://www.ncbi.nlm.nih.gov).

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Target gene	Primer sequence (5'-3')	Accession number	Product size(bp)
GAPDH	F: TACTGGCAAAGTGGACATCG	NM_001190390.1	136
	R: GATGACGAGCTTCCCGTTCT		
GPX-1	F: CGGCTTCCCGTGCAACCAGT	XM_005695962.3	262
	R: GGAGACGTCGTTGCGGCACA		
GPX- 4	F: GGGCCACGTGTGCATCGTCA	GU131344.2	300
	R: GCGTTTCCCAGCATGCCCCT		
CAT	F: CGAGGGCCCCTTCTCGTCCA	GQ204786.1	255
	R: GCCACGAGGGTCACGCACTG		
SOD	F: GCTGTACCAGTGCAGGCCCTC	NM_001285550.1	191
	R: GACCACCATCGTGCGGCCAA		
IL-1β-	F: GACGCAGCCGTGCAGTCAGT	XM_013967700.2	253
	R: CCTCCAGCTGCAGTGTCGGC		
IL-6	F: CTCGCTGTCTCCCTGGGGCT	NM_001285640.1	227
	R: TCTGCCAGTGTCTCCTTGCTGT		
IL-8-	F: TCCTGCTCTCTGCAGCTCTGTGT	XM_005681749.3	234
	R: CCTGCACAACCTTCTGCACCCA		
IL-10	F: CCACTTCCCAGCCAGCCTGC	DQ837159.1	272
	GCAGCCGGAGGGTCTTCAGC		
TNF-α	F: ATGCCCTCAAGGCCAACGGC	XM_005696606.3	228
	R: CAGCCCCCTCTGGGGTCTCC		

5.3.14 Statistical analysis

The data were analyzed through one-way ANOVA with SPSS statistical software (Version 27.0 for Windows; SPSS, Chicago, IL, USA). Duncan's multiple-range test was used to find statistically significant differences. The data were shown as the mean and standard error of the mean (SEM). The significance level was set to $P < 0.05$.

5.4 Results

5.4.1 Growth performance

There was no significant difference ($P > 0.05$) in growth performance among the three groups (Table 5.7); however, the HTBSF showed an increased trend in BWC and ADG.

Table 5.7 Growth performance.

Items	FFS	BSF	HTBSF	SEM	p-Value
DMI, g/d	762.78	761.96	762.74	8.27	0.10
Initial weight,	20.65	19.92	20.33	0.20	0.33
kg	24.36	23.76	24.39	0.35	0.75
Final weight, kg	4.08	4.08	4.57	0.27	0.70
BWC, kg	69.17	69.15	77.50	4.60	0.70
ADG, g	11.17	11.46	10.85	0.43	0.86
FCR					

ADG = average weight gain, BWC = body weight change, DMI = dry matter intake, FCR = feed conversion rate. BSF = black soldier fly, FFS = full-fat soybeans, HTBSF = heat-treated black soldier fly.

Different letters within a row are significantly different ($P < 0.05$).

5.4.2 Apparent digestibility

The apparent digestibility rate of NDF in the HTBSF group was significantly higher ($P < 0.05$) than that of the FFS and BSF groups (Table 5.8), while there were no significant differences ($P > 0.05$) in other apparent digestion parameters among the three groups.

Table 5.8 Apparent digestibility (%).

Items	FFS	BSF	HTBSF	SEM	p-Value
DM	65.93	65.53	65.98	1.56	0.16
OM	71.30	70.82	70.94	1.40	0.12
CP	76.01	75.39	75.27	1.13	0.17
NDF	41.22^{b}	40.43^{b}	44.40^{a}	0.60	< 0.01
ADF	32.13	29.77	32.86	0.63	0.11
GE	68.96	68.98	69.32	1.08	0.10

ADF = acid detergent fiber, CP = crude protein, DM = dry matter, OMD = Organic matter, EE = ether extract, GE = gross energy, NDF = neutral detergent fiber. BSF = black soldier fly, FFS = full-fat soybeans, HTBSF = heat-treated black soldier fly.

Different letters within a row are significantly different ($P < 0.05$).

5.4.3 PH, NH_3-N and BUN

At 0 h, the rumen pH of the HTBSF group was significantly higher ($P < 0.05$) than that of the FFS group (Table 5.9); at 4 h, there were no differences ($P > 0.05$) among the three groups. However, the NH_3-N levels in the 0 h BSF group were significantly higher ($P < 0.05$) than those in the FFS group; at 4 h, NH_3-N levels in the HTBSF group were significantly higher ($P < 0.05$) than those in the FFS and BSF groups. Additionally, BUN levels in the BSF group were significantly higher ($P < 0.05$) than those in the FFS group.

Table 5.9 pH, NH_3-N and BUN.

Items	FFS	BSF	HTBSF	SEM	p-Value
pH					
0-h	6.79^{b}	6.94^{ab}	7.28^{a}	0.04	0.01
4-h	6.48	6.45	6.82	0.05	0.85
NH_3-N, mg/dl					
0-h	7.06^{b}	9.42^{a}	7.93^{ab}	0.43	0.03
4-h	9.38^{b}	9.19^{b}	13.85^{a}	0.79	0.01
BUN, mmol/L					
0- h	4.65^{b}	5.61^{a}	5.12^{ab}	0.17	0.02
4- h	5.97^{b}	7.02^{a}	6.57^{ab}	0.19	0.03

BUN = blood urea nitrogen. BSF = black soldier fly, FFS = full-fat soybeans, HTBSF = heat-treated black soldier fly.

Different letters within a row are significantly different ($P < 0.05$).

5.4.4 VFAs

Overall, there were no differences ($P > 0.05$) in VFA levels among the three groups (Table 5.10). However, isobutyric acid and isovaleric acid levels in the BSF and HTBSF groups were significantly higher ($P < 0.05$) than those in the FFS group at 4 h.

Table 5.10 VFA.

Items	FFS	BSF	HTBSF	SEM	p-Value
Acetic acid, %					
0-h	50.28	48.52	50.96	0.55	0.17
4-h	50.86	49.37	51.01	0.38	0.15
Propionic acid, %					
0-h	26.67	26.59	26.03	0.16	0.22
4-h	29.49	29.20	28.34	0.30	0.29
Butyric acid, %					
0-h	14.26	14.50	14.38	0.18	0.88
4-h	13.59	13.68	13.61	0.17	0.98
Isobutyric acid, %					
0-h	2.33	2.98	2.74	0.12	0.08
4-h	1.49[b]	2.28[a]	2.18[a]	0.11	< 0.01
Isovaleric acid, %					
0-h	3.99	4.64	3.88	0.21	0.30
4-h	1.71[b]	2.30[a]	2.16[a]	0.10	0.04
Valeric acid, %					
0-h	2.47	2.77	1.99	0.16	0.12
4-h	2.85	3.18	2.70	0.11	0.21
A/ P, %					
0-h	1.89	1.83	1.96	0.02	0.10
4-h	1.73	1.69	1.80	0.03	0.23
Total VFA, mmol/L					
0-h	83.92	88.68	83.52	1.61	0.37
4-h	95.043	97.80	97.17	1.34	0.70

A/ P = acetic acid/ propionic acid. BSF = black soldier fly, FFS = full-fat soybeans, HTBSF = heat-treated black soldier fly.

Different letters within a row are significantly different ($P < 0.05$).

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5.4.5 Serum immune parameters

There were no significant differences ($P > 0.05$) in IgA, IL-1β, and TNF-α levels among the three groups (Table 5.11). However, IgG, IgM, IL-6, IL-8, and IL-10 levels in the BSF group were significantly lower ($P < 0.05$) than those in the FFS and HTBSF groups at 0 h but significantly higher ($P < 0.05$) at 4 h.

Table 5.11 Serum immune parameters.

Items	FFS	BSF	HTBSF	SEM	p-Value
IgA, ug/mL					
0- h	936.96	912.24	923.83	7.68	0.45
4- h	918.39	939.41	919.87	12.47	0.77
IgG, ug/mL					
0- h	1915.93^{a}	1781.90^{b}	1931.40^{a}	25.88	0.02
4- h	1816.20^{b}	2131.87^{a}	1865.80^{b}	45.04	< 0.01
IgM, ug/mL					
0- h	738.48^{a}	666.62^{b}	680.58^{b}	11.26	0.01
4- h	628.34^{b}	686.62^{a}	628.93^{b}	10.96	0.03
IL- 6, pg/mL					
0- h	46.94^{a}	39.25^{b}	46.24^{a}	1.08	< 0.01
4- h	39.15^{b}	46.13^{a}	41.45^{b}	0.83	< 0.01
IL- 8, pg/mL					
0- h	41.79^{a}	36.62^{b}	41.72^{a}	0.69	< 0.01
4- h	37.45^{b}	43.47^{a}	37.31^{b}	1.00	< 0.01
IL- 10, pg/mL					
0- h	19.17^{b}	16.00^{c}	23.50^{a}	0.86	< 0.01
4- h	19.80^{a}	19.98^{a}	16.44^{b}	0.50	< 0.01
IL- 1β, pg/mL					
0- h	26.67	26.68	27.11	0.38	0.88
4- h	27.03	27.71	27.11	0.37	0.74
TNF-α, pg/mL					
0- h	70.34	77.39	83.30	4.08	0.46
4- h	73.17	81.55	79.28	1.96	0.20

BSF = black soldier fly, FFS = full-fat soybeans, HTBSF = heat-treated black soldier fly.

Different letters within a row are significantly different ($P < 0.05$).

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5.4.6 Serum antioxidant parameters

At 0 h, there were no significant differences ($P > 0.05$) in SOD levels (Table 5.12). However, at 4 h, the SOD levels in the BSF group were significantly higher ($P < 0.05$) than those in the FFS and HTBSF groups. CAT, GSH-Px, and T-AOC levels in the FFS group were significantly higher ($P < 0.05$) than those in the BSF and HTBSF groups. CAT levels in the HTBSF group were significantly higher ($P < 0.05$) than those in the BSF group at 0 h, while GSH-Px and T-AOC levels were significantly higher ($P < 0.05$) at 4 h compared to the BSF group. MDA levels in the FFS and HTBSF groups were significantly lower ($P < 0.05$) than those in the BSF group. There were no significant differences ($P > 0.05$) in DPPH levels among the three groups.

Table 5.12 Serum antioxidant parameters.

Items	FFS	BSF	HTBSF	SEM	p-Value
SOD, U/ml					
0- h	91.46	97.95	92.19	1.31	0.08
4- h	90.90^{b}	101.17^{a}	89.36^{b}	1.70	< 0.01
CAT, U/ml					
0- h	15.54^{a}	6.22^{c}	8.38^{b}	1.01	< 0.01
4- h	11.17^{a}	7.53^{b}	7.61^{b}	0.62	0.01
GSH-Px, U/ml					
0- h	58.62^{a}	51.63^{b}	53.22^{b}	0.81	< 0.01
4- h	64.66^{a}	52.00^{c}	59.20^{b}	1.33	< 0.01
MDA, nmol/ml					
0- h	4.69^{b}	5.48^{a}	5.03^{b}	0.11	< 0.01
4- h	5.16^{b}	5.83^{a}	5.36^{b}	0.09	< 0.01
DPPH, %					
0- h	89.28	86.72	86.16	0.78	0.23
4- h	84.63	85.08	83.24	0.82	0.66
T-AOC, mmol/ml					
0- h	18.16^{a}	16.14^{b}	16.68^{b}	0.25	< 0.01
4- h	20.36^{a}	16.08^{c}	18.86^{b}	0.48	< 0.01

BSF = black soldier fly, FFS = full-fat soybeans, HTBSF = heat-treated black soldier fly.

Different letters within a row are significantly different ($P < 0.05$).

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5.4.7 Rumen microorganisms

The V3-V4 region of the 16S rRNA gene was sequenced using Illumina technology for all samples. The raw reads data ranged from 78,036 to 81,291. After quality control, the clean tags data ranged from 63,722 to 70,960. After removing chimeras, the valid tags data ranged from 59,369 to 67,338. The number of ASVs in each sample ranged from 580 to 997. For differential analysis of the project samples using ANOVA algorithm, there were 70 differential ASVs, 13 differential genera, and 3 differential phyla identified.

5.4.7.1 Venn plot

The three groups shared 1107 ASVs (amplicon sequence variants) in their microbiota (Figure 5.1). FFS and BSF shared 315 ASVs, FFS and HTBSF shared 291 ASVs, and BSF and HTBSF shared 288 ASVs. FFS had 847 unique ASVs, BSF had 718 unique ASVs, and HTBSF had 762 unique ASVs.

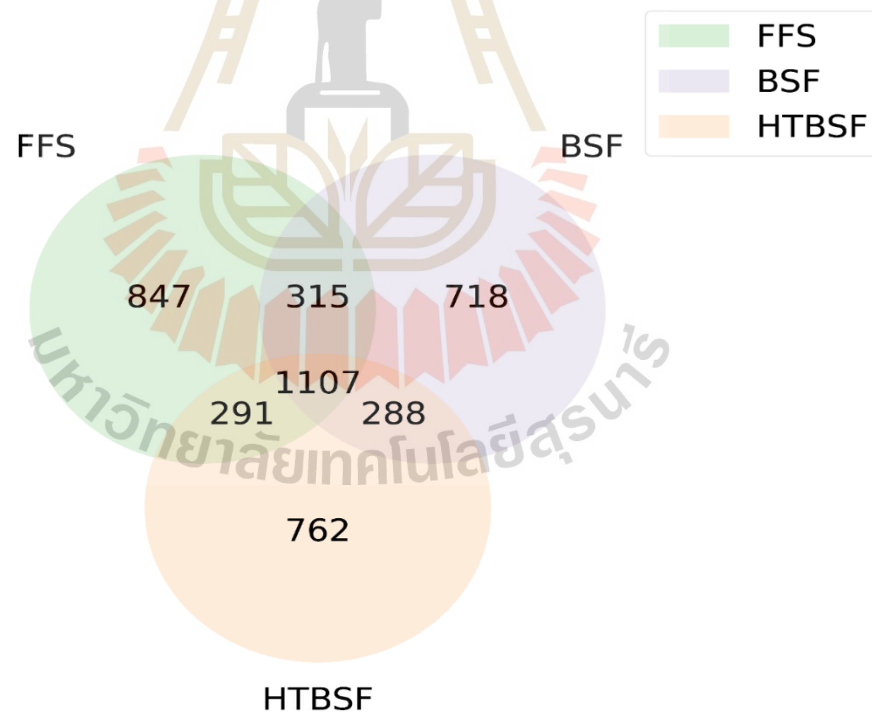

Figure 5.1 Venn plot (BSF = black soldier fly, FFS = full-fat soybeans, HTBSF = heat-treated black soldier fly).

5.4.7.2 Alpha diversity related boxplot analysis

The differences in α-diversity of the rumen microbiota (Figure 5.2), including ACE, Chao1, Goods_coverage, PH_whole_tree, Observed_species, Shannon, and Simpson indices, were not significant (P > 0.05) among the three groups.

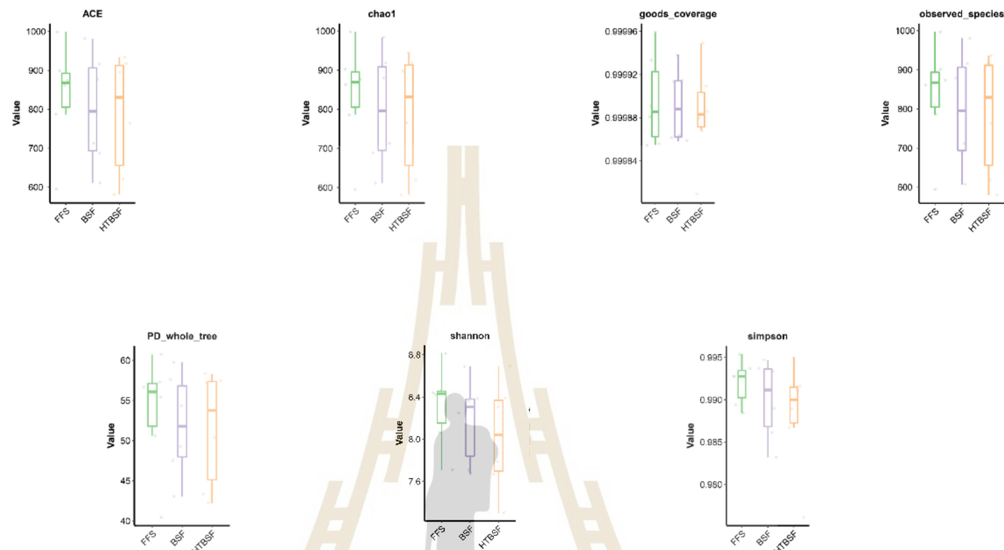

Figure 5.2 Alpha diversity-associated boxplot analysis (BSF = black soldier fly, FFS = full-fat soybeans, HTBSF = heat-treated black soldier fly).

5.4.7.3 Beta diversity analysis

Although the positions of the FFS and HTBSF groups are relatively distant with only two samples each (Figure 5.3A), overall, there was no impact on the relative abundance of the three groups. Moreover, based on the Bray-Curtis dissimilarity measure analysis of β-diversity (Figure 5.3B and C), the microbial communities among the three groups did not separate.

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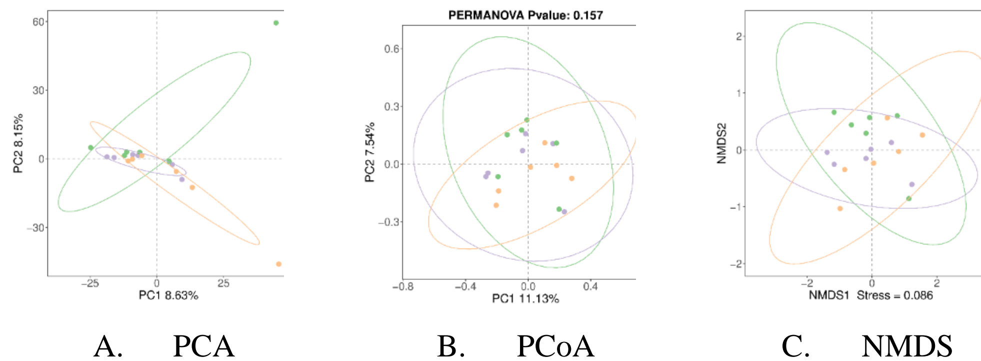
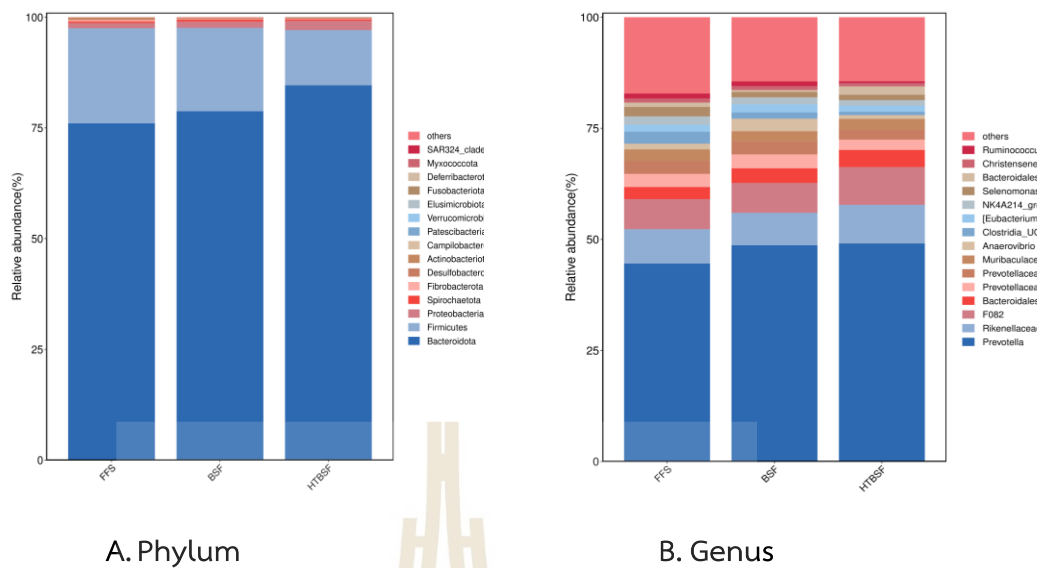


Figure 5.3 Beta diversity analysis (BSF = black soldier fly, FFS = full-fat soybeans, HTBSF = heat-treated black soldier fly).

5.4.7.4 Community structure bar chart

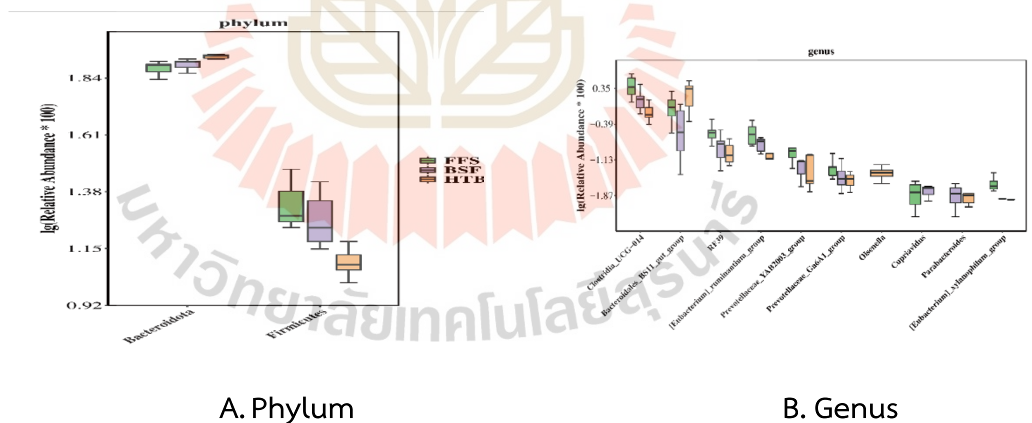
Microbial communities were detected using 16S rRNA, and at the phylum level (Figure 5.4A), the dominant phyla were *Bacteroidota*, *Firmicutes*, *Proteobateria*, *Spirochaetota*, and *Fibrobacterota*. The level of *Bacteroidota* in the HTBSF group was significantly higher (P < 0.05) than that in the FFS and BSF groups (Figure 5.5A), while the level of Firmicutes was significantly lower (P < 0.05) than in these two groups.

At the genus level (Figure 5.4B), the dominant genera were identified as *Prevotella*, *Rikenellaceae_RC9_gut_group*, *F082*, *Bacteroidales_RF16_group*, *Prevotellaceae_UCG_001*, *Prevotellaceae_UCG-003*, *Muribaculaceae*, *Anaerovibrio*, *Clostridia_UCG-014* and *[Eubacterium]_coprostanoligenes_group*. In the FFS group (Figure 5.5B), *Clostridia_UCG-014*, *RF39*, *[Eubacterium]_coprostanoligenes_group*, *Prevotellaceae_YAB2003_group*, *Prevotellaceae_Ga6A1_group*, *Cupriavidus*, and *[Eubacterium]_ xylanophilum_group* were significantly highest (P < 0.05), while *Parabacteroides* was significantly highest (P < 0.05) in the BSF group, and *Bacteroidales_BS11_gut_group* and *Olsenella* were significantly highest (P < 0.05) in the HTBSF group.



A. Phylum B. Genus

Figure 5.4 Microbial community structure bar chart (TOP15). Different colors represent different annotation information, and 'others' denotes all species except the top ones (BSF = black soldier fly, FFS = full-fat soybeans, HTBSF = heat-treated black soldier fly).



A. Phylum B. Genus

Figure 5.5 Boxplot of differential species abundance (TOP10) (BSF = black soldier fly, FFS = full-fat soybeans, HTBSF = heat-treated black soldier fly).

5.4.7.5 Community structure heatmap

The heatmap analysis of microbial community structure revealed significant differences in the relative abundance of species at the phylum level (Figure 5.6A). Specifically, the FFS group showed higher relative abundance of *Firmicutes, Elusimicrobiota, Patescibacteria, Actinobacteriota, Fibrobacterota, and Myxococcota*. The BSF group exhibited higher relative abundance of *Bacteroidota, Proteobacteria, Desulfobacterota, and SAR324_clade (Marine_group_B)*. On the other hand, the HTBSF group displayed higher relative abundance of *Spirochaetota, Desulfobacterota, Fusobacteriota, Campilobacterota,* and *Verrucomicrobiota*. At the genus level (Figure 5.6B), the FFS and BSF groups exhibit similar relative abundances of *Ruminococcus, Prevotellaceae_UCG-003, Prevotellaceae_UCG-001*, and *Christensenellaceae_ R-7_group*. Their differences lie in *Muribaculaceae, Selenomonas, Clostridia_UCG-014, NK4A214_group, Anaerovibrio*, and *[Eubacterium]_ coprostanoligenes_group*. In contrast, the HTBSF group shows higher relative abundances of *F082, Rikenellaceae_RC9_ gut_group, and Bacteroidales_RF16_group*.

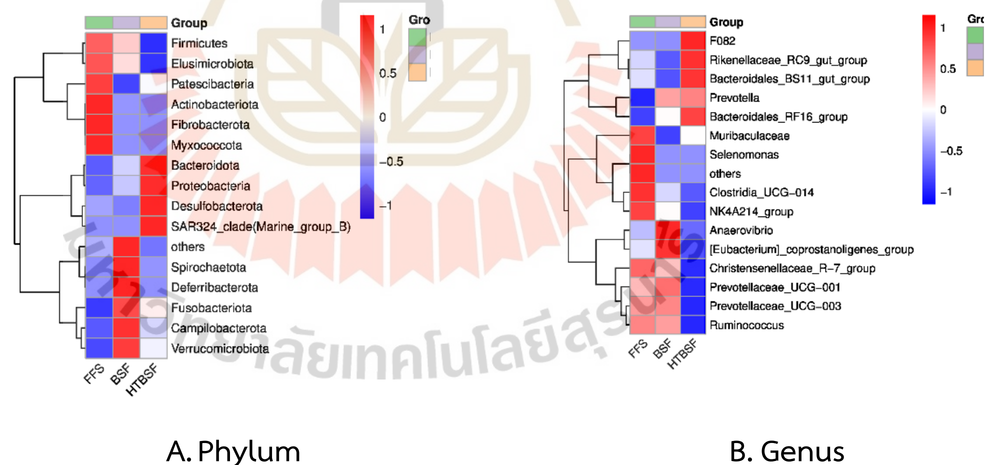
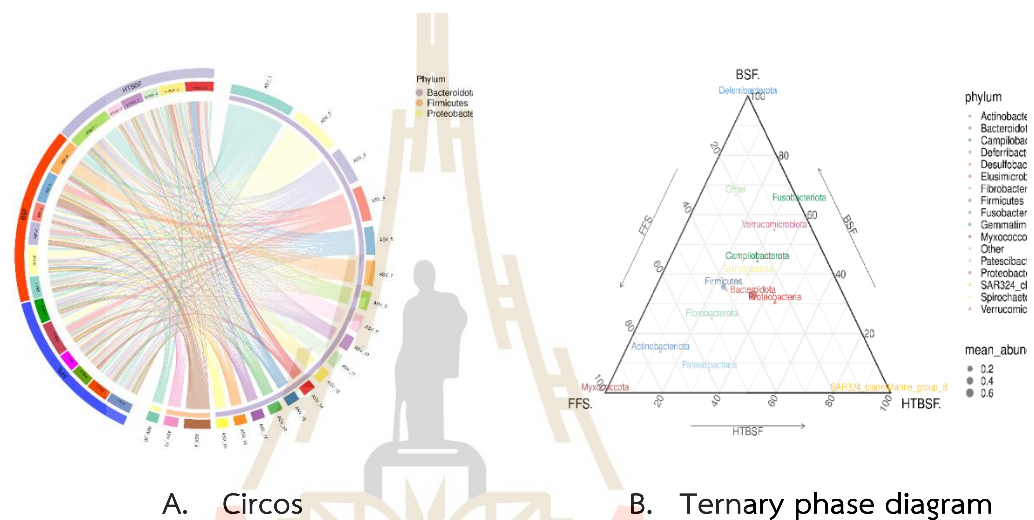


Figure 5.6 Community structure heatmap (TOP15). The left clustering tree represents the clustering of species. "Group" indicates different groups. Red denotes species with relatively high abundance, while blue denotes species with relatively low abundance (BSF = black soldier fly, FFS = full-fat soybeans, HTBSF = heat-treated black soldier fly).

5.4.7.6 **Species relationship diagram**

The Circos plot (Figure 5.7A) and Ternary Plot (Figure 5.7B) primarily depict the dominant microbial communities and their relative abundances at the phylum level in the experimental groups. The dominant microbial communities in these three groups are mainly distributed in the phyla Bacteroidota, Firmicutes, and Proteobacteria. Among these groups, Proteobacteria and Firmicutes exhibit relatively higher relative abundances.



A. Circos B. Ternary phase diagram

Figure 5.7 Species relationship diagram (BSF = black soldier fly, FFS = full-fat soybeans, HTBSF = heat-treated black soldier fly). A.The first circle displays the taxonomic classification at the phylum level and grouping information for each ASV. The second circle shows the percentage abundance of each ASV. The third circle represents the main blocks of ASVs and samples, differentiated by color and labels. The outer scale of the third circle indicates the absolute abundance of ASVs. The fourth circle corresponds to the main blocks (third circle) and displays the abundance of each ASV in each sample, as well as the abundance of each ASV in each sample. The fifth circle corresponds to the ASV and sample sub-blocks (fourth circle), showing the association between ASVs and samples through connecting lines. B. The three vertices represent three groups. In the legend, colored circles represent species classification at the phylum level. In the ternary plot, circles represent species classification at the phylum

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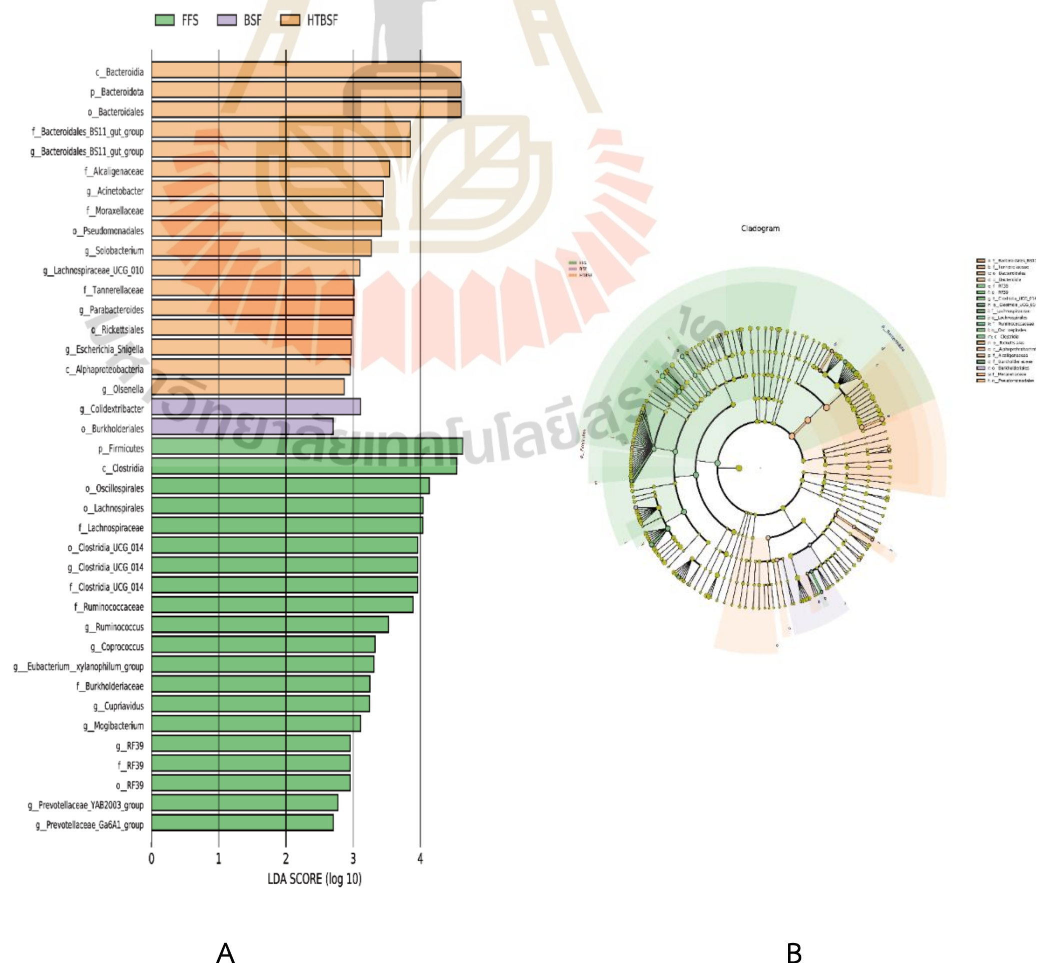
level, and the size of the circles represents the average relative abundance of the species

5.4.7.7 LEfSe analysis

The LEfSe algorithm was used to identify ASV biomarkers. Compared with the FFS group, the relative abundance of species *g__Colidextribacter* and *o__Burkholderiales* was higher in the BSF group (Figure 5.8). Additionally, the HTBSF group exhibits higher relative abundance of species such as *p__Firmicutes, c__Clostridia, o__Oscillospirales, o__Lachnospirales, f__Lachnospiraceae, o__Clostridia_UCG_014, g__Clostridia_UCG_014, f__Clostridia_UCG_014, f__Ruminococcaceae, g__Ruminococcus, g__Coprococcus, g__Eubacterium__coprostanoligenes_group, f__Burkholderiaceae, g__Cupriavidus, g__Mogibacterium, g__RF39, f__RF39, o__RF39, g__Prevotellaceae_YAB2003_group,* and *g__Prevotellaceae_Ga6A1_group.*



A B

Figure 5.8 LEfSe analysis. Linear discriminant analysis effect sizes (LEfSe) were analyzed using a linear discriminant analysis (LDA) score threshold of 2.0 to identify bacterial biomarkers in the rumen of BSF and BTBSF (BSF = black soldier fly, FFS = full-fat soybeans, HTBSF = heat-treated black soldier fly).

5.4.7.8 Random forest analysis

The most important genera in the rumen microbiota were *Ruminococcus, Bacteroidales_BS11_gut_group, Clostridia_UCG-014, Prevotella,* and *UCG-005* (Figure 5.9). Among these, *Prevotella* had the highest relative abundance and exerts the greatest impact on the rumen environment.

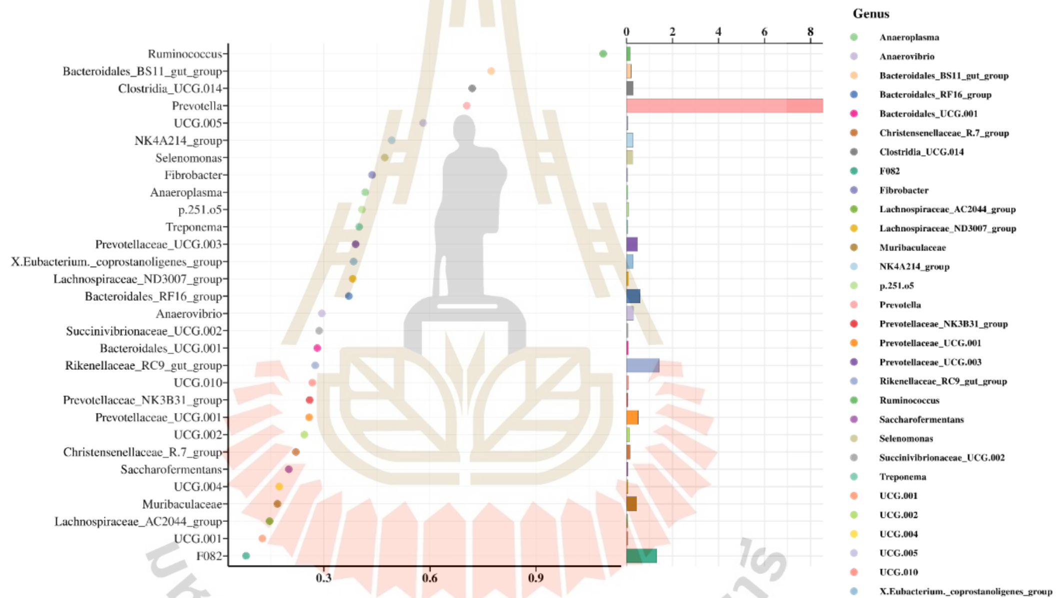


Figure 5.9 Random forest analysis. The left side is the species (variable) importance point plot. The abscissa is the importance measure, and the ordinate is the species names sorted by importance. On the right is a histogram of species' relative abundance. (BSF = black soldier fly, FFS = full-fat soybeans, HTBSF = heat-treated black soldier fly).

5.4.8 Slaughter performance and meat quality

There were no significant differences (P > 0.05) in the moisture, DM, CP, and ash content among the three groups of muscles (Table 5.13). However, the EE content in the FFS and HTBSF groups was significantly higher (P < 0.05) than that in

the BSF group. The dressing percentages in both the BSF and the HTBSF groups were significantly lower (P < 0.05) than those of the FFS group. The carcass weight in the FFS group was significantly higher (P < 0.05) than that in the BSF group. The drip loss and pH 45min levels in the BSF group were significantly higher (P < 0.05) than those in the FFS and HTBSF groups, while there was no significant difference (P > 0.05) between the FFS and HTBSF groups. There were no significant differences (P > 0.05) observed among the three groups in terms of shear force, WHC, cooking loss, and pH 24h. However, the L* values in the HTBSF were significantly higher (P < 0.05) than those in the FFS and BSF groups. The a* and b* values in the FFS and BSF groups were significantly higher (P < 0.05) than those in the HTBSF group.

Table 5.13 Effects of heat treatment BSF on slaughter performance and meat quality.

Items	FFS	BSF	HTBSF	SEM	p-Value
Moisture (%)	72.90	72.30	72.84	0.19	0.40
DM (%)	27.10	27.70	27.16	0.19	0.40
CP (%)	19.52	20.17	19.48	0.03	0.30
EE (%)	3.56^{a}	3.40^{b}	3.55^{a}	0.19	0.02
Ash (%)	4.02	4.14	4.13	0.03	0.25
Dressing percentage (%)	49.53^{a}	45.31^{b}	45.15^{b}	0.53	< 0.01
Carcass weight (kg)	12.73^{a}	11.02^{b}	12.49^{ab}	0.31	0.04
Shear force (N)	16.17	17.92	14.10	0.69	0.08
WHC (%)	87.16	85.04	86.72	0.88	0.61
Cooking loss (%)	42.84	42.12	43.20	0.41	0.59
Drip loss (%)	2.07^{b}	2.56^{a}	2.04^{b}	0.09	0.04
pH_{45min}	6.10^{ab}	6.19^{a}	5.99^{b}	0.03	0.04
pH_{24h}	5.46	5.70	5.53	0.04	0.75
Lightness (*L**)	19.8^{b}	25.2^{ab}	26.6^{a}	1.25	0.03
Redness (*a**)	16.8^{a}	17.4^{a}	13.6^{b}	0.49	< 0.01
Yellowness (*b**)	17.2^{a}	17.3^{a}	12.9^{b}	0.58	< 0.01

Different letters within a row are significantly different (P < 0.05). CP = crude protein, DM = dry matter, EE = ether extract, pH_{45min} = pH at 45 min post slaughter, pH_{24h} = pH at 24 h post slaughter, WHC = water holding capacity. BSF = black soldier fly, FFS = full-fat soybeans, HTBSF = heat-treated black soldier fly.

5.4.9 AA content of LTL

AA content of LTL is shown in Table 5.14. There was only the methionine level in the BSF group was significantly higher ($p < 0.05$) than that in the FFS group. However, differences in other essential AA were non-significant in our study. There were no significant differences in the total amino acid (TAA), EAA, and umami amino acids (UAA) among the three feeding groups.

Table 5.14 Effects of heat treatment BSF on AA content of muscle (mg/100 g).

Items	FFS	BSF	HTBSF	SEM	p-Value
Essential amino acids Arginine					
Histidine	4.61	4.73	4.60	0.05	0.55
Isoleucine	2.24	2.26	2.42	0.04	0.11
Leucine	2.23	2.27	2.25	0.03	0.85
Lysine	5.94	6.04	5.96	0.07	0.83
Methionine	6.74	6.72	6.72	0.06	0.98
Phenylalanine	1.32^{b}	1.58^{a}	1.51^{ab}	0.04	0.04
Threonine	3.08	3.17	3.17	0.04	0.60
Valine	3.40	3.50	3.42	0.05	0.69
Dispensable amino acids	2.61	2.65	2.62	0.03	0.88
Alanine	4.58	4.64	4.58	0.04	0.81
Aspartic acid	7.37	7.49	7.44	0.08	0.86
Glycine	3.62	3.70	3.66	0.03	0.51
Glutamic acid	12.14	12.50	12.43	0.14	0.58
Proline	2.91	2.99	2.78	0.05	0.20
Serine	3.30	3.45	3.32	0.05	0.47
Tyrosine	2.58	2.55	2.46	0.03	0.23
TAA	68.67	70.24	69.34	0.73	0.70
EAA	32.17	32.92	32.68	0.36	0.72
UAA	33.37	34.05	33.74	0.32	0.71

Different letters within a row are significantly different ($P < 0.05$). TAA = total amino acid, EAA = essential amino acids, UAA = umami amino acids (phenylalanine, alanine, aspartic acid, glycine, glutamic acid, tyrosine). BSF = black soldier fly, FFS = full-fat soybeans, HTBSF = heat-treated black soldier fly.

5.4.10 FAs content of LTL

FA content of LTL is shown in Table 5.15. The levels of C10:0, C18:1n9t, C18:2n6c, C20:0, and C18:3n3 in muscle were significantly reduced ($p < 0.05$) in the BSF and HTBSF groups, while the levels of C14:1 and C16:1 were increased ($p < 0.05$). Moreover, the levels of C12:0, C14:0, and C16:0 in the BSF group were significantly higher ($p < 0.05$) than those in the FFS and HTBSF groups. The HTBSF group exhibited the highest ($p < 0.05$) levels of C18:1n9c, while the BSF group showed the lowest levels ($p < 0.05$). Additionally, the levels of C18:2n6t, C18:3n6, and C20:2 significantly increased ($p < 0.05$) in the HTBSF group. The SFA levels were highest ($p < 0.05$) in the BSF group, followed ($p < 0.05$) by the FFS group, and lowest ($p < 0.05$) in the HTBSF group. Furthermore, the BSF group significantly decreased ($p < 0.05$) the PUFA/SFA ratio, but the n-6/n-3 ratio significantly increased ($p < 0.05$) compared to the FFS group.

Table 5.15 Effects of heat treatment BSF on fatty acid content of muscle (mg/100 g).

Items	FFS	BSF	HTBSF	SEM	p-Value
C10:0	0.18^{a}	0.13^{b}	0.11^{b}	0.01	< 0.01
C12:0	0.09^{b}	0.15^{a}	0.11^{ab}	0.01	0.03
C14:0	1.69^{b}	2.27^{a}	1.82^{b}	0.08	< 0.01
C14:1	0.07^{b}	0.11^{a}	0.09^{a}	0.001	< 0.01
C15:0	0.35	0.42	0.32	0.02	0.08
C16:0	21.06^{b}	23.38^{a}	20.73^{b}	0.31	< 0.01
C16:1	1.37^{b}	1.86^{a}	1.82^{a}	0.07	< 0.01
C17:0	0.79^{a}	0.80^{a}	0.05^{b}	0.13	0.02
C17:1	0.66	0.76	0.52	0.06	0.27
C18:0	15.67	15.39	15.53	0.23	0.90
C18:1n9t	1.79^{a}	1.32^{b}	1.26^{b}	0.08	< 0.01
C18:1n9c	51.75^{b}	49.57^{c}	53.62^{a}	0.48	< 0.01
C18:2n6t	0.23^{b}	0.26^{ab}	0.28^{a}	0.01	0.03
C18:2n6c	3.65^{a}	3.01^{b}	3.06^{b}	0.11	0.03
C20:0	0.18^{a}	0.12^{b}	0.09^{b}	0.01	< 0.01
C18:3n6	0.05^{b}	0.03^{b}	0.09^{a}	0.01	0.04
C18:3n3	0.20^{a}	0.15^{b}	0.15^{b}	0.01	< 0.01

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Table 5.15 (Continue).

Items	FFS	BSF	HTBSF	SEM	p-Value
C20:2		0.02 b	0.05 a	0.01	< 0.01
C20:3n6			0.01	0.00	-
C20:4n6	0.30	0.27	0.28	0.02	0.83
SFA	39.99^{b}	42.66^{a}	38.76^{c}	0.44	< 0.01
UFA	60.01^{b}	57.34^{c}	61.24^{a}	0.44	< 0.01
PUFA/SFA	0.11^{a}	0.09^{b}	0.10^{a}	0.003	< 0.01
n-3 PUFA	0.15	0.15	0.15	0.01	0.98
n-6 PUFA	4.20	3.56	3.73	0.12	0.06
n-3/ n-6	0.04	0.04	0.041	0.01	0.18

Different letters within a row are significantly different ($P < 0.05$). PUFA = polyunsaturated fatty acid, SFA = saturated fatty acid, UFA = unsaturated fatty acid. BSF = black soldier fly, FFS = full-fat soybeans, HTBSF = heat-treated black soldier fly.

5.4.11 Mineral content and antioxidant capacity of longissimus LTL

There was only the Cu level in the FFS group was significantly higher ($P < 0.05$) than that in the BSF group (Table 5.16). There were no significant differences ($P > 0.05$) in the levels of other minerals among the three groups. There were no significant differences ($P > 0.05$) observed in the levels of SOD in the muscles among the three groups (Table 9). The levels of CAT, GSH-Px, and T-AOC in the FFS group were significantly higher ($P < 0.05$) than those in the BSF and HTBSF groups.

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Table 5.16 Effects of heat treatment BSF on the mineral content and antioxidant capacity of LTL.

Items	FFS	BSF	HTBSF	SEM	p-Value
Fe, mg/ kg	67.99	59.95	56.46	3.14	0.40
Mn, mg/ kg	0.58	0.42	0.44	0.03	0.11
Cu, mg/ kg	2.84^{a}	2.30^{b}	2.58^{ab}	0.09	0.04
Zn, mg/ kg	109.18	102.87	102.89	2.18	0.50
K, g/ kg	19.43	18.80	19.27	0.20	0.51
Na, g/ kg	2.04	2.21	2.09	0.06	0.62
Ca, mg/ kg	258.30	196.75	239.72	13.89	0.23
Mg, g/ kg	1.17	1.13	1.18	0.01	0.34
P, g/ kg	9.68	9.30	9.54	0.10	0.38
Se, mg/ kg	0.46	0.43	0.45	0.01	0.47
SOD (U/ml)	88.13	94.45	88.69	1.29	0.08
CAT (U/ml)	13.87^{a}	5.72^{b}	6.71^{b}	0.92	< 0.01
GSH-Px (U/ml)	54.29^{a}	47.13^{b}	48.89^{b}	0.84	< 0.01
T-AOC (mmol/ml)	16.00^{a}	13.64^{b}	14.02^{b}	0.31	< 0.01

Different letters within a row are significantly different ($P < 0.05$). GSH-Px = glutathione peroxidase superoxide, SOD = superoxide dismutase, CAT = catalase, T-AOC = total antioxidant capacity. BSF = black soldier fly, FFS = full-fat soybeans, HTBSF = heat-treated black soldier fly.

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5.4.12 Gene expression

The gene expression levels of CAT, GPX-1, and IL-1β were significantly higher ($P < 0.05$) in the HTBSF group than in the FFS and BSF groups (Figure 5.10). GPX-4 and IL-6 related gene expression levels were significantly higher ($P < 0.05$) in the BSF and HTBSF groups compared to the FFS group. The gene expression levels of SOD, IL-8, and TNF-α related genes were significantly higher ($P < 0.05$) in the HTBSF group than in the FFS and BSF groups, while the BSF group gene expression levels were significantly higher ($P < 0.05$) than those in the FFS group. The gene expression levels of IL-10 genes were significantly higher ($P < 0.05$) in the FFS group than in the BSF and

HTBSF groups, with HTBSF gene expression levels of significantly higher (P < 0.05) than those in the BSF group.

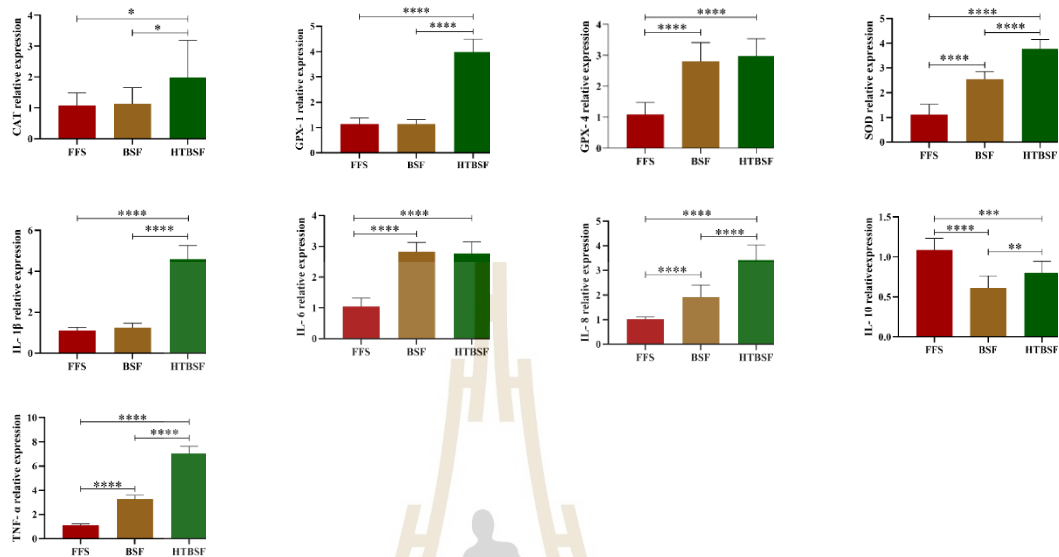

Figure 5.10 Muscle-related gene expression (BSF = black soldier fly, FFS = full-fat soybeans, HTBSF = heat-treated black soldier fly, “*” means P < 0.05, “**” means P < 0.01, and “***” means P < 0.001, “****” means P < 0.0001).

5.5 Discussion

5.5.1 Growth performance

Based on our knowledge, there is currently no research on the growth performance of ruminant animals regarding BSF. In this study, both BSF and HTBSF did not affect the growth performance of goats, indicating that heat-treated BSF did not have a promoting effect on growth performance but showed a trend of increasing ADG. This is consistent with the results of (Biasato Renna et al., 2019), where replacing 5% or 10% of soybean meal with BSF did not affect the growth performance of piglets. (Spranghers et al., 2018) also reported that feeding whole (4% and 8%) and defatted (5.4%) BSF showed no significant difference in the growth performance of piglets. Additionally, although the difference in FCR in this study was not significant, the HTBSF group showed a decreasing trend. Recent studies have found that higher RUP content

can improve feed efficiency in dairy calves (Kazemi-Bonchenari et al., 2015). However, other studies have shown that heat-treated RUP does not improve ADG and FCR, which is consistent with the results of this study (Kazemi-Bonchenari et al., 2016). This may be because providing BSF protein for RUP has little impact on the protein content or amino acid composition in the small intestine.

5.5.2 Apparent digestibility

Previous studies evaluating the addition of insects or their oil in ruminant diets have found that the chitin content and high-fat content of insects lead to a reduction in DM digestibility, hence insects are often considered to have lower nutritional value (Chaudhari et al., 2011). Interestingly, in this study, apart from the significantly higher NDF digestibility rate in the HTBSF group compared to the FFS and BSF groups, there were no significant differences in all other digestibility parameters. Recent *in vitro* studies have found that supplementing BSF at either 1 or 2 week old decreases digestibility rates of DM and OM (A. Jayanegara et al., 2017), which may be attributed to the higher crude fat content in BSF. Fat can coat the surface of fiber cells in the diet and hinder the microbial breakdown of cellulose particles in the rumen (Weimer, 2022). On the contrary, (Kahraman et al., 2023b) found that supplementing 20% and 40% BSF in *in vitro* fermentation increased the 24 h digestibility rate of DM. At 48 h, the highest NDF digestibility rate was observed. The authors attributed this result to the levels of non-fibrous carbohydrates (NFC) and starch in the diet; higher NFC levels can affect the *in vitro* NDF digestibility rate, and a high NFC/NDF ratio is associated with a decrease in NDF digestibility (Homem Junior et al., 2015). However, in this study, there were no differences in digestion parameters between the FFS and BSF groups. This might be attributed to the use of whole-fat soybeans in this study, thus excluding the factor of fat content. It is possible that chitin in BSF and anti-nutritional factors in soybeans share similar anti-nutritional properties (Gu et al., 2010), leading to these comparable digestion outcomes. The motivation for how heat-treated BSF increases NDF digestibility is currently unclear, but it may be due to the structural disruption of chitin after intense pressing during heat treatment, thereby increasing its digestibility.

5.5.3 PH, NH3-N and BUN

Accumulation of VFA and lactic acid in the rumen results in lowered ruminal pH. Prolonged periods of low ruminal pH each day can affect feed intake of goats, microbial metabolism, and feed digestion. In this study, the HTBSF group exhibited significantly higher values at 0 h compared to the FFS group, with no significant differences observed at 4 h. This indicates that the HTBSF group maintained a consistently high ruminal pH over the long term, which is beneficial for enhancing the activity of primary fibrolytic bacteria and promoting ruminal fiber digestion (Erdman, 1988). This explains why the NDF digestibility significantly increased in the HTBSF group. Ruminant animals are inefficient converters of ingested protein into animal products, as a significant portion of this protein is lost in the rumen in the form of NH_3-N. At 0 h, the BSF group had the highest NH_3-N levels, while by 4 h, the HTBSF group exhibited the highest NH_3-N levels. This indicates that the microbial protein absorbability was lower in the BSF group, whereas the degradability and absorbability were highest in the HTBSF group. The research by (Toral et al., 2022) shows that compared to soybean meal, different insect meals result in lower ruminal protein degradation. (Kahraman et al., 2023b) report also indicates that supplementing with 20% BSF reduces ruminal NH_3-N levels. The lower protein degradation in insect feed can be explained by the significant proportion of proteins related to chitin and the chemical composition of insect exoskeletons (Jonas-Levi and Martinez, 2017). Heat treatment may disrupt chitin and exoskeletal structures, leading to increased ruminal protein degradation. BUN is an important indicator of ruminal nitrogen intake because it is positively correlated with ruminal NH3-N concentration (Kohn et al., 2005). Urea nitrogen is also a crucial indicator of nitrogen efficiency because it has a strong linear relationship with urinary nitrogen excretion (Rastgoo et al., 2020). In this study, the BUN level in the BSF group was significantly higher than that in the FFS group, which was positively correlated with NH_3-N concentration. (Phesatcha et al., 2023) found that adding crickets to beef cattle increased the levels of NH3-N and BUN. This outcome may be related to the content of RUP, as soybeans themselves have a high RUP content, and the HTBSF group also has a higher RUP content than the BSF group, thereby reducing the availability of amino acids for ruminal bacteria, resulting in decreased BUN concentration (Burakowska et al., 2021; Jafarpour et al., 2023).

5.5.4 VFAs

VFA is the primary mode of energy absorption in ruminant animals. In this study, there were no significant differences in each short-chain fatty acid and total VFA, except that isobutyric acid and isovaleric acid in the FFS group were significantly lower than those in the BSF and HTBSF groups at 4 h. This indicated that overall supplementation of untreated or heat-treated BSF had no negative impact on ruminal VFA.

5.5.5 Serum immune and antioxidant parameters

Immune and antioxidant markers are important parameters for evaluating the body's ability to resist oxidative stress and inflammation. In this study, the levels of IgG, IgM, IL-6, IL-8, and IL-10 in the BSF group were significantly lower than those in the FFS and HTBSF groups at 0 h. However, at 4 h, they were significantly higher, indicating that the BSF group was unable to enhance immune function over a prolonged period. The results of the BSF group are contrary to previous research findings. (Driemeyer, 2016) found that supplementing 3.5% BSF in piglet diets did not affect serum albumin, IgA, IgG, and IgM levels. (Jin et al., 2016) reported that adding 6% full-fat black soldier fly larvae in weaning pig diets reduced blood urea nitrogen (BUN) and increased serum insulin-like growth factor, but did not significantly affect serum IgG and IgA concentrations. A study reported by (S. Jian et al., 2022) found that supplementing either 20% defatted BSF or 8% full-fat BSF did not affect IgA, IgG, IgM, IL-6, IL-8, IL-8I, IL-10, IL-1β, and TNF-α levels in dogs. These results are consistent with the findings of the HTBSF group. This finding seems difficult to explain, as we did not observe any signs of discomfort or inflammatory diseases in the goats from the BSF and HTBSF groups. In addition, overall, the antioxidant levels in the FFS group were significantly higher than those in the BSF and HTBSF groups. However, some antioxidant parameters in the HFBSF group were still better than those in the BSF group, such as CAT and MDA at 0 h, and GSH-Px and T-AOC at 4 h. This is contrary to previous research results on BSF in animals. (S. Jian et al., 2022) study showed that BSF did not affect the antioxidant parameters in beagle dogs. (Sihem Dabbou et al., 2018a) study found that supplementing 5%, 10%, and 15% BSF linearly increased the levels of GSH-Px in the blood of broiler chickens. The authors attributed this to the fact that insect powder can provide dietary tocopherol (42.72 mg/kg insect powder), which leads to an increase in GSH-Px levels. Although BSF contains antioxidants such as chitin, Se, and vitamin E

(Hender et al., 2021b), the content of antioxidants in soybeans, including isoflavones (such as soy isoflavones, flavonoids, etc.), polyphenolic compounds (such as anthocyanins, isoflavone glycosides, etc.), vitamin C, vitamin E, soy isoflavone glycosides, and phytosterols, is higher (Rizzo, 2020). This results in a higher level of antioxidants in the FFS group compared to the BSF and HTBSF groups. The difference in antioxidant capacity between the HTBSF and BSF groups could be attributed to the structural changes in proteins and starch caused by heat treatment. These changes make proteins and starch easier to be absorbed and utilized by animals, thereby promoting the generation and utilization of antioxidant substances (Deng et al., 2022).

5.5.6 Rumen microorganisms

The chitin, C12:0, and C14:0 contained in BSF are closely related to the diversity of rumen microorganisms. This study did not find differences in α-diversity and β-diversity among the three groups of rumen microbiota, indicating that BSF and HTBSF did not have a significant impact on the abundance and diversity of goat rumen microorganisms. This is consistent with previous reports, showing that BSF did not affect the α-diversity and β-diversity of intestinal microbiota (Dabbou Lauwaerts Ferrocino Biasato Sirri Zampiga Bergagna Pagliasso Gariglio Colombino et al., 2021). The *Firmicutes* and *Bacteroidetes* phyla are major phyla in the rumen, and their bacteria play significant roles in the rumen function and ecology of ruminant animals (Jiao et al., 2015). The dominant phyla in this study are *Bacteroidota, Firmicutes, Proteobacteria, Spirochaetota,* and *Fibrobacterota*. Members of the *Bacteroidota* phylum are primarily responsible for protein hydrolysis and carbohydrate degradation, while members of the *Firmicutes* phylum play a significant role in energy utilization (Gary D. Wu et al., 2011). We found a significant increase in the abundance of *Bacteroidota* and a significant decrease in *Firmicutes* in the HTBSF group. This indicates that the HTBSF group can enhance the utilization of proteins and carbohydrates, possibly due to structural reorganization of proteins and carbohydrates induced by heat treatment, leading to an increase in the EE ratio and the suppression of the *Firmicutes* population (Renna et al., 2022). Because research on Beagle dogs had shown that untreated BSF did not affect the dominant genera at the phylum level (S. Jian et al., 2022). The dominant genera in all three groups were *Prevotella, Rikenellaceae_RC9_gut_group,*

F082, Bacteroidales_RF16_group, Prevotellaceae_UCG_001, Prevotellaceae_UCG-003, Muribaculaceae, Anaerovibrio, Clostridia_UCG-014, and [Eubacterium]_coprostanoligenes_ group. Although there were no significant differences in the dominant genera among the three groups, BSF and HTBSF increased the abundance of *Prevotella*. Protein is crucial as a nitrogen source for the growth of *Prevotella. Prevotellaceae* is mainly involved in pectin and protein metabolism, playing an important role in amino acid metabolism in the rumen of ruminant animals (Walker et al., 2003). Additionally, the *Prevotellaceae* family represents a metabolically and genetically diverse microbial population in the rumen, capable of degrading lignocellulosic materials (Bi et al., 2018). In this study, we found differences in NDF digestion. At the same time, the increase in *Prevotella* abundance may be related to the easier digestion of animal-derived proteins compared to plant-derived proteins (Niu et al., 2024).

5.5.7 Slaughter performance and meat quality

Crude fat, crude protein, and moisture are the main chemical components of muscle. The fat content and fatty acid composition can influence the hardness of meat because different fatty acid has different melting points (Wood et al., 2008). The water content of muscle directly influences the juiciness of the meat, while the crude ash content is related to the mineral and trace element content in the meat (Mir et al., 2017). In this study, besides the significantly lower EE observed in the BSF group, there were no significant differences in moisture, DM, CP, and ash content. This suggests a weaker lipid deposition capacity in the muscles of the BSF group. Given the lack of research on the nutritional composition of ruminant muscle by BSF, and the rich protein and mineral content in chicken and goat meat, we have introduced a discussion on chicken in this section. As Pieterse et al. (E. Pieterse et al., 2019) reported, supplementing BSF had no significant impact on the approximate composition of broiler breast muscle. Aprianto et al. (Aprianto et al., 2023) also obtained a similar result, suggesting that the BSF oil (1% 2% and 3%) feed supplement and approximate broiler muscle compositions were unrelated, however, with an increase in supplementation levels, there was a reduction in the deposition of EE in the muscle. Similarly, BSF reduced abdominal fat in laying hens (Nilugonda et al., 2022) and broilers (Hartinger et al., 2021). Many studies attributed resulted in the high content of C12:0 in BSF because it was found that feeding palm oil, sunflower seed oil, flaxseed,

or flaxseed oil resulted in higher intramuscular fat compared to animals fed coconut oil (also rich in C12:0) or BSF (Suryati et al., 2023). This interpretation was evidently in contrast with our study. Since the muscle EE content of HTBSF did not decrease, but the levels of C12:0 in the diets of both BSF and HTBSF groups were close. This could be attributed to the processing method of the HTBSF group, wherein starch, protein, and fatty acid undergo structural rearrangement at high temperatures, forming a protective barrier that reduced the oxidation of C12:0. According to reports, C12:0 was oxidized to CO2 faster than 18:3n-3 and other long-chain fatty acid, making it less available for storage in tissues or elongation to 14:0 and 16:0 (Dalle Zotte et al., 2018).

Factors influencing meat quality include dressing percentage, color, pH, tenderness, WHC, and muscle fibers. Among them, the dressing percentage is an important index to evaluate carcass quality (Cerdeño et al., 2006). Meat color is a direct factor in assessing meat quality and customer purchasing intention. Muscle pH value is an important indicator reflecting the post-slaughter muscle glycolysis rate in animals. It is related to the WHC and color of goat meat (Y Wang et al., 2020). In this study, the dressing percentage and carcass weight of BSF-fed goats were lower than those of the FFS group, indicating that BSF feeding might have a negative influence on carcass quality. A similar study result was also found by Murawska et al. (Daria Murawska et al., 2021), BSF supplementation led to a decrease in the carcass yield of broiler chickens. This may be the increase of parts not intended for consumption, such as offal and limbs. Although shear force, WHC, and cooking loss showed no difference, the untreated BSF exhibited higher drip loss and pH45min. Interestingly, the HTBSF group exhibited higher L* values, while a* and b* values were lower. This was inconsistent with the findings of research by Herrera et al. (Herrera et al., 2022), in which they concluded that BSF did not affect WHC, pH, and meat color in guinea pig muscles. Pieterse et al.(E. Pieterse et al., 2019) also concluded that BSF did not impact the meat color and pH of chicken muscles. However, there was also a study that indicated that supplementing BSF increased the b* values and pH of chicken muscles (D. Murawska et al., 2021). Biasato et al. (Biasato Dabbou et al., 2019) observed a 10% increase in the a* value of chicken muscles with BSF supplementation, which is consistent with our BSF group results while in contrast with the HTBSF group. We assume that this may be related to the higher deposition of UFA in the LTL muscles

of the HTBSF group. In summary, despite the lower EE content in the LTL of the BSF group, overall, the HTBSF group had a negative impact on meat quality.

5.5.8 AA content of LTL

The types and content of AA determine the nutritional value of proteins. In lamb meat, the types and content of AA is related to factors such as animal species and feed composition (Wood, 2017). Aspartic acid and glutamic acid can undergo the Maillard reaction with reducing sugars, producing aroma (Eric et al., 2013). The umami AA consist of phenylalanine, alanine, aspartic acid, glycine, glutamic acid, and tyrosine, while the sweet-tasting AA include threonine, alanine, glycine, serine, lysine, and proline. Aromatic AA is primarily composed of phenylalanine and tyrosine. The flavor of lamb meat is associated with these three categories of AA, with umami AA being crucial in determining the flavor of lamb meat. The results of this study indicated that there were no differences in TAA, EAA, and UAA among the three groups. This is consistent with the findings of the studies conducted by (Lu et al., 2020) and (Herrera et al., 2022). The only exception was that the BSF exhibits higher levels of methionine compared to the FFS group. However, it was noteworthy that no differences in methionine were found in the daily diet of this study. This may be related to the superior meat quality of the BSF group, as methionine was positively correlated with muscle WHC, color stability, and the prevention of lipid and protein oxidation (Estévez et al., 2020).

5.5.9 FA content of LTL

Monounsaturated fatty acids (MUFA) play a crucial role in reducing the incidence of coronary heart disease and preventing atherosclerosis, while PUFA is involved in the development of the brain and retina. Among PUFA, arachidonic acid (C20:4n6c), linseed oil, and alpha-linolenic acid (C18:3n3) are considered essential FA for the human body, which can only be obtained from food (Kapoor et al., 2021; Lotfi et al., 2021). In this study, although the C18:3n3 content in the BSF and HTBSF groups was lower than that in the FFS group, the C18:1n9c in the HTBSF group was significantly higher than that in the FFS group, while the BSF group was significantly lower than the FFS group. Interestingly, a significant increase of UFA and a significant decrease of SFA in the HTBSF were observed, whereas the BSF group exhibited the opposite pattern. Moreover, in terms of the PUFA/SFA and n-6/n-3 ratios, the BSF group also

demonstrated significantly lower values. Among individual SFA (C12:0-C17:0), the content was consistently the lowest in the HTBSF group. However, no significant differences were observed in the content of these FA in the basic diets of the BSF and HTBSF groups. This indicated that HTBSF could improve the compositional pattern of FA in LTL muscles, promoting the deposition of FA in a more favorable direction. Our results regarding the positive influence of BSF on SFA was also confirmed by other reports (Acuti et al., 2018; Zhou et al., 2018). In the HTBSF group, it may be that heat treatment forms starch-lipid complexes, which could protect the degradation of UFA in the rumen and allow more UFA to be absorbed in the small intestine (Zhou et al., 2018), subsequently increasing the content of UFA in the muscles.

5.5.10 Mineral content and antioxidant capacity of *longissimus* LTL

Generally, several minerals in meat such as Fe, Zn, Na, Ca, Mg, and Se are considered important for human intake. In this study, we observed that, apart from Cu, there were no significant differences in other minerals in LTL meat. This indicated that most minerals in BSF were not absorbed and deposited in muscle tissue, possibly due to the lower bioavailability of minerals in insects (Dicke et al., 2018). Specific proteins (such as hemoglobin and casein) and mineral-binding peptides (casein phosphopeptides) support the absorption and bioavailability of minerals from traditional foods (such as meat and dairy). However, these pathways may not be applicable to the consumption of insects (Ojha et al., 2021).

The oxidation of meat can decrease its hydrolytic sensitivity, weaken protein degradation, and reduce the moisture reserves in myofibers, thereby increasing water loss and brightness in meat (Huff-Lonergan and Lonergan, 2005). Enhancing the antioxidant defense system can protect muscles from damage caused by free radicals, contributing to more effective binding and retention of moisture in the muscles (Zhang et al., 2014). However, this study found that the antioxidant levels in the BSF and HTBSF groups decreased. This contrasts with the results obtained by (Dalle Zotte et al., 2018). The decrease in muscle antioxidant levels in the HTBSF group could be attributed to the decline in meat quality and the increase in UFA contents. Furthermore, there was a downward trend in the levels of antioxidant-related minerals (Zn, Se, etc.) in the muscles of both BSF and HTBSF, which may also contribute to the

decrease in muscle antioxidant levels. In conclusion, supplementing BSF could be detrimental to the antioxidant performance of muscles.

5.5.11 Gene expression

BSF has the ability to resist oxidation and enhance immunity due to their content of chitin and various bioactive substances. Previous studies have clearly elucidated the beneficial scavenging activity of chitin on free radicals (such as ROS) (Ngo et al., 2008). However, the results of this study are inconsistent with previous research findings. Therefore, in order to further investigate the potential antioxidant mechanisms of BSF in goats, we selected several genes related to antioxidation and inflammation to study their mRNA expression levels. This study found that the mRNA expression levels of CAT, GPX-1, GPX-4, and SOD in the BSF and HTBSF groups were significantly higher than those in the FFS group, and the expression levels of GPX-4 and SOD in the HTBSF group were higher than those in the BSF group. This is consistent with other results, indicating that BSF enhances the antioxidant system (D'Antonio et al., 2021), and the expression levels of antioxidant genes in treated BSF are higher. The mRNA expression levels of IL-6, IL-8, IL-1β, and TNF-α in the HTBSF and BSF groups were significantly higher than those in the FFS group, and the HTBSF group showed a significant increase compared to the BSF group. Unfortunately, the IL-10 mRNA expression in the FFS group was significantly higher than that in the BSF and HTBSF groups, but HTBSF still remained higher than the BSF group. Consistent with these findings, feeding different doses of BSF by (Abdel-Latif et al., 2021) showed a significant linear increase in mRNA expression levels of IL-10 and IL-1β in the fish liver. (Stenberg et al., 2019) reported a significant upregulation of IL-1β, IL-8, IL-10, and TNF-α mRNA expression in head kidney leukocytes of Atlantic salmon supplemented with 66% and 100% BSF. (Cardinaletti et al., 2019) observed significant activation of IL-10 and TNF-α mRNA expression in the intestine of rainbow trout supplemented with 25% and 50% whole fat larvae powder. IL-10, as an anti-inflammatory factor, we cannot explain the reason for its downregulation of expression, but overall, both the BST and HTBSF groups have improved immune gene expression, with the HTBSF group showing better results. However, the results of antioxidant and immune levels in the blood and gene expression

levels were contradictory. The lower concentration of antioxidant enzymes in the bloodstream may be due to the rapid metabolism of BSF bioactive substances in goats.

5.6 Conclusion

The growth performance of the three groups of goats was not significantly affected. HTBSF increased the digestibility of NDF and rumen pH. Overall, there was no significant effect on rumen VFA levels in the three groups. At 0 h, the activity of immune enzymes in the blood of the FFS and HTBSF groups was higher than that of the BSF group, but at 4 h, the activity of the BSF group was significantly higher than that of the FFS and HTBSF groups. The serum antioxidant level of the FFS group was significantly higher than that of the BSF and HTBSF groups. The HTBSF group increased the abundance of the phylum *Bacteroidetes* and decreased the abundance of the phylum *Firmicutes*. Both of the BSF and HTBSF groups showed a decrease in slaughter rate, carcass weight, and muscle antioxidant capacity. Additionally, the meat quality parameters of the HTBSF group were the poorest, but it improved the composition of beneficial fatty acids in the muscles for human health, such as C18:2n6t, C18:3n6, and UFA. Compared to the FFS group, both the BSF and HTBSF groups showed increased levels of antioxidant and immune mRNA expression, with the HTBSF group having higher mRNA expression levels than the BSF group.

5.7 References

Abdel-Latif, H. M. R., Abdel-Tawwab, M., Khalil, R. H., Metwally, A. A., Shakweer, M. S., Ghetas, H. A., & Khallaf, M. A. (2021). Black soldier fly (*Hermetia illucens*) larvae meal in diets of European seabass: Effects on antioxidative capacity, non-specific immunity, transcriptomic responses, and resistance to the challenge with Vibrio alginolyticus. Fish & Shellfish Immunology, 111, 111-118. https://doi.org/https://doi.org/10.1016/j.fsi.2021.01.013.

Abdelrahman, M., Wang, W., Lv, H., Di, Z., An, Z., Lijun, W., Shaukat, A., Bo, W., Guangsheng, Z., & Liguo, Y. (2022). Evaluating the Effect of Forage Rape (Brassica Napus) Ensiling Kinetics on Degradability and Milk Performance as Non-Conventional Forage for Dairy Buffalo. Frontiers in Veterinary Science, 9, 926906.

Abril, S., Pinzón, M., Hernández-Carrión, M., & Sánchez-Camargo, A. d. P. (2022). Edible insects in latin america: a sustainable alternative for our food security. Frontiers in nutrition, 9, 904812.

Acuti, G., Cullere, M., Dalle Zotte, A., Giaccone, V., Marangon, A., & Tasoniero, G. (2018). Black soldier fly as dietary protein source for broiler quails: meat proximate composition, fatty acid and amino acid profile, oxidative status and sensory traits. animal, 12(3), 640-647. https://doi.org/10.1017/S1751731117001860.

Agbohessou, P. S., Mandiki, S. N., Gougbédji, A., Megido, R. C., Hossain, M. S., De Jaeger, P., Larondelle, Y., Francis, F., Lalèyè, P. A., & Kestemont, P. (2021). Total replacement of fish meal by enriched-fatty acid *Hermetia illucens* meal did not substantially affect growth parameters or innate immune status and improved whole body biochemical quality of Nile tilapia juveniles. Aquaculture nutrition, 27(3), 880-896.

Allegretti, G., Talamini, E., Schmidt, V., Bogorni, P. C., & Ortega, E. (2018). Insect as feed: An emergy assessment of insect meal as a sustainable protein source for the Brazilian poultry industry. Journal of Cleaner Production, 171, 403-412.

Antoniewicz, A., Van Vuuren, A., Van der Koelen, C., & Kosmala, I. (1992). Intestinal digestibility of rumen undegraded protein of formaldehyde-treated feedstuffs measured by mobile bag and in vitro technique. Animal Feed Science and Technology, 39(1-2), 111-124.

AOAC, C. (2005). Official methods of analysis of the Association of Analytical Chemists International. Official Methods: Gaithersburg, MD, USA.

Aprianto, M. A., Muhlisin, Kurniawati, A., Hanim, C., Ariyadi, B., & Anas, M. A. (2023). Effect supplementation of black soldier fly larvae oil (*Hermetia illucens* L.) calcium salt on performance, blood biochemical profile, carcass characteristic, meat quality, and gene expression in fat metabolism broilers. Poultry Science, 102(10), 102984. https://doi.org/https://doi.org/10.1016/j.psj.2023.102984.

Atole, A. F. F., & Bestil, L. C. (2014). Extrapolating Bypass Protein Potential of Treated Soybean Meal by Degradation in Rumen-Fistulated Brahman Cattle. Annals of Tropical Research, 36(1), 50-62.

Aufrère, J., Graviou, D., Melcion, J.-P., & Demarquilly, C. (2001). Degradation in the rumen of lupin (*Lupinus albus* L.) and pea (*Pisum sativum* L.) seed proteins:

มหาวิทยาลัยเทคโนโลยีสุรนารี

Effect of heat treatment. Animal Feed Science and Technology, 92(3-4), 215-236.

Austin, P., Brine, C., Castle, J., & Zikakis, J. (1981). Chitin: New facets of research. Science, 212(4496), 749-753.

Bachmann, M., Kuhnitzsch, C., Thierbach, A., Michel, S., Bochnia, M., Greef, J., Martens, S., Steinhöfel, O., & Zeyner, A. (2020). Effects of toasting temperature and duration on in vitro ruminal gas production kinetics and post-ruminal crude protein from field pea (*Pisum sativum*) legume grain silages. Livestock science, 233, 103944.

Banks, I. J., Gibson, W. T., & Cameron, M. M. (2014). Growth rates of black soldier fly larvae fed on fresh human faeces and their implication for improving sanitation. Tropical medicine & international health, 19(1), 14-22.

Bellezza Oddon, S., Biasato, I., Imarisio, A., Pipan, M., Dekleva, D., Colombino, E., Capucchio, M. T., Meneguz, M., Bergagna, S., Barbero, R., Gariglio, M., Dabbou, S., Fiorilla, E., Gasco, L., & Schiavone, A. (2021). Black soldier fly and yellow mealworm live larvae for broiler chickens: Effects on bird performance and health status. J Anim Physiol Anim Nutr (Berl), 105 Suppl 1(Suppl 1), 10-18. https://doi.org/10.1111/jpn.13567.

Belverdy, M. S., Khadem, A. A., Alamouti, A. A., Khani, J., Calsamiglia, S., & Jacobs, J. (2021). Use of fat-coated or heat-treated soybean meal for partial replacement of solvent-extracted soybean meal in the diets of early lactation dairy cows. Animal Production Science.

Bi, Y., Zeng, S., Zhang, R., Diao, Q., & Tu, Y. (2018). Effects of dietary energy levels on rumen bacterial community composition in Holstein heifers under the same forage to concentrate ratio condition. BMC Microbiology, 18(1), 69. https://doi.org/10.1186/s12866-018-1213-9.

Biasato, I., Dabbou, S., Gai, F., Gasco, L., Petracci, M., Schiavone, A., Sirri, F., & Zampiga, M. (2019). Black soldier fly defatted meal as a dietary protein source for broiler chickens: effects on carcass traits, breast meat quality and safety. animal, 13(10), 2397-2405. https://doi.org/10.1017/S1751731119000685.

Biasato, I., Ferrocino, I., Colombino, E., Gai, F., Schiavone, A., Cocolin, L., Vincenti, V., Capucchio, M. T., & Gasco, L. (2020). Effects of dietary Hermetia illucens meal

inclusion on cecal microbiota and small intestinal mucin dynamics and infiltration with immune cells of weaned piglets. Journal of animal science and biotechnology, 11(1), 1-11.

Biasato, I., Ferrocino, I., Dabbou, S., Evangelista, R., Gai, F., Gasco, L., Cocolin, L., Capucchio, M. T., & Schiavone, A. (2020). Black soldier fly and gut health in broiler chickens: insights into the relationship between cecal microbiota and intestinal mucin composition. Journal of animal science and biotechnology, 11, 1-12.

Biasato, I., Renna, M., Gai, F., Dabbou, S., Meneguz, M., Perona, G., Martinez, S., Lajusticia, A. C. B., Bergagna, S., Sardi, L., Capucchio, M. T., Bressan, E., Dama, A., Schiavone, A., & Gasco, L. (2019). Partially defatted black soldier fly larva meal inclusion in piglet diets: effects on the growth performance, nutrient digestibility, blood profile, gut morphology and histological features. J Anim Sci Biotechnol, 10, 12. https://doi.org/10.1186/s40104-019-0325-x.

Bolyen, E., Rideout, J. R., Dillon, M. R., Bokulich, N. A., Abnet, C. C., Al-Ghalith, G. A., Alexander, H., Alm, E. J., Arumugam, M., Asnicar, F., Bai, Y., Bisanz, J. E., Bittinger, K., Brejnrod, A., Brislawn, C. J., Brown, C. T., Callahan, B. J., Caraballo-Rodríguez, A. M., Chase, J., . . . Caporaso, J. G. (2019). Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nature Biotechnology, 37(8), 852-857. https://doi.org/10.1038/s41587-019-0209-9.

Bortolini, S., Macavei, L. I., Saadoun, J. H., Foca, G., Ulrici, A., Bernini, F., Malferrari, D., Setti, L., Ronga, D., & Maistrello, L. (2020). *Hermetia illucens* (L.) larvae as chicken manure management tool for circular economy. Journal of Cleaner Production, 262, 121289.

Boucher, S., Calsamiglia, S., Parsons, C., Stern, M., Moreno, M. R., Vázquez-Añón, M., & Schwab, C. G. (2009). In vitro digestibility of individual amino acids in rumen-undegraded protein: The modified three-step procedure and the immobilized digestive enzyme assay. Journal of Dairy Science, 92(8), 3939-3950.

Bovera, F., Lestingi, A., Iannaccone, F., Tateo, A., & Nizza, A. (2012). Use of dietary mannanoligosaccharides during rabbit fattening period: Effects on growth performance, feed nutrient digestibility, carcass traits, and meat quality. Journal of Animal Science, 90(11), 3858-3866. https://doi.org/10.2527/jas.2011-4119.

มหาวิทยาลัยเทคโนโลยีสุรนารี

Buccioni, A., Decandia, M., Minieri, S., Molle, G., & Cabiddu, A. (2012). Lipid metabolism in the rumen: New insights on lipolysis and biohydrogenation with an emphasis on the role of endogenous plant factors. Animal Feed Science and Technology, 174(1), 1-25. https://doi.org/https://doi.org/10.1016/j.anifeedsci.2012.02.009.

Bukkens, S. G. (1997). The nutritional value of edible insects. Ecology of Food and Nutrition, 36(2-4), 287-319.

Bulak, P., Wiącek, D., & Bieganowski, A. (2020). *Hermetia illucens* exhibits bioaccumulative potential for 15 different elements-Implications for feed and food production. The Science of the Total Environment, 723, 138125-138125.

Burakowska, K., Penner, G. B., Korytkowski, Ł., Flaga, J., Kowalski, Z. M., & Górka, P. (2021). Canola meal or soybean meal as protein source and the effect of microencapsulated sodium butyrate supplementation in calf starter mixture. I. Performance, digestibility, and selected blood variables. Journal of Dairy Science, 104(6), 6646-6662. https://doi.org/https://doi.org/10.3168/jds.2020-19779.

Cabezas-Garcia, E., Krizsan, S., Shingfield, K. J., & Huhtanen, P. (2017). Between-cow variation in digestion and rumen fermentation variables associated with methane production. Journal of Dairy Science, 100(6), 4409-4424.

Caimi, C., Gasco, L., Biasato, I., Malfatto, V., Varello, K., Prearo, M., Pastorino, P., Bona, M. C., Francese, D. R., Schiavone, A., Elia, A. C., Dorr, A. J. M., & Gai, F. (2020). Could Dietary Black Soldier Fly Meal Inclusion Affect the Liver and Intestinal Histological Traits and the Oxidative Stress Biomarkers of Siberian Sturgeon (Acipenser baerii) Juveniles? Animals (Basel), 10(1). https://doi.org/10.3390/ani10010155.

Caligiani, A., Marseglia, A., Leni, G., Baldassarre, S., Maistrello, L., Dossena, A., & Sforza, S. (2018). Composition of black soldier fly prepupae and systematic approaches for extraction and fractionation of proteins, lipids and chitin. Food research international, 105, 812-820.

Campbell, M., Ortuño, J., Stratakos, A. C., Linton, M., Corcionivoschi, N., Elliott, T., Koidis, A., & Theodoridou, K. (2020a). Impact of thermal and high-pressure treatments on the microbiological quality and in vitro digestibility of black soldier fly (*Hermetia illucens*) larvae. Animals, 10(4), 682.

Campbell, M., Ortuño, J., Stratakos, A. C., Linton, M., Corcionivoschi, N., Elliott, T., Koidis, A., & Theodoridou, K. (2020b). Impact of Thermal and High-Pressure Treatments on the Microbiological Quality and In Vitro Digestibility of Black Soldier Fly (*Hermetia illucens*) Larvae. Animals, 10(4).

Cardinaletti, G., Randazzo, B., Messina, M., Zarantoniello, M., Giorgini, E., Zimbelli, A., Bruni, L., Parisi, G., Olivotto, I., & Tulli, F. (2019). Effects of Graded Dietary Inclusion Level of Full-Fat Hermetia illucens Prepupae Meal in Practical Diets for Rainbow Trout (Oncorhynchus mykiss). Animals, 9(5).

Castro, S. B., Phillip, L., Lapierre, H., Jardon, P., & Berthiaume, R. (2007). Ruminal degradability and intestinal digestibility of protein and amino acids in treated soybean meal products. Journal of Dairy Science, 90(2), 810-822.

Cerdeño, A., Vieira, C., Serrano, E., Lavín, P., & Mantecón, A. R. (2006). Effects of feeding strategy during a short finishing period on performance, carcass and meat quality in previously-grazed young bulls. Meat Science, 72(4), 719-726. https://doi.org/https://doi.org/10.1016/j.meatsci.2005.10.002.

Chan, J. M., Darke, A. K., Penney, K. L., Tangen, C. M., Goodman, P. J., Lee, G.-S. M., Sun, T., Peisch, S., Tinianow, A. M., & Rae, J. M. (2016). Selenium-or vitamin E–related gene variants, interaction with supplementation, and risk of high-grade prostate cancer in SELECT. Cancer Epidemiology, Biomarkers & Prevention, 25(7), 1050-1058.

Chao, A., & Bunge, J. (2002). Estimating the number of species in a stochastic abundance model. Biometrics, 58(3), 531-539. https://doi.org/10.1111/j.0006-341x.2002.00531.x.

Chaudhari, S. S., Arakane, Y., Specht, C. A., Moussian, B., Boyle, D. L., Park, Y., Kramer, K. J., Beeman, R. W., & Muthukrishnan, S. (2011). Knickkopf protein protects and organizes chitin in the newly synthesized insect exoskeleton. Proceedings of the National Academy of Sciences, 108(41), 17028-17033. https://doi.org/10.1073/pnas.1112288108.

Cheng, J. Y., Chiu, S. L., & Lo, I. M. (2017). Effects of moisture content of food waste on residue separation, larval growth and larval survival in black soldier fly bioconversion. Waste management, 67, 315-323.

มหาวิทยาลัยเทคโนโลยีสุรนารี

Chia, S. Y., Tanga, C. M., Khamis, F. M., Mohamed, S. A., Salifu, D., Sevgan, S., Fiaboe, K. K., Niassy, S., van Loon, J. J., & Dicke, M. (2018). Threshold temperatures and thermal requirements of black soldier fly *Hermetia illucens*: Implications for mass production. PloS one, 13(11), e0206097.

Chippindale, A. K., Leroi, A. M., Kim, S. B., & Rose, M. R. (2004). Phenotypic plasticity and selection in Drosophila life-history evolution. I. Nutrition and the cost of reproduction. In Methuselah flies: A case study in the evolution of aging (pp. 122-144). *World Scientific*.

Chowdhury, M. R., Chanda, S., Shipa, A., Saiyara, T., Chowdhury, Z. J., & Khan, M. M. H. (2022). Effect of heat-treated green tea waste feeding on fermentation kinetics, in vitro degradability, in vivo apparent digestibility, nitrogen balance, and blood metabolites in Black Bengal goat. Anim Sci J, 93(1), e13704. https://doi.org/10.1111/asj.13704.

Churchward-Venne, T. A., Pinckaers, P. J. M., van Loon, J. J. A., & van Loon, L. J. C. (2017). Consideration of insects as a source of dietary protein for human consumption. Nutrition Reviews, 75(12), 1035-1045. https://doi.org/10.1093/nutrit/nux057.

Council, N. R. (2012). Nutrient requirements of swine. Natl. Acad. Press, Washington, DC.

Crosbie, M., Zhu, C., Shoveller, A. K., & Huber, L.-A. (2020). Standardized ileal digestible amino acids and net energy contents in full fat and defatted black soldier fly larvae meals (*Hermetia illucens*) fed to growing pigs. Translational Animal Science, 4(3), txaa104.

Cullere, M., Tasoniero, G., Giaccone, V., Miotti-Scapin, R., Claeys, E., De Smet, S., & Dalle Zotte, A. (2016). Black soldier fly as dietary protein source for broiler quails: apparent digestibility, excreta microbial load, feed choice, performance, carcass and meat traits. animal, 10(12), 1923-1930. https://doi.org/10.1017/S1751731116001270.

D'Antonio, V., Serafini, M., & Battista, N. (2021). Dietary Modulation of Oxidative Stress From Edible Insects: A Mini-Review. Front Nutr, 8, 642551. https://doi.org/10.3389/fnut.2021.642551.

มหาวิทยาลัยเทคโนโลยีสุรนารี

Dabbou, S., Ferrocino, I., Gasco, L., Schiavone, A., Trocino, A., Xiccato, G., Barroeta, A. C., Maione, S., Soglia, D., & Biasato, I. (2020). Antimicrobial effects of black soldier fly and yellow mealworm fats and their impact on gut microbiota of growing rabbits. Animals, 10(8), 1292.

Dabbou, S., Gai, F., Biasato, I., Capucchio, M. T., Biasibetti, E., Dezzutto, D., Meneguz, M., Placha, I., Gasco, L., & Schiavone, A. (2018). Black soldier fly defatted meal as a dietary protein source for broiler chickens: Effects on growth performance, blood traits, gut morphology and histological features. J Anim Sci Biotechnol, 9, 49. https://doi.org/10.1186/s40104-018-0266-9.

Dabbou, S., Gai, F., Biasato, I., Capucchio, M. T., Biasibetti, E., Dezzutto, D., Meneguz, M., Plachà, I., Gasco, L., & Schiavone, A. (2018a). Black soldier fly defatted meal as a dietary protein source for broiler chickens: Effects on growth performance, blood traits, gut morphology and histological features. Journal of Animal Science and Biotechnology, 9(1), 49. https://doi.org/10.1186/s40104-018-0266-9.

Dabbou, S., Gai, F., Biasato, I., Capucchio, M. T., Biasibetti, E., Dezzutto, D., Meneguz, M., Plachà, I., Gasco, L., & Schiavone, A. (2018b). Black soldier fly defatted meal as a dietary protein source for broiler chickens: Effects on growth performance, blood traits, gut morphology and histological features. Journal of animal science and biotechnology, 9, 1-10.

Dabbou, S., Lauwaerts, A., Ferrocino, I., Biasato, I., Sirri, F., Zampiga, M., Bergagna, S., Pagliasso, G., Gariglio, M., & Colombino, E. (2021). Modified black soldier fly larva fat in broiler diet: Effects on performance, carcass traits, blood parameters, histomorphological features and gut microbiota. Animals, 11(6), 1837.

Dabbou, S., Lauwaerts, A., Ferrocino, I., Biasato, I., Sirri, F., Zampiga, M., Bergagna, S., Pagliasso, G., Gariglio, M., Colombino, E., Narro, C. G., Gai, F., Capucchio, M. T., Gasco, L., Cocolin, L., & Schiavone, A. (2021). Modified Black Soldier Fly Larva Fat in Broiler Diet: Effects on Performance, Carcass Traits, Blood Parameters, Histomorphological Features and Gut Microbiota. Animals, 11(6).

Dalle Zotte, A., Cullere, M., Martins, C., Alves, S. P., Freire, J. P. B., Falcão-e-Cunha, L., & Bessa, R. J. B. (2018). Incorporation of Black Soldier Fly (*Hermetia illucens* L.) larvae fat or extruded linseed in diets of growing rabbits and their effects on

มหาวิทยาลัยเทคโนโลยีสุรนารี

meat quality traits including detailed fatty acid composition. Meat Science, 146, 50-58. https://doi.org/https://doi.org/10.1016/j.meatsci.2018.08.002.

Daszkiewicz, T., Murawska, D., Kubiak, D., & Han, J. (2022). Chemical composition and fatty acid profile of the pectoralis major muscle in broiler chickens fed diets with full-fat black soldier fly (*Hermetia illucens*) larvae meal. Animals, 12(4), 464.

Day, L., Cakebread, J. A., & Loveday, S. M. (2022). Food proteins from animals and plants: Differences in the nutritional and functional properties. Trends in Food Science & Technology, 119, 428-442.

de Paiva, P. G., de Jesus, E. F., Del Valle, T. A., de Almeida, G. F., Costa, A. G. B. V. B., Consentini, C. E. C., Zanferari, F., Takiya, C. S., da Silva Bueno, I. C., & Rennó, F. P. (2016). Effects of chitosan on ruminal fermentation, nutrient digestibility, and milk yield and composition of dairy cows. Animal Production Science, 57(2), 301-307.

de Souza Vilela, J., Alvarenga, T. I., Andrew, N. R., McPhee, M., Kolakshyapati, M., Hopkins, D. L., & Ruhnke, I. (2021). Technological quality, amino acid and fatty acid profile of broiler meat enhanced by dietary inclusion of black soldier fly larvae. Foods, 10(2), 297.

de Souza Vilela, J., Andronicos, N. M., Kolakshyapati, M., Hilliar, M., Sibanda, T. Z., Andrew, N. R., Swick, R. A., Wilkinson, S., & Ruhnke, I. (2021). Black soldier fly larvae in broiler diets improve broiler performance and modulate the immune system. Animal Nutrition, 7(3), 695-706. https://doi.org/https://doi.org/10.1016/j.aninu.2020.08.014.

Debruyne, S., Ruiz-González, A., Artiles-Ortega, E., Ampe, B., Van Den Broeck, W., De Keyser, E., Vandaele, L., Goossens, K., & Fievez, V. (2018). Supplementing goat kids with coconut medium chain fatty acids in early life influences growth and rumen papillae development until 4 months after supplementation but effects on in vitro methane emissions and the rumen microbiota are transient. Journal of Animal Science, 96(5), 1978-1995.

Deng, X., Huang, H., Huang, S., Yang, M., Wu, J., Ci, Z., He, Y., Wu, Z., Han, L., & Zhang, D. (2022). Insight into the incredible effects of microwave heating: Driving

มหาวิทยาลัยเทคโนโลยีสุรนารี

changes in the structure, properties and functions of macromolecular nutrients in novel food. Front Nutr, 9, 941527. https://doi.org/10.3389/fnut.2022.941527.

Dias, A., Goes, R., Gandra, J., Takiya, C., Branco, A., Jacaúna, A., Oliveira, R., Souza, C., & Vaz, M. (2017). Increasing doses of chitosan to grazing beef steers: Nutrient intake and digestibility, ruminal fermentation, and nitrogen utilization. Animal Feed Science and Technology, 225, 73-80.

Dicke, M. (2018). Insects as feed and the Sustainable Development Goals. Journal of Insects as Food and Feed, 4(3), 147-156.

Dicke, M., Melse-Boonstra, A., Mwangi, M. N., Oonincx, D. G. A. B., Stouten, T., Veenenbos, M., & van Loon, J. J. A. (2018). Insects as sources of iron and zinc in human nutrition. Nutrition Research Reviews, 31(2), 248-255. https://doi.org/10.1017/S0954422418000094.

Diener, S., Zurbrügg, C., & Tockner, K. (2009). Conversion of organic material by black soldier fly larvae: establishing optimal feeding rates. Waste Management & Research, 27(6), 603-610.

Dijkstra, J., Ellis, J. L., Kebreab, E., Strathe, A. B., López, S., France, J., & Bannink, A. (2012). Ruminal pH regulation and nutritional consequences of low pH. Animal Feed Science and Technology, 172(1), 22-33. https://doi.org/https://doi.org/10.1016/j.anifeedsci.2011.12.005.

Dijkstra, J., Van Gastelen, S., Dieho, K., Nichols, K., & Bannink, A. (2020). Rumen sensors: Data and interpretation for key rumen metabolic processes. Animal, 14(S1), s176-s186.

Do, S., Koutsos, E. A., McComb, A., Phungviwatnikul, T., de Godoy, M. R. C., & Swanson, K. S. (2022). Palatability and apparent total tract macronutrient digestibility of retorted black soldier fly larvae-containing diets and their effects on the fecal characteristics of cats consuming them. Journal of Animal Science, 100(4), skac068. https://doi.org/10.1093/jas/skac068.

DONG, S.-z., Azarfar, A., Yang, Z., LI, S.-l., WANG, Y.-j., & CAO, Z.-j. (2017). Effects of sequence of nylon bags rumen incubation on kinetics of degradation in some commonly used feedstuffs in dairy rations. Journal of Integrative Agriculture, 16(1), 162-168.

มหาวิทยาลัยเทคโนโลยีสุรนารี

Dortmans, B., Diener, S., Bart, V., & Zurbrügg, C. (2017). Black soldier fly biowaste processing: a step-by-step guide. **eawag**.

Driemeyer, H. (2016). Evaluation of black soldier fly (*Hermetia illucens*) larvae as an alternative protein source in pig creep diets in relation to production, blood and manure microbiology parameters Stellenbosch: Stellenbosch University].

Ebeneezar, S., Tejpal, C., Jeena, N., Summaya, R., Chandrasekar, S., Sayooj, P., & Vijayagopal, P. (2021). Nutritional evaluation, bioconversion performance and phylogenetic assessment of black soldier fly (*Hermetia illucens*, Linn. 1758) larvae valorized from food waste. Environmental Technology & Innovation, 23, 101783.

El-Hack, A., Mohamed, E., Shafi, M. E., Alghamdi, W. Y., Abdelnour, S. A., Shehata, A. M., Noreldin, A. E., Ashour, E. A., Swelum, A. A., & Al-Sagan, A. A. (2020). Black soldier fly (*Hermetia illucens*) meal as a promising feed ingredient for poultry: A comprehensive review. Agriculture, 10(8), 339.

Elieh Ali Komi, D., Sharma, L., & Dela Cruz, C. S. (2018). Chitin and its effects on inflammatory and immune responses. Clinical reviews in allergy & immunology, 54, 213-223.

Enser, M., Gulati, S. K., Richardson, I., Scollan, N. D., & Wood, J. D. (2003). Effects of including a ruminally protected lipid supplement in the diet on the fatty acid composition of beef muscle. British Journal of Nutrition, 90(3), 709-716. https://doi.org/10.1079/BJN2003933.

Erdman, R. A. (1988). Dietary Buffering Requirements of the Lactating Dairy Cow: A Review1. Journal of Dairy Science, 71(12), 3246-3266. https://doi.org/https://doi.org/10.3168/jds.S0022-0302(88)79930-0.

Eric, K., Raymond, L. V., Huang, M., Cheserek, M. J., Hayat, K., Savio, N. D., Amédée, M., & Zhang, X. (2013). Sensory attributes and antioxidant capacity of Maillard reaction products derived from xylose, cysteine and sunflower protein hydrolysate model system. Food Research International, 54(2), 1437-1447. https://doi.org/10.1016/j.foodres.2013.09.034.

Erickson, M. C., Islam, M., Sheppard, C., Liao, J., & Doyle, M. P. (2004). Reduction of Escherichia coli O157: H7 and Salmonella enterica serovar Enteritidis in chicken manure by larvae of the black soldier fly. Journal of food protection, 67(4), 685-690.

Estévez, M., Geraert, P.-A., Liu, R., Delgado, J., Mercier, Y., & Zhang, W. (2020). Sulphur amino acids, muscle redox status and meat quality: More than building blocks – Invited review. Meat Science, 163, 108087. https://doi.org/https://doi.org/10.1016/j.meatsci.2020.108087.

Ewald, N., Vidakovic, A., Langeland, M., Kiessling, A., Sampels, S., & Lalander, C. (2020). Fatty acid composition of black soldier fly larvae (*Hermetia illucens*)–Possibilities and limitations for modification through diet. Waste Management, 102, 40-47.

Finke, M. D. (2007). Estimate of chitin in raw whole insects. Zoo biology: published in affiliation with the American zoo and aquarium association, 26(2), 105-115.

Gariglio, M., Dabbou, S., Crispo, M., Biasato, I., Gai, F., Gasco, L., Piacente, F., Odetti, P., Bergagna, S., Placha, I., Valle, E., Colombino, E., Capucchio, M. T., & Schiavone, A. (2019). Effects of the Dietary Inclusion of Partially Defatted Black Soldier Fly (*Hermetia illucens*) Meal on the Blood Chemistry and Tissue (Spleen, Liver, Thymus, and Bursa of Fabricius) Histology of Muscovy Ducks (Cairina moschata domestica). Animals (Basel), 9(6). https://doi.org/10.3390/ani9060307.

Gasco, L., Dabbou, S., Trocino, A., Xiccato, G., Capucchio, M. T., Biasato, I., Dezzutto, D., Birolo, M., Meneguz, M., & Schiavone, A. (2019). Effect of dietary supplementation with insect fats on growth performance, digestive efficiency and health of rabbits. Journal of animal science and biotechnology, 10(1), 1-9.

Ghimire, S. (2021). The effect of black soldier fly (Hermetia illucens) larvae fractions in diets for Atlantic salmon (Salmo salar) on extruder parameters, pellet quality, growth performance and nutrient utilization Norwegian University of Life Sciences, Ås].

Goetsch, A. L., Merkel, R. C., & Gipson, T. A. (2011). Factors affecting goat meat production and quality. Small Ruminant Research, 101(1), 173-181. https://doi.org/https://doi.org/10.1016/j.smallrumres.2011.09.037.

Goh, P. G., & Chou, M. C.-f. (2022). Global Supply Chains in a Glocal World: The Impact of COVID-19 and Digitalisation. **World Scientific**.

Goiri, I., Oregui, L., & Garcia-Rodriguez, A. (2010). Use of chitosans to modulate ruminal fermentation of a 50: 50 forage-to-concentrate diet in sheep. Journal of animal science, 88(2), 749-755.

Gold, M., Cassar, C. M., Zurbrügg, C., Kreuzer, M., Boulos, S., Diener, S., & Mathys, A. (2020). Biowaste treatment with black soldier fly larvae: Increasing performance through the formulation of biowastes based on protein and carbohydrates. Waste Management, 102, 319-329.

Górka, P., Kowalski, Z. M., Zabielski, R., & Guilloteau, P. (2018). Invited review: Use of butyrate to promote gastrointestinal tract development in calves. Journal of Dairy Science, 101(6), 4785-4800. https://doi.org/https://doi.org/10.3168/jds.2017-14086.

Gu, C., Pan, H., Sun, Z., & Qin, G. (2010). Effect of Soybean Variety on Anti-Nutritional Factors Content, and Growth Performance and Nutrients Metabolism in Rat. International Journal of Molecular Sciences, 11(3), 1048-1056.

Hao, L., Xiang, Y., Degen, A., Huang, Y., Niu, J., Sun, L., Chai, S., Zhou, J., Ding, L., Long, R., & Liu, S. (2019). Adding heat-treated rapeseed to the diet of yak improves growth performance and tenderness and nutritional quality of the meat. Anim Sci J, 90(9), 1177-1184. https://doi.org/10.1111/asj.13266.

Hartinger, K., Greinix, J., Thaler, N., Ebbing, M. A., Yacoubi, N., Schedle, K., & Gierus, M. (2021). Effect of Graded Substitution of Soybean Meal by Hermetia illucens Larvae Meal on Animal Performance, Apparent Ileal Digestibility, Gut Histology and Microbial Metabolites of Broilers. Animals (Basel), 11(6). https://doi.org/10.3390/ani11061628.

Henchion, M., Moloney, A. P., Hyland, J., Zimmermann, J., & McCarthy, S. (2021). Review: Trends for meat, milk and egg consumption for the next decades and the role played by livestock systems in the global production of proteins. Animal, 15, 100287. https://doi.org/https://doi.org/10.1016/j.animal.2021.100287.

Hender, A., Siddik, M. A., Howieson, J., & Fotedar, R. (2021a). Black soldier fly, *Hermetia illucens* as an alternative to fishmeal protein and fish oil: impact on growth, immune response, mucosal barrier status, and flesh quality of juvenile barramundi, Lates calcarifer (Bloch, 1790). Biology, 10(6), 505.

Hender, A., Siddik, M. A. B., Howieson, J., & Fotedar, R. (2021b). Black Soldier Fly, *Hermetia illucens* as an Alternative to Fishmeal Protein and Fish Oil: Impact on Growth, Immune Response, Mucosal Barrier Status, and Flesh Quality of Juvenile Barramundi, Lates calcarifer (Bloch, 1790). Biology, 10(6).

Herrera, E., Petrusan, J.-I., Salvá-Ruiz, B., Novak, A., Cavalcanti, K., Aguilar, V., Heinz, V., & Smetana, S. (2022). Meat Quality of Guinea Pig (Cavia porcellus) Fed with Black Soldier Fly Larvae Meal (*Hermetia illucens*) as a Protein Source. Sustainability, 14(3). https://doi.org/10.3390/su14031292.

Hervás, G., Boussalia, Y., Labbouz, Y., Della Badia, A., Toral, P. G., & Frutos, P. (2022). Insect oils and chitosan in sheep feeding: Effects on in vitro ruminal biohydrogenation and fermentation. Animal Feed Science and Technology, 285, 115222.

Hill, T. C. J., Walsh, K. A., Harris, J. A., & Moffett, B. F. (2003). Using ecological diversity measures with bacterial communities. FEMS Microbiology Ecology, 43(1), 1-11. https://doi.org/10.1111/j.1574-6941.2003.tb01040.x.

Holmes, L. (2010). Role of abiotic factors on the development and life history of the black soldier fly, *Hermetia illucens* (L.)(Diptera: Stratiomyidae).

Goh, P. G., & Chou, M. C.-f. (2022). Global Supply Chains in a Glocal World: The Impact of COVID-19 and Digitalisation. **World Scientific**.

Goiri, I., Oregui, L., & Garcia-Rodriguez, A. (2010). Use of chitosans to modulate ruminal fermentation of a 50: 50 forage-to-concentrate diet in sheep. Journal of animal science, 88(2), 749-755.

Gold, M., Cassar, C. M., Zurbrügg, C., Kreuzer, M., Boulos, S., Diener, S., & Mathys, A. (2020). Biowaste treatment with black soldier fly larvae: Increasing performance through the formulation of biowastes based on protein and carbohydrates. Waste Management, 102, 319-329.

Górka, P., Kowalski, Z. M., Zabielski, R., & Guilloteau, P. (2018). Invited review: Use of butyrate to promote gastrointestinal tract development in calves. Journal of Dairy Science, 101(6), 4785-4800. https://doi.org/https://doi.org/10.3168/jds.2017-14086

Gu, C., Pan, H., Sun, Z., & Qin, G. (2010). Effect of Soybean Variety on Anti-Nutritional Factors Content, and Growth Performance and Nutrients Metabolism in Rat. International Journal of Molecular Sciences, 11(3), 1048-1056.

Hao, L., Xiang, Y., Degen, A., Huang, Y., Niu, J., Sun, L., Chai, S., Zhou, J., Ding, L., Long, R., & Liu, S. (2019). Adding heat-treated rapeseed to the diet of yak improves

มหาวิทยาลัยเทคโนโลยีสุรนารี

growth performance and tenderness and nutritional quality of the meat. Anim Sci J, 90(9), 1177-1184. https://doi.org/10.1111/asj.13266.

Hartinger, K., Greinix, J., Thaler, N., Ebbing, M. A., Yacoubi, N., Schedle, K., & Gierus, M. (2021). Effect of Graded Substitution of Soybean Meal by *Hermetia illucens* Larvae Meal on Animal Performance, Apparent Ileal Digestibility, Gut Histology and Microbial Metabolites of Broilers. Animals (Basel), 11(6). https://doi.org/10.3390/ani11061628.

Henchion, M., Moloney, A. P., Hyland, J., Zimmermann, J., & McCarthy, S. (2021). Review: Trends for meat, milk and egg consumption for the next decades and the role played by livestock systems in the global production of proteins. Animal, 15, 100287. https://doi.org/https://doi.org/10.1016/j.animal.2021.100287.

Hender, A., Siddik, M. A., Howieson, J., & Fotedar, R. (2021a). Black soldier fly, Hermetia illucens as an alternative to fishmeal protein and fish oil: impact on growth, immune response, mucosal barrier status, and flesh quality of juvenile barramundi, Lates calcarifer (Bloch, 1790). Biology, 10(6), 505.

Hender, A., Siddik, M. A. B., Howieson, J., & Fotedar, R. (2021b). Black Soldier Fly, Hermetia illucens as an Alternative to Fishmeal Protein and Fish Oil: Impact on Growth, Immune Response, Mucosal Barrier Status, and Flesh Quality of Juvenile Barramundi, Lates calcarifer (Bloch, 1790). Biology, 10(6).

Herrera, E., Petrusan, J.-I., Salvá-Ruiz, B., Novak, A., Cavalcanti, K., Aguilar, V., Heinz, V., & Smetana, S. (2022). Meat Quality of Guinea Pig (*Cavia porcellus*) Fed with Black Soldier Fly Larvae Meal (Hermetia illucens) as a Protein Source. Sustainability, 14(3). https://doi.org/10.3390/su14031292.

Hervás, G., Boussalia, Y., Labbouz, Y., Della Badia, A., Toral, P. G., & Frutos, P. (2022). Insect oils and chitosan in sheep feeding: Effects on in vitro ruminal biohydrogenation and fermentation. Animal Feed Science and Technology, 285, 115222.

Hill, T. C. J., Walsh, K. A., Harris, J. A., & Moffett, B. F. (2003). Using ecological diversity measures with bacterial communities. FEMS Microbiology Ecology, 43(1), 1-11. https://doi.org/10.1111/j.1574-6941.2003.tb01040.x.

Holmes, L. (2010). Role of abiotic factors on the development and life history of the black soldier fly, *Hermetia illucens* (L.)(Diptera: Stratiomyidae).

Je, J.-Y., Park, P.-J., & Kim, S.-K. (2004). Free radical scavenging properties of hetero-chitooligosaccharides using an ESR spectroscopy. Food and Chemical Toxicology, 42(3), 381-387.

Jeong, C. D., Mamuad, L. L., Kim, S. H., Choi, Y. J., Soriano, A. P., Cho, K. K., Jeon, C. O., Lee, S. S., & Lee, S. S. (2015). Effect of Soybean Meal and Soluble Starch on Biogenic Amine Production and Microbial Diversity Using In vitro Rumen Fermentation. Asian-Australas J Anim Sci, 28(1), 50-57. https://doi.org/10.5713/ajas.14.0555.

Jian, S., Zhang, L., Ding, N., Yang, K., Xin, Z., Hu, M., Zhou, Z., Zhao, Z., Deng, B., & Deng, J. (2022). Effects of black soldier fly larvae as protein or fat sources on apparent nutrient digestibility, fecal microbiota, and metabolic profiles in beagle dogs. Front Microbiol, 13, 1044986. https://doi.org/10.3389/fmicb.2022.1044986.

Jian, S., Zhang, L., Ding, N., Yang, K., Xin, Z., Hu, M., Zhou, Z., Zhao, Z., Deng, B., & Deng, J. (2022). Effects of black soldier fly larvae as protein or fat sources on apparent nutrient digestibility, fecal microbiota, and metabolic profiles in beagle dogs. Frontiers in Microbiology, 13, 1044986.

Jiao, J., Huang, J., Zhou, C., & Tan, Z. (2015). Taxonomic Identification of Ruminal Epithelial Bacterial Diversity during Rumen Development in Goats. Applied and Environmental Microbiology, 81(10), 3502-3509. https://doi.org/10.1128/ AEM.00203-15.

Jin, X. H., Heo, P. S., Hong, J. S., Kim, N. J., & Kim, Y. Y. (2016). Supplementation of Dried Mealworm (Tenebrio molitor larva) on Growth Performance, Nutrient Digestibility and Blood Profiles in Weaning Pigs. Asian-Australas J Anim Sci, 29(7), 979-986. https://doi.org/10.5713/ajas.15.0535.

Jonas-Levi, A., & Martinez, J.-J. I. (2017). The high level of protein content reported in insects for food and feed is overestimated. Journal of Food Composition and Analysis, 62, 184-188. https://doi.org/https://doi.org/10.1016/j.jfca.2017.06.004.

Kaewwongsa, W., Traiyakun, S., Yuangklang, C., Wachirapakorn, C., & Paengkoum, P. (2011). Protein enrichment of cassava pulp fermentation by *Saccharomyces cerevisiae*. Journal of Animal and Veterinary Advances, 10(18), 2434-2440.

Kahraman, O., Gülşen, N., İnal, F., Alataş, M. S., İnanç, Z. S., Ahmed, İ., Şişman, D., & Küçük, A. E. (2023a). Comparative Analysis of In Vitro Fermentation Parameters in Total Mixed Rations of Dairy Cows with Varied Levels of Defatted Black

Soldier Fly Larvae (*Hermetia illucens*) as a Substitute for Soybean Meal. Fermentation, 9(7), 652. https://www.mdpi.com/2311-5637/9/7/652.

Kahraman, O., Gülşen, N., İnal, F., Alataş, M. S., İnanç, Z. S., Ahmed, İ., Şişman, D., & Küçük, A. E. (2023b). Comparative Analysis of In Vitro Fermentation Parameters in Total Mixed Rations of Dairy Cows with Varied Levels of Defatted Black Soldier Fly Larvae (*Hermetia illucens*) as a Substitute for Soybean Meal. Fermentation, 9(7).

Kahraman, O., Gülşen, N., İnal, F., Alataş, M. S., İnanç, Z. S., Ahmed, İ., Şişman, D., & Küçük, A. E. (2023c). Comparative Analysis of In Vitro Fermentation Parameters in Total Mixed Rations of Dairy Cows with Varied Levels of Defatted Black Soldier Fly Larvae (*Hermetia illucens*) as a Substitute for Soybean Meal. Fermentation, 9(7). https://doi.org/10.3390/fermentation9070652.

Kamau, E., Mutungi, C., Kinyuru, J., Imathiu, S., Tanga, C., Affognon, H., Ekesi, S., Nakimbugwe, D., & Fiaboe, K. (2018). Moisture adsorption properties and shelf-life estimation of dried and pulverised edible house cricket *Acheta domesticus* (L.) and black soldier fly larvae *Hermetia illucens* (L.). Food research international, 106, 420-427.

Kang, S., Wanapat, M., Phesatcha, K., Norrapoke, T., Foiklang, S., Ampapon, T., & Phesatcha, B. (2016). Using krabok (Irvingia malayana) seed oil and Flemingia macrophylla leaf meal as a rumen enhancer in an in vitro gas production system. Animal Production Science, 57(2), 327-333.

Kapoor, B., Kapoor, D., Gautam, S., Singh, R., & Bhardwaj, S. (2021). Dietary Polyunsaturated Fatty Acids (PUFAs): Uses and Potential Health Benefits. Curr Nutr Rep, 10(3), 232-242. https://doi.org/10.1007/s13668-021-00363-3.

Karlsson, L., Ruiz-Moreno, M., Stern, M., & Martinsson, K. (2012). Effects of temperature during moist heat treatment on ruminal degradability and intestinal digestibility of protein and amino acids in hempseed cake. Asian-Australasian journal of animal sciences, 25(11), 1559.

Karlsson, L., Ruiz-Moreno, M., Stern, M. D., & Martinsson, K. (2012). Effects of Temperature during Moist Heat Treatment on Ruminal Degradability and Intestinal Digestibility of Protein and Amino Acids in Hempseed Cake. Asian-Australas J Anim Sci, 25(11), 1559-1567. https://doi.org/10.5713/ajas.2012.12213.

Kawasaki, K., Hashimoto, Y., Hori, A., Kawasaki, T., Hirayasu, H., Iwase, S.-i., Hashizume, A., Ido, A., Miura, C., & Miura, T. (2019). Evaluation of black soldier fly (*Hermetia illucens*) larvae and pre-pupae raised on household organic waste, as potential ingredients for poultry feed. Animals, 9(3), 98.

Kazemi-Bonchenari, M., Alizadeh, A. R., Tahriri, A. R., Karkoodi, K., Jalali, S., & Sadri, H. (2015). The Effects of Partial Replacement of Soybean Meal by Xylose-Treated Soybean Meal in the Starter Concentrate on Performance, Health Status, and Blood Metabolites of Holstein Calves. Italian Journal of Animal Science, 14(1), 3680. https://doi.org/10.4081/ijas.2015.3680.

Kazemi-Bonchenari, M., Mirzaei, M., Jahani-Moghadam, M., Soltani, A., Mahjoubi, E., & Patton, R. A. (2016). Interactions between levels of heat-treated soybean meal and prilled fat on growth, rumen fermentation, and blood metabolites of Holstein calves1. Journal of Animal Science, 94(10), 4267-4275. https://doi.org/10.2527/jas.2016-0514.

Kim, D., Mizinga, K., Kube, J., Friesen, K., McLeod, K., & Harmon, D. (2014). Influence of monensin and lauric acid distillate or palm oil on in vitro fermentation kinetics and metabolites produced using forage and high concentrate substrates. Animal Feed Science and Technology, 189, 19-29.

Kohn, R. A., Dinneen, M. M., & Russek-Cohen, E. (2005). Using blood urea nitrogen to predict nitrogen excretion and efficiency of nitrogen utilization in cattle, sheep, goats, horses, pigs, and rats1. Journal of Animal Science, 83(4), 879-889. https://doi.org/10.2527/2005.834879x.

Krizsan, S. J., Gidlund, H., Fatehi, F., & Huhtanen, P. (2017). Effect of dietary supplementation with heat-treated canola meal on ruminal nutrient metabolism in lactating dairy cows. Journal of Dairy Science, 100(10), 8004-8017. https://doi.org/https://doi.org/10.3168/jds.2017-12625.

Kröger, S., Heide, C., & Zentek, J. (2020). Evaluation of an extruded diet for adult dogs containing larvae meal from the Black soldier fly (*Hermetia illucens*). Animal Feed Science and Technology, 270, 114699. https://doi.org/https://doi.org/10.1016/j.anifeedsci.2020.114699.

มหาวิทยาลัยเทคโนโลยีสุรนารี

Kryukov, G. V., Castellano, S., Novoselov, S. V., Lobanov, A. V., Zehtab, O., Guigó, R., & Gladyshev, V. N. (2003). Characterization of mammalian selenoproteomes. Science, 300(5624), 1439-1443.

Lalander, C., Diener, S., Magri, M. E., Zurbrügg, C., Lindström, A., & Vinnerås, B. (2013). Faecal sludge management with the larvae of the black soldier fly (*Hermetia illucens*)-From a hygiene aspect. Science of the Total Environment, 458, 312-318.

Lalander, C., Diener, S., Zurbrügg, C., & Vinnerås, B. (2019). Effects of feedstock on larval development and process efficiency in waste treatment with black soldier fly (*Hermetia illucens*). Journal of Cleaner Production, 208, 211-219.

Larouche, J., Deschamps, M.-H., Saucier, L., Lebeuf, Y., Doyen, A., & Vandenberg, G. W. (2019). Effects of killing methods on lipid oxidation, colour and microbial load of black soldier fly (*Hermetia illucens*) larvae. Animals, 9(4), 182.

Li, J., Shi, B., Yan, S., Jin, L., Guo, Y., Xu, Y., Li, T., & Guo, X. (2013). Effects of dietary supplementation of chitosan on humoral and cellular immune function in weaned piglets. Animal Feed Science and Technology, 186(3-4), 204-208.

Liang, J., Fang, W., Wang, Q., Zubair, M., Zhang, G., Ma, W., Cai, Y., & Zhang, P. (2021). Metagenomic analysis of community, enzymes and metabolic pathways during corn straw fermentation with rumen microorganisms for volatile fatty acid production. Bioresource technology, 342, 126004.

Liu, Q., Tomberlin, J. K., Brady, J. A., Sanford, M. R., & Yu, Z. (2008). Black soldier fly (Diptera: Stratiomyidae) larvae reduce Escherichia coli in dairy manure. Environmental entomology, 37(6), 1525-1530.

Liu, S., Sun, J., Yu, L., Zhang, C., Bi, J., Zhu, F., Qu, M., Jiang, C., & Yang, Q. (2012). Extraction and characterization of chitin from the beetle Holotrichia parallela Motschulsky. Molecules, 17(4), 4604-4611.

Liu, X., Chen, X., Wang, H., Yang, Q., ur Rehman, K., Li, W., Cai, M., Li, Q., Mazza, L., & Zhang, J. (2017). Dynamic changes of nutrient composition throughout the entire life cycle of black soldier fly. PloS one, 12(8), e0182601.

Ljøkjel, K., Harstad, O. M., Prestløkken, E., & Skrede, A. (2003a). In situ digestibility of protein in barley grain (*Hordeum vulgare*) and peas (*Pisum sativum* L.) in dairy

มหาวิทยาลัยเทคโนโลยีสุรนารี

cows: influence of heat treatment and glucose addition. Animal Feed Science and Technology, 107(1-4), 87-104.

Ljøkjel, K., Harstad, O. M., Prestløkken, E., & Skrede, A. (2003b). In situ digestibility of starch in barley grain (*Hordeum vulgare*) and peas (*Pisum sativum* L.) in dairy cows: influence of heat treatment and glucose addition. Animal Feed Science and Technology, 107(1), 105-116. https://doi.org/https://doi.org/10.1016/S0377-8401(03)00122-6.

Longvah, T., Mangthya, K., & Ramulu, P. (2011). Nutrient composition and protein quality evaluation of eri silkworm (*Samia ricinii*) prepupae and pupae. Food Chemistry, 128(2), 400-403. https://doi.org/https://doi.org/10.1016/j.foodchem.2011.03.041.

Lotfi, K., Salari-Moghaddam, A., Yousefinia, M., Larijani, B., & Esmaillzadeh, A. (2021). Dietary intakes of monounsaturated fatty acids and risk of mortality from all causes, cardiovascular disease and cancer: A systematic review and dose-response meta-analysis of prospective cohort studies. Ageing Research Reviews, 72, 101467. https://doi.org/https://doi.org/10.1016/j.arr.2021.101467.

Lu, R., Chen, Y., Yu, W., Lin, M., Yang, G., Qin, C., Meng, X., Zhang, Y., Ji, H., & Nie, G. (2020). Defatted black soldier fly (*Hermetia illucens*) larvae meal can replace soybean meal in juvenile grass carp (*Ctenopharyngodon idellus*) diets. Aquaculture Reports, 18, 100520. https://doi.org/https://doi.org/10.1016/j.aqrep.2020.100520.

Lu, S., Taethaisong, N., Meethip, W., Surakhunthod, J., Sinpru, B., Sroichak, T., Archa, P., Thongpea, S., Paengkoum, S., Purba, R. A., & Paengkoum, P. (2022). Nutritional Composition of Black Soldier Fly Larvae (*Hermetia illucens* L.) and Its Potential Uses as Alternative Protein Sources in Animal Diets: A Review. Insects, 13(9).

Lu, S., Taethaisong, N., Meethip, W., Surakhunthod, J., Sinpru, B., Sroichak, T., Archa, P., Thongpea, S., Paengkoum, S., & Purba, R. A. P. (2022). Nutritional composition of black soldier fly larvae (*Hermetia illucens* L.) and its potential uses as alternative protein sources in animal diets: A review. Insects, 13(9), 831.

Lund, P., Weisbjerg, M. R., & Hvelplund, T. (2008). Profile of digested feed amino acids from untreated and expander treated feeds estimated using in situ methods in

dairy cows. Livestock Science, 114(1), 62-74. https://doi.org/https://doi.org/10.1016/j.livsci.2007.04.012.

Ma, J., Lei, Y., Rehman, K. u., Yu, Z., Zhang, J., Li, W., Li, Q., Tomberlin, J. K., & Zheng, L. (2018). Dynamic effects of initial pH of substrate on biological growth and metamorphosis of black soldier fly (Diptera: Stratiomyidae). Environmental entomology, 47(1), 159-165.

Madsen, J., Hvelplund, T., & Weisbjerg, M. (1997). Appropriate methods for the evaluation of tropical feeds for ruminants. Animal Feed Science and Technology, 69(1-3), 53-66.

Mallin, M. A., & Cahoon, L. B. (2003). Industrialized animal production-a major source of nutrient and microbial pollution to aquatic ecosystems. Population and Environment, 24(5), 369-385.

Mantovani, H. C., Huws, S. A., Creevey, C. J., Oyama, L. B., Mizrahi, I., Denman, S. E., Popova, M., Muñoz-Tamayo, R., Forano, E., & Waters, S. M. (2019). Addressing global ruminant agricultural challenges through understanding the rumen microbiome: past, present, and future.

Manzocchi, E., Ferlay, A., Mendowski, S., Chesneau, G., Chapoutot, P., Lemosquet, S., Cantalapiedra-Hijar, G., & Nozière, P. (2023). Extrusion of lupines with or without addition of reducing sugars: Effects on the formation of Maillard reaction compounds, partition of nitrogen and Nε-carboxymethyl-lysine, and performance of dairy cows. Journal of Dairy Science, 106(11), 7675-7697.

Maxin, G., Ouellet, D. R., & Lapierre, H. (2013). Ruminal degradability of dry matter, crude protein, and amino acids in soybean meal, canola meal, corn, and wheat dried distillers grains. Journal of Dairy Science, 96(8), 5151-5160. https://doi.org/https://doi.org/10.3168/jds.2012-6392.

McCann, J. C., Luan, S., Cardoso, F. C., Derakhshani, H., Khafipour, E., & Loor, J. J. (2016). Induction of subacute ruminal acidosis affects the ruminal microbiome and epithelium. Frontiers in Microbiology, 7, 701.

McLeod, A. (2011). World livestock 2011-livestock in food security. *Food and agriculture organization of the united nations (FAO).*

Meneguz, M., Gasco, L., & Tomberlin, J. K. (2018). Impact of pH and feeding system on black soldier fly (*Hermetia illucens*, L; Diptera: Stratiomyidae) larval development. PloS one, 13(8), e0202591.

Meneguz, M., Schiavone, A., Gai, F., Dama, A., Lussiana, C., Renna, M., & Gasco, L. (2018). Effect of rearing substrate on growth performance, waste reduction efficiency and chemical composition of black soldier fly (*Hermetia illucens*) larvae. Journal of the Science of Food and Agriculture, 98(15), 5776-5784.

Mir, N. A., Rafiq, A., Kumar, F., Singh, V., & Shukla, V. (2017). Determinants of broiler chicken meat quality and factors affecting them: a review. J Food Sci Technol, 54(10), 2997-3009. https://doi.org/10.1007/s13197-017-2789-z.

Mok, W. K., Tan, Y. X., & Chen, W. N. (2020). Technology innovations for food security in Singapore: A case study of future food systems for an increasingly natural resource-scarce world. Trends in Food Science & Technology, 102, 155-168. https://doi.org/https://doi.org/10.1016/j.tifs.2020.06.013.

Molosse, V. L., Pereira, D. A. B., Rigon, F., Loregian, K. E., Magnani, E., Marcondes, M. I., Branco, R. H., Benedeti, P. D. B., & Paula, E. M. (2023). Use of Heating Methods and Xylose to Increase Rumen Undegradable Protein of Alternative Protein Sources: 2) Cottonseed Meal. Animals, 13(1).

Müller, A., Wolf, D., & Gutzeit, H. O. (2017). The black soldier fly, Hermetia illucens–a promising source for sustainable production of proteins, lipids and bioactive substances. Zeitschrift für Naturforschung C, 72(9-10), 351-363.

Mupangwa, J., Ngongoni, N., Topps, J., Acamovic, T., & Hamudikuwanda, H. (2003). Rumen degradability and post-ruminal digestion of dry matter, nitrogen and amino acids in three tropical forage legumes estimated by the mobile nylon bag technique. Livestock Production Science, 79(1), 37-46.

Murawska, D., Daszkiewicz, T., Sobotka, W., Gesek, M., Witkowska, D., Matusevicius, P., & Bakula, T. (2021). Partial and Total Replacement of Soybean Meal with Full-Fat Black Soldier Fly (*Hermetia illucens* L.) Larvae Meal in Broiler Chicken Diets: Impact on Growth Performance, Carcass Quality and Meat Quality. Animals (Basel), 11(9). https://doi.org/10.3390/ani11092715.

Murawska, D., Daszkiewicz, T., Sobotka, W., Gesek, M., Witkowska, D., Matusevičius, P., & Bakuła, T. (2021). Partial and Total Replacement of Soybean Meal with Full-

Fat Black Soldier Fly (*Hermetia illucens* L.) Larvae Meal in Broiler Chicken Diets: Impact on Growth Performance, Carcass Quality and Meat Quality. Animals, 11(9).

Myers, H. M., Tomberlin, J. K., Lambert, B. D., & Kattes, D. (2014). Development of black soldier fly (Diptera: Stratiomyidae) larvae fed dairy manure. Environmental entomology, 37(1), 11-15.

Nana, P., Kimpara, J. M., Tiambo, C. K., Tiogue, C. T., Youmbi, J., Choundong, B., & Fonkou, T. (2018). Black soldier flies (*Hermetia illucens* Linnaeus) as recyclers of organic waste and possible livestock feed. International Journal of Biological and Chemical Sciences, 12(5), 2004-2015.

Nayohan, S., Susanto, I., Permata, D., Pangesti, R. T., Rahmadani, M., & Jayanegara, A. (2022). Effect of dietary inclusion of black soldier fly larvae (*Hermetia illucens*) on broiler performance: A meta-analysis. E3S Web of Conferences,

Nevin, K., & Rajamohan, T. (2006). Virgin coconut oil supplemented diet increases the antioxidant status in rats. Food Chemistry, 99(2), 260-266.

Newton, G., Sheppard, D., Watson, D., Burtle, G., Dove, C., Tomberlin, J., & Thelen, E. (2005). The black soldier fly, *Hermetia illucens*, as a manure management/resource recovery tool. Symposium on the state of the science of Animal Manure and Waste Management,

Ngo, D.-H., & Kim, S.-K. (2014a). Antioxidant effects of chitin, chitosan, and their derivatives. Advances in food and nutrition research, 73, 15-31.

Ngo, D.-H., & Kim, S.-K. (2014b). Chapter Two - Antioxidant Effects of Chitin, Chitosan, and Their Derivatives. In S.-K. Kim (Ed.), Advances in Food and Nutrition Research (Vol. 73, pp. 15-31). *Academic Press*. https://doi.org/https://doi.org/10.1016/ B978-0-12-800268-1.00002-0.

Ngo, D.-N., Kim, M.-M., & Kim, S.-K. (2008). Chitin oligosaccharides inhibit oxidative stress in live cells. Carbohydrate Polymers, 74(2), 228-234. https://doi.org/https://doi.org/10.1016/j.carbpol.2008.02.005.

Nguyen, T. T., Tomberlin, J. K., & Vanlaerhoven, S. (2015). Ability of black soldier fly (Diptera: Stratiomyidae) larvae to recycle food waste. Environmental entomology, 44(2), 406-410.

Nilugonda, A., Sankaralingam, S., Anitha, P., Mathew, D. K. D., & Aswathi, P. B. (2022). Influence of Black Soldier Fly (*Hermetia illucens*) larvae feeding on carcass characteristics of Gramasree hens. Journal of Veterinary and Animal Sciences, 53(3). https://doi.org/10.51966/jvas.2022.53.3.429-434.

Niu, D., Zhang, M., Mujumdar, A. S., & Yu, D. (2024). Novel Drying Technologies for Future Animal- and Plant-Derived Protein Foods: Research Progress, Challenges, and Potential Applications. Food and Bioprocess Technology. https://doi.org/10.1007/s11947-024-03375-3.

Nossa, C. W., Oberdorf, W. E., Yang, L., Aas, J. A., Paster, B. J., Desantis, T. Z., Brodie, E. L., Malamud, D., Poles, M. A., & Pei, Z. (2010). Design of 16S rRNA gene primers for 454 pyrosequencing of the human foregut microbiome. World J Gastroenterol, 16(33), 4135-4144. https://doi.org/10.3748/wjg.v16.i33.4135.

Nur Atikah, I., Alimon, A. R., Yaakub, H., Abdullah, N., Jahromi, M. F., Ivan, M., & Samsudin, A. A. (2018). Profiling of rumen fermentation, microbial population and digestibility in goats fed with dietary oils containing different fatty acids. BMC Vet Res, 14(1), 344. https://doi.org/10.1186/s12917-018-1672-0.

Nyakeri, E., Ogola, H., Ayieko, M., & Amimo, F. (2017). An open system for farming black soldier fly larvae as a source of proteins for smallscale poultry and fish production. Journal of Insects as Food and Feed, 3(1), 51-56.

Ojha, S., Bekhit, A. E.-D., Grune, T., & Schlüter, O. K. (2021). Bioavailability of nutrients from edible insects. Current Opinion in Food Science, 41, 240-248. https://doi.org/https://doi.org/10.1016/j.cofs.2021.08.003.

Onsongo, V., Osuga, I., Gachuiri, C., Wachira, A., Miano, D., Tanga, C., Ekesi, S., Nakimbugwe, D., & Fiaboe, K. (2018). Insects for income generation through animal feed: effect of dietary replacement of soybean and fish meal with black soldier fly meal on broiler growth and economic performance. Journal of economic entomology, 111(4), 1966-1973.

Oonincx, D. G., & De Boer, I. J. (2012). Environmental impact of the production of mealworms as a protein source for humans–a life cycle assessment. PloS one, 7(12), e51145.

Oonincx, D. G., Van Broekhoven, S., Van Huis, A., & van Loon, J. J. (2015). Feed conversion, survival and development, and composition of four insect species on diets composed of food by-products. PloS one, 10(12), e0144601.

Ørskov, E.-R., & McDonald, I. (1979). The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. The Journal of Agricultural Science, 92(2), 499-503.

Paengkoum, P. (2010). Effects of neem (*Azadirachta indica*) and leucaena (*Leucaena leucocephala*) fodders on digestibility, rumen fermentation and nitrogen balance of goats fed corn silage. Journal of Animal and Veterinary Advances, 9(5), 883-886.

Paengkoum, P., Chen, S., & Paengkoum, S. (2019). Effects of crude protein and undegradable intake protein on growth performance, nutrient utilization, and rumen fermentation in growing Thai-indigenous beef cattle. Tropical animal health and production, 51(5), 1151-1159.

Paengkoum, P., Liang, J., Jelan, Z., & Basery, M. (2006). Utilization of steam-treated oil palm fronds in growing goats: 1. supplementation with dietary urea. Asian-australasian journal of animal sciences, 19(9), 1305-1313.

Paengkoum, P., Thongpea, S., & Paengkoum, S. (2017). Utilization of concentrate supplements containing varying levels of cassava leaf pellet by growing goats fed a basal diet of pangola hay. Indian Journal of Animal Research, 51(6).

Parisi, G., Tulli, F., Fortina, R., Marino, R., Bani, P., Dalle Zotte, A., De Angelis, A., Piccolo, G., Pinotti, L., & Schiavone, A. (2020). Protein hunger of the feed sector: The alternatives offered by the plant world. Italian Journal of Animal Science, 19(1), 1204-1225.

Park, H. H. (2016). Black soldier fly larvae manual.

Park, P.-J., Je, J.-Y., & Kim, S.-K. (2003). Free radical scavenging activity of chitooligosaccharides by electron spin resonance spectrometry. Journal of agricultural and food chemistry, 51(16), 4624-4627.

Patra, A. (2016). Recent advances in measurement and dietary mitigation of enteric methane emissions in ruminants. Front Vet Sci 2016; 3: 39.

มหาวิทยาลัยเทคโนโลยีสุรนารี

Patra, A., Park, T., Kim, M., & Yu, Z. (2017). Rumen methanogens and mitigation of methane emission by anti-methanogenic compounds and substances. Journal of Animal Science and Biotechnology, 8, 1-18.

Patra, A. K. (2009). A meta-analysis on effects of supplementing low-quality roughages with foliages from browses and tree fodders on intake and growth in sheep. Livestock Science, 121(2-3), 239-249.

Patra, A. K. (2013). The effect of dietary fats on methane emissions, and its other effects on digestibility, rumen fermentation and lactation performance in cattle: A meta-analysis. Livestock science, 155(2-3), 244-254.

Patra, A. K., & Aschenbach, J. R. (2018). Ureases in the gastrointestinal tracts of ruminant and monogastric animals and their implication in urea-N/ammonia metabolism: A review. Journal of Advanced Research, 13, 39-50.

Petlum, A., Paengkoum, P., Liang, J., Vasupen, K., & Paengkoum, S. (2019). Molecular weight of condensed tannins of some tropical feed-leaves and their effect on in vitro gas and methane production. Animal Production Science, 59(12), 2154-2160.

Phesatcha, B., Phesatcha, K., Matra, M., & Wanapat, M. (2023). Cricket (*Gryllus bimaculatus*) meal pellets as a protein supplement to improve feed efficiency, ruminal fermentation and microbial protein synthesis in Thai native beef cattle. Anim Biosci, 36(9), 1384-1392. https://doi.org/10.5713/ab.23.0107.

Pieterse, E., Erasmus, S. W., Uushona, T., & Hoffman, L. C. (2019). Black soldier fly (*Hermetia illucens*) pre-pupae meal as a dietary protein source for broiler production ensures a tasty chicken with standard meat quality for every pot. J Sci Food Agric, 99(2), 893-903. https://doi.org/10.1002/jsfa.9261.

Pieterse, E., Erasmus, S. W., Uushona, T., & Hoffman, L. C. (2019). Black soldier fly (*Hermetia illucens*) pre-pupae meal as a dietary protein source for broiler production ensures a tasty chicken with standard meat quality for every pot. Journal of the Science of Food and Agriculture, 99(2), 893-903.

Prandi, B., Zurlini, C., Maria, C. I., Cutroneo, S., Di Massimo, M., Bondi, M., Brutti, A., Sforza, S., & Tedeschi, T. (2021). Targeting the nutritional value of proteins from legumes by-products through mild extraction technologies. Frontiers in nutrition, 8, 695793.

มหาวิทยาลัยเทคโนโลยีสุรนารี

Purba, R. A. P., & Paengkoum, P. (2019). Bioanalytical HPLC method of Piper betle L. for quantifying phenolic compound, water-soluble vitamin, and essential oil in five different solvent extracts. Journal of Applied Pharmaceutical Science, 9(5), 033-039.

Purba, R. A. P., Yuangklang, C., Paengkoum, S., & Paengkoum, P. (2021). Piper oil decreases in vitro methane production with shifting ruminal fermentation in a variety of diets. Int. J. Agric. Biol, 25, 231-240.

Quintieri, L., Nitride, C., De Angelis, E., Lamonaca, A., Pilolli, R., Russo, F., & Monaci, L. (2023). Alternative Protein Sources and Novel Foods: Benefits, Food Applications and Safety Issues. Nutrients, 15(6). https://doi.org/10.3390/nu15061509.

Rabani, V., Cheatsazan, H., & Davani, S. (2019). Proteomics and lipidomics of black soldier fly (Diptera: Stratiomyidae) and blow fly (Diptera: Calliphoridae) larvae. Journal of Insect Science, 19(3), 29.

Rastgoo, M., Kazemi-Bonchenari, M., HosseinYazdi, M., & Mirzaei, M. (2020). Effects of corn grain processing method (ground versus steam-flaked) with rumen undegradable to degradable protein ratio on growth performance, ruminal fermentation, and microbial protein yield in Holstein dairy calves. Animal Feed Science and Technology, 269, 114646. https://doi.org/https://doi.org/10.1016/j.anifeedsci.2020.114646.

Ravi, H. K., Degrou, A., Costil, J., Trespeuch, C., Chemat, F., & Vian, M. A. (2020). Effect of devitalization techniques on the lipid, protein, antioxidant, and chitin fractions of black soldier fly (*Hermetia illucens*) larvae. European Food Research and Technology, 246, 2549-2568.

Ravi, H. K., Guidou, C., Costil, J., Trespeuch, C., Chemat, F., & Vian, M. A. (2021). Novel insights on the sustainable wet mode fractionation of black soldier fly larvae (*Hermetia illucens*) into lipids, proteins and chitin. Processes, 9(11), 1888.

Rawski, M., Mazurkiewicz, J., Kierończyk, B., & Józefiak, D. (2020). Black soldier fly full-fat larvae meal as an alternative to fish meal and fish oil in Siberian sturgeon nutrition: The effects on physical properties of the feed, animal growth performance, and feed acceptance and utilization. Animals, 10(11), 2119.

Renna, M., Coppa, M., Lussiana, C., Le Morvan, A., Gasco, L., & Maxin, G. (2022). Full-fat insect meals in ruminant nutrition: in vitro rumen fermentation characteristics and lipid biohydrogenation. Journal of Animal Science and Biotechnology, 13(1), 138. https://doi.org/10.1186/s40104-022-00792-2.

Rhee, K. S., Waldron, D. F., Ziprin, Y. A., & Rhee, K. C. (2000). Fatty acid composition of goat diets vs intramuscular fat. Meat Science, 54(4), 313-318. https://doi.org/https://doi.org/10.1016/S0309-1740(99)00094-7.

Rigon, F., Pereira, D. A. B., Loregian, K. E., Magnani, E., Marcondes, M. I., Branco, R. H., Benedeti, P. D. B., & Paula, E. M. (2023). Use of Heating Methods and Xylose to Increase Rumen Undegradable Protein of Alternative Protein Sources: 1) Peanut Meal. Animals, 13(1).

Rinaudo, M. (2013). Materials based on chitin and chitosan. Bio‐Based Plastics: Materials and Applications, 63-87.

Rizzo, G. (2020). The Antioxidant Role of Soy and Soy Foods in Human Health. Antioxidants, 9(7).

Romano, N., Fischer, H., Kumar, V., Francis, S. A., & Sinha, A. K. (2022). Productivity, conversion ability, and biochemical composition of black soldier fly (*Hermetia illucens*) larvae fed with sweet potato, spent coffee or dough. International Journal of Tropical Insect Science, 42(1), 183-190.

Saucier, L., M'ballou, C., Ratti, C., Deschamps, M.-H., Lebeuf, Y., & Vandenberg, G. (2022). Comparison of black soldier fly larvae pre-treatments and drying techniques on the microbial load and physico-chemical characteristics. Journal of Insects as Food and Feed, 8(1), 45-64.

Schiavone, A., Cullere, M., De Marco, M., Meneguz, M., Biasato, I., Bergagna, S., Dezzutto, D., Gai, F., Dabbou, S., & Gasco, L. (2017). Partial or total replacement of soybean oil by black soldier fly larvae (*Hermetia illucens* L.) fat in broiler diets: Effect on growth performances, feed-choice, blood traits, carcass characteristics and meat quality. Italian Journal of Animal Science, 16(1), 93-100.

Schiavone, A., Dabbou, S., De Marco, M., Cullere, M., Biasato, I., Biasibetti, E., Capucchio, M., Bergagna, S., Dezzutto, D., & Meneguz, M. (2018). Black soldier fly larva fat inclusion in finisher broiler chicken diet as an alternative fat source. Animal, 12(10), 2032-2039.

Schiavone, A., Dabbou, S., Petracci, M., Zampiga, M., Sirri, F., Biasato, I., Gai, F., & Gasco, L. (2019). Black soldier fly defatted meal as a dietary protein source for broiler chickens: Effects on carcass traits, breast meat quality and safety. Animal, 13(10), 2397-2405.

Schiavone, A., De Marco, M., Martínez, S., Dabbou, S., Renna, M., Madrid, J., Hernandez, F., Rotolo, L., Costa, P., & Gai, F. (2017). Nutritional value of a partially defatted and a highly defatted black soldier fly larvae (*Hermetia illucens* L.) meal for broiler chickens: apparent nutrient digestibility, apparent metabolizable energy and apparent ileal amino acid digestibility. Journal of animal science and biotechnology, 8(1), 1-9.

Scott, G., & Awika, J. M. (2023). Effect of protein–starch interactions on starch retrogradation and implications for food product quality. Comprehensive Reviews in Food Science and Food Safety, 22(3), 2081-2111.

Sekaran, U., Lai, L., Ussiri, D. A. N., Kumar, S., & Clay, S. (2021). Role of integrated crop-livestock systems in improving agriculture production and addressing food security – A review. Journal of Agriculture and Food Research, 5, 100190. https://doi.org/https://doi.org/10.1016/j.jafr.2021.100190.

Seyedalmoosavi, M. M., Mielenz, M., Veldkamp, T., Das, G., & Metges, C. C. (2022). Growth efficiency, intestinal biology, and nutrient utilization and requirements of black soldier fly (*Hermetia illucens*) larvae compared to monogastric livestock species: a review. J Anim Sci Biotechnol, 13(1), 31. https://doi.org/10.1186/s40104-022-00682-7.

Shah, A. A., Totakul, P., Matra, M., Cherdthong, A., Hanboonsong, Y., & Wanapat, M. (2022). Nutritional composition of various insects and potential uses as alternative protein sources in animal diets. Animal Bioscience, 35(2), 317.

Shah, A. M., Qazi, I. H., Matra, M., & Wanapat, M. (2022). Role of Chitin and Chitosan in Ruminant Diets and Their Impact on Digestibility, Microbiota and Performance of Ruminants. Fermentation, 8(10), 549.

Sheppard, D. C., Tomberlin, J. K., Joyce, J. A., Kiser, B. C., & Sumner, S. M. (2002). Rearing methods for the black soldier fly (Diptera: Stratiomyidae). Journal of medical entomology, 39(4), 695-698.

มหาวิทยาลัยเทคโนโลยีสุรนารี

Shumo, M., Osuga, I. M., Khamis, F. M., Tanga, C. M., Fiaboe, K. K., Subramanian, S., Ekesi, S., van Huis, A., & Borgemeister, C. (2019). The nutritive value of black soldier fly larvae reared on common organic waste streams in Kenya. Scientific reports, 9(1), 1-13.

Siddiqui, S. A., Ristow, B., Rahayu, T., Putra, N. S., Yuwono, N. W., Mategeko, B., Smetana, S., Saki, M., Nawaz, A., & Nagdalian, A. (2022). Black soldier fly larvae (BSFL) and their affinity for organic waste processing. Waste Management, 140, 1-13.

Singh, A., & Kumari, K. (2019). An inclusive approach for organic waste treatment and valorisation using Black Soldier Fly larvae: A review. Journal of Environmental Management, 251, 109569.

Smets, R., Verbinnen, B., Van De Voorde, I., Aerts, G., Claes, J., & Van Der Borght, M. (2020). Sequential extraction and characterisation of lipids, proteins, and chitin from black soldier fly (*Hermetia illucens*) larvae, prepupae, and pupae. Waste and Biomass Valorization, 11(12), 6455-6466.

Sogari, G., Amato, M., Biasato, I., Chiesa, S., & Gasco, L. (2019a). The potential role of insects as feed: A multi-perspective review. Animals, 9(4), 119.

Sogari, G., Amato, M., Biasato, I., Chiesa, S., & Gasco, L. (2019b). The Potential Role of Insects as Feed: A Multi-Perspective Review. Animals, 9(4).

Spranghers, T., Michiels, J., Vrancx, J., Ovyn, A., Eeckhout, M., De Clercq, P., & De Smet, S. (2018). Gut antimicrobial effects and nutritional value of black soldier fly (*Hermetia illucens* L.) prepupae for weaned piglets. Animal Feed Science and Technology, 235, 33-42.

Spranghers, T., Noyez, A., Schildermans, K., & De Clercq, P. (2017). Cold hardiness of the black soldier fly (Diptera: Stratiomyidae). Journal of economic entomology, 110(4), 1501-1507.

Spranghers, T., Ottoboni, M., Klootwijk, C., Ovyn, A., Deboosere, S., De Meulenaer, B., Michiels, J., Eeckhout, M., De Clercq, P., & De Smet, S. (2017). Nutritional composition of black soldier fly (*Hermetia illucens*) prepupae reared on different organic waste substrates. Journal of the Science of Food and Agriculture, 97(8), 2594-2600.

St-Hilaire, S., Sheppard, C., Tomberlin, J. K., Irving, S., Newton, L., McGuire, M. A., Mosley, E. E., Hardy, R. W., & Sealey, W. (2007). Fly prepupae as a feedstuff for rainbow

มหาวิทยาลัยเทคโนโลยีสุรนารี

trout, Oncorhynchus mykiss. Journal of the world aquaculture society, 38(1), 59-67.

Starowicz, M., & Zieliński, H. (2019). How Maillard Reaction Influences Sensorial Properties (Color, Flavor and Texture) of Food Products? Food Reviews International, 35(8), 707-725. https://doi.org/10.1080/87559129.2019.1600538.

Statistics, F. G. F. International Feed Industry Federation; 2021. In.

Stenberg, O. K., Holen, E., Piemontese, L., Liland, N. S., Lock, E.-J., Espe, M., & Belghit, I. (2019). Effect of dietary replacement of fish meal with insect meal on in vitro bacterial and viral induced gene response in Atlantic salmon (Salmo salar) head kidney leukocytes. Fish & Shellfish Immunology, 91, 223-232. https://doi.org/ https://doi.org/10.1016/j.fsi.2019.05.042.

Stern, M., Santos, K., & Satter, L. (1985). Protein degradation in rumen and amino acid absorption in small intestine of lactating dairy cattle fed heat-treated whole soybeans. Journal of Dairy Science, 68(1), 45-56.

Subuh, A., Rowan, T., & Lawrence, T. (1996). Effect of heat or formaldehyde treatment on the rumen degradability and intestinal tract apparent digestibility of protein in soya-bean meal and in rapeseed meals of different glucosinolate content. Animal Feed Science and Technology, 57(3), 257-265.

Subuh, A. M. H., Rowan, T. G., & Lawrence, T. L. J. (1996). Effect of heat or formaldehyde treatment on the rumen degradability and intestinal tract apparent digestibility of protein in soya-bean meal and in rapeseed meals of different glucosinolate content. Animal Feed Science and Technology, 57(3), 257-265. https://doi.org/ https://doi.org/10.1016/0377-8401(95)00857-8.

Suong, N. T. M., Paengkoum, S., Schonewille, J. T., Purba, R. A. P., & Paengkoum, P. (2022). Growth performance, blood biochemical indices, rumen bacterial community, and carcass characteristics in goats fed anthocyanin-rich black cane silage. Frontiers in Veterinary Science, 9, 880838.

Suryati, T., Julaeha, E., Farabi, K., Ambarsari, H., & Hidayat, A. T. (2023). Lauric Acid from the Black Soldier Fly (*Hermetia illucens*) and Its Potential Applications. Sustainability, 15(13).

Swiatkiewicz, S., Swiatkiewicz, M., Arczewska-Wlosek, A., & Jozefiak, D. (2015). Chitosan and its oligosaccharide derivatives (chito-oligosaccharides) as feed supplements in poultry and swine nutrition. Journal of animal physiology and animal nutrition, 99(1), 1-12.

Synowiecki, J., & Al-Khateeb, N. A. (2003). Production, properties, and some new applications of chitin and its derivatives.

Tabata, E., Kashimura, A., Kikuchi, A., Masuda, H., Miyahara, R., Hiruma, Y., Wakita, S., Ohno, M., Sakaguchi, M., & Sugahara, Y. (2018). Chitin digestibility is dependent on feeding behaviors, which determine acidic chitinase mRNA levels in mammalian and poultry stomachs. Scientific Reports, 8(1), 1461.

Tabata, E., Kashimura, A., Wakita, S., Ohno, M., Sakaguchi, M., Sugahara, Y., Kino, Y., Matoska, V., Bauer, P. O., & Oyama, F. (2017a). Gastric and intestinal proteases resistance of chicken acidic chitinase nominates chitin-containing organisms for alternative whole edible diets for poultry. Scientific Reports, 7(1), 6662. https://doi.org/10.1038/s41598-017-07146-3.

Tabata, E., Kashimura, A., Wakita, S., Ohno, M., Sakaguchi, M., Sugahara, Y., Kino, Y., Matoska, V., Bauer, P. O., & Oyama, F. (2017b). Gastric and intestinal proteases resistance of chicken acidic chitinase nominates chitin-containing organisms for alternative whole edible diets for poultry. Scientific reports, 7(1), 1-11.

Taelman, S. E., De Meester, S., Van Dijk, W., da Silva, V., & Dewulf, J. (2015). Environmental sustainability analysis of a protein-rich livestock feed ingredient in The Netherlands: Microalgae production versus soybean import. Resources, Conservation and Recycling, 101, 61-72. https://doi.org/https://doi.org/10.1016/j.resconrec.2015.05.013.

Tahamtani, F. M., Ivarsson, E., Wiklicky, V., Lalander, C., Wall, H., Rodenburg, T. B., Tuyttens, F. A., & Hernandez, C. E. (2021). Feeding live Black Soldier Fly larvae (*Hermetia illucens*) to laying hens: effects on feed consumption, hen health, hen behavior, and egg quality. Poultry Science, 100(10), 101400.

Tian, X., Li, J.-X., Luo, Q.-Y., Wang, X., Xiao, M.-M., Zhou, D., Lu, Q., & Chen, X. (2022). Effect of supplementation with selenium-yeast on muscle antioxidant activity, meat quality, fatty acids and amino acids in goats. Frontiers in veterinary science, 1683.

Tian, X., Li, J., Luo, Q., Wang, X., Wang, T., Zhou, D., Xie, L., Ban, C., & Lu, Q. (2022a). Effects of Purple Corn Anthocyanin on Growth Performance, Meat Quality, Muscle Antioxidant Status, and Fatty Acid Profiles in Goats. Foods, 11(9).

Tian, X., Li, J., Luo, Q., Wang, X., Wang, T., Zhou, D., Xie, L., Ban, C., & Lu, Q. (2022b). Effects of Purple Corn Anthocyanin on Growth Performance, Meat Quality, Muscle Antioxidant Status, and Fatty Acid Profiles in Goats. Foods, 11(9), 1255. https://www.mdpi.com/2304-8158/11/9/1255.

Tian, X., Lu, Q., Paengkoum, P., & Paengkoum, S. (2020). Effect of purple corn pigment on change of anthocyanin composition and unsaturated fatty acids during milk storage. Journal of dairy science, 103(9), 7808-7812.

Tian, X. Z., Li, J. X., Luo, Q. Y., Wang, X., Xiao, M. M., Zhou, D., Lu, Q., & Chen, X. (2021). Effect of Supplementation With Selenium-Yeast on Muscle Antioxidant Activity, Meat Quality, Fatty Acids and Amino Acids in Goats. Front Vet Sci, 8, 813672. https://doi.org/10.3389/fvets.2021.813672.

Tian, X. Z., Lu, Q., Paengkoum, P., & Paengkoum, S. (2020). Short communication: Effect of purple corn pigment on change of anthocyanin composition and unsaturated fatty acids during milk storage. Journal of Dairy Science, 103(9), 7808-7812. https://doi.org/https://doi.org/10.3168/jds.2020-18409.

Tiengtam, N., Paengkoum, P., Sirivoharn, S., Phonsiri, K., & Boonanuntanasarn, S. (2017). The effects of dietary inulin and Jerusalem artichoke (Helianthus tuberosus) tuber on the growth performance, haematological, blood chemical and immune parameters of Nile tilapia (*Oreochromis niloticus*) fingerlings. Aquaculture Research, 48(10), 5280-5288.

Tomberlin, J. K., Adler, P. H., & Myers, H. M. (2009). Development of the black soldier fly (Diptera: Stratiomyidae) in relation to temperature. Environmental entomology, 38(3), 930-934.

Toral, P. G., Hervás, G., González-Rosales, M. G., Mendoza, A. G., Robles-Jiménez, L. E., & Frutos, P. (2022). Insects as alternative feed for ruminants: comparison of protein evaluation methods. Journal of Animal Science and Biotechnology, 13(1), 21. https://doi.org/10.1186/s40104-021-00671-2.

Traksele, L., Speiciene, V., Smicius, R., Alencikiene, G., Salaseviciene, A., Garmiene, G., Zigmantaite, V., Grigaleviciute, R., & Kucinskas, A. (2021). Investigation of in vitro

มหาวิทยาลัยเทคโนโลยีสุรนารี

and in vivo digestibility of black soldier fly (*Hermetia illucens* L.) larvae protein. Journal of Functional Foods, 79, 104402. https://doi.org/https://doi.org/10.1016/j.jff.2021.104402.

Tschirner, M., & Simon, A. (2015). Influence of different growing substrates and processing on the nutrient composition of black soldier fly larvae destined for animal feed. Journal of Insects as Food and Feed, 1(4), 249-259.

Tyshko, N. V., Zhminchenko, V. M., Nikitin, N. S., Trebukh, M. D., Shestakova, S. I., Pashorina, V. A., & Sadykova, E. O. (2021). The comprehensive studies of *Hermetia illucens* larvae protein's biological value. Problems of nutritions, 90, 49-58.

Üstüner, T., Hasbenlí, A., & Rozkošný, R. (2003). The first record of *Hermetia illucens* (Linnaeus, 1758)(Diptera, Stratiomyidae) from the Near East. Studia dipterologica(1).

Uyanga, V. A., Ejeromedoghene, O., Lambo, M. T., Alowakennu, M., Alli, Y. A., Ere-Richard, A. A., Min, L., Zhao, J., Wang, X., & Jiao, H. (2023). Chitosan and chitosan-based composites as beneficial compounds for animal health: Impact on gastrointestinal functions and biocarrier application. Journal of Functional Foods, 104, 105520.

Van Huis, A., & Oonincx, D. G. (2017). The environmental sustainability of insects as food and feed. A review. Agronomy for Sustainable Development, 37(5), 1-14.

Van Soest, P. v., Robertson, J. B., & Lewis, B. A. (1991). Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. Journal of Dairy Science, 74(10), 3583-3597.

Vargas, J. E., Andrés, S., López-Ferreras, L., Snelling, T. J., Yáñez-Ruíz, D. R., García-Estrada, C., & López, S. (2020). Dietary supplemental plant oils reduce methanogenesis from anaerobic microbial fermentation in the rumen. Scientific Reports, 10(1), 1613.

Veldkamp, T., & Bosch, G. (2015). Insects: a protein-rich feed ingredient in pig and poultry diets. Animal Frontiers, 5(2), 45-50.

Vendramini, T. H. A., Takiya, C. S., Silva, T., Zanferari, F., Rentas, M. F., Bertoni, J., Consentini, C. E. C., Gardinal, R., Acedo, T. S., & Rennó, F. P. (2016). Effects of a

blend of essential oils, chitosan or monensin on nutrient intake and digestibility of lactating dairy cows. Animal Feed Science and Technology, 214, 12-21.

Vorlaphim, T., Paengkoum, P., Purba, R. A. P., Yuangklang, C., Paengkoum, S., & Schonewille, J. T. (2021). Treatment of rice stubble with *Pleurotus ostreatus* and urea improves the growth performance in slow-growing goats. Animals, 11(4), 1053.

Walker, N. D., McEwan, N. R., & Wallace, R. J. (2003). Cloning and functional expression of dipeptidyl peptidase IV from the ruminal bacterium Prevotella albensis M384(T). Microbiology (Reading), 149(Pt 8), 2227-2234. https://doi.org/10.1099/mic.0.26119-0.

Wanapat, M., Chumpawadee, S., & Paengkoum, P. (2000). Utilization of urea-treated rice straw and whole sugar cane crop as roughage sources for dairy cattle during the dry season. Asian-australasian journal of animal sciences, 13(4), 474-477.

Wang, H., ur Rehman, K., Feng, W., Yang, D., ur Rehman, R., Cai, M., Zhang, J., Yu, Z., & Zheng, L. (2020). Physicochemical structure of chitin in the developing stages of black soldier fly. International journal of biological macromolecules, 149, 901-907.

Wang, J., Zhao, S., Min, G., Qiao, D., Zhang, B., Niu, M., Jia, C., Xu, Y., & Lin, Q. (2021). Starch-protein interplay varies the multi-scale structures of starch undergoing thermal processing. International Journal of Biological Macromolecules, 175, 179-187.

Wang, S., Kreuzer, M., Braun, U., & Schwarm, A. (2017). Effect of unconventional oilseeds (safflower, poppy, hemp, camelina) on in vitro ruminal methane production and fermentation. Journal of the Science of Food and Agriculture, 97(11), 3864-3870.

Wang, Y.-S., & Shelomi, M. (2017). Review of Black Soldier Fly (Hermetia illucens) as Animal Feed and Human Food. Foods, 6(10).

Wang, Y., Wang, Q., Dai, C., Li, J., Huang, P., Li, Y., Ding, X., Huang, J., Hussain, T., & Yang, H. (2020). Effects of dietary energy on growth performance, carcass characteristics, serum biochemical index, and meat quality of female Hu lambs. Animal Nutrition, 6(4), 499-506. https://doi.org/https://doi.org/10.1016/j.aninu.2020.05.008.

มหาวิทยาลัยเทคโนโลยีสุรนารี

Weimer, P. J. (2022). Degradation of Cellulose and Hemicellulose by Ruminal Microorganisms. Microorganisms, 10(12).

Wood, J. D. (2017). Chapter 20 - Meat Composition and Nutritional Value. In F. Toldra´ (Ed.), Lawrie´s Meat Science (Eighth Edition) (pp. 635-659). *Woodhead Publishing*. https://doi.org/https://doi.org/10.1016/B978-0-08-100694-8.00020-0.

Wood, J. D., Enser, M., Fisher, A. V., Nute, G. R., Sheard, P. R., Richardson, R. I., Hughes, S. I., & Whittington, F. M. (2008). Fat deposition, fatty acid composition and meat quality: A review. Meat Science, 78(4), 343-358. https://doi.org/https://doi.org/10.1016/j.meatsci.2007.07.019.

Woodley, N. E. (2011). A world catalog of the Stratiomyidae (Insecta: Diptera): a supplement with revisionary notes and errata. Myia, 12, 379-415.

Wu, G. D., Chen, J., Hoffmann, C., Bittinger, K., Chen, Y.-Y., Keilbaugh, S. A., Bewtra, M., Knights, D., Walters, W. A., & Knight, R. (2011). Linking long-term dietary patterns with gut microbial enterotypes. Science, 334(6052), 105-108.

Wu, G. D., Chen, J., Hoffmann, C., Bittinger, K., Chen, Y.-Y., Keilbaugh, S. A., Bewtra, M., Knights, D., Walters, W. A., Knight, R., Sinha, R., Gilroy, E., Gupta, K., Baldassano, R., Nessel, L., Li, H., Bushman, F. D., & Lewis, J. D. (2011). Linking Long-Term Dietary Patterns with Gut Microbial Enterotypes. Science, 334(6052), 105-108. https://doi.org/10.1126/science.1208344.

Xiao, J., Khan, M. Z., Ma, Y., Alugongo, G. M., Ma, J., Chen, T., Khan, A., & Cao, Z. (2021). The Antioxidant Properties of Selenium and Vitamin E; Their Role in Periparturient Dairy Cattle Health Regulation. Antioxidants, 10(10).

Yadav, S., & Jha, R. (2019). Strategies to modulate the intestinal microbiota and their effects on nutrient utilization, performance, and health of poultry. Journal of animal science and biotechnology, 10(1), 1-11.

Yang, H. W., Hsu, C. K., & Yang, Y. F. (2014). Effect of thermal treatments on anti-nutritional factors and antioxidant capabilities in yellow soybeans and green-cotyledon small black soybeans. Journal of the Science of Food and Agriculture, 94(9), 1794-1801.

มหาวิทยาลัยเทคโนโลยีสุรนารี

Yildirim‐Aksoy, M., Eljack, R., & Beck, B. H. (2020). Nutritional value of frass from black soldier fly larvae, *Hermetia illucens*, in a channel catfish, Ictalurus punctatus, diet. Aquaculture nutrition, 26(3), 812-819.

Younes, I., & Rinaudo, M. (2015). Chitin and Chitosan Preparation from Marine Sources. Structure, Properties and Applications. Marine Drugs, 13(3), 1133-1174.

Yu, G., Chen, Y., Yu, Z., & Cheng, P. (2009). Research progress on the larvae and prepupae of black soldier fly *Hermetia illucens* used as animal feedstuff. Chinese bulletin of entomology, 46(1), 41-45.

Zhang, H., & Zhang, H. (2019). Food Power in the Context of Sino-American Rivalry. Securing the 'Rice Bowl' China and Global Food Security, 207-233.

Zhang, J., Huang, L., He, J., Tomberlin, J. K., Li, J., Lei, C., Sun, M., Liu, Z., & Yu, Z. (2010). An artificial light source influences mating and oviposition of black soldier flies, *Hermetia illucens*. Journal of insect science, 10(1).

Zhang, L., Wang, Y. X., Zhou, Y., Zheng, L., Zhan, X. A., & Pu, Q. H. (2014). Different sources of maternal selenium affect selenium retention, antioxidant status, and meat quality of 56-day-old offspring of broiler breeders. Poultry Science, 93(9), 2210-2219. https://doi.org/https://doi.org/10.3382/ps.2013-03605.

Zheng, L., Li, Q., Zhang, J., & Yu, Z. (2012). Double the biodiesel yield: Rearing black soldier fly larvae, *Hermetia illucens*, on solid residual fraction of restaurant waste after grease extraction for biodiesel production. Renewable energy, 41, 75-79.

Zhou, F., Tomberlin, J. K., Zheng, L., Yu, Z., & Zhang, J. (2013). Developmental and waste reduction plasticity of three black soldier fly strains (Diptera: Stratiomyidae) raised on different livestock manures. Journal of medical entomology, 50(6), 1224-1230.

Zhou, J. S., Liu, S. S., Ji, H., & Yu, H. B. (2018). Effect of replacing dietary fish meal with black soldier fly larvae meal on growth and fatty acid composition of Jian carp (*Cyprinus carpio* var. Jian). Aquaculture Nutrition, 24(1), 424-433. https://doi.org/10.1111/anu.12574.

Zuki, N. M., Ibrahim, N., Mat, K., Rusli, N., Harun, H., Mahmud, M., Samat, N., & Al-Amsyar, S. (2021). Hybrid treatment of black soldier fly larvae (*Hermetia

มหาวิทยาลัยเทคโนโลยีสุรนารี

illucens) as a sustainable and efficient protein source in poultry diets. IOP Conference Series: Earth and Environmental Science.

CHAPTER VI
OVERALL CONCLUSION

The results of this study can be divided into three main parts:

The first part aimed to investigate the potential impact of supplementing different levels of BSF on the growth performance, serum antioxidants, and rumen microbiota of goats. The results indicated that supplementing with BSF did not affect the growth performance and rumen microbiota of goats. However, it is noteworthy that BSF5 and BSF10 were beneficial, as they increased antioxidant levels and the proportion of short-chain fatty acid. In contrast, supplementation with BSF15 resulted in decreased digestibility, antioxidant levels, and VFA parameters. Therefore, we recommend limiting the addition of BSF in goat diets to no more than 10%.

The second part 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. The results indicate that compared to the BS group, heat-treated BSF showed increased rumen dry matter degradability and effective degradability. The 14BSC group exhibited increased rumen crude protein degradability and degradation kinetic parameters, while the 12BSC group showed a decrease in these parameters. Furthermore, the crude protein 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. Additionally, heat treatment was beneficial in increasing the Idg and IDCP of BSF, with the 14BSC treatment showing a significantly better effect than 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.

The third part aims to evaluate the effects of heat treatment on BSF regarding growth performance, serum antioxidants and immunity, rumen microorganisms, meat quality, and related gene expression in goats. The results indicate that the growth

performance of the three groups of goats was not significantly affected. HTBSF increased the digestibility of NDF and rumen pH. Overall, there was no significant effect on rumen VFA levels in the three groups. At 0 h, the activity of immune enzymes in the blood of the FFS and HTBSF groups was higher than that of the BSF group, but at 4 hours, the activity of the BSF group was significantly higher than that of the FFS and HTBSF groups. The serum antioxidant level of the FFS group was significantly higher than that of the BSF and HTBSF groups. The HTBSF group increased the abundance of the phylum *Bacteroidetes* and decreased the abundance of the phylum *Firmicutes*. Both the BSF and HTBSF groups showed a decrease in slaughter rate, carcass weight, and muscle antioxidant capacity. Additionally, the meat quality parameters of the HTBSF group were the poorest, but it improved the composition of beneficial fatty acid in the muscles for human health, such as C18:2n6t, C18:3n6, and UFA. Compared to the FFS group, both the BSF and HTBSF groups showed increased levels of antioxidant and immune mRNA expression, with the HTBSF group having higher mRNA expression levels than the BSF group.

Based on the above, under the conditions of this study, the supplementation level of BSF in goat diets should not exceed 10%, and feeding them after treatment at 140°C yields better results.

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BIOGRAPHY

Shengyong Lu was born on May 22th, 1994, in Guizhou, China. In 2014, he obtained his Bachelor of Science in Animal Science, College of Animal Science, Guizhou University. In 2021, he received his Master of Animal Husbandry, College of Animal Science, Guizhou University. In 2017, he was awarded a scholarship by the Shengyong Lu gratefully recognizes the Suranaree University of Technology scholarship for External Grants and Scholarships for Graduate Students (SUT-OROG scholarship) as a source of funding for his Doctor of Philosophy (Ph.D. degree) study in Animal Production Technology at the School of Animal Technology and Innovation, Institute of Agricultural Technology, Suranaree University of Technology, Nakhon Ratchasima.

During his Ph.D. study, he has published four articles "**Lu S**, Taethaisong N, Meethip W, et al. (2022). Nutritional composition of black soldier fly larvae (*Hermetia illucens* L.) and its potential uses as alternative protein sources in animal diets: A review. **Insects**. 13(9): 831." "**Lu S**, Chen S, Li H, et al. (2022). Sustainable valorization of tomato pomace (*Lycopersicon esculentum*) in animal nutrition: a review. **Animals.** 12(23): 3294." "**Lu S**, Chen S, Paengkoum S, et al. Effects of Black Soldier Fly (*Hermetia illucens* L., BSF) Larvae Addition on In Vitro Fermentation Parameters of Goat Diets. **Insects**.15(5): 343." and "**Lu S**, Chen S, Paengkoum S, et al. (2024). Effects of black soldier fly larvae (*Hermetia illucens* L.) as feed supplements on muscle nutrient composition, meat quality, and antioxidant capacity in Qianbei goat. **Animal Bioscience**. (accept)."