INSTITUTE OF HEALTH SCIENCES
DEPARTMENT OF ANIMAL NUTRITION AND NUTRITIONAL DISEASES
PhD THESIS

Replacement of Soybean Meal with Varying Inclusions of Black Soldier Fly Larvae in Albino Wistar Rats as A Model for Monogastric Animals

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ÖZET

TEK MİDELİ HAYVANLAR İÇİN MODEL OLARAK ALBİNO WİSTAR RATLARIN BESLENMESİNDE SOYA KÜSPESİ YERİNE FARKLI DÜZEYLERDE KARA ASKER SİNEĞİ LARVALARININ KULLANILMASININ ETKİSİ

Dünya genelinde gıda yetersizliği ve gıda güvenliği dikkate alındığında alternatif yem kaynakları pazarın önde gelen tercihlerindendir. Kara Asker Sineği ise uygun maliyeti, ekolojik olması, sürdürülebilirliği ayrıca iş gücü ve arazi ihtiyacının düşük olması sebebiyle son derece dikkat çeken bir üründür. Kara Asker Sineği larvalarının getirdiği kazanımlar hayvan sağlığının sürdürülebilirliği açısından da bir gereklilik haline gelmiştir. AB, bu ürünün kümes hayvanları, balıklar, domuzlar, kediler, köpekler, bıldırcınlar, ördekler, sürüngenler, kuşlar ve laboratuvar hayvanlarını beslemek için kullanımına müsaade vermiştir. Kara Asker Sineği larvası, hayvan beslemenin yanı sıra tarımda da kullanılarak biyolojik atıkların oluşumunu azaltmakta, organik gübre ve biyoyakıt ihtiyacını karşılamaktadır. Bu çalışmanın amacı, Wistar albino ratların beslenmesinde farkli duzeylerde soya küspesinin yerine kullanilan Kara Asker Sineği larvalarının performans, kan parametreleri ve histopatolojik parametrelere etkilerini tespit etmek için yapılmıştır.

Bu çalışmada yarısı dişi yarısı erkek olmak üzere toplam 80 rat kullanılmıştır. Tüm ratlar 5 farklı deney grubuna ayrılıp her deney grubu 8 erkek ve 8 dişi olmak üzere toplam 16 rattan oluşmaktadır. Alt gruplar her birinde 2 rat olacak şekilde ayrı kafeslere alınmışlardır. Ratların rasyonundaki soya küspesi, %0 (kontrol), %5, %10, %15 ve %20 oranlarında Kara Asker Sineği larvası unu ile değiştirilmiştir. Kara Asker Sineği larvasının canlı ağırlık artışı, ortalama yem tüketimi, hematolojik ve serum biyokimya parametreleri, doku ve serum oksidatif stres parametreleri ve histopatolojik bulgular araştırılmıştır. Çalışma sonunda tüm ratlardan kan ve doku örnekleri alınarak hematoloji ve serum biyokimya parametreleri analiz edildi. Kan ve doku (karaciğer, böbrek ve kalp) örneklerinin bir kısmı biyokimya laboratuvarında serum oksidatif parametrelerinin analizi için -18 °C'de saklandı. Karaciğer, böbrek, kalp, testisler, yumurtalıklar, uterus ve duodenum gibi dokular formalin içeren steril kavanozlara alındı ve histopatolojik inceleme için analiz edildi.

Çalışmada kullanılan yemler, soya küspesi ve Kara Asker Sineği larvasından alınan örnekler besin madde profili analizi için Tübitak Marmara Araştırma Merkezi'ne gönderilmiştir. Yem örnekleri ilgili laboratuvarda kuru madde, ham kül, ham protein, ham yağ, ham lif, yağ asidi bileşimi ve amino asit bileşimi açısından analiz edilmiştir.

İstatistiksel analizlerden önce değerler üzerinde normallik testi yapılmış, veriler deney grupları arasındaki farkı analiz etmek için IBM SPSS (V. 24.0) programı tarafından gerçekleştirilen “Tek Yönlü Varyans Analizi (ANOVA)” ve “Kruskal Wallis Testi” kullanılarak analiz edilmiştir. Korelasyon analizi için ise “Pearson Testi” uygulanmıştır. İstatistik sonuçlarına bakıldığında, kontrol grubu ile kıyaslanan deney grupları için ağırlık artışı ve ortalama yem tüketiminde önemlilik bulunmamıştır ($p > 0.05$). Ayrıca yapılan analizlerde kontrol grubu ile kıyaslandığında, MCHC, PDW, MPV, P-LCR, ürik asit, ALT, ALP, LDL, HDL, total kolesterol, karaciğer ve böbrek MDA ve GSH ve doku TOS ve TAS parametrelerinde erkek ve dişi ratlar da cinsiyet dikkate alınmaksızın beraber değerlendirildiğinde önemli düzeyde farklılıklar görülmüştür ($p < 0.05$). Bununla beraber cinsiyete özgü etki olarak MCV, MCHC, PLT, RDW-CV, LDL, HDL, ALP erkek ratlarda, total kolesterol, lenfosit, PDW, PCT, kreatinin, total protein, ALT, kalsiyum, fosfor, OSI, GSH (kalp) ise dişi ratlarda istatistiksel olarak anlamlı bulunmuştur ($p < 0.05$). Ayrıca serumda, ürik asit, TOS ve TAS; böbrek ve karaciğerde MDA ve GSH; bağırsaklarda ise villus uzunluğu hem erkek hem de dişi ratlarda önemli düzeyde farklılıklar oluşmuşken histopatolojik bulgular açısından önemli farklılıklar görülmemiştir.

Sonuç olarak, rasyona soya küspesinin yerine %20'ye kadar Kara Asker Sineği larvasının dâhil edilmesinin, ratlarda olumsuz bir etkisinin olmadığı tespit edilmiştir. Bununla birlikte kan biyokimya parametreleri, sindirim sistem parametreleri ve oksidatif parametreler açısından bakıldığında %20'ye kadar Kara Asker Sineği kullanımı ratlar için tavsiye edilebilir.

Anahtar kelimeler: Kara Asker Sineği, Rat, Sağlık, Soya küspesi

ABSTRACT

REPLACEMENT OF SOYBEAN MEAL WITH VARYING INCLUSIONS OF BLACK SOLDIER FLY LARVAE IN ALBINO WISTAR RATS AS A MODEL FOR MONOGASTRIC ANIMALS

Regarding universal food and feed security, alternate feed resources are the leading market stance. Black Soldier Fly (BSF) is the most well-reputed because of its cost-effectiveness, ecological, sustainability, and less labor and land intensive. Subsequently, the payback of BSF larvae for health sustainability has become a great necessity. EU has legalized it to feed poultry, fish, pigs, cats, dogs, quails, ducks, reptiles, birds, and laboratory animals. BSF larvae farming helps to reduce organic waste removal and in return it provides animal feeding, organic fertilizer and biodiesel as end products. The objective of this study was to evaluate the effect of feeding varying inclusions of BSF larvae to Wistar albino rats as a replacement to soybean meal on blood chemistry, oxidative biomarkers and tissue histopathology parameters.

The number of total rats was 80 in this study. Half of the rats were male, and half of them were female. All rats were divided into five groups; each group contained eight subgroups, of which the first four contained male rats and the following four included female rats. In this way, each group had 16 total rats, 8 male and 8 female, and each subgroup consisted of two rats in one cage. The rats were fed with 0, 5, 10, 15, and 20% inclusions of BSF larvae meal as replacement to soybean meal. The effect of BSF larvae on body weight gain, average feed intake, hematological and serum biochemical parameters, tissue and serum oxidative stress parameters, and histopathological findings were seen. At the end of the study, the blood and tissue samples were collected from all rats. The blood samples were immediately analysed for hematological and serum biochemical parameters. Some of the blood and tissue (liver, kidney, and heart) samples were stored at -18 °C for serum oxidative parameters analysis which was performed at the Biochemistry lab. Tissues including liver, kidney, heart, testis, ovaries, uterus and duodenum were collected in sterile jars containing formalin and were analysed for histopathalogical analaysis.

The experimental feeds, soybean and BSF larvae samples were sent to the TUBITAK Marmara Research Centre for their nutritional profile analysis. The feed samples were analysed for dry matter, crude ash, crude protein, crude fat, crude fiber, fatty acid and amino acid composition.

The statistical analysis showed weight gain and average feed intake to be non-significant ($p > 0.05$) for all experimental groups compared to control. For statistical analysis, first of all, the normality test of the data was performed by descriptive statistics. On the basis of the normality test, the data was further analysed by One way Analysis of Variance (ANOVA) or Kruskal Wallis test (KWH) which were performed by IBM SPSS (V. 24.0) to analyse the difference between experimental groups. For Correlational analysis Pearson's test was performed. The statistical analysis showed that MCHC, PDW, MPV, P-LCR, uric acid, ALT, ALP, LDL, HDL, cholesterol total, liver, and kidney MDA and GSH, and tissue TOS and TAS were significant ($p < 0.05$) compared to the control as the male and female combined effect. However, as a gender-specific effect, MCV, MCHC, PLT, RDW-CV, LDL, non-HDL, ALP and cholesterol total in male rats and lymphocytes, PDW, PCT, creatinine, protein total, ALT, calcium, phosphorous, OSI, GSH (heart) in female rats were statistically significant ($p < 0.05$) compared to the control. Moreover, uric acid, TOS and TAS in serum, MDA and GSH in kidney and liver, and villus length were significant in both male and female rats and there were not any considerable histopathological changes.

Convincingly, up to 20% inclusion of BSF larvae as replacement to soybean meal is effective for feeding rats without causing any negative effect on health and nutrient absorption. An improvement in blood chemistry, intestinal histopathology and oxidative parameters in response to partial inclusion of BSFL up to 20% is recommend for use in lab animals.

Key words: BSF larva, Rat, Health, Soybean meal

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ABBREVIATIONS

AOAC: Association of Official Agricultural Chemists

ALP: Alkaline Phosphatase

ALT: Alanine Transaminase

AST: Aspartate Aminotransferase

BA: Basophils

BSE: Bovine spongiform Encephalopathy

BSF: Black Soldier Fly

CO_2: Carbon Dioxide

CH_4: Methane

CLP-1: Cecropin-Like Peptide-1

DDGs: Dry Distiller's Grains

DLP-3: Defensin-Like Peptide-3

DLP-4: Defensin-Like Peptide-4

DTNB: 5,5'-Dithiobis-(2-Nitrobenzoic Acid)/ Ellman's Reagent

EC: European Commission

EDTA: Ethylene Diamine Tetra Acetic Acid

EU: European Union

FAO: Food and Agricultural Organization

GHG: Greenhouse Gases

GPx: Glutathione Peroxidase

GSH: Reduced Glutathione

GSSG: Glutathione Disulphide

HACCP: Hazard Analysis Critical Control Points

HCL: Hydrochloric Acid

HCT: Haematocrit

HDL: High-Density Lipoprotein

HGB: Haemoglobin

HPLC: High-Performance Liquid Chromatography

IgG: Immunoglobulin-G

IUPAC: International Union of Pure and Applied Chemistry

LDL: Low-Density Lipoprotein

LY: Lymphocytes

MCHC: Mean Corpuscular Hemoglobin Concentration

MCH: Mean Corpuscular Hemoglobin

MCV: Mean Corpuscular Volume

MDA: Malondialdehyde

MDR: Multi-Drug Resistant

MO: Monocytes

MPV: Mean Platelet Volume

MRSA: Methicillin-Resistant *Staphylococcus aureus*

MUFA: Monounsaturated fatty acids

NEU: Neutrophils

NOx: Nitric Oxide

N_2O: Nitrous Oxide

n-RBC: Nucleated Red Blood Cells

NRC: National Research Council

OSI: Oxidative Status Index

PAPs: Processed Animal Proteins

PCT: Procalcitonin Test

PDW: Platelet Distribution Width

P-LCR: Platelet Large Cell Ratio

PLT: Platelets

PUFA: Polyunsaturated Fatty Acids

RBC: Red Blood Cells

RDW-CV: Red Cell Distribution Width- Coefficient of Variance

RDW- SD: Red Cell Distribution Width- Standard Deviation

SDS: Sodium Dodecyl Sulphate

SOD: Superoxide Dismutase

TAS: Total Antioxidative Status

TBA: Thiobarbituric Acid

TOS: Total Oxidative Status

UFLC: Ultra-Fast Liquid Chromatography

UNEP: United Nations Environment Program

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1. INTRODUCTION

1.1. Importance of BSF

The momentum for food and feed production sustainability has urged several modifications in industrialization. The government and private sectors must examine production technologies considering ecological dynamics, which sequentially control goods and services manufacturing, distribution, and authorization. Similarly, an uninterrupted global supply of food and feed and control of environmental pollution by technological advancement and scientific trials to regulate sustainability and animal health without any food scarcity for the human population is mandatory. The upsurge in the global populace brings about more organic waste due to unsystematic production systems and the disposal of various kinds of eatables enriched with valuable nutrients (Parisi et al., 2020). Advancement in the human population has changed consumer preferences as more are inclined to animal proteins. However, this trend is causing more ecological hazards and less food safety (Langyan et al., 2022). The proteins from animals and plants are insufficient to meet the human population's demands. Moreover, protein resources necessitate the advent of value maintenance chains and approachability to disputes such as consumer acceptance, food security, food safety, and economics (Henchion et al., 2017).

Feed ingredients that can potentially decrease global warming impacts are beneficial alternatives to conventional animal feed resources as they are economical and comprise a better nutritional profile. One option for fish and soybean meal replacement is BSF larvae reported in many scientific experiments. Fish and soybean are not only stapled food for humans but also expensive for animal feeding, and soybean cultivation causes environmental pollution. Insects are the best creatures to utilize organic wastes efficiently. Insects can be grown on a large scale with potentially less land and water requirements and cheaper organic wastes, including fruits and vegetables, kitchen wastes, and manure. In this way, it decreases the ecological loads and feed costs and improves the health of various pet animals, poultry birds, wild animals, lab animals, and pigs (Vauterin et al., 2021). BSF larvae have fascinated the dedication of several scientists and academics. The level of protein and fat in BSF larvae is extraordinary, roughly 40% and 30%, correspondingly, and it varies depending on the substrate used

for the rearing of larvae and the harvesting stage of metamorphosis. BSF protein-rich ingredient is an excellent protein and fat basis for feeding broilers, layers, fish, shrimp, quails, pigs, cats, dogs, pet birds, rats, mice, rabbits, wild lab models, lizards, and frogs (Prasetya et al., 2021). Although many studies on livestock and aquaculture animals show the potential of BSF larvae, no study has been conducted until now on rats as an alternative feeding ingredient to soybean meal. The current study aimed to evaluate the use of BSF larvae as a protein source in rat diets as an alternative to soybean meal. The purpose of using rats was to minimize the cost of feeding lab animals by introducing novel, nutritionally enriched, and alternative cheaper feed resources and as a model for monogastric animals. The intention of feeding BSF larvae to rats in this study was to evaluate the effects on average weight gain and feed intake; hematological and serum biochemical parameters; tissues and serum oxidative biomarkers, and histopathological traits.

1.2. BSF history

Even though BSF larvae have obtained the responsiveness of researchers for the last decade passionately, they are not an up-to-the-minute innovation. In 1916, L.H. Dunn found a putrefying corpse of an adult man in Panama enclosed entirely by BSF larvae in countless quantities. Scholars and technologists facing BSF larvae had different interpretations of them (Diener, 2010). In a study, BSF larvae restricted the house fly from oviposition in poultry droppings, one of its benefits for decreasing the house fly population (Furman et al., 1959). Later it was reported that the BSF converts the organic wastes into valuable, highly nutritious larvae that serve as potential natural feedstuff and reduce the ecological encumbrances. Such organic waste reconditioning may be one way of lessening waste dumping difficulties (Hale, 1973). The prospect of practicing BSF and insect farming for environmental and waste recycling settlements continued to amass after a well-known researcher, DeFoliart, reported in 1989 that essentially every ingredient of organic derivation, together with cellulose, for nurturing all species of insects is valuable. In the meantime, several types of research on the flies congregate on how BSF larvae determine advantages and what products be able to attain from BSF larvae farming.

1.3. BSF traits

The Black Soldier Fly (*Hermetia illucens*) goes to order, Diptera and family, Stratiomyidae. Its size is between 13 mm to 20 mm (Ma et al., 2018). BSF has two wings; this pair is called halters, and the BSF adult fly does not have a proboscis, the functional mouth part (Diclaro and Kaufman, 2012). BSF is inherited in America's tropical, subtropical, and high-temperature areas and migrated to tropical and hot countries with 28-32 °C temperatures (Barry, 2004). One benefit of adult BSF is that BSF does not have functional mouthparts; therefore, adult BSF flies survive without feeding anything and utilize the fats deposited during their larval phase (Tomberlin et al., 2002). Figure 1.1 shows the morphometry of the adult BSF.

Figure 1.1: Adult Black Soldier Fly

1.4. BSF life cycle

BSF goes through different platforms to complete its life cycle. The lengthiest stages are pupa and larval, whereas egg and adult stages are somewhat diminutive. Adults start mating within two days after the pupa stage ends, and the mating continues for not more than one week. Male flies find the female flies behind the bushes or near leaves, and females lay eggs on organic wastes so that their progenies can eat from the first day, and one female fly can lay up to 600-900 eggs (Diclaro and Kaufman, 2009). After the egg-laying, hatching begins on the fifth day under all optimum circumstances. Maggots descend keenly on a diet as soon as instars hatched from the ova (Booth and Sheppard, 1984). Gut microbes in larvae enable them to digest waste.

The most dynamic stage of BSF metamorphosis is the larva stage. Fatty reserves for adult flies develop during the larval stage. Before transferring to pre-pupae, larvae feed for two weeks (Yang andTomberlin, 2020). The pre-pupa step is the last phase of waste conversion. Nevertheless, all the former phases are correspondingly significant, although unrelated to biomass transformation (Hoffmann et al., 2021).

Their color variation categorizes pre-pupae as white to dark brown. Their affinity to roam from the larval territory to somewhere it can pupate after, preferably a desiccated and shadowy chamber. This relocation is dynamic; for instance, it consents for their collection for breeding into adults or handing out into animal feed and biofuel. Pre-pupae can ascend slopes of approximately 40o and creep 100 meters up to find a relevant space to pupate after (Singh et al., 2022). The pupation phase is the last stage before the emergence of adult BSF, and it generally proceeds for roughly two. On the other hand, the timeframe of the pupa stage can differ depending upon the predominant circumstances (Craig Sheppard et al., 1994). The life cycle of BSF larvae is shown in Figure 1.2, and the mating behavior of adult BSF is shown in Figure 1.3.

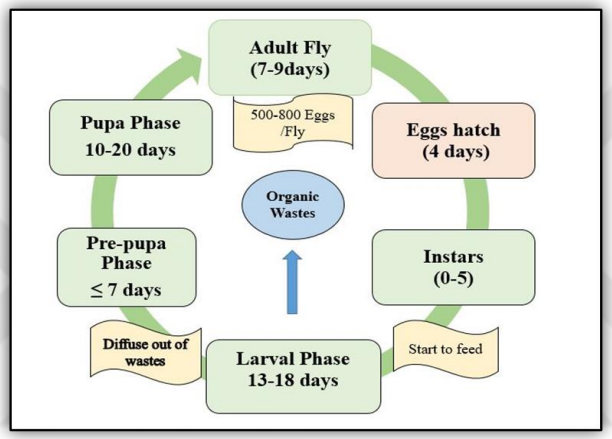

Figure 1.2: Metamorphosis of BSF

Figure 1.3: Mating behavior of adult BSF

1.5. Optimum growth conditions

Anyhow the toughness and flexibility of the BSF and various ecological situations are obligatory at all phases of the life cycle. Larvae movement drops discreetly at less than 10°C, whereas at more than 40°C, larvae creep out of the cages (Harnden and Tomberlin, 2016). The larvae of BSF are less than 1 mm lengthwise just after hatching and gain a length of a maximum of 3.6 cm and a weight of a maximum of 300-350 mg, provided that the temperature, light, and humidity are optimal (Tomberlin and Sheppard, 2002).

There exists a maximum of 44% lipids in BSF larvae, and 38% of the lipids in BSF larvae are lauric acid. Lauric acid acts as an antibacterial, antiviral, and antiprotozoal triglyceride. This is why even BSF larvae feed on pathogenic organic wastes, but its natural immune system is excellent (Ushakova et al., 2016). The melting point of lauric acid is 42°C which is why BSF does not tolerate more than 40°C. For better biomass production of larvae, 27-30°C is the best range (Mutafela, 2015). The larvae produced at 27.5°C were 30% more heavy and 5% lengthier than those grown at 30°C (Opare et al., 2022). In another study, female larvae gained 18% more weight at 27°C than larvae grew at 32°C (Shumo et al., 2019). Some researchers have reported that larvae creep out very damp feedstuffs and that a desiccated feed results in little biomass transformation. The humidity of 70% in feed for larvae is experiential to sustain the best biomass progression (Zhou et al., 2013).

Larvae with feed containing 70% humidity have shown a 93% adult development rate and eight days durability of adult BSF flies. However, the development rate of larvae and survivability of adult flies is 58% and seven days, respectively, at 40% humidity in feed and 17% and five days at 25% humidity in feed (Holmes et al., 2012). BSF larvae feed on various kinds of wastes fluctuating from plant to animal-based (Popa and Green, 2012). BSF larvae utilize the scraps more efficiently because of activities of α and β-galactosidase, leucine arylamidase, α-fucosidase, and α-mannosidase in their gut (Kim et al., 2011). BSF larvae can feed upon poultry feed and manure, pig and cow dung, and kitchen and vegetable wastes but all types of manure are prohibited from feeding larvae because of the unethical approach.

Kitchen wastes produce the bulkiest and longest larvae because it has comparatively more fat content (Nguyen et al., 2015). Batch feeding is more effective for heavier pre-pupa within a few days than daily feeding (Mutafela, 2015). The amount of waste for larvae varies concerning the types of feed. For instance, 100 mg per larva per day of poultry feed to larvae is enough. However, 30 mg of cuisine waste is enough for one larva per day (Diener et al., 2009). The optimum growth conditions of BSF larvae are shown in Table 1.1.

Table 1.1: BSF larvae requisites for optimum growth (Fazli Qomi et al., 2021)

Metamorphosis		Optimal Necessities			
		Temp. (^{o}C)	Humidity (%)	Nutriments	Restricted diets
Eggs	0 - 4 days	> 28	> 62	No need	Not required
Larvae	1 - 4 days	27 - 30	67 - 74	A mixture of alfalfa meal, corn meal, wheat bran, and water	No feces, carcasses, meat products, free of toxic metals
Larvae	5 - 14 days	28 - 32	68 - 76	Kitchen wastes, vegetable and fruit wastes, poultry feed	No feces, corpses, meat products, free of toxic metals
Pre-pupa and pupa phase	11 - 15 days	25 - 28	Low	Little need	Less required
Adult flies	< 8 days	27	35 - 85	Only water	Not required

*Temp: Temperature

1.6. Worldwide food threats

Since the global populace in 2017 reached 7.7 billion people, the United Nations forecasts that it could rise to 9.8 billion in 2050 and more than 11 billion in the 21st century. With a surplus of 2 billion people waiting for 2050, the sustenance related to nutriment is encouraged because FAO reported that in 2017 almost 950 million humans were half-starved (FAO, 2018). All at once, an augmented accumulation of carbon-based gasses has kept additional agronomic areas for constructing non-food biomass. However, emergent revenues in several states have increased meat consumption, necessitating more terrestrial farming (Calicioglu et al., 2019). Additional distress is that the excessive quantities of food are worthless universally and go to waste. Probably one-third of the foodstuff is discarded as leftovers (Green et al., 2018). Although poor schemas and establishments for organic waste handling are the foremost reasons for economic damage in underdeveloped republics; for instance, in European countries in 2016, almost 95 million tonnes of market and cafeteria waste were produced (Usubiaga et al., 2018). Advising the food waste chain of command, as FAO (2013) offered, inhibition and lessening food waste are the best operative behaviours for managing organic waste concerning ecological, communal, and financial dynamics. The subsequent and superlative possibility in the chain of command is to recycle this organic waste (Cristóbal et al., 2018).

Thus, it must be painstaking to utilize the sustenance waste (not appropriate for humanoids and the environment) as feedstuff for animals earlier transporting it for incorporation into biogas or burning (Corbould, 2013). Although pigs have remained an animal nourished with significant quantities of food leftovers, a novel clutch of omnivorous creatures has recently acquired increasing consideration called insects (Arnold et al., 2022). With an increase in global population, a rise in upcoming scarcity for nutriment supplies like rice (43%), maize (66%), soybean (56%), and wheat (40%) are possible (Ray et al., 2013). These estimates highlight the necessity for novel reasonable food and feed bases that have an optimum nutrient profile for animal feeding and production (Ritchie et al., 2018). Eatable insects are new bases for substitute protein for animal and human feeding.

They are commercial and universally discrete, the basis for effectual food conversion, have a short breeding period, and comprise a unique profile of nutrients (Lange and Nakamura, 2021). Consequently, insects could be industrialized and recycled to discourse difficulties of protein scarcities in food and feed productiveness (Nowak et al., 2016; Meyer-Rochow et al., 2021). Above 1800 eatable insect types worldwide have satisfactory nutritious worth to support human undernourishment (Weru et al., 2021). The nutritional substances in insects comprise excellent protein and fat quality with higher percentages that can make supernumerary to outdated protein bases in poultry, aquaculture, pigs, quails, pet birds, cats, and dog feed production (Van et al., 2017; Bazoche and Poret, 2021; Gałęcki et al., 2021; Guiné et al., 2021). Nonetheless, some insect classes are deficient in transforming several organic wastes and need explicit nourishment to be operational bio-converters (Morales and Wolff, 2010). These insects are annoyances and vectors of humanoid and animal pathogens (Gurung et al., 2019; Gupta and Nair, 2020). The schematic diagram indicating the steps to control global food security is shown in Figure 1.4.

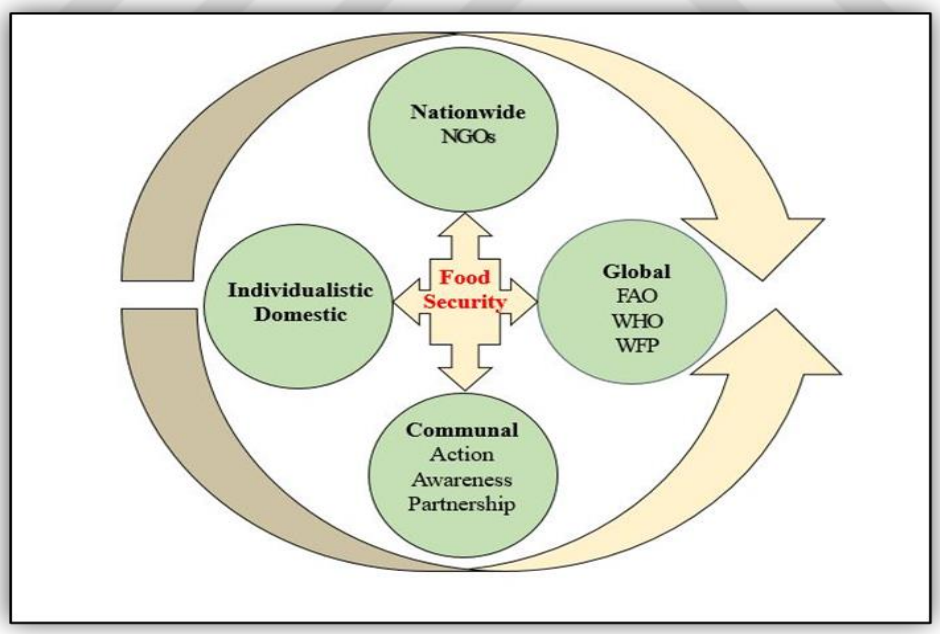

Figure 1.4: Steps to control global food security.

1.7. Unprocessed organic waste hazards

The surplus of carbon-based wastes influences the environment's well-being with the financial crisis. However, well-organized waste removal and recycling system are advantageous; meanwhile, it provides jobs and produces revenue for the official and private divisions (Diener et al., 2011a). Inappropriate management of organic wastes causes many environmental effluences, including aquatic, terrestrial, and airborne. It pollutes water and land and produces steady water reserves for mating mosquitos, pollutant microbes, and poisonous gases (Zhang et al., 2020). Cholera and typhoid are human diseases because of polluted water and land resources (Srigirisetty et al., 2017). Public health-related studies have shown that improper sanitation and cleanliness because of poorly handled organic wastes cause many diseases in Africa and underdeveloped regions. Rats, houseflies, mosquitos, dengue virus, *Salmonella typhimurium*, *Vibrio cholera*, volatile gaseous compounds, heavy metals, and many more are the entities of the rotten and inefficiently disposed of wastes (Liu and Zheng, 2020).

Furthermore, the putrefaction of solid waste emits agonizing smells, and burning produces air-contaminating loathsome vapors (Ferronato and Torretta, 2019). United Nations Environment Program (UNEP) stated that probably 11.3 billion tons of waste production per annum globally. It releases around 5% of greenhouse gas emissions, causing ozone depletion and global warming (Canton, 2021). Because of poor monetary policies for organic waste handling, it is primarily deserted in landfills causing excessive leachate and biogas production comprising 65% methane and carbon dioxide (CO_2) and 5-10% of greenhouse gases (GHG) (Mozhiarasi and Natarajan, 2022). Therefore, there is a demand for innovative, lucrative, and environmentally friendly waste management approaches, and insect farming by using organic waste is one of the most favorable and profitable businesses nowadays (Surendra et al., 2016; Yang et al., 2019; Pereira et al., 2020; Zhang et al., 2021). The hazards of organic wastes are shown in Figure 1.5.

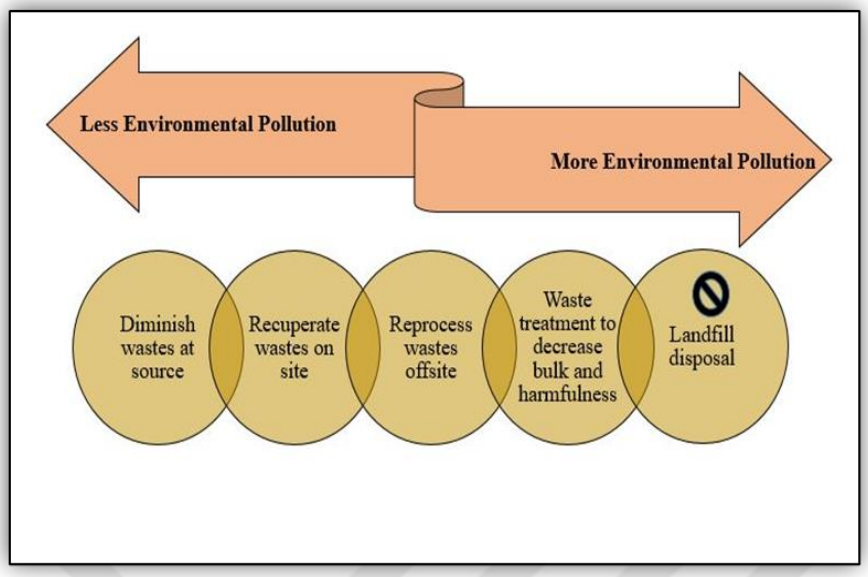

Figure 1.5: Sustainable environmental policies.

1.8. Biomass transformation by BSF

Overall solid waste comprises 78% of the total globally. Organic litter can be efficiently transformed into animal feed by side-to-side bio-conversion. This is a practice in which organic resources are transformed into valuable energy by biotic mediators such as microbes and insects (Zhu et al., 2011; Basim et al., 2012). Biotransformation is worthy as it encompasses the usage of the sarcophagus, for example, insect larvae, to decompose the organic leftover into eco-friendly by-products. For instance, earthworm (*Lumbriculus variegatus*) is a possible alternative for nutrient production from biological waste (Emamjomeh et al., 2018).

An additional choice is the decaying of biological surplus through common housefly (*Musca domestica),* yellow mealworm (*Tenebrio molitor),* BSF (*Hermetia illucens),* and crickets to produce feed for poultry, pets, pigs, quails, and fish with less ecological and health risks (Cappellozza et al., 2019). BSF larvae can transform several organic leftovers into highly esteemed and less detrimental biomass while releasing fewer greenhouse gases and ammonia (Van Huis et al., 2013). Due to the exceptional conformation of larval gut microbes, BSF larvae can eat a variety of foodstuffs.

Studies have revealed a range of biomass or waste reduction or transformation capability of larvae, which is 50% (Newton et al., 2005). Organic waste conversion or reduction is 65-75%, as stated by (Diener et al., 2011b), and 78%, as stated by (Li et al., 2011). These proportions of transformations, nevertheless, were experimental on different waste sources. The statement that with time researchers are reporting higher waste conversion competencies, there is confidence that waste reduction outputs could improve with ongoing research and enhanced optimization of BSF farming or housing education and infrastructure. The benefits of BSF larvae production by feeding organic wastes is shown in Figure 1.6.

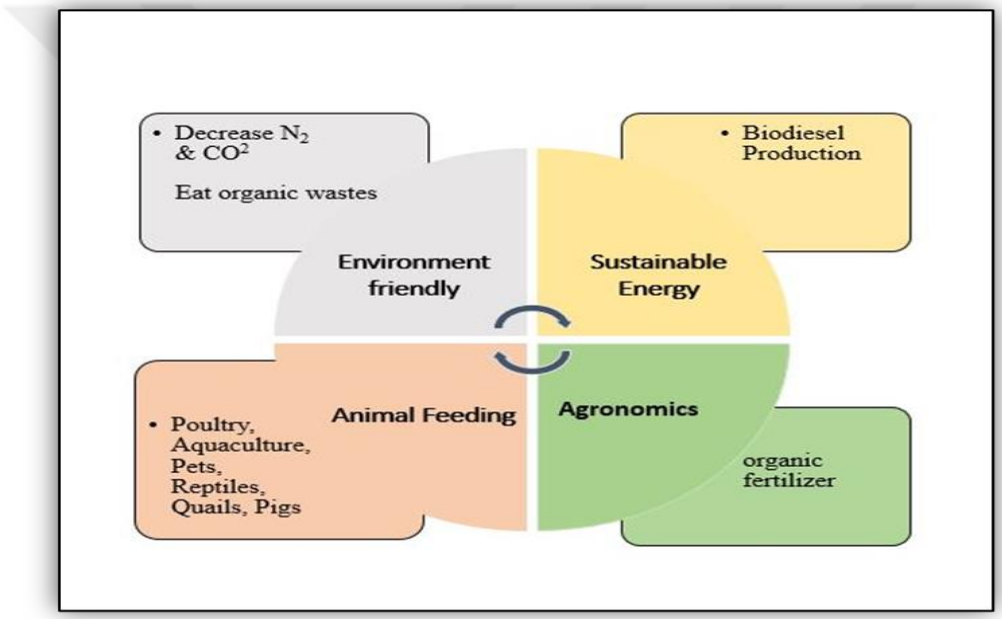

Figure 1.6: Benefits of BSF larvae production from wastes

1.9. Odor lessening

Odor lessening is one of the benefits resulting from BSF larvae. Bulky masses of larvae reared on organic wastes cause waste processing or transformation. Additionally, the larvae ventilate, unfertile organic leftover, and overcome bacterial progression (Mutiar and Yulhendri, 2021). With such a tendency of salient features, the production of odor decreases.

1.10. Housefly eradication

Unlike adult BSF, the common housefly (*Musca domestica*) has a proboscis, a feeding mouth part. Hence it eats all over their life. Fundamentally, housefly hunts for nourishment, not only biological leftovers but any food basis as well as humans' diet.
It has direct contact with humans and spreads diseases like salmonellosis and typhoid fever (Issa, 2019). Adult BSF flies have no functional mouth part and only rely on fat reserves in their body during their little life span. It does not pursue to arrive in human households or cafeterias, unlike houseflies, but survives its lifetime distant from humans (Barry, 2004). BSF decreases the housefly population exceptionally well because the existence of BSF prevents the common housefly from oviposition, thus causing population control. It is recognized in a trial that BSF colonization of poultry and pig manure ensured the prospective to decrease the common housefly populace by 95-100% (Sheppard and Newton, 2000).

1.11. Low pathogenicity

Adult BSF transmits no pathogen to another organism, and it appears BSF is a regular source of organic waste control. BSF encompass antibiotics-like compounds naturally that prevent them from disease dissemination (Sheppard et al., 2007). Many academics describe BSF larvae decreasing *E. coli O157:H7* and *Salmonella enterica serovar Enteritidis* in adulterated chicken manure (Zhou et al., 2013). Research on antimicrobial peptides in BSF larvae has shown that they are exceptionally safe from contamination (Joosten et al., 2020). The lack of well-known sickness in BSF larvae is surprising since mealworm (*Tenebrio molitor*) and insect species have confronted numerous transmissible infections (Eilenberg et al., 2015).

BSF larvae confronted with various microbes showed a considerable increase in phenol-oxidase action (Zdybicka-Barabas et al., 2017). BSF larvae produce antimicrobial proteins in response to the microbes in biomass (Vogel et al., 2018). For instance, in an experimental trial, the number of *Salmonella* and *Escherichia coli* was decreased in broiler feces (Erickson et al., 2004).

1.12. Microbial reduction character of BSF

Many researchers propose that insects might be recycled as a substitute for antibiotics or food preservatives, decreasing the requirement for antibiotics in poultry and humans. The investigators recommend that chitin endorse insects' antibacterial action. Insects comprise several antimicrobial peptides that, if administered to rats and lab animals, possibly will improve innovative approaches to fight multiple drug resistance in humans (Bessa et al., 2020). Several investigators have described that BSF larvae are augmented by lauric acid; hence, it presents good bacteriostatic impacts on various microorganisms (Spranghers et al., 2018). Lauric acid is probably 48% of the total fatty acids profile of the BSF larvae (Schreven et al., 2021).

In a research trial, the growth inhibition effect of lauric acid was presented against *Bacteroides* and *Clostridium* in rats (Matsue et al., 2019). Similarly, BSF fat decreased the number of *Streptococcus* and *Lactobacilli* in weaned piglets, so it was assumed that similar effects might be seen in other animals and humans (Martins et al., 2018). Many academics recommend that chitin probably endorses antibacterial properties in insects. BSF larvae extracts have excellent activity against gram-negative and gram-positive bacteria. For instance, the growths of *Neisseria gonorrhoeae*, *Shigella sonnei,* and *Klebsiella pneumoniae* have been reported to be decreased by methanol extracts of BSF larvae (Choi and Jiang, 2014). Likewise, the growths of *Staphylococcus aureus* and *Pseudomonas aeruginosa* were reduced because of lyophilized BSF extracts (Park et al., 2014). The larval hemolymph has also shown bacteriostatic action against *K. pneumoniae* (Choi et al., 2018). For wound healing, BSF extracts were used in-vitro, indicating growth stopover against *Pseudomonas fluorescence* (Moretta et al., 2020). Various antimicrobial peptides in BSF larvae are produced naturally because of the more significant microbial population in wastes.

The provision of lignin, chitin, cellulose, beverage grains, and sunflower oil displayed a diet reliant on the action of antimicrobial peptides (Shah and Çetıngül, 2022b). In a study of mice-induced infection with *K. pneumoniae,* hexanedioic acid was used for treatment and showed improvement (Chu et al., 2014). Defensin-like peptide 4 (DLP4) is extracted from BSF larvae hemolymph. It reduces colonies of methicillin-resistant

bacteria (MRSA) (Park et al., 2015). Similarly, defensin-like peptide 3 (DLP3) and cecropinlike peptide 1 (CLP1) extracted from BSF larvae reduce colonies of gram-negative and gram-positive microbes (Park and Yoe, 2017). *Helicobacter pylori* have resistant to metronidazole, and BSF larval peptides have constantly shown activity against this bacterium (Alvarez et al., 2019).

E. coli inhabiting the urinary tract of rats and multiple drug-resistant (MDR) bacteria have been reduced in number under treatment with cecropins extracted from BSF larvae (Brady et al., 2019; Kalsy et al., 2020). Hidefensin-1 and Hidiptericin-1 are extracted from BSF larvae and have been shown to inhibit the growth of *Streptococcus pneumoniae* and *E. coli* (Xu et al., 2020). Recombinant attacin-like peptides reduced the growth of *E. coli* and MRSA (Shin and Park, 2019). These peptides have antibacterial action and have been reported as antiviral (Feng et al., 2020). Based on the research above, BSF larvae obviously preclude animals and humans from many types of infections, improve the immune system and substitute antibiotics supplementation in feed, and undoubtedly decrease antibiotic resistance in animals and humans.

1.13. Potential cost-effective effects

Black soldier fly larvae in bio-waste control are comparatively highly economical and feasible compared to dumping and high-cost waste removal strategies. The byproducts obtained from BSF (fats, proteins, compost/frass, biodiesel) are almost 200% more economically valued than unprocessed organic wastes (Tomberlin and Sheppard, 2001). Moreover, the BSF technology needs improvements to recompense waste assortment and organization expenses.

1.14. Sustainable energy source

The utilization and demand for viable energy supply are rising globally as fossil fuel capitals gradually decline. People's intention is clear now why the trend has changed from fossil fuels to more sustainable and substitute fuels, i.e., biodiesel. It is an auspicious substitute for petroleum, and its popularity in public is improved globally in many republics (Zheng et al., 2012). Nevertheless, biodiesel is probably reluctant because of its more costly usage. Biodiesel assembly has similarly upraised arguments on an endless supply chain, chiefly owing to its production from edible oils. At the

same time, the food stream is not, however, sufficient (Li et al., 2011). Singh et al., (2020) motivated that even though people desire biodiesel worldwide and its balance up, using comestible oils for biodiesel is objectionable. Food quantity should not be disturbed by biodiesel production; food is an elementary human prerequisite. Supporting investigation in other viable energy bases has been a requirement.

Several replacements have better feedback for biodiesel, for instance, *Jatropha curcas* seed oil (Okullo, 2017; Laborde et al., 2019), brown grease (Kolet et al., 2020), waste grease (Li et al., 2012), muskmelon seed oil, microalgae (Chisti, 2007) and *Madhuca Indica* (Chowdhury et al., 2021). BSF larvae's perspective on promoting sustainable energy and less usage of fossil fuels and its practice in biodiesel is worthy (Kamari et al., 2020). BSF larvae can reprocess waste into energy, decreasing the ecological effluence of compost, kitchen, and vegetable and fruit wastes.

The lifespan of BSF larvae is very short, and it does not need extensive use of land, long lifecycle products, and expensive technology for biodiesel production. In a trial, 2000 BSF larvae were fed with 1000g of feed mixture containing 30% rice straw and 70% kitchen waste, and BSF larvae produced 44g of biodiesel (Franco et al., 2021). In a comparative trial, 72g of BSF larvae reared on cow dung produced 16g of biodiesel (Wong et al., 2018), and this perspective is a requisite to be explored furthermore.

1.15. Ecological paybacks of BSF

Feed and food challenges will cause an eco-friendly effect of up to 85% by 2050, owing to the absence of advanced adaptation and moderation developments (Wiseman et al., 2019). Livestock is the remotest biologically harmful of all the evolutionary activities with massive impacts on global warming, and it contributes to 77% of total agronomic gaseous releases (Springmann et al., 2018). Due to poor feed efficiency, less duodenal fermentation in livestock animals, and more gaseous discharge in feces, a widespread biological discredit as land scarcity, damage to regular habitations, conservatory properties, and constant burden on marine and land-dwelling species, there is the necessity of alternative feed ingredients (Sakadevan and Nguyen, 2017). Cafeteria and market waste have consideration for control due to unwelcomed biological, public, and well-being effects (Teigiserova et al., 2020). The EU anticipated organic wastes to

amass more than 100 million tons per annum, indicating that they should be recycled efficiently (Guo et al., 2021). Assuming to the European Union Waste Framework Directive (2008), the productive placement of biological wastes has essentially been the notable scheme to decrease the natural threats in Europe.

The ecological friendliness of insects as food and feed is considered far and wide as an alternative supportable, biologically, and low-cost adequate protein and fat source, organic fertilizer, and biodiesel source (Gahukar, 2016). The conversion of organic wastes to BSF larvae and its influence on greenhouse gas production varies for various organic substrates for rearing (Rahmi et al., 2020). Likewise, the productivity of larvae and the nutritional profile of larvae go concerning waste type (Raksasat et al., 2020). Compared to the degradation of wastes by aerobic microbes, BSF larvae use the waste material in a week and produce 27% CO2. In contrast, microbes consume the same waste food in 45 days and emit 50% CO2 (Perednia et al., 2017).

In another study, larvae production is a total of 45% of the organic waste. BSF reared on kitchen and market wastes produce significantly less methane (CH4) and nitrous oxide (N2O), whose emission is very high in the cultivation of natural protein crops, i.e., soybean (Bosch et al., 2019). There is less land use for larvae production and efficient utilization of manure and municipal sewerage. A study shows that one kg of larvae emits 2.1 kilograms of CO2, and one kg of larvae requires 0.05 m2 of land (Salomone et al., 2017). Furthermore, if BSF larvae feed on beet pulp, sorghum, poultry feed, and dry distillers' grains (DDGs), no significant environmental hazards will happen. BSF larvae decrease the residues of tetracycline and insecticides from cow dung (Lalander et al., 2016; Caligiani et al., 2018).

BSF larvae have the competence to decrease environmental contamination caused by manure up to the extent of 65%. In an experiment, BSF larvae reared on pig manure reduced nitrogen, phosphorus, and potassium by up to 70%, 53%, and 45%, respectively. At the same time, other environmental elements, including calcium, cadmium, boron, aluminium, copper, iron, lead, and nickel, were reduced by up to 80% (El-Dakar et al., 2021). Similarly, nitrogen emission decreased in cow manure by up to 50%, and phosphorus decreased to 70% in cow manure when offered for BSF rearing

(Chirere et al., 2021). BSF larvae process organic leachate that is expensive to compost and pollutes the habitats of aquatic and land-dwelling animals because of its richness in nitrogen and carbon. BSF larvae can efficiently process leachate from organic sources into valuable animal feedstuff (Popa and Green, 2012).

1.16. Production and de-fattening methods

There are optimal parameters for BSF larvae breeding and harvesting that must be well-ordered for the appropriate breeding of adult flies and ova hatching. The nutritional profile differs regarding the type of waste for feeding larvae. In an experiment pointing to breeding methods of BSF, the most typical larvae density was two larvae for every one gram of the waste, and optimal oviposition is 8500/m^3 under a sunlight disclosure. The pre-pupae delivered with euphorbia leaves, colza oil, and soybean meal were enriched in C18:2n-6, which is vital for the development of tilapia. Similarly, the pre-pupae fed with fish intestines, leftovers, and soybean meal is high in C20:5n-3, that are vigorous for humans. Furthermore, for optimal productivity of BSF larvae, 26.8 ± 0.50 °C and light for at least 12 hours are obligatory. The asking price of one kg of pure organic larvae is 1.82 USD (Gougbedji et al., 2021). The moisture content in wastes used for feeding the larvae is also crucial to be optimal for production.

For instance, in a research trial, it was seen that at 44.9% of moisture content, the show was 0.62 kg for one kg of the waste, while it was 0.52 when the substrate moisture was 74.9% (Bekker et al., 2021). High-fat content in BSF larvae causes excessive lipid peroxidation imparting the larvae's rancid odour and texture, and desired fat level in larvae is 30%. Besides this, it can cause rubbing and obstruction of the pelleting machines' sieve and produce easily breakable pellets. An experienced technique for defatted larvae is mechanical removal by bolt pressure. For extraction, after grinding and pressing the larvae at 100°C, the fat is removed, and the by-product is protein cakes. More processing causes the separation of protein meals, and the thick liquid fat comes out. At 100°C, the fat is squeezed from the larvae by a screw press, giving cake and liquid. Further processing separates protein meal from the cake and BSF larvae fat from the press liquid (Matthäus et al., 2019). An alternative method to this is solvent removal coupled with a microwave. The rotor-stator homogenizer ruptures the larvae cell wall. The slurry of larvae dries through a thermal process. Then the dried larvae

powder is placed in a cone containing hexane. The liquid part is the supernatant and contains solvent and fat, while the pellet is protein powder obtained after centrifugation (Feng et al., 2019). The selection of the biomass processing technique depends upon the usage of the end products.

Fat and chitin extraction is the maximum in chemical and stepwise methods. The quality of the proteins differs in various extraction methods. By chemical extraction, the degree of hydrolysis of the protein is 15%, and it is appropriate to practice it as a raw substantial for bioplastic synthesis. The protein obtained from the stepwise extraction method is excellent, and its degree of hydrolysis is less than 3%. However, a protein obtained by enzymatic technique is also better in quality. So, these can be used to feed animals (Caligiani et al., 2018; Rosa et al., 2020). A comparative study reports various solvents for fat extraction. In this study, 2-methyloxolane (2-MeO) produces excellent fat quality with better bioactivity and loftier protein powder quality than n-hexane (Ravi et al., 2019). In comparison, in the case of inorganic solvents, it was 35%, and lactic acid in the organic solvent category produced up to 86% pure fats and 62% proteins without any impurities (Soetemans et al., 2019).

Some proteases fasten lipid removal, amongst which the more efficient is protamex, which produces two times fatter than when there is no enzymatic usage. The enzymes used for the extraction are proteases, pepsin, papain, and pancreatin. However, proteases from *Bacillus licheniformis* have presented the most excellent activity concerning nitrogen mined from biomass and the degree of hydrolysis (Su et al., 2019). Another way to obtain fats from the larvae is the CO_2 extraction technique. In this technique, 11-19 mm grounded larvae are conveyed to a CO_2 extraction machine at a pressure of 352 bar for six hours (Kim et al., 2019). Another method understood for larvae fat removal is wet mode fractionation. BSF larvae go to a steam boiler, which is heated to make a slurry-like shape. Then pressing is performed to obtain larvae pulp and cake. The homogenized pulp transfers to incubators after adding enzymes, and later, centrifugation is performed. The aqueous phase has lipids, while the solid part is probably 60% chitin (Ravi et al., 2021). Production methods and extraction yields of BSF larvae are shown in Figure 1.7 and Figure 1.8, respectively.

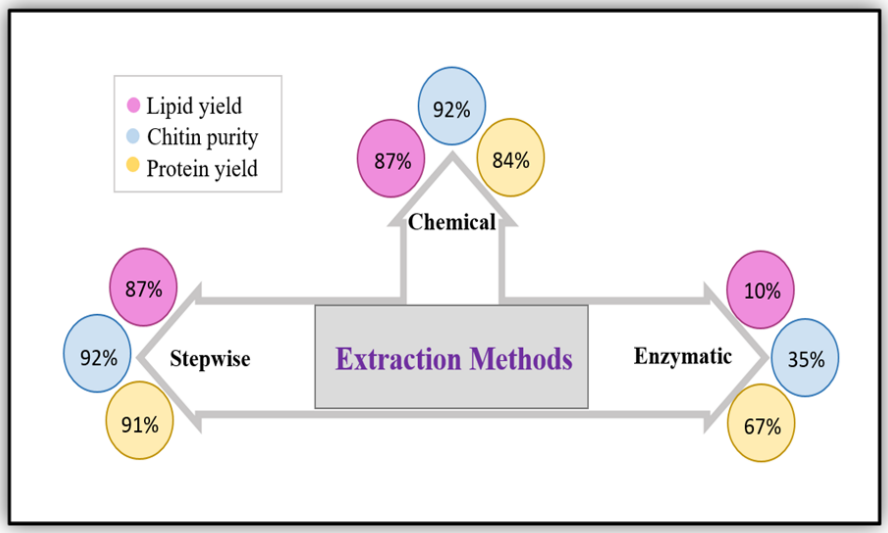

Figure 1.7: Extraction yields of BSF by three different methods.

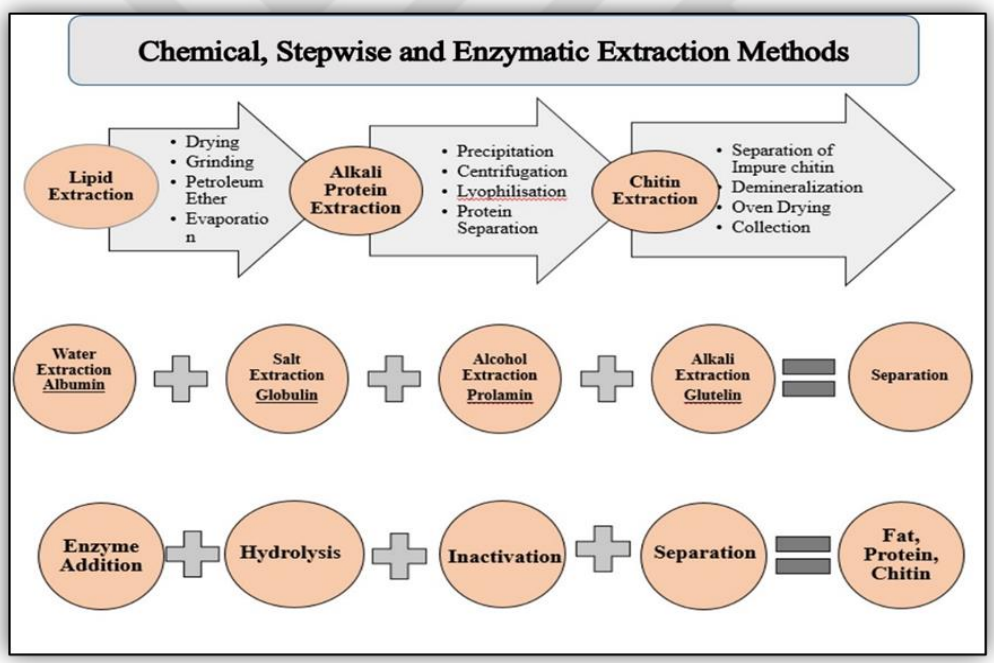

Figure 1.8: BSF fractioning methods.

1.17. EU authorization context

The regulating policies of BSF larvae as feed for animals usually differ between nation-states worldwide, and consumer liking and recognition of insect utilization varies (Lähteenmäki-Uutela et al., 2017). Due to bovine spongiform encephalopathy (BSE), processed proteins from animal sources (PAPs) are not suitable to use according to the

directive of the EU (EC 1069/2001) in 2009. 2002 came up with all probable feeding resources in the directive with their complete inclusions in the ration. All unwanted ingredients were also outlined (European Parliament and the Council of the EU, 2002). Revision of Annex I to IV of EC Regulation No: 999/2001 designed a new directive 2002/32/EC correspondent to EC regulation No: 56/2013 recognized that non-ruminant-originated processed proteins are allowed in aquaculture (European Parliament, 2013).

However, the usage of insects was not straightforward in the European Commission's encyclopedia. Subsequently, EC modified annexes X, XIV, and XV of EC: 142/2011 and Annexes I, IV of EC: 999/2001 on 24 May 2017, and Annex II of regulation EC: 2017/893 explicitly indicated the proteins obtained from insects are suitable for feeding of aquatic animals used for human food and farmed animals excluding fur animals, and it permitted the insect species for animal feeding including *Acheta domesticus*, *Gryllus assimilis* and *Gryllodes sigillatus* (Crickets); *Tenebrio molitor* and *Alphitobius diaperinus* (mealworms); *Musca domestica* (housefly larvae) and *Hermetia illucens* (BSF) larvae (European Commission, 2017).

Additionally, in the directive, the fat removed from these insects was permitted to feed all animals. Adaptation to the wastes, Annex III of directive EC: 767/2009 states that feces of cows, poultry, and pigs, along with fish offal and animal's intestines, are prohibited from feeding larvae (European Commission and Report, 2010), and it states same in the directive of European Commission, 2014. Likewise, in EC: 1069/2009, it was reported that insects fall in the category of farmed animals, and their feeding, any feces from humans and animals, and meaty or fleshy commodities are strictly prohibited (European Parliament and Council, 2009). Recently EU has declared that it is allowed to use processed animal protein (PAP) derived from BSF larvae for the feeding of farmed poultry, pigs, aquaculture, all fur animals, and pets based upon the condition that the substrate for the feeding of BSF larvae is not from meat or feces (Regulation 2021/1372, 2021). The allowed and banned substrates for feeding BSF larvae are shown in Figure 1.9.

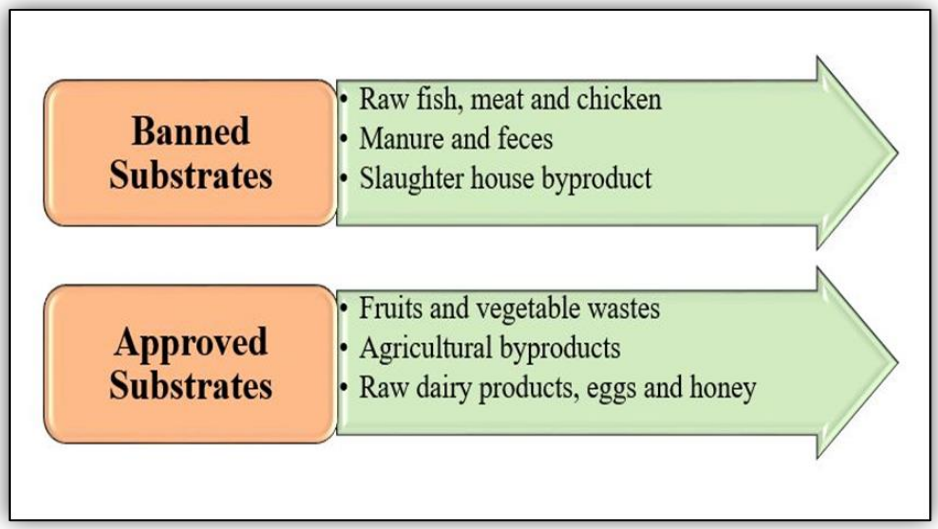

Figure 1.9: EU-authorized substrates for BSF larvae production

1.18. Nutritional potential of BSF larvae

BSF larvae have ample protein, fat, chitin, and calcium, and it has more lysine and methionine content than soybean; hence, it is a valuable feedstuff as an alternative to fish meal and soybean meal for various animals. The pre-pupa of BSF contains almost 40-45% protein and 30-35% fat. BSF larvae have a nutritional profile that reinforces the prospect of consuming it in aquatic, livestock, and pet food (Khan, 2018). The nutritive perception of BSF differs regarding the substrates recycled for its growing stage during production (Malla and Opeyemi, 2018).

1.18.1. BSF larvae protein

The crude protein content in BSF larvae upsurges impartially after hatching, progressively declining; for instance, on the 14th day of the metamorphosis, it is 40%. Afterward, it goes up to 45% and 55% at the pre-pupa and pupa phases correspondingly (Liu et al., 2017). Some researchers report that fat extraction from the BSF increases the protein content compared to the full-fat BSF larvae (Veldkamp and Bosch, 2015). For example, after the complete removal of fat from the larvae, the protein content reaches 65%, and the same larva with total fat was reported to have 55% protein (Schiavone et al., 2017b; Crosbie et al., 2020), which is very similar to meat and bone meal, and fish meal.

One study has shown the protein content of BSF larvae to be 34% identical to the plant-derived stuff comprising cottonseed, wheat distillers, and sunflower meal (Sauvant et al., 2004). Lysine, methionine, and threonine are insufficient in grains, and their by-products and these amino acids are abundant in BSF larvae protein meal (FAO, 2013). In contrast to 60% corn gluten, there has been higher content of lysine, arginine, and leucine reported (Liu et al., 2017). Histidine is an amino acid that is four times higher in BSF larvae than in fish meals (Elwert et al., 2010). The content of alanine, proline, and tyrosine is more in BSF when compared to fish and soybean meals (Taufek et al., 2021).

1.18.2. BSF larvae fat

Researchers have reported that the fat profile of the BSF is more efficient and bioactive compared to the fat obtained from the palm kernel and soybean, and it has more efficiency for production and better digestion. It improves the intestinal health of consumers (Gasco et al., 2019a). The trend of fat is opposite to protein during metamorphosis. Larvae hatch from the ova and contain almost 5% fat, increasing progressively. The time comes that at the pupa phase, it is 30% (McGuckin et al., 2011). The enzymes, including fatty acid synthase, acetyl-CoA carboxylase, and dehydrogenase, increase progressively with the development (Giannetto et al., 2020).

Nature has equipped BSF larvae with an antimicrobial fatty acid called lauric acid, which comprises 36-49% of the overall fat content of larvae (Oonincx et al., 2015). Myristic acid is a saturated fatty acid in BSF larvae associated with soybean meal (Leni et al., 2017). The level of saturated fatty acids (SFA) in BSF is the highest, monounsaturated fatty acids (MUFA) is moderate, while polyunsaturated fatty acids (PUFA) is approximately 15% (Hoc et al., 2020; Zarantoniello et al., 2020). Linoleic acid and α-linolenic acid on the 7^{th} day of metamorphosis are 32% and 1.7%, respectively. At the end of the second week, it is 6.99% and 1.49%, correspondingly (Paul et al. Oleic acid is a monounsaturated omega-9 fatty acid, up to 15% of the total fat in BSF larvae (Michaelsen et al., 2009). In a study, the rabbits were fed a diet having fat derived from BSF larvae and mealworms. The results showed that rabbit meat was less susceptible to oxidation, and the content of malondialdehyde was reduced in tissues (Gasco et al., 2019b).

1.181.3. Vitamins and minerals

Larvae of BSF comprise a unique content of phosphorous, iron, calcium, zinc, and Vitamin E, which has tremendous implications for feeding animals (Liland et al., 2017). During the prepupa phase of the larvae, vitamin E is 3.3 mg/100g and gets high on the 14^{th} day at 6.8 mg/100g. In the first stages of the metamorphosis, phosphorous and calcium are twice as compared to the pupa phase.

At the same time, zinc, iron, and sodium are high in the final phase of metamorphosis, i.e., at the pupa stage (Liu et al., 2017). In a study, feeding horse feces resulted in 916 mg/100 g of phosphorous at the pupa phase, and feeding chicken feed to larvae resulted in 319 mg/100 g of phosphorous. However, it is prohibited to use feces to feed the larvae intended to produce farmed animals (Moula et al., 2018). The general nutritional profile of BSF larvae is shown in Table 1.2.

Table 1.2: Low and high values of significant nutrients in BSF Larvae in two older studies.

	(Dortmans, 2015)		(Spranghers et al., 2017)	
Nutrients	**Low**	**High**	**Low**	**High**
Total solids	22	29.5	38.2	41.1
Protein %	30.9	52.99	40	42.2
Fat %	26.2	40.8	33.7	38.7
Ash %	7.4	14.7	2.8	19.8
Chitin %	1.3	6.3	5.7	6.8

1.19. SWOT exploration of BSF globally and in Turkiye

SWOT represents the strengths, weaknesses, opportunities, and threats. This sort of analysis represents all aspects of BSF in the animal feed industry without any unfairness.

1.19.1. Strengths

- Substitute basis of inexpensive and valuable protein for animal feeding.
- It decreases the organic waste bulk efficiently along with reducing odor glitches.
- Offers appreciated byproducts (agronomic fertilizer, fat, protein, biodiesel).
- Prospective to increase more financial worth of the waste by comparing composting.
- Stable resource of organic waste for the sustainable production of BSF larvae.
- The more natural diet of poultry, pet birds, quails, cats, dogs, and pigs.
- Reduce the environmental pollution and global warming caused by a bulk of carbon-based wastes and extensive cultivation of the soybean.
- Larvae might not need auxiliary dispensation, and they can be fed to all animals except herbivores.

1.19.2. Weakness

- Potential marketplace build-up for the BSF larvae and its byproducts.
- Even though the EU legally accepts BSF larvae, the marketplace in Turkiye for larvae is significantly less developed.
- Controlled monitoring of breeding and growth parameters.

1.19.3. Opportunities

- Because of enough organic waste all over the year, BSF larvae production is possible very cheap.
- It is prospective for BSF fat to be utilized in other capacities like biodiesel production.
- The compost obtained from the BSF larvae acts as organic fertilizer, and it is very high in phosphorous and best for crops.

- The pre-pupae might be processed into valuable protein and can be exported to other countries where extensive usage of insect-based proteins exists.

1.19.4. Threats

- It might take time for the industry to flourish in Turkiye, even though this sector is well developed in other developed and devolving countries; therefore, if the fully up-scale of its farming is done, there might be severe business risks.
- Utilization of manure and meat cadavers is prohibited; nevertheless, the producers prefer to use it because of less production cost causing the quality of the larvae to be depraved.

1.20. BSF use in poultry

Insects are incredibly explored as potential protein superfluous ingredients in poultry feed (Gasco et al., 2020), giving positive reliability to the productivity and welfare distinction of the chickens (Khusro et al., 2012; Veldkamp et al., 2012; Józefiak and Engberg, 2015; Józefiak et al., 2016) but prodigious an unattractive concern on nutrient digestibility, possibly owing to chitin existing in their exoskeleton (Hossein Barghaman et al., 2019). There have been limited studies about the impact of chitin and chitosan from insects on gut health, morphology, and gene expression. In a study on broilers, chitin and chitosan were provided from crickets and shrimps, and the results showed that the villus height of jejunum was improved, and crypt depth was decreased, and caused no significant effect on body weight gain (Ibitoye et al., 2019). Chitin can be an electrifying compound as it overwhelms a domineering character in clarifying the health of fowls obliged by diet-covering insects (Józefiak et al., 2019).

In an experiment, layers were provided for BSF larvae as a complete substitute to soybean meal, and the consequences were an increased percentage of butyric acid, a nutrient preventing gastric mucosa from erosions, due to bacteriological alterations by chitin in caecum as linked to the control group (Borrelli et al., 2017). Similarly, Leiber et al., 2018 accompanied a study on poultry birds where BSF larvae meal was fed as a partial alternative to soybean. It resulted in no significant discrepancy between all experimental groups of layers on egg quality and feed efficiency.

Among all experimental groups of broilers, there was no considerable dissimilarity in carcass quality and weight gain. Hy-line brown hens fed fat-free BSF larvae moderately as an alternative to soybean, resulting in improved quality of eggs compared to control (Secci et al., 2020). In another study, no considerable benefits were reported compared to the control regarding egg production, haugh unit, and feed intake. In contrast, body weight, egg yolk color, and eggshell thickness increased while shaver layers were fed with defatted BSF as 100% substituted to soybean meal (Mwaniki et al., 2020). According to (Moniello et al., 2019), egg quality and amount of butyrate and acetate increase in hens given BSF larvae.

An experiment on broilers fed with BSF larvae as an alternative to the fishmeal resulted in an upsurge in the crude protein of meat, more muscles on breast cuts, and a non-significant effect on abdominal and thigh amid all experimental clutches (Mlaga et al., 2020). In another study on broilers, soybean meal replacement with BSF larvae resulted in unaltered carcass parameters, including color, juiciness, pH, cooking loss, flavor, tenderness, and thawing loss among all tentative groups (Pieterse et al., 2019). According to Jansen (2018), BSF larvae with total fat showed no abnormalities in eggs and increased the hens' egg-laying capacity. A study about BSF larvae feeding to turkey resulted in an improved biological response, immune eminence, and meat quality and no negative impact on productivity and carcass quality except gizzard weight, which was higher in treatment groups as compared to the control group (Lalev et al., 2020). Various other trials indicate the favourable impact of insects in poultry without negatively impacting their growth and health. BSF larvae have been used in the poultry industry and have gained an optimistic approach for usage in turkeys, quails, ducks, aquaculture, cats, dogs, pet birds, and pigs.

1.21. BSF use in aquaculture

Insects are the most appreciated ingredients for usage in aquatic food creatures. Among all the insects, the nutritious summary of the BSF larvae is healthier than fishmeal (Çetingül and Shah, 2022). Moreover, Insects are easy to nurture, the source for improvement in the reduction of substantial ecological pollution and encompass a better nutrient profile paralleled to conservative feedstuffs (Ferrer et al., 2019).

Several types of research indicate the prominence of the BSF larvae in aquaculture. In a study for the feeding of climbing perch (*Anabas testudineus),* the fish meal was substituted fully and partially by BSF larvae and pre-pupa. The weight and crude protein percentage were comparable to the control; however, protein efficiency and crude fat% in meat samples increased in the group where fishmeal substitutes with BSF larvae at 100% (Vongvichith et al., 2020). In another study, juvenile Jian carp, for 59 days as a replacement to fishmeal, a fully defatted BSF larvae meal for nurturing, improved anti-oxidation without any difference in gut lipase and protease activities (Li et al., 2017).

BSF larvae as a feed for juvenile Grass carp for 56 days resulted in no difference in feed efficiency and weight gain. However, fatty acids in meat and anti-oxidation were improved in groups given higher percentages of BSF (Lu et al., 2020). In another trial, Japanese seabass fish was fed with fatless BSF larvae meal as a fill-in for fishmeal. It resulted in no different results on weightiness, histopathological findings, anti-oxidation, and immune responses. However, BSF decreased lipids deposition in the fish's liver compared to the control (Wang et al., 2019).

Juvenile rainbow trout were fed with BSF larvae meal instead of fishmeal, showing a surplus of *Bacillaceae*, *Selenomonas, Pectinatus, Megasphaera, actinobacteria,* and *Zymophilus* in BSF-fed groups (Huyben et al., 2019). Partly defatted BSF larvae were used instead of fishmeal in rainbow trout, and it summed up no adverse effect on productivity and rise in Hydroxyproline in groups given BSF larvae (Dumas et al., 2018). Muin et al. (2017) reported that 50% replacement of fishmeal endorses no negative impacts on fish production. BSF larvae have also presented excellent results in Nile tilapia, supporting better feed conversion and product quality than when fed soybean and fishmeal (Devic et al., 2018). In the lab model of fish called zebrafish, BSF larvae, as an alternative to fishmeal, lead to superior fatty acid production in zebrafish, promoting its health and immune response (Zarantoniello et al., 2018). Several researchers have publicized that a 50% replacement of fishmeal with BSF larvae meal demonstrated outstanding efficiency concerning the bioavailability of nutrients from the BSF larvae, productivity, and health benefits (Henry et al., 2015; Ssepuuya et al., 2017;

Zarantoniello et al., 2019; Zarantoniello et al., 2020). Various other trials designate the favorable impact of insects in aquaculture without negatively impacting the growth and health of aquatic food creatures.

1.22. BSF and other insects for feeding dogs and cats

The proteins derived from animals are not merely feedstuffs for dogs and cats. However, researchers are searching for novel ingredients that are digestible smoothly and beneficial for immunity but also come across the marketplace demand for sustainability and eco-friendly. Insects have become proficient as an increasing protein basis in current years. However, their appropriateness as a protein basis for dogs remained unexplored. The flavour of canine feed prepared from BSF larvae looks like a cheese and beef mixture. "Yora food" for pets is the world's most ecological meal derived from insects purely for pets in the UK. Its director reported that dogs love to eat it. A unique feedstuff that has gained attention in the pet food business is BSF larvae processed protein and fat (Ahmed et al., 2022).

The Chief of "Mars Pet care" reported insects as an alternate basis of protein for pets presently and in prospect, and it is the giant merchandise of insect-based feeds for pets globally (Wilkie, 2018). In the modern era, BSF larvae (*Hermetia illucens*) have been considered an excellent substitute for feedstuff and are prodigious awareness of pet food productiveness. The central HACCP regulated and authorized industrial unit in Dongen, Netherlands, stated in 2015 that food prepared for dogs using BSF larvae and potatoes is less allergic (Os, 2015). BSF larvae are desiccated, heat preserved, and crushed into fine particles for pet food formulation comprising 45% to 55% crude protein (Bosch, 2016). In a study on dogs, 5, 10, and 20% replacements of poultry by-product meal and corn meal with BSF larvae meal and the 1, 2.5, and 5% replacement of poultry fat with BSF larvae fats resulted in no apparent difference among all groups weights, feed intake and blood hematological and biochemical parameters. The investigation on stool consistency revealed healthy-shaped and muggy stool. The digestibility of all nutrients was also generally unchanged. BSF larvae and fat did not disturb overall well-being and might be safe and sound in dog diets (Freel et al., 2021)**.**

In another trial, BSF larvae were supplemented to nine female beagle dogs for six weeks at inclusion levels of 0%, 1%, and 2%. Albumin and calcium levels in the blood and serum of the dogs increased. The dry matter and protein were highly digestible in insect-based groups. Moreover, a decrease in tumor necrotic factor-α and an increase in glutathione peroxidase (GPx) and Superoxide dismutase (SOD) in BSF larvae-fed groups were noticed. In short, it was concluded that because of better digestibility, total anti-oxidation status, and anti-inflammatory action, BSF larvae are auspicious substitutes for dogs (Lei et al., 2019). In a study, BSF larvae were fed to the dogs, and the feces of the dogs were evaluated for microbiological analysis. The results showed that the stool's physical nature improved with improved consistency and less odor. Moreover, the number of pathogenic bacteria in dogs' feces decreased because BSF larvae have antibacterial compounds in their hemolymph, and extracts from their bodies are valuable antimicrobial active products (Attia Jaber, 2012).

A study showed that the microbes in dogs could digest the indigestible residues of insect-derived foods (Bosch et al., 2016). In a study, the BSF larvae meal was replaced with the lamb meal in a dog's diet, and the results showed that fecal production is reduced in dogs. The results showed that BSF larvae meal in dry dog food was endured without conflicting effects and did not alter immunological effects compared to an excellent dry diet with lamb meal, demonstrating that BSF larvae meal might be considered as an alternative protein basis for dog nourishment (Kröger et al., 2020). In a study on dogs, venison meal and BSF larvae meal were fed, and the results showed that the protein and calcium digestibility of BSF larvae was better than the control diet. However, the digestibility of fiber was less in BSF-fed dogs. Digestibility examination of dog feed comprising insect meal as the sole source of protein displayed promising outcomes, representing its appropriateness as a supportable protein basis for dogs (Penazzi et al., 2021). In an in-vitro study, the antioxidative status of the BSF larvae protein and its hydrolysates was evaluated compared to the fishmeal and chicken meal and resulted in the BSF larvae showing a higher total antioxidative rate. The consequences of the trial specified that BSF larvae might be possibly involved in feeding pets and aquaculture as immunity maintenance ingredients (Mouithys-Mickalad et al., 2020). In a study, the amino acid profile and crude protein level of twenty species

of plants, eighteen aquatic algae, and five insects were evaluated. Excepting taurine levels in BSF larvae, all insects surpassed NRC necessities for canine and feline. Taurine levels in insects vary; however high, the highest level of taurine was in flesh flies and ants. Preliminary outcomes recommend that insects and some aquatic algae, including *mazaella*, red algae, *porphyra*, and *chondracanthus*, might be realistic as replacements for conventional protein and complementary taurine sources in feeds for pets (McCusker et al., 2014). Similarly, another study reported that the BSF and housefly larvae pupae are high in protein and contain high amino acid scores but are less digestible compared to the crickets and cockroaches (Bosch et al., 2014).

In another study on dogs, pierced and intact, Madagascar cockroaches and super-warm larvae meal were offered as 7.5% and 15%. The results showed that the fecal consistency and digestibility for dry matter, crude protein, and crude fats were more than 80% for all groups (Lisenko et al., 2018a). In a study, 20 dogs with a history of atopic dermatitis were fed a diet comprised of locusts, mealworms, and crickets. The results showed that skin lesions and coat quality improved significantly; however, the pruritus score remained unchanged, and the explored insect-derived protein was an excellent substitute for dogs having food sensitivity (Böhm et al., 2018). A study was conducted on 35 dogs to evaluate the attractiveness of mealworms, BSF larvae, cockroaches, and cricket-based diets based on smell. The results showed that the dogs love to smell and eat the insect's derived diets compared to conventional commercial diets indicating the potential of insects for dogs (Kierończyk et al., 2018).

In another trial, crickets were included at 0-24% for 32 healthy dogs to explore their benefits on gastric microbes. An increase happened in *Lachnospiraceae*, *Catenibacterium*, and *Faecalitalea*, while a reduction in *Faecalibacterium* and *Bacteroides*. Moreover, the outcomes showed that crickets are as healthy as conventional feedstuffs for dogs (Jarett et al., 2019). Housefly larvae meal was supplemented to weaned beagle dogs for six weeks with 0% and 5%. Hematological and serum biochemical parameters showed no prominent dissimilarity among all groups. The levels of c-reactive proteins, lysozymes, and antibodies against canine parvovirus and canine distemper were also optimum in all experimental groups.

However, the MDA levels decreased in insect-fed groups compared to the control. In short, housefly larvae meal has the potential to be replaced with conventional feedstuffs in dogs (Hong et al., 2020). Crickets are an adequate constituent for feeding dogs. Feeding of beagles with 0, 8, 16, and 24% meals derived from crickets for five weeks decreased the total gut digestibility with increasing inclusion. Moreover, it resulted in more than 80% digestibility of all nutrients and healthier levels of hematology and serum parameters (Kilburn et al., 2020). A study on beagle dogs fed with BSF larvae meal as a replacement to poultry meal showed that BSF larvae-fed dogs had more apparent protein digestibility at 82.5% while poultry meal-derived diet had 80.6%. Likewise, the apparent digestibility of fat was more in BSF-fed dogs, and it was 94.65%, which was 91.5% in poultry meal-fed dogs. The consistency of dogs' feces stayed on a healthiness scale, while the dry matter of the feces in poultry meal-fed dogs was more than 33.2%, while it was 28.2% in BSF-fed dogs (El-Wahab et al., 2021).

Similarly, in another study, dogs were fed with silkworms and house crickets as a replacement for poultry meal for one month, and the results showed that 14% and 20% inclusion were better, respectively, without having any harmful effects on health and nutrient digestibility (Areerat et al., 2021). Likewise, for three months, fermented oats were substituted with BSF larvae meal for 20 female dogs. It resulted in no significant change in feed intake, body weights, BCS, fecal consistency, and skin shining of the dogs. However, the provision of BSF larvae for three months significantly decreased serum cholesterol (Seo et al., 2021).

Cats are solely meat-eaters and, in the wild environment, depend exclusively on a meat-based diet to meet their exclusive nutritional necessities. Nevertheless, there is less information concerning the sound effects of BSF larvae meal on the healthiness of adult cats. Insects are a regular dietary constituent for various wild animals. Feeding BSF larvae to adult cats at 4.5% for 20 days resulted in 5% of body weight loss and a high rise in alanine, potassium, sodium, and chloride, and a decrease in albumin, calcium, amylase, cholesterol, lymphocytes, red blood cells, total proteins, and total solids (Pezzali and Shoveller, 2021).

Feeding BSF larvae to cats instead of a chicken meal for 28 days resulted that the diets being highly digestible, and the extent of digestibility was not significantly different in all experimental groups. Likewise, the fecal scoring was generally the same in all groups (Hu et al., 2020). A total of 4,060 owners of dogs and cats were selected for a survey about their pets feeding plans and interests. The owners explained that insect-based feeds are usually as appetizing as conventional feeds (Knight and Satchell, 2021). Another study evaluated that the digestibility of protein and fat from BSF larvae meal was high at 86% and 90%, respectively (Gligorescu et al., 2020). Feeding *Lucilia Caesar* larvae to cats as an alternative protein source resulted in better health because its excellent nutritional profile is auspicious for cats and dogs as feedstuff (Nadtochii et al., 2019). A meta-analysis of wild and street cats reported that they need 52% of protein and 46% of fats, and insects comprise this as a prospective ingredient to improve their immunity and diminish nutritional deprivation (Plantinga et al., 2011).

Feeding 7.5% and 15% inclusions of *Madagascar* cockroach, *Zophobas morio,* and speckled cockroach to cats resulted in 15% inclusion imparted no adverse outcome on welfare intestinal health (Lisenko et al., 2018b). For consumer acceptance, some studies were performed in America about the usability of BSF larvae meals. Two investigations evaluated that the American inhabitants showed they are eager to consume insects directly, ingesting animals nourished by insects and feeding insects to their domesticated dogs. Contributors were more passionate about attempting food finished with insect meals instead of consuming intact insects, using a similar framework developed for the suitability of insects in dog foodstuff (Higa et al., 2021). Various other trials designate the favourable impact of insects in cats and dogs without negatively impacting their growth and health.

1.23. BSF and other insects practiced in rabbits and reptiles

Various types of research show the significance of BSF larvae in poultry and aquaculture. However, there is limited research evaluating its future benefits in laboratory animals, including rabbits, guinea pigs, rats, and reptiles (Higa et al., 2021). Feeding BSF larvae, lucerne, meat, and bone meal to Guinea pigs for two months resulted in no prominent dissimilarity in weight gain in rats for all sorts of diets. However, the increase in weight was high in guinea pigs fed with BSF larvae and lucerne. Concerning the fatty acid profile in meat, the level of polyunsaturated fatty acids was high in BSF larvae-fed groups and increased more in rats than in guinea pigs. There were low levels of saturated fatty acids in lucerne-fed groups. Compared to guinea pigs, the levels of monounsaturated fatty acids were high in rats for the group fed with BSF larvae, while BSF larvae fed guinea pigs had the highest content of trans-fatty acids. Concerning health benefits, the BSF larvae have a higher content of saturated fatty acids, MUFA, and PUFA (De Cuyper et al., 2020).

Feeding BSF larvae to juvenile alligators compared to fishmeal resulted in better growth of alligators and more economical feedstuff (Bodri and Cole, 2007). The insects' feeding efficiency differs with handling methods. Feeding crickets and live, intact, and mashed BSF larvae to mountain frogs (*Leptodactylus fallux*) resulted in a significant decrease in dry matter digestibility of intact larvae and the presence of 74% larvae in feces without digestion. In comparison, it was 76.5% and 84.7% for mashed BSF larvae and crickets, respectively (Dierenfeld and King, 2008). The exoskeleton of BSF larvae comprises excellent content of CaCO3 (Van der Fels-Klerx et al., 2020). Nature has provided BSF larvae with 2.5:1 of calcium and phosphorous (Barragan-Fonseca et al., 2017). The bioavailability of calcium is highest if the outer covering of their Skelton is ruptured mechanically. In a trial, there was 44.2% calcium absorption in blood for intact larvae, but the absorption increased in the case of mashed larvae, which was 88.7%. Feeding larvae in the texture of slurry increases the absorption of all nutrients. Mashed larvae have higher digestibility of phosphorous and calcium, i.e., 90% (Dierenfeld and King, 2008). BSF larvae lack vitamin A, which increases by providing Vitamin A in the substrate, and the larvae offered for feeding to rats caused no deficiency of vitamin A (Boykin and Mitchell, 2020c).

Feeding mashed and intact BSF larvae reared by supplemented sources of BSF larvae to leopard geckos resulted in no symptoms of vitamin A deficiency (Boykin et al., 2020b). Mostly captive snakes eat rodents, and they can sometimes be injured if the prey bites the snakes. Moreover, high calories can cause snake fat (Moon et al., 2019). Feeding BSF larvae sausages to babies of corn snakes resulted in no significant difference in weight gain, and nutrient digestibility was high in insect-based diets (Boykin et al., 2020a). Feeding fats of mealworms and BSF larvae instead of soybean to rabbits resulted in non-significant effects on body weights, FCR, dry matter digestibility, hematology, and gastrointestinal histopathology between all groups (Gasco et al., 2019). A few other trials entitle the favourable impact of insects in lab animals without negatively impacting their growth and health.

The study aimed to evaluate the use of BSF larvae as a protein source in rat diets as an alternative to soybean meal. The purpose of using rats was to minimize the cost of feeding lab animals by introducing novel, nutritionally enriched, and alternative cheaper feed resources and as a model for monogastric animals. The intention of feeding BSF larvae to rats in this study was to evaluate the effects on average weight gain and feed intake; hematological and serum biochemical parameters; tissues and serum oxidative biomarkers, and histopathological traits.

2. MATERIALS AND METHODS

2.1. Experimental design and project approval

The present research was accomplished under project number: 21. SAĞ.BIL.15 at the Experimental Animals Application and Research Centre, Afyon Kocatepe University, after the approval of the Local Ethics Board (AKUHADYEK) Turkiye under Endorsement No: 49533702/43, on AKUHADYEK Reference No: 38-21.

In this study, 80 Wistar albino rats were obtained from the Experimental Animals Application and Research Centre to conduct this study. Before the start of the study, all the rats were weighed, and the rats were evenly distributed into groups according to their mean body weights. The rats were given an adaptation period of two weeks, and during the adaptation, the rats were fed with experimentally formulated diets. The total duration of the study was 77 days (63 days experimental period and 14 days adaptation period). During the study, rats were provided 16 hours of light and 8 hours of dark periods in cages.

The parameters evaluated in this study were:

- Assessment of the production parameters of rats fed with 0%, 5%, 10%, 15%, and 20% replacement of soybean meal with BSF larvae in their diets.
- Evaluation of the hematological and serum biochemical parameters of rats.
- Measure the effects of BSF larvae feeding on oxidative stress biomarkers, i.e., MDA, GSH, NOx, TAS, TOS, and OSI in serum and kidney, liver, and heart.
- Microscopic examination for the histopathological findings in the duodenum, liver, heart, kidneys, testis, and ovaries.

2.2. Animal grouping

The number of total rats was 80. Half of the rats were male, and half of them were female. All rats were divided into five groups; each group contained eight subgroups, of which the first four contained male rats and the following four included female rats. In this way, each group had 16 total rats, eight males, and eight females, and each subgroup consisted of two rats in one cage.

2.3. Diet protocol

The groups were named A, B, C, D, and E, representing 0%, 5%, 10%, 15%, and 20% replacement of soybean meal with BSF larvae meal. Group A obliged the control group, while groups B, C, D, and E were provided with BSF larvae meal, as shown in Table 2.1. All groups were provided with an isocaloric and isonitrogenous diet. At the start, all water boxes were cleaned entirely, then after starting the study, water and experimental diets were provided ad-libitum every week during the trial period. The 16 days old harvested BSF larvae were obtained from the commercial marketplace in the Hatay district of Turkiye nearby the Syrian border. Before purchasing the larvae, it was ensured that vegetables, fruits, and kitchen wastes were used for rearing them. After the BSF larvae were obtained, they were stored at -18°C.

Table 2.1: Dietary treatment protocol of the study

Group	Treatment
BSF 0%	Normal Diet
BSF 5%	Diet having 5% replacement of soybean meal with BSF larvae meal
BSF 10%	Diet having 10% replacement of soybean meal with BSF larvae meal
BSF 15%	Diet having 15% replacement of soybean meal with BSF larvae meal
BSF 20%	Diet having 20% replacement of soybean meal with BSF larvae meal

* 0% BSF larvae, Control group *BSF 5, 10, 15 and 20% are the treatment groups

2.4. Experimental feed preparation

First the feeding ingredients were weighed, grounded, and mixed according to ingredient composition of the experimental diets as shown in Table 2.2. After that, according to the formulation, the feeding ingredients were shifted to the pellet machine for making 5 mm thick pellets. The pellets were made at a private feed factory. After the pellets were dried, they were transferred to the Animal Nutrition and Nutritional Diseases Laboratory for storage at -18°C to avoid any loss of nutrient profile. The required amount of the pellets was kept in oven for 12 hours at 50°C for every week before offering to the rats.

Table 2.2: Ingredient composition of the experimental diets

Ingredient	BSF 0%	BSF 5%	BSF 10%	BSF 15%	BSF 20%
Barley	3.44	4.8	2.56	0	0.68
Wheat Bran	7.6	7.2	7.6	3.6	6.2
Wheat Grain	14	13.2	14	13.4	13.8
Corn	6	5.2	5.52	10.4	7.4
Sunflower Meal 34% CP	0	0.96	1.72	4.24	3.8
Soybean Meal 44% CP	8	6	4	2	0
DCP	0.06	0	0	0.02	0
Limestone	0.76	0.532	0.496	0.236	0.016
Sea Salt	0.04	0.008	0.004	0.004	0.004
Vit-Mineral Premix	0.1	0.1	0.1	0.1	0.1
BSF Larvae	0	2	4	6	8
Total (Kg)	**40**	**40**	**40**	**40**	**40**

*Experimental feed formulation of control group (0% BSF) and treatment groups (5, 10, 15 and 20% groups). **DCP: Dicalcium Phosphate *The 40 kg of the feed was prepared for each group because it was the required amount of the experiment

2.5. Nutrient composition of feed, BSFL, and soybean meal

The samples of experimental compound feed, soybean meal, BSF larvae were sent to the TÜBİTAK Marmara Research Centre for analysing their nutrient profile. The analysed nutrient composition of the feed by TÜBİTAK Marmara Research Centre is shown in Table 2.3, and the nutrient composition of BSF larvae and soybean meal analysed by TÜBİTAK Marmara Research Centre is shown in Table 2.4.

The dry matter and ash of the feed samples was measured at the laboratory of nutrition at Afyon Kocatepe University. For determining dry matter, hot air oven method, AOAC 925.09, was used (AOAC International, 1990). For this purpose, the samples were first finely grounded and were put for 48 hours at 70°C and three consecutive readings were noted. The crude ash in the feed samples, BSF larvae, and soybean was determined by the incineration method, AOAC 923.03, (AOAC International, 1990), the finely grounded samples were put in a muffle furnace at 500°C for 8 hours, and the residual was determined as the inorganic minerals.

All other nutrients, amino acid composition and fatty acid composition in the feed samples were analysed by TÜBİTAK Marmara Research Centre. The crude fiber in the experimental feeds, BSF larvae, and soybean meal was determined by Weende method (AOAC 962.09) and the analysis was performed at the TÜBİTAK Marmara Research Centre. The crude protein in the experimental feeds, BSF larvae, and soybean meal was determined by the Kjeldahl method (AOAC 960.52) as described by (Chromý et al., 2015) and the analysis was performed at the TÜBİTAK Marmara Research Centre.

The composition of amino acids in BSF larvae and soybean meal was analysed by D.05.G106 in-house method, UFLC-UV (Ultra-fast liquid chromatography) as described by Czauderna et al., 2002. The tryptophan in BSF larvae and soybean meal was analysed by the D.05.G418 in-house method, HPLC-FLD. The analysis was performed at the TÜBİTAK Marmara Research Centre. The composition of amino acids in BSF larvae and soybean meal is shown in Table 2.5. The crude fat in experimental diets, BSF larvae, and soybean was extracted and preserved using n-hexane according to the Soxhlet method described by Sofyan et al., 2021.

For calculating the fatty acid composition in all experimental diets, BSF larvae meal, and soybean meal, the International Union of Pure and Applied Chemistry (IUPAC) IID-19 method was used as described by Mieth (1980). The analysis was performed at the TÜBİTAK Marmara Research Centre. The fatty acid composition of all experimental diets containing 0, 5, 10, 15 and 20% BSF larvae meal, soybean meal, and BSF larvae meal is shown in Table 2.6.

Table 2.3: Analyzed nutrient profile of the experimental feeds.

Nutrients	0% BSF	5% BSF	10% BSF	15% BSF	20% BSF
Dry Matter %	86.80	88.07	89.55	90.69	91.14
Crude Ash %	6.25	5.42	5.32	4.72	4.54
Crude Fiber %	4.58	6.42	5.74	6.22	6.06
Crude Fat %	2.71	4.31	6.37	8.52	10.93
Crude Protein %	20.81	22.00	20.50	20.75	20.75
***ME (Mcal/Kg)**	2,750	2,750	2,750	2,750	2,750

*ME: Metabolizable energy is calculated

Table 2.4: Analyzed nutrient composition of the soybean and BSF larvae meal.

Nutrients Composition	Soybean Meal	BSF Larvae Meal
Dry Matter %	88.65	93.20
Crude Ash %	10.04	8.60
Crude Fiber %	6.24	10.09
Crude Fat %	2.28	36.83
Crude Protein %	41.85	42.38

*Nutrient composition of BSF larvae meal and soybean meal determined by AOAC method.

Table 2.5: Amino acid composition of soybean meal and BSF larvae meal, mg/100 mg.

Amino Acid Composition	Soybean Meal	BSF larvae Meal
L-Alanine (Ala)	1702	3091
Glycine (Gly)	2039	3105
L-Valine (Val)	1600	2538
L-Leucine (Leu)	2851	2986
L-Isoleucine (lle)	1882	2020
L-Threonine (Thr)	682	997
L- Serine (Ser)	1817	2385
L-Proline (Pro)	2644	3433
L-Arginine (Arg)	2147	1818
L-Aspartic Acid (Asp)	4259	1285
L-Methionine (Met)	183	414
L-Glutamic acid (Glu)	8038	3858
L-Phenylalanine (Phe)	2058	1744
L-Lysine (Lysine)	5060	6476
L-Histidine (His)	1129	1613
L-Tyrosine (Tyr)	1285	2984
Tryptophan	624	640

*Amino acid composition of BSF larvae meal and soybean meal determined by D.05.G106 in-house method, UFLC-UV (Ultra-fast liquid chromatography) and Tryptophan was analysed by D.05.G418 in-house method, HPLC-FLD

Table 2.6: Fatty acid composition of experimental feeds, soybean meal and BSF larvae meal.

Fatty acid %	0% BSF	5% BSF	10% BSF	15% BSF	20% BSF	BSF Larvae Meal	Soybean meal
Capric Acid (C10:0)	0.04	0.44	0.49	0.56	0.63	0.9	-
Lauric Acid (C12:0)	2.75	31.32	35.58	39.83	44.39	47.32	0.41
Myristic Acid (C14:0)	0.77	7.12	8.18	9.21	9.42	10.16	0.15
Myristoleic Acid (C14:1)	-	-	-	0.19	0.21	0.25	-
Pentadecanoic Acid (C15:0)	0.06	0.09	0.09	0.09	0.09	0.15	0.03
Palmitic Acid (C16:0)	15.89	16.34	16.11	15.76	15.22	13.96	13.96
Palmitoleic Acid (C16:1)	0.27	1.84	2.11	2.43	2.46	2.64	0.12
Heptadecanoic Acid (C17:0)	0.08	0.08	0.08	0.08	0.07	0.11	0.1
Stearic Acid (C18:0)	1.92	2.06	2.09	2.34	1.94	2.14	3.77
Oleic Acid (C18:1n9c)	20.27	13.99	13.06	12.56	10.64	10.87	18.84
Linoleic Acid (C18:2n6c)	50.51	23.1	18.94	14.3	11.65	5.91	51.23
Arachidonic Acid (C20:0)	0.34	0.13	0.12	0.1	0.08	0.13	0.35
***cis*-11-Eicosenoic acid (C20:1)**	0.48	0.19	0.14	0.08	-	0.03	0.15
***α*-Linolenic acid (C18:3n3)**	3.65	1.65	1.25	0.79	0.65	0.6	7.81
Heneicosylic acid (C21:0)	-	0.14	0.15	0.21	0.13	0.13	-
Behenic Acid (C22:0)	0.17	0.08	0.06	0.04	0.3	0.09	0.38
Lignoceric acid (C24:0)	0.15	0.06	0.03	0.01	-	0.09	0.18

*0% BSF (Control), 5, 10, 15 and 20% BSF larvae (Treatment groups)

2.6. Feed intake and weight gain

According to the groups and subgroups, a specific amount of feed of 600 grams was offered weekly per subgroup/cage. From these cages, a typical experimental feed for every experimental group was provided to each subgroup at a specific time. To calculate feed consumption at the end of the week, the remaining feed in the subgroups was weighted, and total feed intake was calculated using the formula. Feed consumption: Total feed offered at the start of the week − Residual feed at the end of the week. In each subgroup, the initial body weight of all individual rats was recorded on the zero days of the study, and body weight was also recorded for all nine weeks for all rats.

2.7. Sample collections and analysis

The samples required for the analysis of various parameters were collected before the study, during the study, and at the end of the study to enhance the efficacy of the study and to determine all possible effects of BSF larvae meal as a substitute for soybean meal. The experimental compound feeds, BSF larvae and soybean samples were sent to the TUBITAK, Marmara Research Centre, Turkiye, to determine the dry matter, crude ash, crude protein, crude fat, crude fiber, total amino acids, and fatty acids composition. Blood samples were collected from each rat at the end of the study. Blood was contained in a 10 ml syringe from the heart of each rat, and then after blood aspiration, it was put in two types of blood tubes. One tube contained anticoagulant Ethylenediaminetetraacetic acid (EDTA), and the other tube was without coagulant or anticoagulant. After blood collection, serum was separated from blood using the centrifugation method at 3000 rpm for 10 minutes, and for further analyses, serum was stored at -20 °C.

2.7.1. Samples of blood and tissues for redox parameters

At the end of the study, blood and tissue samples were obtained from all rats for biochemical and redox parameters and histopathological examination. The collected tissues were the liver, kidney, heart, duodenum, testis, and ovaries. The samples were collected in sterile plastic containers for tissue biochemical parameters, while the tissues were collected in glass containers containing 10% formalin for histopathological

examination. At the end of the study, the blood was obtained directly from the heart of all rats under anaesthesia by exsanguination technique. It was immediately delivered to the biochemistry lab under a cold chain. First, centrifugation was performed at 4000 rpm for 15 minutes using Orto-Alresa® Digicen 21R centrifuge machine and stored at -20°C. The MDA, GSH, NOx, TAS, and TOS assays were performed for blood samples thawed with an appropriate procedure on the analysis day.

The liver, kidney, and heart tissues for biochemical analysis were collected after cutting the animals, washed in standard saline solution, and stored at -20°C to be ready for analysis. The tissues were moved to 4°C for 12 hours before the analysis until they were thawed. A 1:10 (w/v) dilution was prepared for these issues, then homogenization (IKA-T18 Ultra Turrax®) was performed in phosphate buffer (50mM, pH: 7). Moreover, the resulting suspension was treated with a second homogenization phase in an ultrasonicate (Bandelin Sonopuls; 20 kHz power). In the end, centrifugation (5000 rpm, +4°C, and 15 min) was performed in this homogenate. The supernatant portion was used for GSH and MDA analysis.

2.7.2. Hematological parameters analysis

Automated Hematology Analyzer XN-L series was used to process the blood samples in EDTA tubes for hematological parameters, including white blood cells (WBC), neutrophils (NEU), lymphocytes (LY), monocytes (MO), basophils (BA), eosinophils (EO), red blood cells (RBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelets (PLT), platelets distribution width (PDW), mean platelet volume (MPV), platelet- large cell ratio (P-LCR), procalcitonin test (PCT), red cell distribution width- coefficient of variance (RDW-CV), red cell distribution width-standard deviation (RDW-SD), and nucleated red blood cells (n-RBC).

2.7.3. Biochemical parameters analysis

Automatic biochemistry analyser BK-400 was used to calculate the serum biochemical parameters. The samples in vacutainer tubes were centrifuged at 3000 rpm for 10 minutes. Supernatants were transferred to Eppendorf tubes and stored at -20 °C till

biochemical analyses. Creatinine, total protein, uric acid, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), calcium, phosphorous, total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), non-HDL, and immunoglobulin-G (IgG) concentrations were determined.

2.7.4. Techniques for redox parameters

For MDA and GSH analysis, there are different techniques which are elaborated below.

2.7.4.1. MDA analysis

For analysing MDA levels in liver, kidney, and heart tissue, a colorimetric test was performed called the double warming method, described by Draper and Hadley, 1990. In standard, it is centred on the reaction of MDA with thiobarbituric acid (TBA) to produce specific and individual absorbance at a particular wavelength for tissue samples (Janero, 1990). This procedure involves the following steps:

- First, 0.2 ml of the supernatant from each sample was placed in the glass tube.
- After that, 0.2 ml of 8.1% sodium dodecyl sulphate (SDS) was added.
- After that, 1.5 ml of 20% glacial acetic acid was added.
- After that, 1.5 ml of 8% TBA was added.
- After that, 0.6 ml of distilled water was added.
- Subsequently, these add-ons, the glass tubes, were simmered at 95 °C for 1 hour and instantaneously cooled later.
- At that point, 1 ml of distilled water and 5 ml of n-butanal pyridine mixture were added.
- After that, the tubes were shaken for homogenous mixing for 2 minutes by using vortex mixer.
- After that, all the samples were centrifuged at 4000 rpm for 10 minutes.
- After centrifugation, the organic portion was discarded.
- The absorbance of the pink-coloured supernatant portion at 532 nm was measured by UV-1201 Spectrophotometer, Shimadzu, Japan.

2.7.4.2. GSH analysis

GSH levels were calculated in the liver, kidney, and heart tissues using the colorimetric assay, also called Ellman's method. It is essentially cantered on the catalytic reaction of Ellman reagent ($C_{14}H_8N_2O_8S_2$) or (DTNB) with reduced glutathione (GSH) and glutathione disulphide (GSSG) as described by Hissin and Hilf, 1976. Ellman reagent counts the sulfhydryl or thiol groups in samples. This procedure involves the following steps:

- First, 0.2 ml of every tissue homogenate was placed in sterile glass tubes.
- After that, 3 ml of precipitant (1 g EDTA + 90 g NaCl + 5 g metaphosphoric acid was dissolved in 300 ml of distilled water) was added and mixed in it.
- After waiting for 5 minutes, it was filtered through ordinary filter paper.
- Then 2ml of the filtrate was transferred to another tube.
- After that, 8 ml of phosphate buffer was added.
- After that, 0.5 ml of DTNB reagent was added.
- After that, the samples were mixed with reagents homogenously.
- 0.2 ml of GSH standard + 1.8 ml distilled water + 3 ml precipitator was used instead of tissue homogenizer in the standard tube.
- 3 ml of precipitator + 2 ml of distilled water was added to the blank tube and filtered through ordinary filter paper.
- It was then evaluated within 10 minutes at 412 nm by using a UV-1201 Spectrophotometer (Shimadzu, Japan).

2.7.4.3. TOS, TAS, and OSI

TOS and TAS were calculated by colorimetric assay kits (Rel Assay Diagnostics, Gaziantep, Turkiye). TOS is a quantitative test that amounts to the real presence of oxidative bodies in biological samples. However, TAS is a quantitative test that amounts to the total presence of antioxidant bodies in biological samples. Sampling, Standard dilutions, and incubation were accomplished following the guidelines in the lab booklet of the kits. The oxidation of reduced Fe^{+2} in the kit to Fe^{+3} by oxidative substances in the samples was calculated spectrophotometrically at 660 nm as μmol/L. Moreover, the OSI was considered according to the formula described by Esen et al., 2012; OSI = [(TOS) / (TAS x 100)].

2.7.5. Histopathological analysis

The histopathological analysis was conducted for each rat's vital organs (liver, kidney, heart, testis, ovaries, and duodenum) which were collected and cleaned with normal saline. They were observed macroscopically for abnormalities like lesions over control. After that immediately, organs were stored in plastic cups containing 10% formalin for fixation. The old formalin was removed after 24 hours, and fresh 10% formalin was added to plastic cups. For dehydration, ascending grades of alcohol were used, and paraffin wax was used for embedding liquid. After being washed overnight in tap water, it was kept in 50, 70, 80, and 96, and absolute alcohol, xylol, paraffin with xylol, and paraffin melted at 56-58 ℃ for 2 hours and then blocked-in paraffin. Samples cut at 5-micron thickness from each paraffin block with a microtome (Leica, RM 2245) was taken to slides utilizing a water bath (Leica, HI 1210). It was dried in an oven for ten minutes (Thermo, Heraterm) and made ready for use in histopathological methods. All sections were stained according to the hematoxylin-eosin (HE) staining method by passing through absolute, 96, 80, 70, and 50 alcohol series and xylol series. The stained preparations were examined under a binocular headlight microscope (Nikon, Eclipse Ci, Tokyo, Japan).

Microscopic pictures were taken from the necessary preparations, and measurements were made from 10 different regions of the intestinal preparations. (Nikon DS FI3, microscopic digital camera systems, Tokyo, Japan). Moreover, two slides were made for every rat, and every slide enclosed two sections for every organ from different positions. Tissue damage in the observed organs was recorded in various grades, as described by (Karimi et al., 2020).

2.8. Solutions preparation

Some of the solutions were prepared for various procedures according to the requirements, and the recipes for their preparation are mentioned in this section.

2.8.1. Phosphate buffer solution

Phosphate buffer saline was prepared using sodium chloride, potassium chloride, disodium hydrogen phosphate, and potassium dihydrogen phosphate Table 2.7. The pH was adjusted to 7.4 by using hydrochloric acid (HCl).

Table 2.7: Composition of phosphate buffer solution.

Serial No.	Ingredients	Quantity (g/L)
1	Sodium chloride	8
2	Potassium chloride	0.2
3	Disodium hydrogen phosphate	1.42
4	Potassium dihydrogen phosphate	0.24

2.8.2. Normal saline

To prepare a 0.9% solution of normal saline, 9 g of sodium chloride was dissolved in distilled water, and its volume was raised to 1000 mL and then stored in the reagent bottle composition of normal saline is shown in Table 2.8.

Table 2.8: Composition of phosphate buffer solution.

Serial No.	Ingredients	Quantity (g/L)
1	Sodium chloride	9
2	Distilled water	1000

2.8.3. SDS solution

8.1% SDS solution was prepared by dissolving a measured amount of SDS in the deionized water. The composition of the 8.1% SDS Solution is shown in Table 2.9.

Table 2.9: Composition of 8.1% SDS solution.

Serial No.	Ingredients	Quantity
1	SDS	2.02g
2	Distilled Water	Up to 250 ml

*Sodium dodecyl sulphate (SDS)

2.8.4. Pyridine and n-butanol solution

To prepare this solution, the measured amount of n-butanol was mixed with the calculated amount of pyridine. The composition of pyridine and n-butanol solution is shown in Table 2.10.

Table 2.10: Composition of pyridine and n-butanol solution.

Serial No.	Ingredients	Quantity
1	n-butanol	750ml
2	Pyridine	50ml

2.8.5. Precipitator solution for GSH analysis

To prepare the desired precipitator solution, Ethylenediaminetetraacetic acid (EDTA), sodium chloride, and meta-phosphoric acid were added to the calculated deionized water and mixed well. The composition of the precipitator solution for GSH is shown in Table 2.11.

Table 2.11: Composition of precipitator for Elman's GSH analysis.

Serial No.	Ingredients	Quantity
1	EDTA	1g
2	Sodium Chloride	90g
3	Meta-phosphoric Acid	5g
4	Deionized Water	300ml

2.8.6. 20% glacial acetic acid solution

To prepare 20% glacial acetic acid solution, 20 ml of acetic acid was taken in a beaker. After that, 80 ml of ultrapure distilled water was added to the beaker and mixed homogeneously. The composition is shown in Table 2.12.

Table 2.12: Composition of 20% glacial acetic acid.

Serial No.	Ingredients	Quantity
1	Glacial acetic acid	20 ml
2	Ultrapure distilled water	80ml

2.8.7. 8% TBA solution

To prepare 8% TBA solution, 8 g of TBA was taken in a beaker. After that, 100 ml of ultrapure distilled water was added to the beaker and mixed homogeneously on a magnetic stirrer. The composition of 8% TBA is shown in Table 2.13.

Table 2.13: Composition of 8% TBA solution.

Serial No.	Ingredients	Quantity
1	TBA	8 gm
2	Ultrapure distilled water	100 ml

*TBA (Thiobarbituric acid)

2.9. Glassware and equipment

The list of all glassware used in the study is shown in Table 2.14. However, the list of all equipment used in the study is shown in Table 2.15.

Table 2.14: Names of glassware used in the study.

Serial No.	Glassware
01	Borosilicate Glass Beakers
02	Glass Vials
03	Glass Measuring Cylinder
04	Pasteur Pipette
05	Reagent Bottles
06	Round bottom Flasks
07	Micro Column Condenser
08	Glass Slides for Histopathology
09	Test Tubes
10	Pyrex Round-Bottom Centrifuge Glass Tubes (Sigma-Aldrich)

*All of this glassware was used for dry matter and crude ash analysis, serum and tissue oxidative parameters

Table 2.15: Names of the equipment used in the study.

Serial No.	Equipment
1	Electrical weighing balance (Shimadzu)
2	Freezer at -40 °C (Model: MDF-594, Ultra-low, SANYO, Japan)
3	Cold cabinet at 4 °C (Model: MPR-1410, SANYO, Japan)
5	Vacuum Drying Oven (VO-200Memmert, Germany)
6	Muffle Furnace (Vulcan 3-400, Nebertherm GmbH, Germany)
8	Orto-Alresa® Digicen 21R centrifuge (Spain)
9	UV-Vis Spectrophotometer (Shimadzu, UV-1201, Japan)
10	IKA-T18 Ultra Turrax® Tissue Homogenizer (Turkiye)
11	Ultrasonicator (Bandelin Sonopuls; 20 kHz power (Germany)
13	pH meter (Coring)
14	Micropipettes (Witeg)
16	Colorimetric Assay Kit (Rel Assay Diagnostics®, Gaziantep, Turkiye)
17	Magnetic Hot Plate Induction Stirrer (IKA, Germany)
18	Nikon DS FI3, microscopic digital camera systems, Tokyo, Japan
19	Microtome (Leica, RM 2245)
20	Water bath (Leica, HI 1210)
21	Slide Warmer (Qor Labs, Japan)
22	Embedding Cassettes (Sigma-Aldrich)
23	Oven, Thermo, Herateram
24	Atomic Absorption Spectrophotometer (Hitachi-ZA8200, Japan)
25	Light Microscope, Nikon, Eclipse Ci, Tokyo, Japan

*All of this equipment were used for dry matter and crude ash analysis, serum and tissue oxidative parameters and histopathology

2.10. Statistical analysis

For statistical analysis, IBM SPSS Statistics 26 software was used. First, the data samples' normality was checked by a descriptive normality test. If the sample normality significance was > 0.05, One-way ANOVA was applied, and if the sample normality was < 0.05, Kruskal-Wallis Test was used. After selecting the dependent and independent variables, the polynomial cubic effect was seen as the contrast effect. For post hoc analysis, Duncan and Tamhane's T2 was applied. Moreover, a descriptive and homogeneity of variance test were performed at the end. One-way ANOVA was used to compare the control data with the groups fed with varying percentages of BSF larvae meal, where $p < 0.05$ was measured as significant while $p < 0.01$ was highly significant. However, $p > 0.05$ was declared as non-significant. For correctional analysis SPSS Pearson's test was performed and the correlation was checked for each statistical parameter.

3. RESULTS

3.1. Average body weight

The average body weights of all rats in all groups were measured during the study trial of 9 weeks. The data was recorded every week. Moreover, the difference in weights of the rats during the 0-9th Week was also measured. The statistical analysis showed no significant difference in all groups for initial and final weight gain and difference in initial and final weights of the rats among all groups ($p > 0.05$), as shown in Table 3.1 and Figure 3.1.

Table 3.1: Initial and final weights for all groups and average weight difference.

BSF	Initial BW	Final BW	Difference in weights
0%	257.32 ± 9.88	300.07 ± 16.41	42.75 ± 8.74
5%	258.44 ± 7.96	290.5 ± 16.15	32.07 ± 12.66
10%	261.69 ± 7.01	305.44 ± 14.87	43.75 ± 9.21
15%	260.5 ± 7.37	309.69 ± 13.36	49.19 ± 7.31
20%	262.63 ± 6.97	303.75 ± 11.91	41.13 ± 7.03
p-Combined	*0.989*	*0.912*	*0.768*
Linear	*0.613*	*0.568*	*0.635*
Quadratic	*0.935*	*0.950*	*0.977*
Cubic	*0.962*	*0.456*	*0.222*

*Data is represented as least squares. The values are means of 8 replicate cages per diet with 2 rats (n= 16).

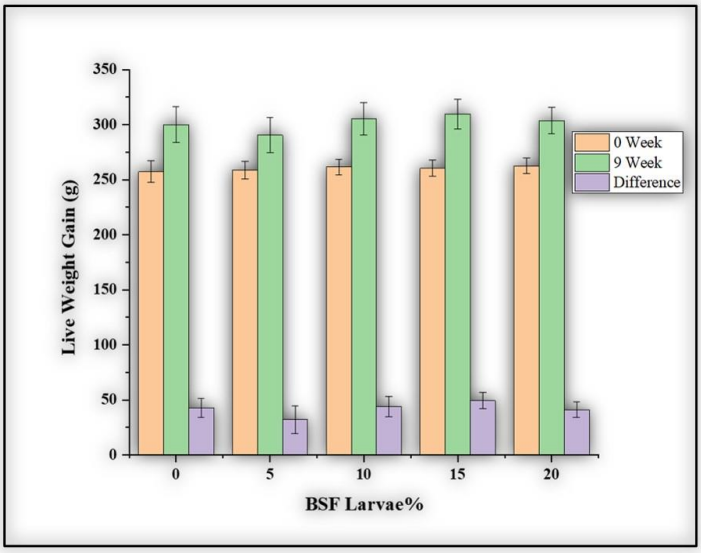

Figure 3.1: Live weight gain in all groups

3.2. Average daily feed consumption

All groups' average daily feed consumption was statistically analysed for all nine weeks of the study. The results showed that there was no statistically significant difference in all experimental groups of the rats ($p > 0.05$) and the feed consumption remained very constant for all rats in all groups throughout the study.

It was seen that the nutrient value of the experimental diets containing BSF larvae was very similar to the control diet, and it did not hold back the rats from eating less as compared to the control because the feed intake of all experimental groups was very similar to the control group. The average daily feed intake of all rats and their p-value is shown in Table 3.2 and Figure 3.2.

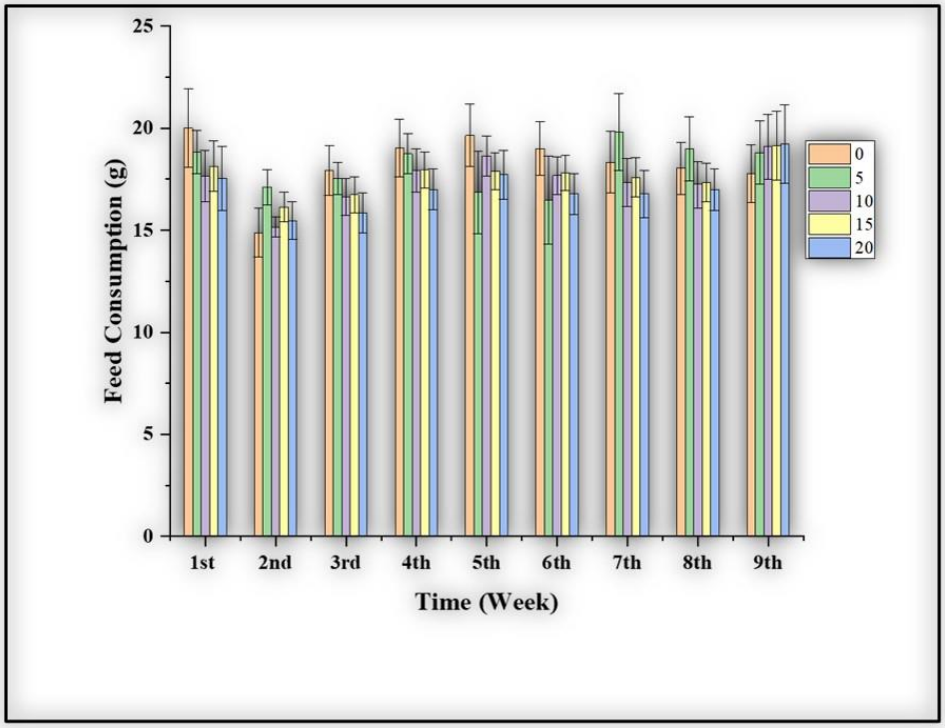

Figure 3.2: Average daily feed consumption for all groups.

Table 3.2: Average daily feed consumption for all groups weekly

Week	BSF larvae inclusion levels					*p*			
	0%	**5%**	**10%**	**15%**	**20%**	***Combined***	***Linear***	***Quadratic***	***Cubic***
1st	20.05 ± 1.92	18.83 ± 1.07	17.65 ± 1.26	18.14 ± 1.25	17.54 ± 1.58	0.74	0.23	0.61	0.82
2nd	14.88 ± 1.19	17.12 ± 0.86	15.15 ± 0.49	16.14 ± 0.73	15.47 ± 0.93	0.38	0.95	0.39	0.36
3rd	17.92 ± 1.21	17.54 ± 0.78	16.63 ± 0.9	16.73 ± 0.88	15.85 ± 0.99	0.58	0.12	0.98	0.89
4th	19.03 ± 1.42	18.74 ± 0.98	17.91 ± 1.06	17.95 ± 0.88	17.10 ± 1.00	0.71	0.17	0.92	0.9
5th	19.65 ± 1.53	16.86 ± 2.02	18.65 ± 0.98	17.89 ± 0.91	17.72 ± 1.19	0.68	0.53	0.61	0.37
6th	19.12 ± 1.32	16.49 ± 2.16	17.68 ± 0.92	17.81 ± 0.86	16.78 ± 1.01	0.71	0.47	0.71	0.26
7th	18.33 ± 1.51	19.8 ± 1.88	17.34 ± 1.18	17.59 ± 0.97	16.78 ± 1.16	0.59	0.23	0.72	0.52
8th	18.03 ± 1.29	19.01 ± 1.58	17.24 ± 1.14	17.34 ± 0.93	16.99 ± 1.02	0.76	0.34	0.87	0.56
9th	17.77 ± 1.42	18.8 ± 1.54	19.1 ± 1.59	19.14 ± 1.68	19.22 ± 1.92	0.96	0.54	0.73	0.89
0-9th	18.31 ± 1.43	18.14 ± 1.44	17.49 ± 1.06	17.64 ± 1.01	17.06 ± 1.20	0.67	0.39	0.72	0.61

*Data is represented as least squares. The values are means of 8 replicate cages per diet with 2 rats (n= 16). *Black Soldier Fly 0% (Control), BSF 5%, 10%, 15% and 20% are the treatment groups.

3.3. Hematological parameters (Combined effect)

The statistical analysis of haematological parameters including WBC, NEU, LY, MO, BA, EO, RBC, HGB, HCT, MCV, MCH, MCHC, PLT, PDW, MPV, P-LCR, PCT, RDW-CV, RDW-SD, and n-RBC was conducted for male and female rats as combined and gender-specific effect. The combined statistical analysis of all the 80 rats for haematological parameters that all parameters other than MCHC, PDW, MPV and P-LCR remained statistically non-significant for all groups in the study ($p > 0.05$). MCHC was changed significantly ($p < 0.05$) as a combined and linear effect compared to the control. Kruskal Wallis test showed that PDW, MPV and P-LCR were increased significantly for 20% group compared to the control ($p < 0.05$). All of the other parameters remained non-significant in all groups ($p > 0.05$). Table 3.3 shows the combined SPSS analysis of the hematological parameters.

Table 3.3: Haematological parameters for all groups, male and female combined. The unit of measurement for WBC, NEU, LY, MO, BA, EO and PLT (10^3/uL), for RBC (10^6/uL), for HGB and MCHC (g/dL), for HCT, P-LCR, PCT, RDW-CV (%), for MCV, PDW, MPV and RDW-SD (fL), for MCH (pg).

Parameters	BSF 0%	BSF 5%	BSF 10%	BSF 15%	BSF 20%	Test name	*p*	*Linear*	*Quadratic*	*Cubic*	Posthoc
WBC	3.38 ± 0.33	3.25 ± 0.29	3.21 ± 0.36	2.97 ± 0.26	3.78 ± 0.35	KWH	*0.512*	-	-	-	-
NEU	0.81 ± 0.06	0.76 ± 0.07	0.79 ± 0.07	0.72 ± 0.06	0.81 ± 0.12	KWH	*0.899*	-	-	-	-
LY	2.35 ± 0.24	2.36 ± 0.24	2.24 ± 0.29	2.04 ± 0.2	2.71 ± 0.26	KWH	*0.403*	-	-	-	-
MO	0.19 ± 0.13	0.08 ± 0.01	0.12 ± 0.04	0.18 ± 0.09	0.21 ± 0.08	KWH	*0.323*	-	-	-	-
BA	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	KWH	*0.768*	-	-	-	-
EO	0.05 ± 0.01	0.06 ± 0.02	0.06 ± 0.02	0.04 ± 0.01	0.07 ± 0.01	KWH	*0.275*	-	-	-	-
RBC	8.33 ± 0.14	8.17 ± 0.15	8.23 ± 0.14	8.27 ± 0.11	8.38 ± 0.11	ANOVA	*0.76*	*0.62*	*0.25*	*0.72*	Duncan
HGB	14.33 ± 0.15	14.15 ± 0.2	14.13 ± 0.18	14.25 ± 0.11	14.38 ± 0.18	KWH	*0.973*				-
HCT	46.95 ± 0.57	46.7 ± 0.79	45.9 ± 0.67	46.26 ± 0.37	46.48 ± 0.56	ANOVA	*0.77*	*0.47*	*0.35*	*0.82*	Duncan
MCV	56.43 ± 0.48	57.22 ± 0.45	55.87 ± 0.46	56.06 ± 0.5	55.54 ± 0.62	KWH	*0.131*				-
MCH	17.24 ± 0.18	17.35 ± 0.11	17.2 ± 0.19	17.25 ± 0.15	17.17 ± 0.17	ANOVA	*0.93*	*0.63*	*0.71*	*0.79*	-
MCHC	30.53 ± 0.15^{ab}	30.33 ± 0.17^{a}	30.8 ± 0.13^{b}	30.82 ± 0.12^{b}	30.95 ± 0.18^{b}	ANOVA	***0.03***	***0.01***	*0.69*	*0.23*	Duncan
PLT	837.57 ± 49.42	877 ± 49.48	911.69 ± 34.01	862.13 ± 28.35	828.25 ± 54.56	KWH	*0.626*	-	-	-	-
PDW	8.79 ± 0.24^{ab}	8.77 ± 0.13^{ab}	8.56 ± 0.14^{a}	9.02 ± 0.13^{b}	9.42 ± 0.26^{b}	KWH	***0.014***	-	-	-	-
MPV	7.94 ± 0.11^{ab}	8.12 ± 0.08^{ab}	7.89 ± 0.09^{a}	8.17 ± 0.09^{ab}	8.3 ± 0.15^{b}	KWH	***0.034***	-	-	-	-
P-LCR	10.37 ± 0.84^{ab}	11.51 ± 0.65^{ab}	9.8 ± 0.71^{a}	11.64 ± 0.63^{ab}	13.27 ± 1.21^{b}	KWH	***0.033***	-	-	-	-
PCT	0.67 ± 0.04	0.72 ± 0.05	0.72 ± 0.03	0.71 ± 0.03	0.69 ± 0.05	ANOVA	*0.81*	*0.71*	*0.26*	*0.68*	Duncan
RDW-CV	17.01 ± 0.61	16.94 ± 0.44	16.77 ± 0.59	16.99 ± 0.5	17.49 ± 0.5	KWH	*0.739*	-	-	-	-
RDW-SD	25.8 ± 0.46	26.47 ± 0.67	25.43 ± 0.34	25.55 ± 0.39	25.87 ± 0.43	KWH	*0.757*	-	-	-	-

3.3.1. WBC, NEU, MO, and LY (Gender specific)

All of the hematological parameters were statistically analyzed for males and females separately. The statistical analysis of WBC shows that the level of it was statistically non-significant in all groups ($p > 0.05$). Similarly, the statistical analysis of NEU shows that the level of it was statistically non-significant in all groups ($p > 0.05$). Likewise, the statistical analysis of LY shows that its level of it was statistically non-significant in all male rats ($p > 0.05$) while in female rats it was shown significant by Kruskal Wallis test ($p < 0.05$). The statistical analysis of MO shows that it was statistically non-significant in male and female rats ($p > 0.05$) compared to the control. The values for male rats is shown in Table 3.4 and for female rats it is shown in Table 3.5 and Figure 3.3.

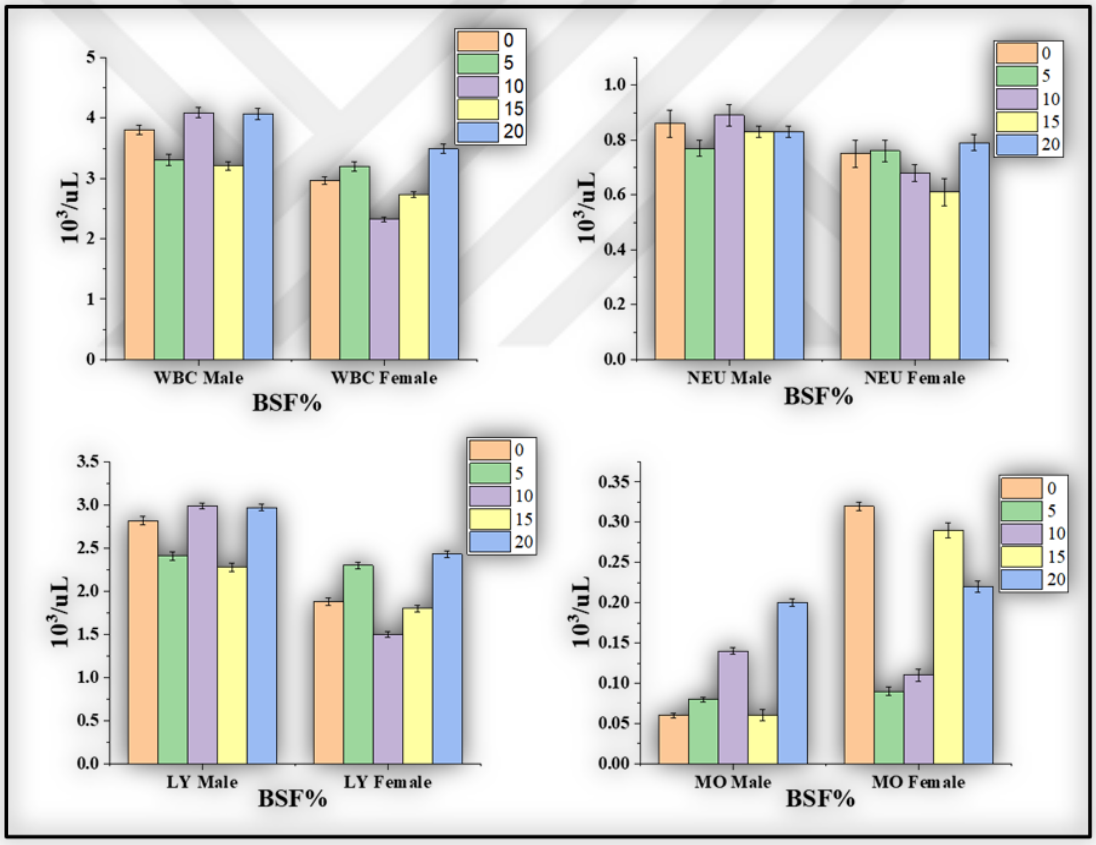

Figure 3.3: Effect of BSF larvae inclusions on WBC, NEU, MO, and LY.

3.3.2. BA, EO, RBC and HGB (Gender specific)

The statistical analysis of BA shows that its level of it was statistically non-significant in all groups ($p > 0.05$). The statistical analysis of EO shows that the level of it was statistically non-significant in male and female rats ($p > 0.05$). Similarly, the statistical analysis of RBC shows that the level of it was statistically non-significant in male and female rats ($p > 0.05$). Likewise, the statistical analysis of HGB shows that its level of it was statistically non-significant in male and female rats ($p > 0.05$). The values for male rats is shown in Table 3.4 and for female rats it is shown in Table 3.5 and Figure 3.4.

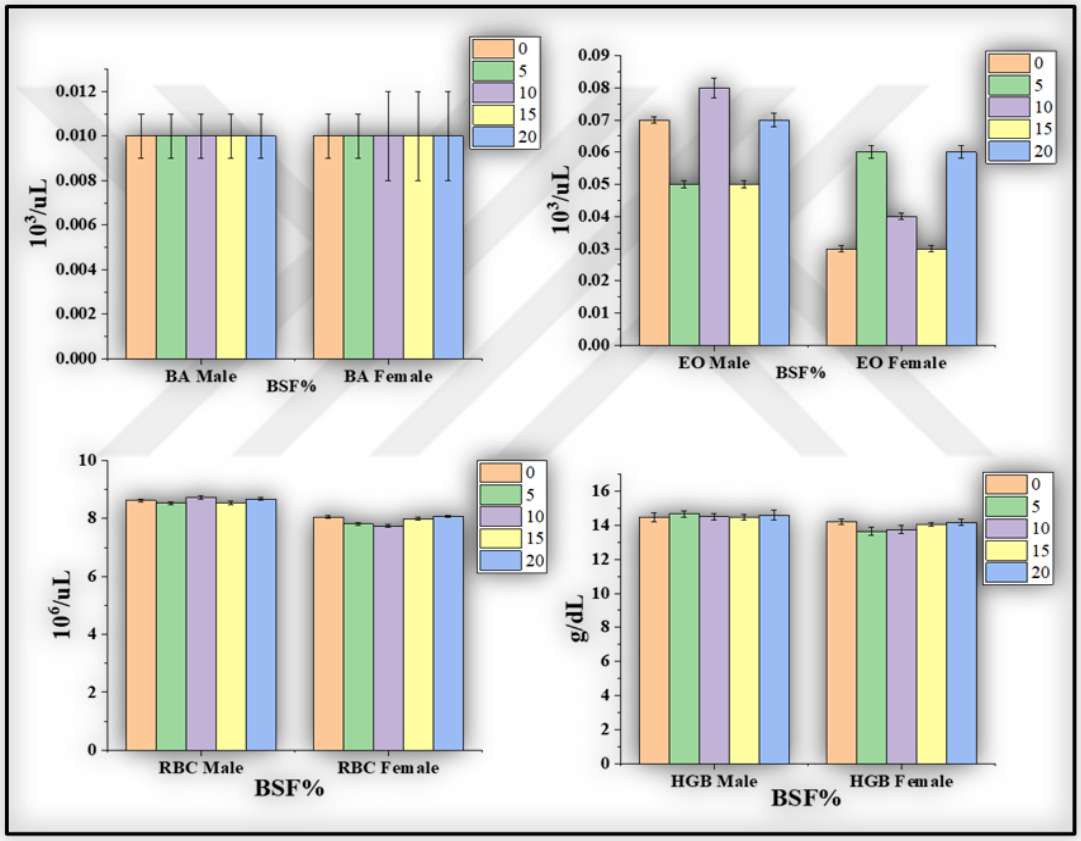

Figure 3.4: Effect of BSF larvae inclusions on BA, EO, HGB, and RBC.

3.3.3. HCT, MCV, MCH, MCHC (Gender specific)

The statistical analysis of HCT shows that it was statistically non-significant in all groups of male and female rats ($p > 0.05$). The statistical analysis of MCV shows that it significantly increased in the 5% group compared to all other groups in male rats ($p < 0.05$). However, MCV in female rats was statistically non-significant ($p > 0.05$). The statistical analysis of MCH shows that the level of it was statistically non-significant in male and female rats ($p > 0.05$). The statistical analysis of MCHC shows that it was statistically significant in male rats in all treatment groups compared to the contorl ($p < 0.05$) and it was statistically non-significant in female rats ($p > 0.05$). The values for male rats is shown in Table 3.4 and for female rats it is shown in Table 3.5 and Figure 3.5.

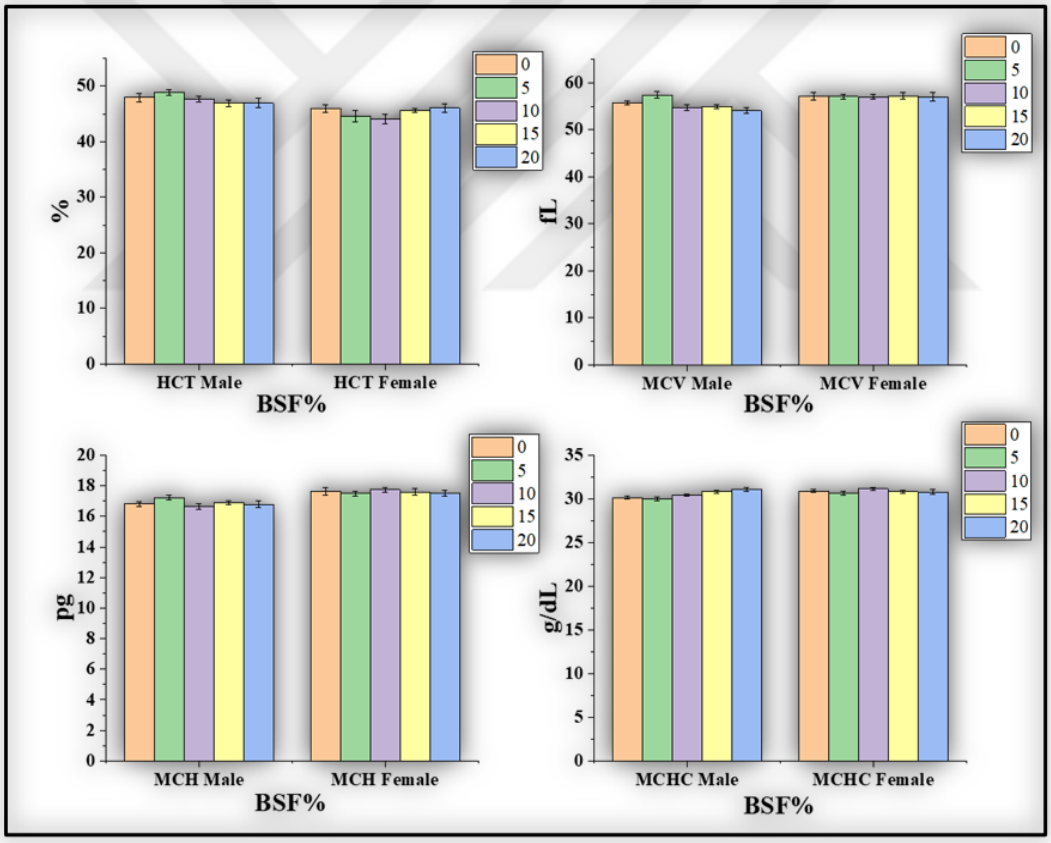

Figure 3.5: Effect of BSF larvae inclusions on HCT, MCV, MCH and MCHC

3.3.4. PLT, PDW, MPV, P-LCR (Gender specific)

The statistical analysis of the rats for PLT indicates that it was statistically significant in male rats ($p < 0.05$) as cubic effect, and it was non-significant for female rats ($p > 0.05$). The statistical analysis shows that PDW was statistically non-significant in male rats ($p > 0.05$) while it was linearly increased in female rats ($p < 0.05$) and maximum of the increase was seen in 20% group. MPV and P-LCR were statistically non-significant in male and female rats ($p > 0.05$). The values for male rats is shown in Table 3.4 and for female rats it is shown in Table 3.5 and Figure 3.6.

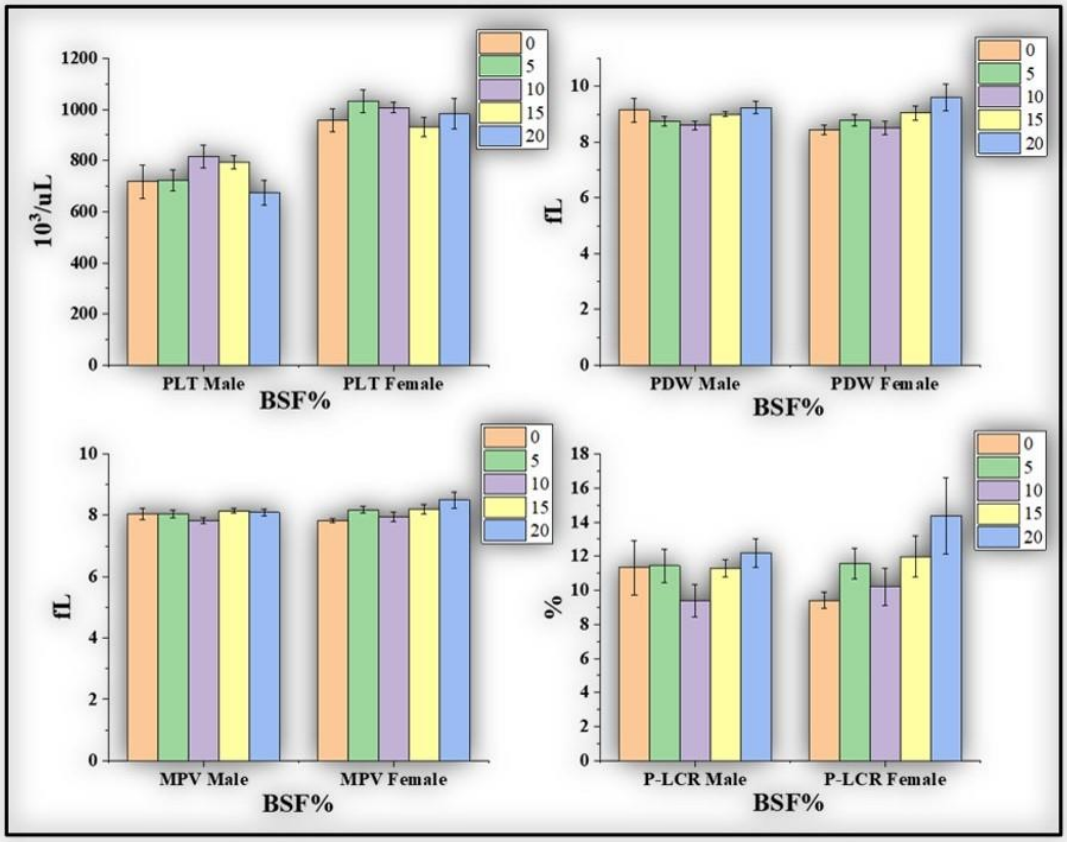

Figure 3.6: Effect of BSF larvae inclusions on PLT, PDW, MPV, and P-LCR.

3.3.5. PCT, RDW-CV, RDW-SD (Gender specific)

The statistical analysis of PCT showed that it was statistically non-significant in male rats ($p > 0.05$), while the level of PCT in female rats increased significantly in the 5% and 20% groups compared to the control as cubic effect ($p < 0.05$). The statistical analysis of RDW-CV showed that it was statistically significant in male rats ($p < 0.05$) compared to the control while in female rats it was non-significant ($p > 0.05$). The statistical analysis of RDW-SD showed that it was non-significant in male and female rats for all groups ($p > 0.05$). The values for male rats is shown in Table 3.4 and for female rats it is shown in Table 3.5 and Figure 3.7.

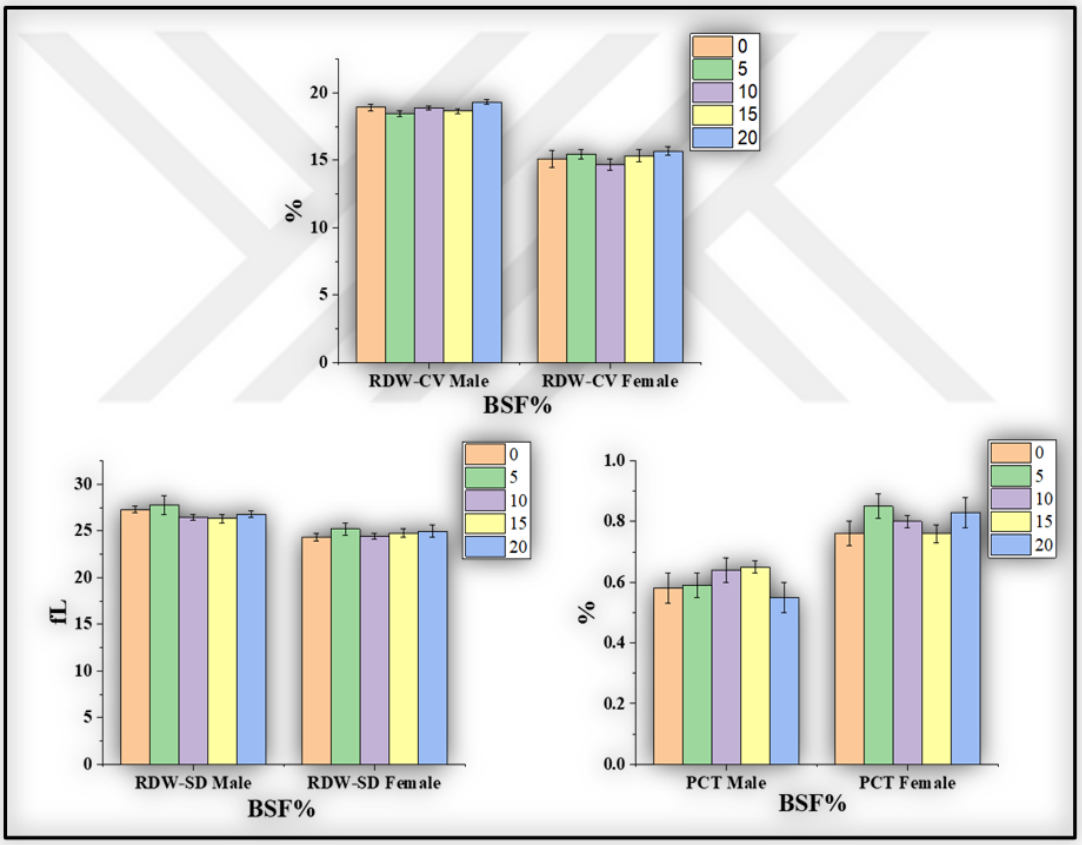

Figure 3.7: Effect of BSF larvae inclusions on PCT, RDW-CV, RDW-SD.

Table 3.4: Male haematological parameters.

Parameters	BSF 0%	BSF 5%	BSF 10%	BSF 15%	BSF 20%	Analysis	*p*	*Linear*	*Quadratic*	*Cubic*	Posthoc
WBC	3.8 ± 0.37	3.31 ± 0.42	4.09 ± 0.45	3.21 ± 0.4	4.07 ± 0.62	KWH	*0.57*	-	-	-	-
NEU	0.86 ± 0.08	0.77 ± 0.11	0.89 ± 0.08	0.83 ± 0.09	0.83 ± 0.2	KWH	*0.659*	-	-	-	-
LY	2.82 ± 0.3	2.41 ± 0.36	2.99 ± 0.34	2.28 ± 0.32	2.97 ± 0.44	KWH	*0.4*	-	-	-	-
MO	0.06 ± 0.02	0.08 ± 0.02	0.14 ± 0.08	0.06 ± 0.02	0.08 ± 0.14	KWH	*0.577*	-	-	-	-
BA	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	KWH	*0.983*	-	-	-	-
EO	0.07 ± 0.02	0.05 ± 0.01	0.08 ± 0.03	0.05 ± 0.01	0.07 ± 0.02	KWH	*0.907*	-	-	-	-
RBC	8.62 ± 0.16	8.53 ± 0.16	8.72 ± 0.06	8.54 ± 0.1	8.68 ± 0.1	ANOVA	*0.755*	*0.710*	*0.839*	*0.924*	Tamhane
HGB	14.47 ± 0.26	14.67 ± 0.19	14.5 ± 0.17	14.45 ± 0.15	14.59 ± 0.29	ANOVA	*0.947*	*0.956*	*0.988*	*0.420*	Duncan
HCT	47.95 ± 0.8	48.84 ± 0.5	47.67 ± 0.56	46.92 ± 0.56	46.92 ± 0.87	ANOVA	*0.243*	*0.068*	*0.595*	*0.194*	Duncan
MCV	55.68 ± 0.36^{ab}	57.38 ± 0.73^{b}	54.73 ± 0.53^{ab}	54.94 ± 0.42^{ab}	54.05 ± 0.57^{a}	ANOVA	***0.001***	***0.002***	*0.254*	*0.062*	Duncan
MCH	16.82 ± 0.15	17.22 ± 0.16	16.65 ± 0.17	16.9 ± 0.1	16.8 ± 0.22	ANOVA	*0.170*	*0.509*	*0.756*	*0.234*	Duncan
MCHC	30.15 ± 0.19^{ab}	30 ± 0.17^{a}	30.43 ± 0.11^{ab}	30.83 ± 0.18^{ab}	31.12 ± 0.23^{b}	KWH	***0.002***	-	-	-	-
PLT	779.88 ± 13.82^{ab}	722 ± 41.6^{ab}	816.63 ± 44.19^{b}	793.13 ± 26.9^{b}	673.51 ± 49.55^{a}	ANOVA	*0.211*	*0.241*	*0.094*	***0.044***	Duncan
PDW	9.14 ± 0.43	8.75 ± 0.17	8.6 ± 0.16	9 ± 0.1	9.24 ± 0.21	ANOVA	*0.308*	*0.553*	*0.051*	*0.598*	Tamhane
MPV	8.04 ± 0.18	8.05 ± 0.12	7.83 ± 0.1	8.15 ± 0.07	8.09 ± 0.11	KWH	*0.242*				-
P-LCR	11.34 ± 1.59	11.44 ± 0.99	9.4 ± 0.96	11.3 ± 0.5	12.17 ± 0.84	ANOVA	*0.376*	*0.646*	*0.166*	*0.738*	Duncan
PCT	0.58 ± 0.05	0.59 ± 0.04	0.64 ± 0.04	0.65 ± 0.02	0.55 ± 0.05	KWH	*0.19*	-	-	-	-
RDW-CV	18.92 ± 0.25^{ab}	18.45 ± 0.21^{a}	18.87 ± 0.17^{ab}	18.65 ± 0.17^{ab}	19.3 ± 0.18^{b}	KWH	***0.048***	-	-	-	-
RDW-SD	27.27 ± 0.35	27.74 ± 1	26.44 ± 0.32	26.33 ± 0.46	26.79 ± 0.33	KWH	*0.273*	-	-	-	-
IgG	0.009 ± 0.0048	0.003 ± 0.0016	0.005 ± 0.0027	0.009 ± 0.004	0.005 ± 0.0027	KWH	*0.746*	-	-	-	-

[*a, b] Values with different superscripts in the same row are significantly different ($p \leq 0.05$) and ($p \leq 0.01$).*White blood cells (WBC), neutrophils (NEU), lymphocytes (LY), monocytes (MO), basophils (BA), eosinophils (EO), red blood cells (RBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelets (PLT), platelets distribution width (PDW), mean platelet volume (MPV), platelet- large cell ratio (P-LCR), procalcitonin test (PCT), red cell distribution width- coefficient of variance (RDW-CV), red cell distribution width- standard deviation (RDW-SD), and nucleated red blood cells (n-RBC). *Data is represented as least squares. The values are means of 8 replicate cages per diet with 2 rats (n= 16). *Black Soldier Fly 0% (Control), BSF 5%, 10%, 15% and 20% are the treatment groups.

Table 3.5: Female haematological parameters.

Parameters	BSF 0%	BSF 5%	BSF 10%	BSF 15%	BSF 20%	Test	*p*	*Linear*	*Quadratic*	*Cubic*	Posthoc
WBC	2.97 ± 0.52	3.2 ± 0.43	2.32 ± 0.34	2.73 ± 0.34	3.49 ± 0.34	KWH	*0.067*	-	-	-	-
NEU	0.75 ± 0.08	0.76 ± 0.09	0.68 ± 0.1	0.61 ± 0.07	0.79 ± 0.13	ANOVA	*0.671*	*0.827*	*0.332*	*0.260*	Duncan
LY	1.88 ± 0.31^{ab}	2.31 ± 0.33^{b}	1.51 ± 0.27^{a}	1.81 ± 0.21^{ab}	2.43 ± 0.24^{b}	KWH	***0.046***	-	-	-	-
MO	0.32 ± 0.25	0.09 ± 0.02	0.11 ± 0.03	0.29 ± 0.17	0.22 ± 0.06	KWH	*0.198*	-	-	-	-
BA	0.01 ± 0.01	0.01 ± 0.01	0 ± 0	0.01 ± 0.01	0.01 ± 0.01	KWH	*0.421*	-	-	-	-
EO	0.03 ± 0.01	0.06 ± 0.02	0.04 ± 0.01	0.03 ± 0.01	0.06 ± 0.02	KWH	*0.062*	-	-	-	-
RBC	8.05 ± 0.16	7.81 ± 0.15	7.74 ± 0.12	7.99 ± 0.1	8.08 ± 0.13	KWH	*0.382*	-	-	-	-
HGB	14.19 ± 0.17	13.64 ± 0.23	13.75 ± 0.26	14.05 ± 0.1	14.17 ± 0.19	KWH	*0.375*	-	-	-	-
HCT	45.94 ± 0.66	44.57 ± 1.04	44.13 ± 0.84	45.6 ± 0.36	46.04 ± 0.71	KWH	*0.296*	-	-	-	-
MCV	57.18 ± 0.82	57.07 ± 0.55	57 ± 0.51	57.18 ± 0.72	57.03 ± 0.83	KWH	*0.948*	-	-	-	-
MCH	17.65 ± 0.26	17.49 ± 0.13	17.75 ± 0.17	17.6 ± 0.21	17.53 ± 0.17	KWH	*0.629*	-	-	-	-
MCHC	30.9 ± 0.15	30.65 ± 0.26	31.17 ± 0.14	30.82 ± 0.16	30.79 ± 0.29	KWH	*0.66*	-	-	-	-
PLT	957.75 ± 44.43	1032 ± 43.56	1006.75 ± 20.52	931.13 ± 36.88	983 ± 58.81	ANOVA	*0.496*	*0.711*	*0.555*	*0.102*	Duncan
PDW	8.44 ± 0.17^{a}	8.78 ± 0.21^{ab}	8.52 ± 0.24^{ab}	9.04 ± 0.25^{ab}	9.6 ± 0.48^{b}	ANOVA	***0.046***	***0.007***	*0.256*	*0.486*	Duncan
MPV	7.83 ± 0.07	8.18 ± 0.11	7.95 ± 0.15	8.19 ± 0.16	8.5 ± 0.27	KWH	*0.104*	-	-	-	-
P-LCR	9.4 ± 0.48	11.58 ± 0.9	10.2 ± 1.08	11.98 ± 1.19	14.38 ± 2.25	KWH	*0.198*	-	-	-	-
PCT	0.76 ± 0.04^{a}	0.85 ± 0.04^{b}	0.8 ± 0.02^{ab}	0.78 ± 0.03^{ab}	0.83 ± 0.05^{b}	ANOVA	***0.036***	*0.518*	*0.763*	***0.036***	Duncan
RDW-CV	15.1 ± 0.64	15.43 ± 0.35	14.68 ± 0.42	15.32 ± 0.47	15.67 ± 0.31	ANOVA	*0.604*	*0.481*	*0.398*	*0.583*	Duncan
RDW-SD	24.33 ± 0.38	25.19 ± 0.66	24.42 ± 0.29	24.77 ± 0.49	24.95 ± 0.65	KWH	*0.759*	-	-	-	-
IgG	0.0038 ± 0.002	0.0038 ± 0.002	0.0025 ± 0.002	0.0025 ± 0.002	0.0038 ± 0.003	KWH	*0.967*	-	-	-	-

[*a, b] Values with different superscripts in the same row are significantly different ($p \leq 0.05$) and ($p \leq 0.01$). *White blood cells (WBC), neutrophils (NEU), lymphocytes (LY), onocytes (MO), basophils (BA), eosinophils (EO), red blood cells (RBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelets (PLT), platelets distribution width (PDW), mean platelet volume (MPV), platelet- large cell ratio (P-LCR), procalcitonin test (PCT), red cell distribution width- coefficient of variance (RDW-CV), red cell distribution width- standard deviation (RDW-SD), and nucleated red blood cells (n-RBC). *Data is represented as least squares. The values are means of 8 replicate cages per diet with 2 rats (n= 16). *Black Soldier Fly 0% (Control), BSF 5%, 10%, 15% and 20% are the treatment groups.

3.4. Serum biochemical parameters (Combined effect)

All the biochemical parameters in serum were analysed for males and females combined and gender-specific way. However, in this section, the combined effect is explained. The statistical analysis of creatinine, total protein, uric acid, ALT, AST, ALP, calcium, phosphorous, cholesterol total, cholesterol LDL, cholesterol HDL, non-HDL and IgG was performed. The statistical analysis of creatinine, total protein, AST, calcium, phosphorous, non-HDL, IgG showed that all of these parameters remained non-significant for all experimental groups ($p > 0.05$). The statistical analysis of uric acid showed that it was very significantly decreased in treatment groups compared to the control ($p < 0.05$) and maximum of the drop in uric acid was found in 20% group following 15%, 10%, and 5% respectively. The statistical analysis of ALT showed that it was also significantly increased in treatment groups compared to the control and maximum of the increase was observed in 10% BSF group followed by 20% group ($p < 0.05$). However, AST (SGOT) was statistically non-significant in all groups ($p > 0.05$). In this study, it was seen that ALP was significantly increased in 5, 10, 15, and 20% groups compared to the control as linear effect ($p < 0.05$). The statistical analysis of total cholesterol indicated that it increased significantly in the 5, 10, 15, and 20% groups compared to the control and maximum of increase was seen in 20% group ($p < 0.05$). The study's statistical analysis showed that the LDL levels increased significantly compared to the control as linear effect and maximum of it was increased in 20% group compared to the control ($p < 0.05$) but the values were within normal range. The statistical analysis of HDL showed that it was also significantly increased for the 10, 15, and 20% groups compared to the control and maximum of the increase in HDL was seen in 15% group followed by 20% group. The levels of all biochemical parameters in serum are shown in Table 3.6.

Table 3.6: Biochemical serum parameters for all groups, male and female combined.

Parameters	BSF 0%	BSF 5%	BSF 10%	BSF 15%	BSF 20%	Test	*p*	*Linear*	*Quadratic*	*Cubic*
Creatinine	0.38 ± 0.03	0.33 ± 0.03	0.41 ± 0.03	0.38 ± 0.03	0.37 ± 0.03	KWH	*0.405*	-	-	-
Total Protein	6.73 ± 0.21	6.21 ± 0.48	6.86 ± 0.24	6.72 ± 0.21	6.96 ± 0.26	KWH	*0.542*	-	-	-
Uric Acid	1.2 ± 0.06^{c}	1.01 ± 0.1^{bc}	0.9 ± 0.1^{b}	0.82 ± 0.1^{ab}	0.72 ± 0.06^{a}	KWH	***<0.001***	-	-	-
ALT (SGPT)	24.8 ± 6.9^{a}	38.5 ± 4.0^{ab}	70.07 ± 20^{b}	35.44 ± 3.9^{ab}	55.8 ± 6.0^{b}	KWH	***0.003***	-	-	-
AST (SGOT)	109.0 ± 17	80.01 ± 8.55	128.32 ± 28	82.5 ± 11.3	93.3 ± 11.2	KWH	*0.72*	-	-	-
ALP	77.9 ± 6.8^{a}	99 ± 16.5^{ab}	113 ± 12^{b}	123.5 ± 12^{bc}	141.4 ± 19^{b}	ANOVA	***0.03***	***0.001***	*0.84*	*0.73*
Calcium	10.26 ± 0.1	9.74 ± 0.66	10.35 ± 0.1	10.35 ± 0.11	10.4 ± 0.17	KWH	*0.407*	-	-	-
Phosphorous	3.78 ± 0.21	3.77 ± 0.31	3.6 ± 0.15	3.87 ± 0.19	4.08 ± 0.15	KWH	*0.308*	-	-	-
Cholesterol Total	59.8 ± 3.1^{a}	62.7 ± 6.4^{ab}	70.2 ± 4^{b}	77.52 ± 6.1^{b}	75.73 ± 3.7^{b}	KWH	***0.007***	-	-	-
LDL	7.2 ± 1.04^{a}	8.98 ± 1.4^{ab}	10.5 ± 1^{ab}	11.9 ± 1.18^{b}	12.73 ± 1.3^{b}	ANOVA	***0.019***	***0.001***	*0.676*	*0.932*
HDL	45.8 ± 2.5^{a}	45.79 ± 4.8^{ab}	53.4 ± 3^{b}	59.51 ± 4.6^{b}	56.6 ± 3.4^{b}	KWH	***0.021***	-	-	-
Non-HDL	14.0 ± 1.45	17 ± 2.24	16.81 ± 1.5	18.01 ± 1.95	19.08 ± 1.77	KWH	*0.101*	-	-	-
IgG	299.3 ± 11	257.19 ± 20	290.32 ± 8	290.19 ± 12	292.94 ± 1	KWH	*0.587*	-	-	-

*[a, b, c] Values with different superscripts in the same row are significantly different ($p \leq 0.05$) and ($p \leq 0.01$). *Alanine aminotransferase (ALT), Aspartate aminotransferase AST), Alkaline phosphatase (ALP), High-density lipoprotein (HDL), Low-density lipoprotein (LDL), immunoglobulin-G (IgG) *Data is represented as least squares. The values are means of 8 replicate cages per diet with 2 rats (n= 16). *Black Soldier Fly 0% (Control), BSF 5%, 10%, 15% and 20% are the treatment groups. *The unit of measurement for creatinine, uric acid, calcium, phosphorous, IgG, LDL, HDL, total cholesterol, and non-HDL is mg/dl and for total protein is g/dl, for AST, ALP, ALT is IU/L.

3.4.1. Creatinine, uric acid, total protein and ALT (Gender specific)

The statistical analysis of the creatinine showed that creatinine level was non-significant for all groups ($p > 0.05$) in male rats, while there was a significant cubic effect in female rats ($p < 0.05$). In female rats, the creatinine level significantly decreased in the 5% group compared to the control group. Protein total was non-significant for male rats ($p > 0.05$) while it was significantly changed for female rats as linear effect ($p < 0.05$). Uric acid was statistically significant in both male and female rats compared to the control group ($p < 0.05$). The statistical analysis indicated that ALT was non-significant for male rats ($p > 0.05$) while in female rats, there was significant increase in ALT compared to control in all treatment groups as linear and cubic effect ($p < 0.05$). The values for male rats are shown in Table 3.7 and for female rats Table 3.8 and Figure 3.8.

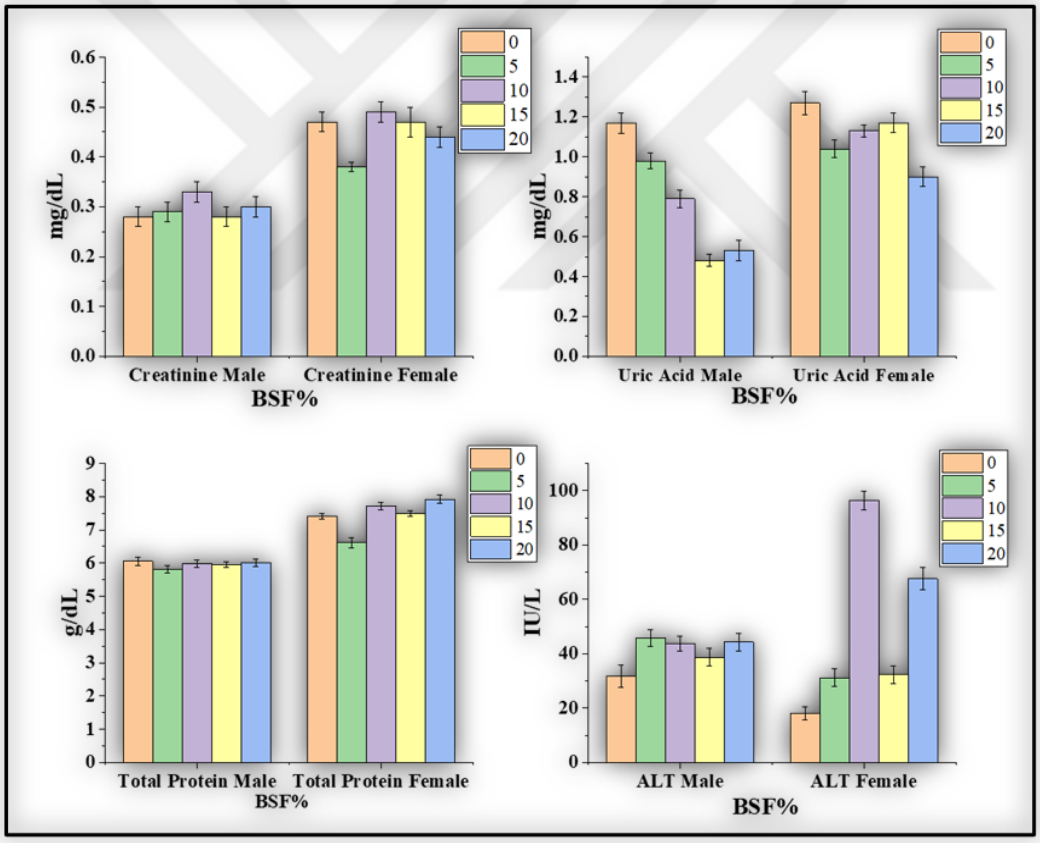

Figure 3.8: Effect of BSF larvae meal on creatinine, uric acid, total protein and ALT.

3.4.2. AST, ALP, calcium and phosphorous (Gender specific)

The levels of AST in male and female rats were non-significant ($p > 0.05$). In this study, it was seen that ALP was significantly increased in male rats in 5, 10, 15, and 20% groups compared to the control as linear and cubic effect ($p < 0.05$) and maximum of the increase was observed in 20% group compared to the control. However, there was no significant effect on female rat's ALP levels ($p > 0.05$). The calcium and phosphorous were non-significant for male rats ($p > 0.05$) and they were significant in female rats ($p < 0.05$). The values for male rats are shown in Table 3.7 and for female rats Table 3.8 and Figure 3.9.

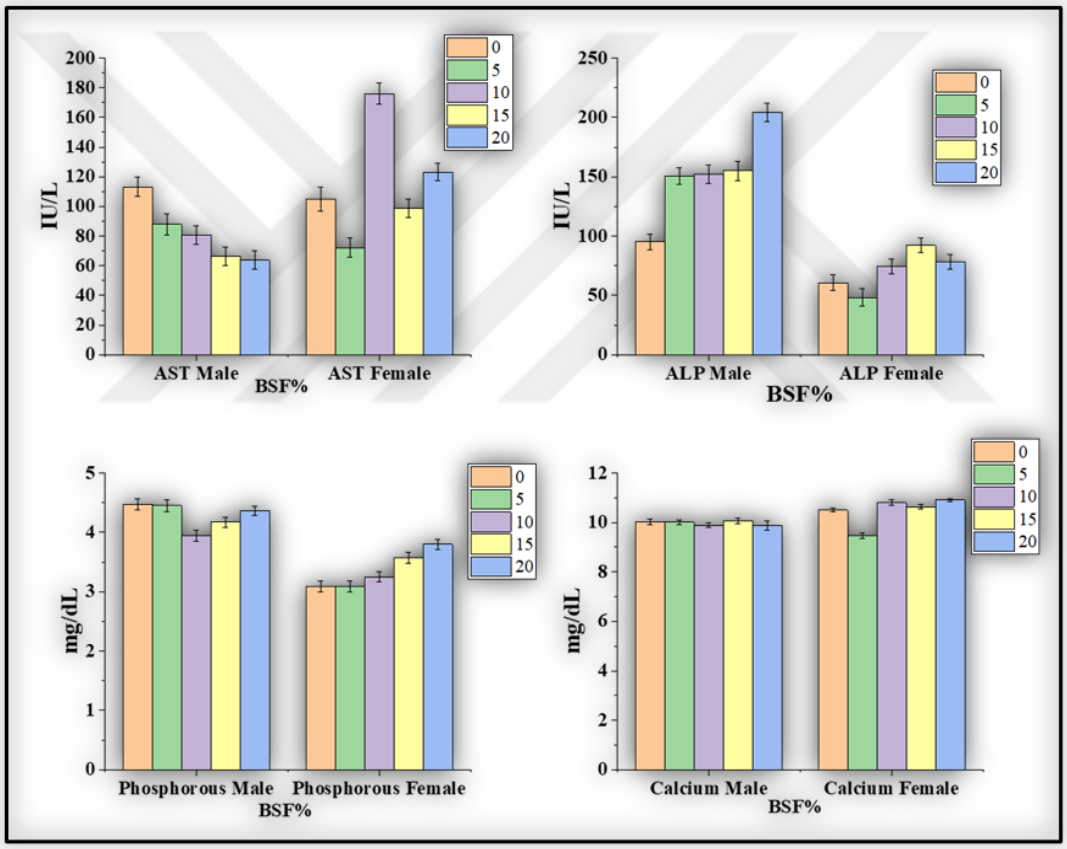

Figure 3.9: Effect of BSF larvae meal on AST, ALP, phosphorous and calcium

3.4.3. Cholesterol total, LDL, HDL and non-HDL (Gender specific)

The statistical analysis of total cholesterol indicates that it increased significantly in 5, 10, 15, and 20% groups in male rats compared to the control as a linear effect ($p < 0.05$) and it was non-significant in female rats ($p > 0.05$). The LDL was increased significantly in male rats linearly compared to the control ($p < 0.05$) and it was also non-significant in female rats ($p > 0.05$). HDL cholesterol was statistically non-significant for male and female rats ($p > 0.05$). The level of non-HDL was increased significantly in male rats as linear and cubic effect ($p < 0.05$). However, the level of non-HDL in female rats was non-significant for all experimental groups ($p > 0.05$) and it was significantly increased in all treatment groups compared to the control as linear and cubic effect ($p < 0.05$) and maximum of the increase was seen in 20% group followed by 5, 10 and 15% groups respectively. The values for male and female rats are shown in Table 3.7 and for female rats Table 3.8 and Figure 3.10.

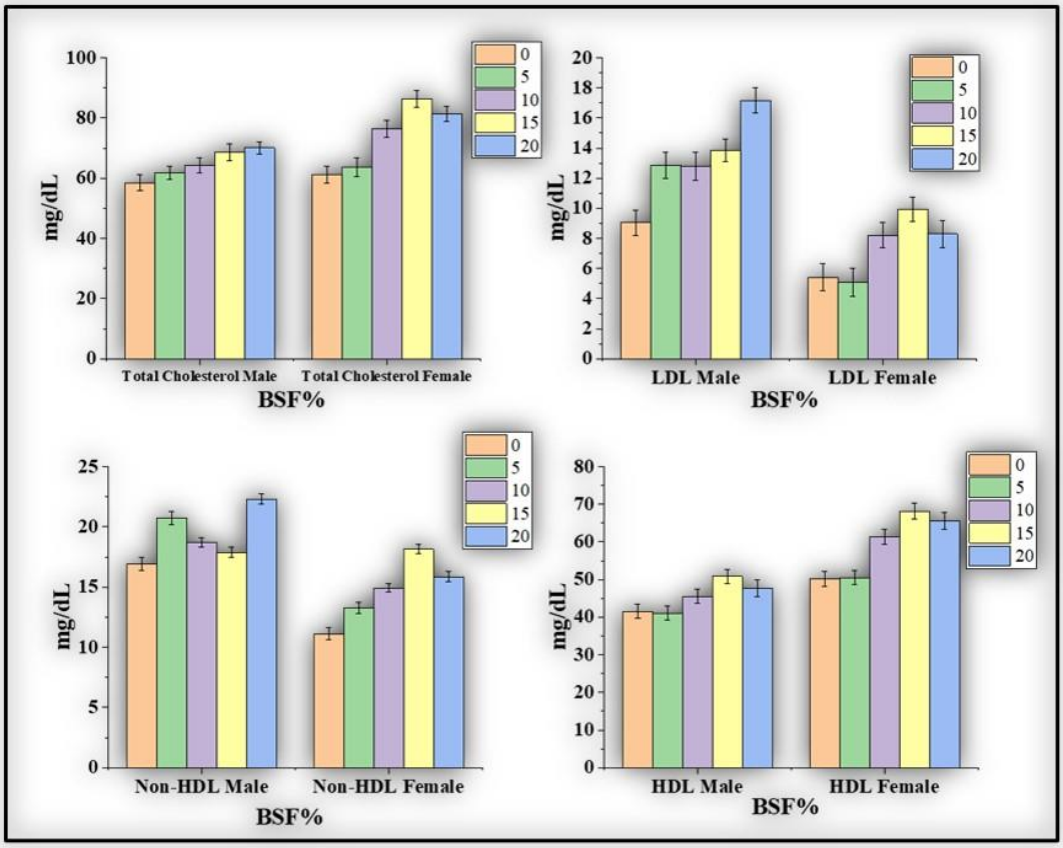

Figure 3.10: Effect of BSF larvae meal total cholesterol, LDL, HDL, and non-HDL

3.4.4. IgG (Gender specific)

Immunoglobulin-G (IgG) level in blood represents the presence of infections. The statistical analysis shows that it was non-significant for all experimental groups ($p > 0.05$) both in male and female rats. The values for male rats are shown in Table 3.7 and for female rats Table 3.8 and Figure 3.11.

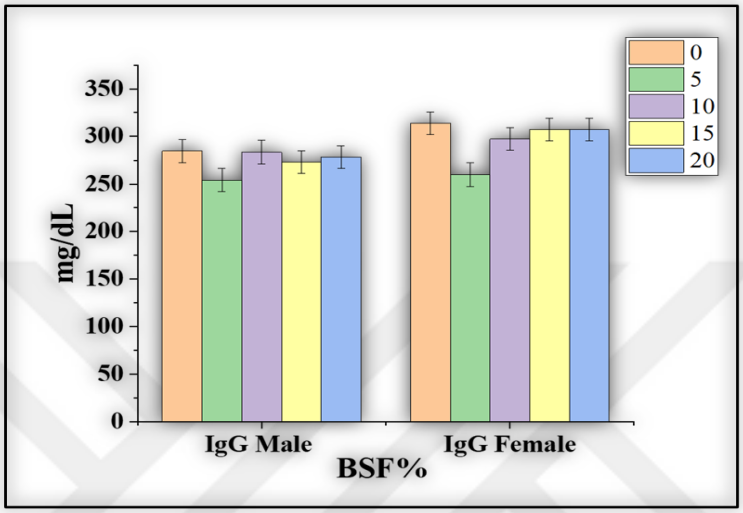

Figure 3.11: Effect of BSF larvae meal on IgG

Table 3.7: Male biochemical parameters

Parameters	Control	5%	10%	15%	20%	Test	*p*	*Linear*	*Quadratic*	*Cubic*	Posthoc
Creatinine	0.28 ± 0.02	0.29 ± 0.02	0.33 ± 0.02	0.28 ± 0.02	0.3 ± 0.02	KWH	*0.149*	-	-	-	-
Protein Total	6.05 ± 0.13	5.81 ± 0.11	5.99 ± 0.11	5.95 ± 0.08	6.01 ± 0.12	KWH	*0.339*	-	-	-	-
Uric Acid	1.17 ± 0.1[b]	0.98 ± 0.15[b]	0.79 ± 0.09[ab]	0.48 ± 0.02[a]	0.53 ± 0.05[ab]	KWH	< ***0.01***	-	-	-	-
ALT	31.75 ± 8.23	45.88 ± 4.5	43.75 ± 3.57	38.63 ± 7.19	44.25 ± 7.36	KWH	*0.589*	-	-	-	-
AST	113.25 ± 32.36	87.88 ± 12.64	80.75 ± 8.9	66.38 ± 11.47	63.63 ± 6.86	KWH	*0.728*	-	-	-	-
ALP	95.13 ± 7.16[a]	150.63 ± 18.5[b]	152.13 ± 13.8[b]	154.88 ± 9.96[b]	204.38 ± 18.91[c]	ANOVA	< ***0.01***	***< 0.01***	*0.843*	***0.034***	Duncan
Calcium	8.9 ± 1.04	10.01 ± 0.09	9.89 ± 0.08	10.06 ± 0.11	9.89 ± 0.19	KWH	*0.596*		-	-	-
Phosphorous	4.47 ± 0.15	4.45 ± 0.19	3.94 ± 0.2	4.17 ± 0.22	4.36 ± 0.22	ANOVA	*0.270*	*0.410*	*0.119*	*0.446*	Duncan
Cholesterol Total	58.42 ± 2.68[a]	61.8 ± 4.21[ab]	64.22 ± 5.21[ab]	68.7 ± 4.25[b]	70.04 ± 3.38[b]	ANOVA	*0.247*	***0.024***	*0.894*	*0.866*	Duncan
Cholesterol LDL	9.04 ± 1.12[a]	12.88 ± 1.63[ab]	12.8 ± 1.42[ab]	13.85 ± 0.85[bc]	17.17 ± 1.34[c]	ANOVA	***0.003***	***< 0.01***	*0.988*	*0.140*	Duncan
Cholesterol HDL	41.5 ± 2.38	41.08 ± 2.87	45.53 ± 5.04	50.83 ± 4.12	47.74 ± 3.14	ANOVA	*0.288*	*0.061*	*0.744*	*0.256*	Duncan
Non-HDL	16.92 ± 1.42[a]	20.73 ± 1.5[b]	18.69 ± 0.88[ab]	17.88 ± 0.69[ab]	22.3 ± 1.55[b]	ANOVA	***0.030***	*0.049*	*0.605*	***< 0.01***	Duncan
IgG	284.63 ± 18.21	254.25 ± 18.36	283.5 ± 13.71	273.25 ± 16.26	278.5 ± 21.47	ANOVA	*0.748*	*0.905*	*0.636*	*0.438*	Duncan

*[a, b, c] Values with different superscripts in the same row are significantly different ($p \leq 0.05$) and ($p \leq 0.01$). *Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Alkaline phosphatase (ALP), High-density lipoprotein (HDL), Low-density lipoprotein (LDL), immunoglobulin-G (IgG) *Data is represented as least squares. The values are means of 8 replicate cages per diet with 2 rats (n= 16). *Black Soldier Fly 0% (Control), BSF 5%, 10%, 15% and 20% are the treatment groups. *The unit of measurement for creatinine, uric acid, calcium, phosphorous, IgG, LDL, HDL, total cholesterol, and non-HDL is mg/dl and for total protein is g/dl, for AST, ALP, ALT is IU/L.

Table 3.8: Female biochemical parameters

Parameters	Control	5%	10%	15%	20%	Test	*p*	*Linear*	*Quadratic*	*Cubic*	Posthoc
Creatinine	0.47 ± 0.02^{ab}	0.38 ± 0.06^{a}	0.49 ± 0.02^{ab}	0.47 ± 0.03^{ab}	0.44 ± 0.02^{ab}	ANOVA	*0.066*	*0.612*	*0.367*	***0.037***	Duncan
Protein Total	7.41 ± 0.09^{ab}	6.61 ± 0.96^{a}	7.72 ± 0.12^{b}	7.49 ± 0.08^{ab}	7.92 ± 0.12^{b}	ANOVA	*0.288*	***0.008***	*0.662*	*0.067*	Duncan
Uric Acid	1.27 ± 0.06^{b}	1.04 ± 0.18^{ab}	1.13 ± 0.1^{ab}	1.17 ± 0.11^{b}	0.9 ± 0.05^{a}	KWH	***0.023***	*0.08*	*0.752*	*0.074*	-
ALT	33.33 ± 6.17^{ab}	35.57 ± 4.56^{ab}	53.63 ± 14.39^{b}	32.25 ± 3.53^{a}	67.5 ± 7.85^{b}	ANOVA	***0.044***	***0.001***	*0.204*	***0.02***	Duncan
AST	104.88 ± 14.02	72.13 ± 11.66	175.88 ± 52.9	98.75 ± 18.53	123 ± 15.74	KWH	*0.226*	*0.471*	*0.517*	*0.686*	-
ALP	60.75 ± 7.91	48.38 ± 8.91	74.38 ± 8.87	92.13 ± 17.82	78.5 ± 10.83	ANOVA	*0.094*	*0.054*	*0.652*	*0.132*	Duncan
Calcium	10.5 ± 0.08^{a}	10.8 ± 0.09^{b}	10.8 ± 0.12^{b}	10.64 ± 0.1^{ab}	10.91 ± 0.06^{b}	ANOVA	*0.469*	***0.026***	*0.488*	***0.014***	Duncan
Phosphorous	3.09 ± 0.16^{a}	3.10 ± 0.49^{ab}	3.25 ± 0.16^{ab}	3.57 ± 0.29^{b}	3.8 ± 0.15^{b}	ANOVA	*0.298*	***0.025***	*0.775*	*0.350*	Duncan
Cholesterol Total	61.23 ± 5.82	63.75 ± 12.73	76.35 ± 6.27	86.33 ± 11.13	81.42 ± 6.2	KWH	*0.057*	-	-	-	-
Cholesterol LDL	5.42 ± 1.56	5.08 ± 1.51	8.2 ± 1.45	9.94 ± 2.04	8.29 ± 0.9	KWH	*0.069*	-	-	-	-
Cholesterol HDL	50.1 ± 3.99	50.49 ± 9.22	61.43 ± 3.81	68.19 ± 7.36	65.57 ± 4.35	KWH	*0.068*	-	-	-	-
Non-HDL	11.13 ± 2.15	13.27 ± 3.91	14.93 ± 2.9	18.14 ± 3.98	15.85 ± 2.83	KWH	*0.367*	-	-	-	-
IgG	314 ± 13.26	260.13 ± 38.13	297.13 ± 11.28	307.13 ± 19.25	307.38 ± 11.66	KWH	*0.791*	-	-	-	-

*$^{a, b, c}$ Values with different superscripts in the same row are significantly different ($p \leq 0.05$) and ($p \leq 0.01$). *Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Alkaline phosphatase (ALP), High-density lipoprotein (HDL), Low-density lipoprotein (LDL), immunoglobulin-G (IgG) *Data is represented as least squares. The values are means of 8 replicate cages per diet with 2 rats (n= 16). *Black Soldier Fly 0% (Control), BSF 5%, 10%, 15% and 20% are the treatment groups. *The unit of measurement for creatinine, uric acid, calcium, phosphorous, IgG, LDL, HDL, total cholesterol, and non-HDL is mg/dl and for total protein is g/dl, for AST, ALP, ALT is IU/L.

3.5. Oxidative stress parameters in serum (Combined effect)

The statistical analysis shows that TOS was statistically non-significant for all groups ($p > 0.05$). Moreover, the statistical analysis of TAS shows that it was also statistically non-significant for all groups ($p > 0.05$). The statistical analysis shows that OSI was also statistically non-significant for all groups compared to the control ($p > 0.05$). MDA, GSH, and NOx was also measured in the serum. The statistical analysis shows that these parameters were also statistically non-significant for all groups compared to the control ($p > 0.05$). The levels of all parameters related to oxidative stress in serum Table 3.9.

Table 3.9: MDA, GSH, TOS, TAS and OSI in serum as combined effect in all rats.

BSF Inclusion	MDA (nmol/L)	GSH (mg/dL)	NOx (µmol/L)	TOS (µmol/L)	TAS (mmol/L)	OSI
0%	5.30 ± 0.25	8.55 ± 0.95	90.64 ± 8.85	25.85 ± 5.20	0.61 ± 0.10	810.06 ± 237.67
5%	5.09 ± 0.52	7.25 ± 0.93	105.71 ± 21.57	29.80 ± 5.81	0.70 ± 0.12	593.06 ± 184.51
10%	5.58 ± 0.34	7.99 ± 0.99	91.84 ± 8.32	30.28 ± 6.42	0.65 ± 0.09	791.98 ± 218.02
15%	5.37 ± 0.29	8.68 ± 0.90	96.87 ± 9.12	37.96 ± 7.05	0.78 ± 0.10	723.38 ± 165.07
20%	5.56 ± 0.37	8.19 ± 0.89	94.81 ± 11.72	32.36 ± 5.81	0.79 ± 0.14	1049.59 ± 308.11
p-Combined	*0.87*	*0.83*	*0.93*	*0.714*	*0.706*	*0.712*
Linear	*0.484*	*0.815*	*0.99*	*0.275*	*0.196*	*0.401*
Quadratic	*0.939*	*0.655*	*0.751*	*0.603*	*0.994*	*0.34*
Cubic	*0.808*	*0.276*	*0.594*	*0.611*	*0.934*	*0.977*
Posthoc	Tamhane	Tamhane	Duncan	Duncan	Duncan	Duncan

*Data is represented as least squares. The values are means of 8 replicate cages per diet with 2 rats (n= 16). *Black Soldier Fly 0% (Control), BSF 5%, 10%, 15% and 20% are the treatment groups. *MDA (malondialdehyde), GSH (Reduced glutathione), NOx (Nitric oxide), TOS (Total oxidative status), TAS (Total antioxidative status), OSI (Oxidative status index)

3.5.1. MDA and GSH in tissues (Combined effect)

The statistical analysis shows that MDA in the liver was significantly decreased in 5, 10, 15, and 20% groups compared to the control ($p < 0.05$) as linear effect and it was seen that maximum of the drop was seen in 20% group followed by 15, 10 and 5% groups respectively. Similarly, the statistical analysis shows that MDA in kidneys was significantly decreased in 5, 10, 15, and 20% groups compared to the control ($p < 0.05$) as linear effect and maximum drop was seen in 20% group followed by 10, 5 and 15% groups respectively. The statistical analysis shows that MDA in the heart was statistically non-significant for all groups compared to the control ($p > 0.05$).

The statistical analysis shows that the GSH in the liver increased significantly in all experimental groups compared to the control ($p < 0.05$). GSH in the liver was increased to the maximum in 15% group followed by 20, 10 and 5% groups respectively. The statistical analysis shows that the GSH in the kidney was increased significantly in all experimental groups compared to the control ($p < 0.05$). GSH in the kidney was increased to the maximum extent in 10% group followed by 20, 15 and 5% groups. The statistical analysis shows that the GSH in the heart was also non-significant for all groups compared to the control ($p > 0.05$). The levels of oxidative stress parameters in tissues are shown in Table 3.10

Table 3.10: The levels of MDA and GSH in liver, kidney, and heart as combined effect.

BSF Inclusion	MDA (Liver)	MDA (Kidney)	MDA (Heart)	GSH (Liver)	GSH (Kidney)	GSH (Heart)
0 %	240.02 ± 26.9^{b}	465.42 ± 46.41^{b}	169.23 ± 14.9	4.24 ± 0.14^{ab}	5.54 ± 0.77^{a}	4.65 ± 0.28
5 %	174.4 ± 17.91^{b}	363.63 ± 28.3^{ab}	164.9 ± 21.33	4.16 ± 0.35^{a}	5.75 ± 0.46^{ab}	4.35 ± 0.43
10 %	171.23 ± 11.0^{b}	350.95 ± 18.64^{a}	166.74 ± 16.2	4.99 ± 0.26^{ab}	7.23 ± 0.51^{b}	4.92 ± 0.46
15 %	153.58 ± 11.8^{ab}	370.78 ± 19.4^{ab}	156.0 ± 15.11	5.26 ± 0.41^{b}	6.25 ± 0.53^{b}	3.96 ± 0.19
20 %	136.09 ± 7.6^{a}	330.8 ± 22.8^{a}	208.8 ± 16.59	5.16 ± 0.4^{b}	6.4 ± 0.75^{b}	3.8 ± 0.34
p-Combined	***<0.01***	***<0.01***	*0.221*	***0.047***	***0.033***	*0.139*
Linear	***<0.01***	***<0.01***	*0.194*	***<0.01***	***0.025***	*0.05*
Quadratic	*0.138*	*0.192*	*0.114*	*0.629*	*0.265*	*0.338*
Cubic	*0.111*	*0.139*	*0.29*	*0.221*	*0.945*	*0.943*

*[a, b] Values with different superscripts in the same row are significantly different ($p \leq 0.05$) and ($p \leq 0.01$). *Data is represented as least squares. The values are means of 8 replicate cages per diet with 2 rats (n= 16). *Black Soldier Fly 0% (Control), BSF 5%, 10%, 15% and 20% are the treatment groups. *MDA (malondialdehyde), GSH (Reduced glutathione) * The unit of measurement for MDA is nmol/g and for GSH is U/g.

3.5.2. TOS and TAS in Serum (Gender specific)

The statistical analysis shows that TOS was statistically significant in tissues of male rats compared to the control ($p < 0.05$) as a combined, linear, and quadratic effect. Moreover, it was seen that for the 15% group, it increased to a maximum extent in male rats. Similarly, the statistical analysis of female rats shows that in tissues TOS increased significantly at 15% and 20% compared to the control ($p < 0.05$). Conversely, it was significantly decreased in the 5 and 10% groups compared to the control ($p < 0.05$).

The statistical analysis of TAS in tissues shows that it was significantly increased in male rats in all experimental groups compared to the control as a quadratic effect ($p < 0.05$). The TAS level was higher in the male 15% group compared to the other experimental groups and control ($p < 0.05$). Similarly, for female rats, TAS was increased significantly for all experimental groups compared to the control linearly ($p < 0.05$). It was higher in the 20% group than in the other experimental and control groups ($p < 0.05$). The statistical analysis showed that TOS was higher in male rats as compared to female rats, and TAS was higher in female rats as compared to male rats. The levels of TOS and TAS are shown in Table 3.11 and Figure 3.12.

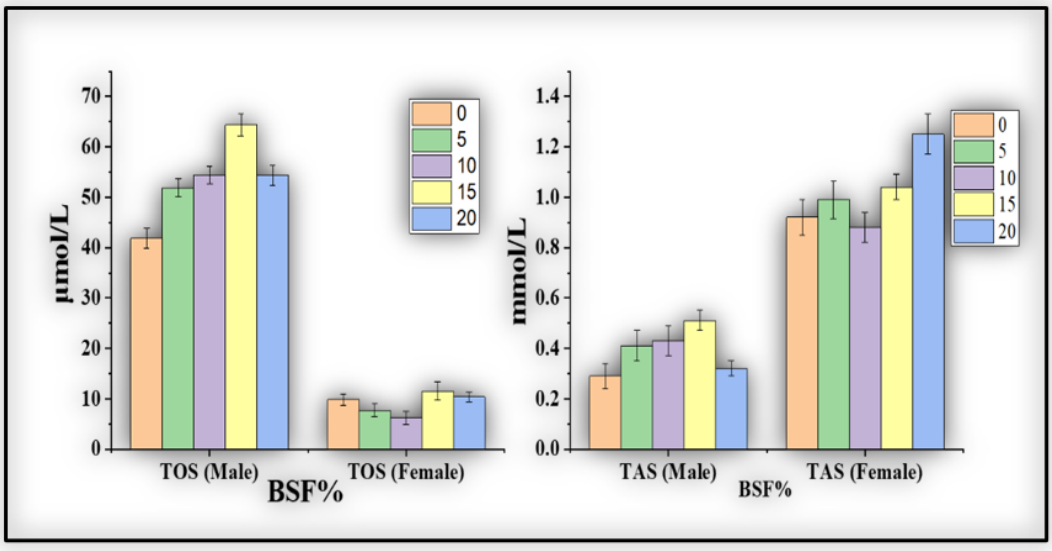

Figure 3.12: Effect of BSF larvae meal on TOS and TAS in tissues

3.5.3. OSI and NOx in serum (Gender specific)

The statistical analysis shows that OSI in tissues was non-significant for male rats ($p > 0.05$). In female rats, it decreased significantly in 5%, 10%, and 20% groups compared to the control as cubic effect ($p < 0.05$). The statistical analysis shows that NOx in tissues remained non-significant for all groups in male and female rats ($p < 0.05$). However, compared to the control for male and female rats, the means of NOx for all experimental groups were higher, indicating good health. The levels of OSI and NOx are shown in Table 3.11 and Figure 3.13.

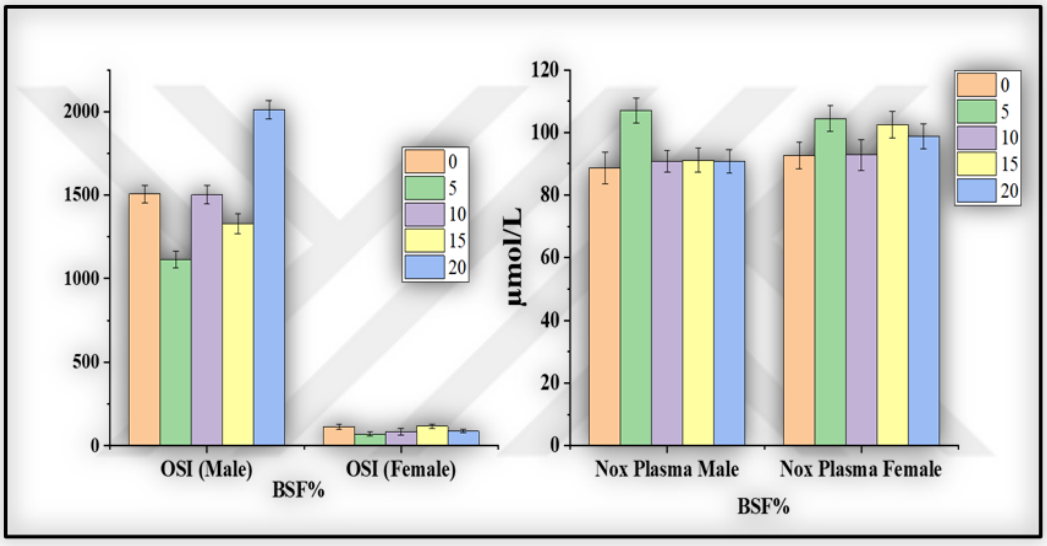

Figure 3.13: Effect of BSF larvae meal on OSI and NOx in tissues

Table 3.11: TOS, TAS, NOx and OSI in male and female rats

Parameter	0% BSF	5% BSF	10% BSF	15% BSF	20% BSF	*p*	*Linear*	*Quadratic*	*Cubic*
TOS (M)	41,88 ± 18,11^{a}	51,88 ± 5,3^{ab}	54,38 ± 8,21^{b}	64,38 ± 9,04^{b}	54,38 ± 6,23^{b}	***0,010***	-	-	-
TOS (F)	9.82 ± 1.18^{b}	7.71 ± 1.27^{ab}	6.18 ± 1.37^{a}	11.54 ± 1.82^{b}	10.33 ± 0.87^{b}	***0.048***	*0.258*	*0.090*	*0.099*
TAS (M)	0,33 ± 0,17^{ab}	0,46 ± 0,15^{b}	0,42 ± 0,15^{b}	0,5 ± 0,1^{b}	0,31 ± 0,08^{a}	***0,028***	-	-	-
TAS (F)	0.92 ± 0.09^{ab}	0.99 ± 0.16^{ab}	0.88 ± 0.11^{a}	1.04 ± 0.14^{ab}	1.25 ± 0.12^{b}	*0.0218*	***0.045***	*0.216*	*0.538*
NOx (M)	88.68 ± 16.43	107.02 ± 41.19	90.86 ± 15.52	91.2 ± 16.49	90.86 ± 22.14	*0.983*	*0.883*	*0.820*	*0.663*
NOx (F)	92.61 ± 8.02	104.41 ± 17.23	92.81 ± 7.45	102.54 ± 8.68	98.76 ± 9.71	*0.907*	*0.763*	*0.809*	*0.775*
OSI (M)	1506.08 ± 321.54	1116.47 ± 259.77	1501.72 ± 243.7	1330.17 ± 106.64	2011.59 ± 377.26	*0.246*	*0.171*	*0.135*	*0.929*
OSI (F)	114,02 ± 46,66^{b}	79,6 ± 20,09^{a}	82,22 ± 55,7^{ab}	116,58 ± 39,76^{b}	87,58 ± 27,08^{ab}	***0,035***	*0.751*	***0.040***	*0.121*

*TOS (M): Total Oxidative Status in male rats *TOS (F): Total Oxidative Status in female rats * TAS (M): Total Antioxidative Status in male rats * TAS (F): Total Antioxidative Status in female rats; *NOx (M): Nitric oxide in male rats;* NOx (F): Nitric oxide in female rats; *OSI (M): Oxidative status index in male rats;* OSI (F): Oxidative status index in female rats

3.5.4. MDA and GSH in serum (Gender specific)

The statistical analysis of MDA in serum shows the statistically non-significant effect of BSF larvae on MDA in plasma compared to the control for all male and female rats ($p > 0.05$). The statistical analysis of GSH in serum shows a non-significant effect of BSF larvae on GSH in plasma compared to the control for all male and female rats ($p > 0.05$). However, the level of MDA in plasma was higher in female rats than in male rats, and the level of GSH in plasma was higher in male rats than in female rats. The MDA and GSH in the serum of male and female rats are shown in Table 3.12 and Figure 3.14.

Table 3.12: The levels of MDA and GSH in the serum of male and female rats

	MDA (Plasma) (nmol/mL)		GSH (Plasma) (mg/dL)	
BSF Inclusion	**Male**	**Female**	**Male**	**Female**
0	4.42 ± 0.09	6.17 ± 0.20	11.86 ± 0.73	5.25 ± 0.42
5	4.30 ± 0.09	5.88 ± 0.98	10.47 ± 0.53	4.04 ± 0.67
10	4.40 ± 0.22	6.75 ± 0.22	11.48 ± 0.83	4.50 ± 0.16
15	4.37 ± 0.07	6.36 ± 0.26	11.86 ± 0.60	5.50 ± 0.47
20	4.22 ± 0.10	6.91 ± 0.19	11.22 ± 0.37	5.15 ± 0.78
p-Combined	*0.757*	*0.553*	*0.512*	*0.311*
Linear	*0.385*	*0.206*	*0.960*	*0.459*
Quadratic	*0.655*	*0.819*	*0.716*	*0.273*
Cubic	*0.385*	*0.885*	*0.094*	*0.085*
Posthoc	Duncan	Duncan	Duncan	Duncan

*Data is represented as least squares. The values are means of 8 replicate cages per diet with 2 rats (n= 16). *Black Soldier Fly 0% (Control), BSF 5%, 10%, 15% and 20% are the treatment groups. *MDA (malondialdehyde), GSH (Reduced glutathione)

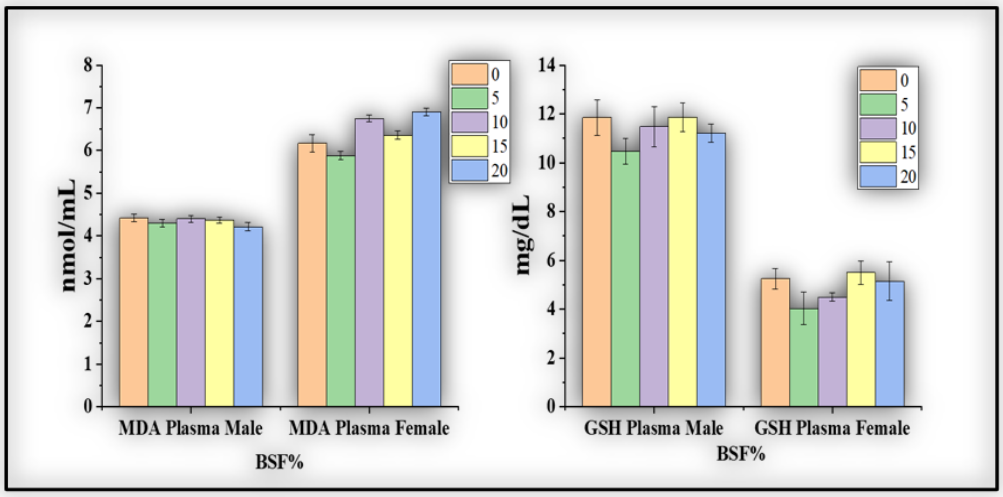

Figure 3.14: Effect of BSF larvae meal on MDA and GSH in plasma

3.5.5. MDA and GSH in the liver (Gender specific)

The statistical analysis showed that the level of MDA in tissues was statistically significant in male rats compared to the control in all experimental groups ($p < 0.05$). It was seen that the level of MDA in the liver was significantly decreased as compared to the control for the increasing concentration of BSF larvae % as a combined, linear, and quadratic effect ($p < 0.05$). The maximum drop of MDA in the liver was seen in the 20% group compared to the control group. Similarly, in female rats, the statistical analysis showed that the level of MDA in the liver was statistically decreased for all experimental groups compared to the control ($p < 0.05$). The maximum drop was seen in the 5% group.

The statistical analysis showed that the level of GSH was significant for male rats in all experimental groups compared to the control ($p < 0.05$). In female rats, it was seen that level of GSH was significantly increased linearly with an increasing inclusion of BSF larvae in all experimental groups compared to the control ($p < 0.05$). The maximum increase in GSH in the liver of female rats was observed in the 20% group compared to the control group. The levels of MDA in male rats were more than in female rats, and the level of GSH in female rats was higher than in male rats. The levels of MDA and GSH in the liver are shown in Table 3.13 and Figure 3.15.

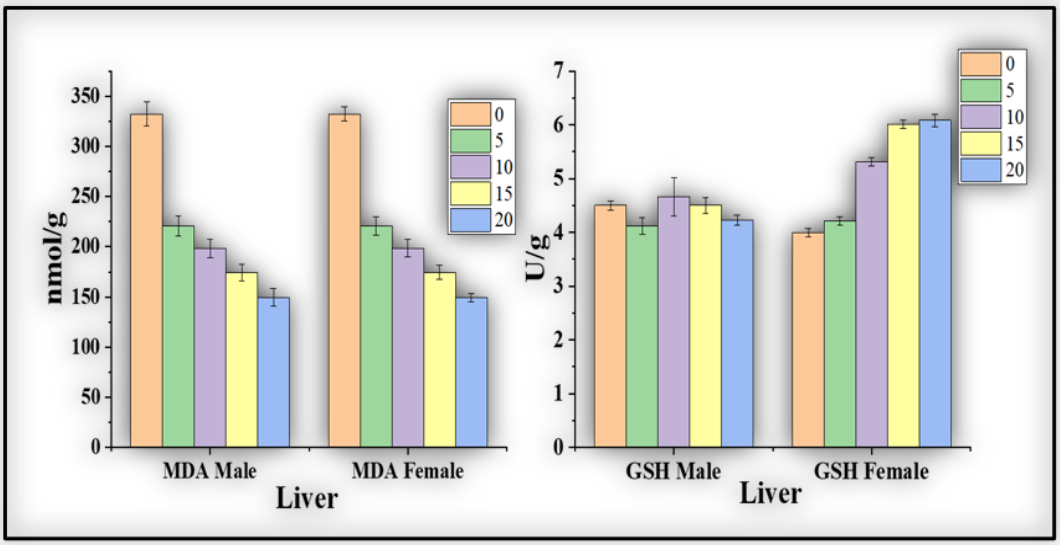

Figure 3.15: Effect of BSF larvae meal on MDA and GSH in the liver

3.5.6. MDA and GSH in Kidney (Gender specific)

The statistical analysis showed that the level of MDA in the kidneys of male rats was significantly decreased for all treatment groups as compared to the control ($p < 0.05$) as a combined, linearly, and quadratic effect and a maximum drop in MDA in the kidney of male rats was seen in the 10% group as compared to the control. Moreover, the level of MDA in the kidney of female rats were also significantly decreased compared to the control ($p < 0.05$). The statistical analysis shows that the level of GSH in the kidney of male rats was significantly different for all treatment groups as compared to the control ($p < 0.05$) as a combined and linear effect. It was seen that the level of GSH in the kidney was significantly increased with increasing percentages of BSF larvae ($p < 0.05$). The maximum increase was seen in the 20% group compared to the control group. However, the level of GSH in female rats was also significant in treatment groups compared to the control as a cubic effect ($p < 0.05$). The maximum of GSH in the kidney of female rats was increased in the 10% group compared to the control ($p < 0.05$). The level of MDA in the kidney of male rats was higher compared to the female rats, and the level of GSH in female rats was higher than that of the male rats. The MDA and GSH in the kidney are shown in Table 3.13 and Figure 3.16.

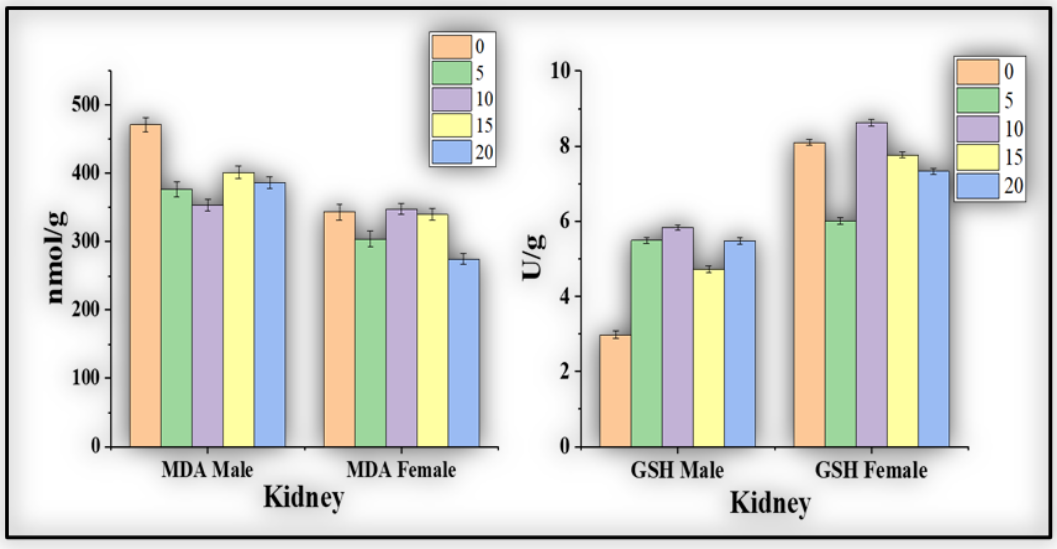

Figure 3.16: Effect of BSF larvae meal on MDA and GSH in the kidney

3.5.7. MDA and GSH in heart (Gender specific)

The statistical analysis of MDA in the heart indicated that it was statistically non-significant for all male and female groups compared to the control ($p > 0.05$). Moreover, the level of GSH in the heart of male rats was also statistically non-significant compared to the control ($p > 0.05$). However, GSH in the female rats' hearts was statistically significant compared to the control ($p < 0.05$) as cubic effect. It was seen that its level was significantly decreased in the 20% group compared to the control ($p < 0.05$). The MDA and GSH in the kidney are shown in Table 3.13 and Figure 3.17.

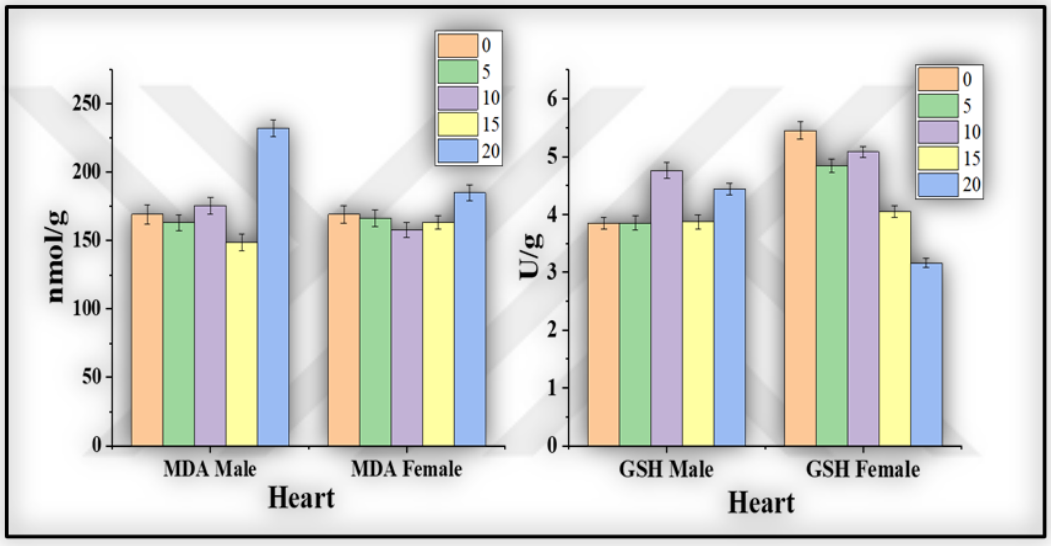

Figure 3.17: Effect of BSF larvae meal on MDA and GSH in the heart

Table 3.13: Levels of MDA and GSH in kidney, liver and heart of male and female rats

Parameters	0% BSF	5% BSF	10% BSF	15% BSF	20% BSF	Test	*p*	*Linear*	*Quadratic*	*Cubic*
MDA-K (M)	538.71 ± 61.28^{b}	377.1 ± 68.72^{a}	353.78 ± 41.96^{a}	401.75 ± 86.48^{ab}	386.76 ± 88.54^{a}	ANOVA	***<0.001***	***0.003***	***0.001***	***0.018***
MDA-K (F)	392.52 ± 81.85^{b}	347.60 ± 73.10^{ab}	348.12 ± 100.65^{ab}	339.79 ± 57.74^{ab}	274.83 ± 53.08^{a}	KWH	***0.043***	-	-	-
MDA-L (M)	332.13 ± 71.42^{b}	220.86 ± 37.97^{ab}	198.55 ± 30.15^{ab}	174.23 ± 56.94^{ab}	149.58 ± 37.76^{a}	KWH	***<0.001***	-	-	-
MDA-L (F)	147.91 ± 7.11^{b}	105.94 ± 15.82^{a}	143.92 ± 14.01^{b}	132.92 ± 8.59^{ab}	122.6 ± 4.1^{ab}	ANOVA	*0.063*	*0.495*	*0.726*	***0.027***
MDA-H (M)	169.23 ± 28.17	163.24 ± 34.45	175.56 ± 30.7	148.91 ± 25.42	232.53 ± 29.08	ANOVA	*0.342*	*0.240*	*0.215*	*0.334*
MDA-H (F)	169.23 ± 12.82	166.57 ± 27.62	157.91 ± 12.63	163.24 ± 17.82	185.22 ± 13.16	ANOVA	*0.852*	*0.613*	*0.347*	*0.689*
GSH-K (M)	3.40 ± 0.56^{a}	5.48 ± 0.2^{b}	5.83 ± 0.3^{b}	4.71 ± 0.2^{ab}	5.46 ± 1.4^{b}	KWH	***0.002***	-	-	-
GSH-K (F)	8.1 ± 0.59^{b}	6.01 ± 0.91^{a}	8.62 ± 0.66^{b}	7.77 ± 0.7^{ab}	7.33 ± 0.33^{ab}	ANOVA	*0.084*	*0.914*	*0.943*	***0.047***
GSH-L (M)	332.13 ± 25.26^{c}	220.87 ± 13.43^{b}	198.55 ± 10.67^{ab}	174.23 ± 20.14^{ab}	149.58 ± 13.35^{a}	ANOVA	***<0.01***	***<0.01***	***0.013***	*0.114*
GSH-L (F)	3.99 ± 0.38^{a}	4.81 ± 1.10^{ab}	5.30 ± 1.05^{b}	6.00 ± 2.06^{b}	6.07 ± 1.75^{b}	ANOVA	***0.030***	***0.02***	*0.489*	*0.835*
GSH-H (M)	3.85 ± 0.17	3.85 ± 0.34	4.76 ± 0.82	3.87 ± 0.17	4.44 ± 0.42	ANOVA	*0.483*	*0.407*	*0.694*	*0.701*
GSH-H (F)	5.44 ± 0.98^{b}	5.52 ± 0.94^{b}	5.08 ± 1.33^{b}	4.04 ± 0.96^{ab}	3.15 ± 1.20^{a}	KWH	***0.003***	-	-	-

*$^{a, b}$ Values with different superscripts in the same row are significantly different ($p \leq 0.05$) and ($p \leq 0.01$). *Data is represented as least squares. The values are means of 8 replicate cages per diet with 2 rats (n= 16). *Black Soldier Fly 0% (Control), BSF 5%, 10%, 15% and 20% are the treatment groups. *MDA-K (M)=Malondialdehyde in kidney of male rats; MDA-K (F)=Malondialdehyde in kidney of female rats. *MDA-L (M)=Malondialdehyde in liver of male rats; MDA-L (F)=Malondialdehyde in liver of female rats. *MDA-H) =Malondialdehyde in heart of male rats; MDA-H (F)=Malondialdehyde in heart of female rats. *GSH-K (M)=Glutathione in kidney of male rats; GSH-K (F)=Glutathione in kidney of female rats *GSH-L (M)=Glutathione in liver of male rats; GSH-L (F)=Glutathione in liver of female rats. *GSH-H (M)=Glutathione in heart of male rats; GSH-H (F)=Glutathione in heart of female rats

3.6. Histopathology

The general histopathology of the duodenum, liver, kidney, testis, ovary, heart, and uterus was performed. The liver (Figure 3.19, A1-A5), kidney (Figure 3.19, B1-B5), testis (Figure 3.19, C1-C5), and intestines (Figure 3.21, A1-A5) in the male rats showed standard and typical histological structure. No histopathological findings were found. Moreover, the statistical analysis of villus length from duodenum shows that it was statistically significant for male rats as linear and combined effect ($p < 0.05$) in all treatment groups compared to the control, as shown in Table 3.14 and Figure 3.18.

The liver (Figure 3.20, A1-A5), heart (Figure 3.20, C1-C5), uterus (Figure 3.20, E1-E5), and intestines (Figure 3.21, A1-A5) were normal in rats in this group. Renal tubular lumens had hyaline cylinders in all treatment groups (Figure 3.20, B2-B5), and oedema was observed in the ovarian interstitial cells (Figure 3.20, D3-D5). No other histopathological findings were found. Moreover, in female rats, the villus length was also statistically significant ($p < 0.05$) in all treatment groups compared to the control as a combined, linear, quadratic effect. The statistical analysis is shown in Table 3.14, and the graphical representation is in Figure 3.18.

Table 3.14: The values of villus length in male and female rats

BSF Inclusion (%)		**Villus Length**	
		Male	**Female**
Groups	**0**	786.31 ± 25.45^{a}	677.53 ± 21.66^{a}
	5	990.82 ± 16.22^{ab}	876.81 ± 10.29^{b}
	10	1138.35 ± 11.54^{c}	1025.46 ± 15.41^{c}
	15	1252.25 ± 33.37^{d}	1067.56 ± 50.43^{c}
	20	1435.63 ± 18.51^{e}	1100.08 ± 21.10^{c}
p	***Combined***	***<0.001***	***<0.001***
	Linear	***<0.001***	***<0.001***
	Quadratic	*0.369*	***<0.001***
	Cubic	*0.08*	*0.64*

*[a, b, c, d. e] Values with different superscripts in the same row are significantly different ($p \leq 0.01$). *Data is represented as least squares. The values are means of 8 replicate cages per diet with 2 rats (n= 16). *Black Soldier Fly 0% (Control), BSF 5%, 10%, 15% and 20% are the treatment groups.

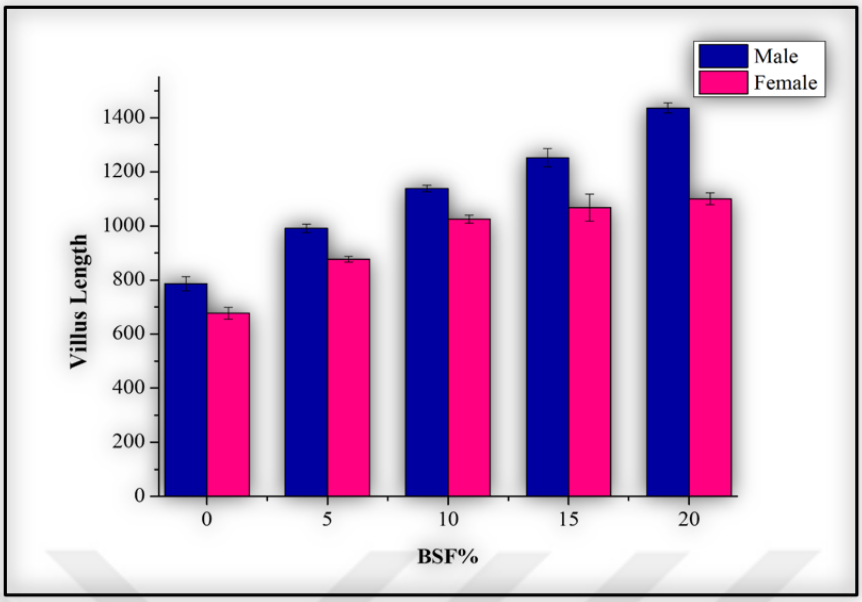

Figure 3.18: Effect of BSF larvae meal on villus length of the duodenum

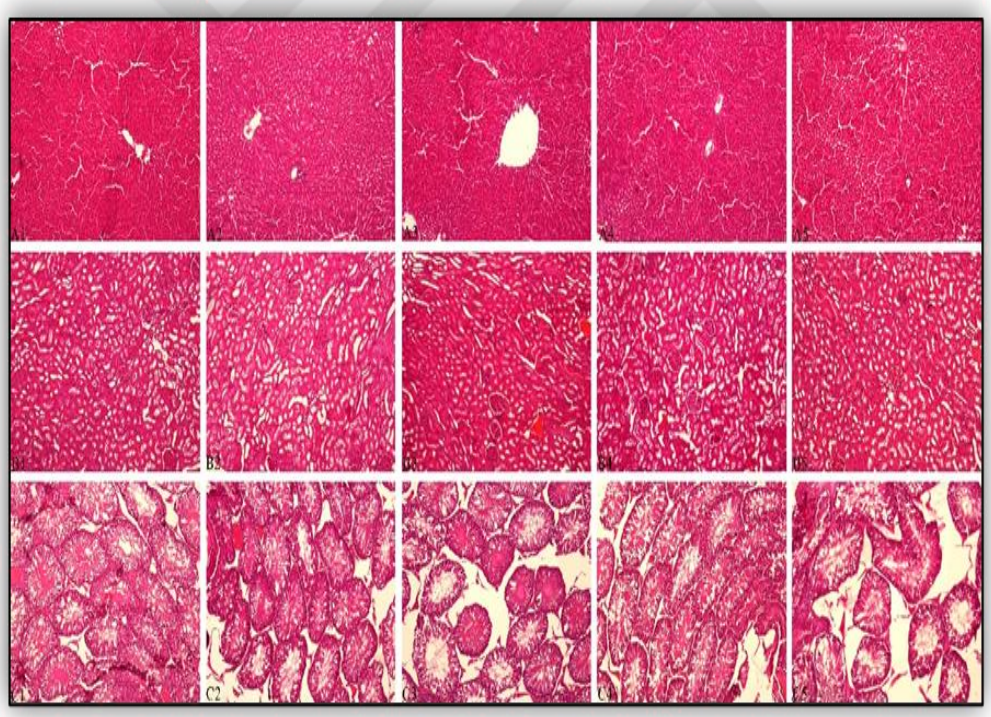

Figure 3.19: Effect of BSF larvae meal on the liver (A1-A5), kidney (B1-B5) and Testis (C1-C2) of male rats and there was no pathological finding.

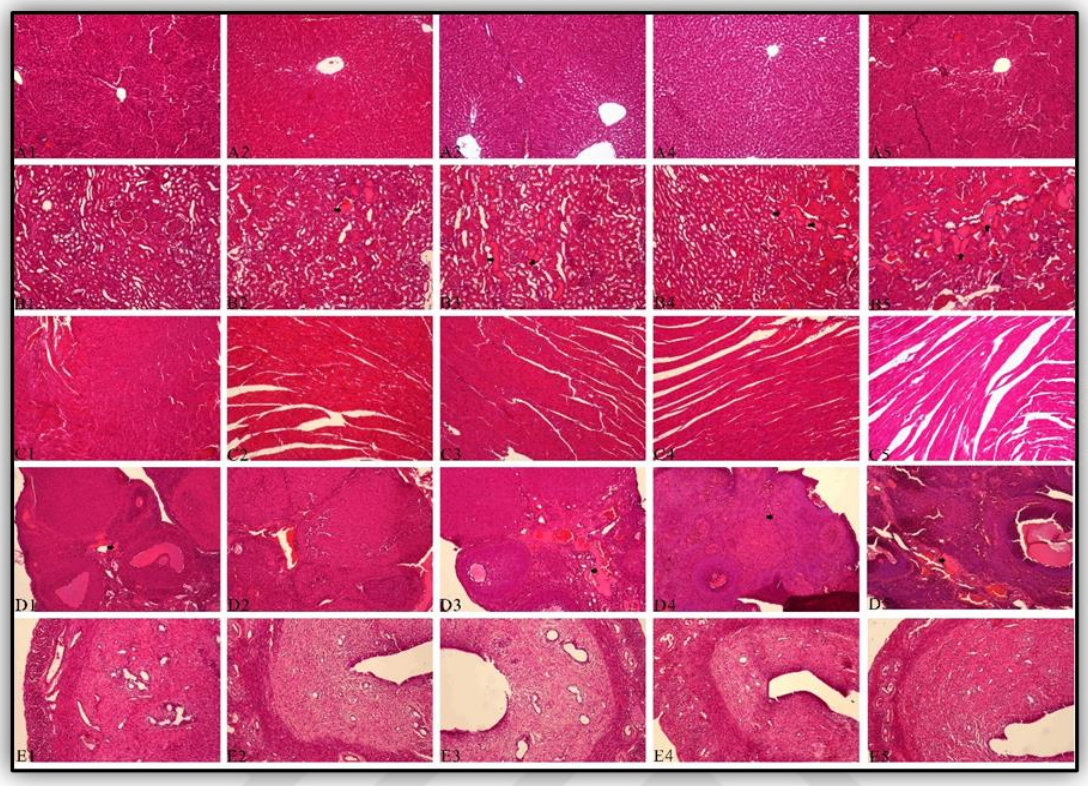

Figure 3.20: Effect of BSF larvae meal on histopathological findings in the liver (A1-A5), kidney (B1-B5), heart (C1-C5), ovaries (D1-D5), and uterus (E1-E5) of female rats and there was hyaline deposition at a smaller level in kidney and slightly edema in ovaries and no significant pathological finding.

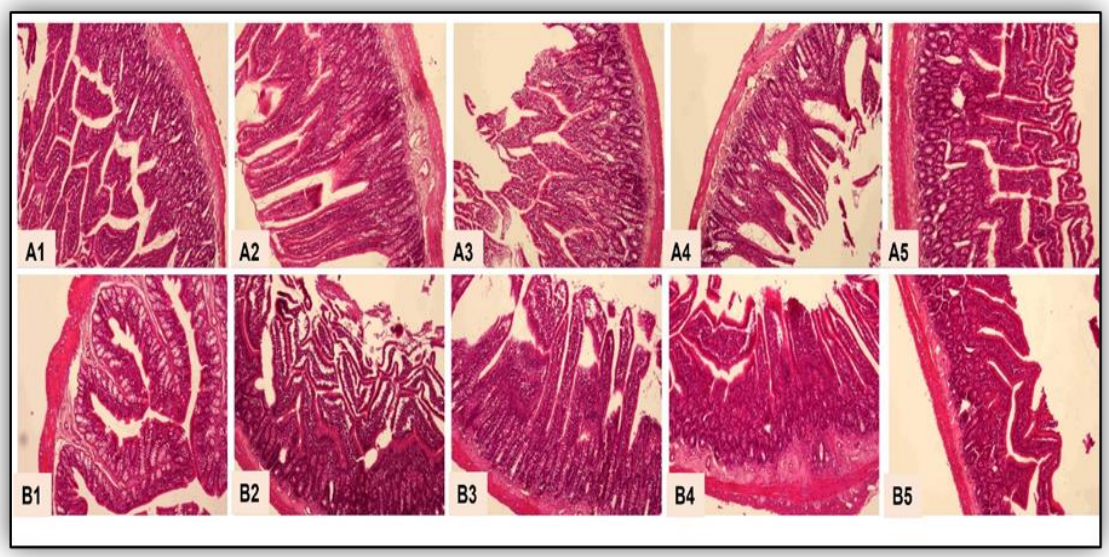

Figure 3.21: Effect of BSF larvae meal on the intestines of male and female rats, A1-A5 (Male), B1-B5 (Female)

3.7. Pearson's correlational analysis

3.7.1. Correlational analysis of male biochemical parameters

SPSS Pearson Correlational analysis showed that in male rats there was moderate correlation of IgG and HDL with total protein; moderate correlation of LDL and AST with uric acid; moderate correlation of calcium and phosphorous with AST; moderate correlation of non-HDL with phosphorous and total cholesterol; strong correlation of non-HDL, LDL and HDL with total cholesterol and a weak correlation of AST with ALT. The Pearson's correlational analysis of male biochemical parameters is shown in Table 3.15.

3.7.2. Correlational analysis of female biochemical parameters

SPSS Pearson Correlational analysis showed that in female rats there was negative weak correlation of calcium and phosphorous with creatinine; a moderate correlation of HDL, non-HDL, cholesterol total, calcium and ALT with protein total; negative weak correlation of ALT with uric acid; a weak and moderate correlation of HDL, non-HDL, cholesterol total with calcium; a strong correlation of non-HDL, HDL and LDL with cholesterol total; strong correlation of HDL and non-HDL with LDL; positive strong correlation of HDL with non-HDL and a weak negative correlation of IgG with non-HDL and HDL as shown in Table 3.16.

Table 3.15: Pearson Correlational (R^2) analysis for male biochemical parameters

Parameters	Creatinine	PT	UA	ALT	AST	ALP	Calcium	Phosphorous	TC	LDL	HDL	Non-HDL	IgG
Creatinine	1	.195	.027	.101	.031	.195	.116	-.232	.233	.179	.226	.094	.105
Protein Total		1	.176	.165	.197	.090	-.024	-.173	.224	-.051	**.321***	-.187	**.588****
Uric Acid			1	-.068	**.438****	-.286	-.069	.099	-.232	-**.343***	-.220	-.103	.182
ALT				1	**.313***	.367*	-.214	-.017	.025	.005	.057	-.076	.310
AST					1	-.187	**-.475****	**.313***	.000	-.144	.004	-.013	.280
ALP						1	.142	-.291	.126	.245	.111	.079	.308
Calcium							1	-.131	.081	.128	.064	.070	-.126
Phosphorous								1	.073	.208	-.077	**.422****	-.212
TC									1	**.807****	**.944****	**.464****	-.219
LDL										1	**.609****	**.783****	-.306
HDL											1	.145	-.090
Non-HDL												1	**-.414****
IgG													1

** Correlation is significant at the 0.01 level (2-tailed). *Correlation is significant at the 0.05 level (2-tailed). * Protein Total (PT) *Uric Acid (UA) *Total Cholesterol (TC) *Alanine aminotransferase (ALT) *Aspartate aminotransferase (AST) b*Alkaline phosphatase (ALP) *High-density lipoprotein (HDL) *Low-density lipoprotein (LDL) *Immunoglobulin-G (IgG)

Table 3.16: Pearson Correlational (R^2) analysis for female biochemical parameters

Parameters	Creatinine	PT	Uric Acid	ALT	AST	ALP	Calcium	Phosphorous	TC	LDL	HDL	Non-HDL	IgG
Creatinine	1	-.078	.142	-.113	.137	-.139	**-.364***	**-.390***	-.058	.074	-.023	-.111	.047
Protein Total		1	-.168	**.524****	.123	.082	**.706****	.089	**.524****	.312	**.513****	**.475****	.038
Uric Acid			1	**-.329***	.254	.040	-.042	.076	-.025	.090	-.063	.046	.171
ALT				1	.238	.149	.273	.076	.193	.147	.167	.214	-.100
AST					1	.089	.095	.135	.161	.204	.105	.239	.046
ALP						1	.166	.240	-.057	.007	-.048	-.064	.083
Calcium							1	.282	**.462****	**.329***	**.479****	**.371***	-.232
Phosphorous								1	.109	.112	.115	.085	.165
Cholesterol									1	**.900****	**.975****	**.917****	**-.365***
Total													
LDL										1	**.898****	**.788****	-.275
HDL											1	**.805****	**-.349***
Non-HDL												1	**-.347***
IgG													1

** Correlation is significant at the 0.01 level (2-tailed) *Correlation is significant at the 0.05 level (2-tailed). * Protein Total (PT) *Total Cholesterol (TC) *Alanine aminotransferase (ALT) *Aspartate aminotransferase (AST) b*Alkaline phosphatase (ALP) *High-density lipoprotein (HDL) *Low-density lipoprotein (LDL) *Immunoglobulin-G (IgG)

3.7.3. Correlational analysis of male hematological parameters

SPSS Pearson Correlational analysis showed in male rats' a strong correlation of WBC with NEU, LY, MO, EO and IgG and a moderate correlation of WBC with BA, RBC and a negative correlation of WBC with MCV and MCH was seen. A weak correlation of NEU with EO and RBC and moderate with IgG and LY was seen. LY showed moderate correlation with MO, BA, EO, RBC and IgG and negative correlation with MCH. MO showed strong correlation with EO and IgG. RBC showed strong correlation with HGB, HCT and weak moderate correlation with PCT and RDW-CV and a negative correlation with MCV, MCH and P-LCR. HGB showed strong correlation with HCT and moderate one with MCH. HCT showed moderate correlation with MCV and MCH and a negative one with MCHC. MCV showed strong correlation with MCH, RDW-SD and a negative correlation with MCHC and RDW-CV. MCH showed a strong correlation with RDW-SD and a negative correlation with PLT and RDW-CV. PLT showed a negative correlation with PDW, MPV, P-LCR, RDW-SD and a strong correlation with PCT. PDW showed a strong correlation with MPV, P-LCR, n-RBC and a negative correlation with PCT. P-LCR showed a negative correlation with PCT and a moderate correlation with RDW-SD. MPV showed a strong correlation with P-LCR, a weak correlation with RDW-SD and a negative correlation with PCT. The Pearson's correlational analysis of male haematological parameters is shown in Table 3.17.

3.7.4. Correlational analysis of female hematological parameters

WBC showed a strong correlation with NEU, LY, MO, RBC, HCT and RDW-CV and a moderate correlation with BA, HGB and a negative correlation with MCH. NEU showed a strong correlation with LY and HGB and a negative weak correlation with MO and BA. LY showed a moderate correlation with MO, EO, RBC, HGB and HCT. LY showed a moderate correlation with MO, EO, RBC, HGB, HCT, RDW-CV and a weak correlation with MCH. MO showed a strong correlation with BA, RBC, RDW-CV and a negative correlation with MCH. BA showed a weak correlation with RDW-CV. RBC showed strong correlation with HGB, HCT, RDW-CV and a moderate negative correlation with MCH and RDW-SD. HGB showed a strong correlation with HCT. MCV showed a strong correlation with MCH, RDW-SD and a negative correlation with RDW-CV.

MCH showed a negative correlation with RDW-CV and a positive correlation with RDW-SD. MCHC showed a negative moderate correlation with RDW-SD. PDW showed a moderate correlation with RDW-CV. RDW-CV showed a moderate correlation with RDW-SD. The Pearson's correlational analysis of female hematological parameters is shown in Table 3.18.

3.7.5. Correlational analysis of oxidative parameters in serum

SPSS Pearson's correlational analysis showed that in male rats' serum, MDA had a moderate correlation with GSH in male rats. GSH in female rats showed a moderate correlation with OSI in female rats. NOx in female rats showed moderate correlation with TOS and TAS in female rats. TOS in male rats showed moderate correlation with TAS in female rats. TOS in female rats showed a moderate correlation with TAS in female rats and a strong correlation with OSI in female rats. TAS in male rats showed a negative strong correlation with OSI in males and TAS in female rats showed moderate correlation with OSI in female rats. The Pearson's correlational analysis of male and female oxidative parameters is shown in Table 3.19.

3.7.6. Correlational analysis of oxidative parameters in tissues

MDA in liver of male rats showed a moderate correlation with MDA in kidney of male rats and MDA in kidney of male rats showed a moderately negative correlation with GSH in kidney of male rats. MDA in heart of female rats showed a moderately negative correlation with GSH in kidney of female rats. MDA in kidney of female rats showed a moderately positive correlation with GSH in heart and negative correlation with GSH in liver of female rats. The Pearson's correlational analysis of male and female tissue oxidative parameters is shown in Table 3.20 and Table 3.21, respectively.

Table 3.17: Pearson Correlational (R^2) analysis for male hematological parameters

	WBC	NEU	LY	MO	BA	EO	RBC	HGB	HCT	MCV	MCH	MCHC	PLT	PDW	MPV	P-LCR	PCT	RDW-CV	RDW-SD	n-RBC	IgG
WBC	1	**.702****	**.980****	**.489****	**.391***	**.529****	**.401***	.064	.125	**-.316***	**-.458****	-.125	.276	.008	-.091	-.092	.181	.263	-.289	.220	**.509****
NEU		1	**.608****	.165	.170	**.322***	**.357***	.173	.214	-.165	-.267	-.084	.119	-.087	-.178	-.178	.082	.231	-.184	-.123	**.564****
LY			1	**.431****	**.388***	**.464****	**.385***	.056	.112	-.311	**-.441****	-.118	.261	.068	-.035	-.033	.153	.273	-.252	.260	**.439****
MO				1	.300	**.669****	.100	-.013	-.003	-.114	-.154	-.015	.259	-.181	-.211	-.203	.202	-.034	-.188	.027	**.349***
BA					1	.088	**.380***	.204	.197	-.191	-.203	.020	.204	-.145	-.081	-.121	.271	**.362***	.032	.038	**.459****
EO						1	**.320***	.228	.163	-.179	-.126	.125	.194	-.120	-.199	-.178	.172	.123	-.300	-.019	**.409****
RBC							1	**.743****	**.672****	**-.354***	**-.312***	.105	.213	-.237	-.306	**-.352***	**.341***	**.355***	**-.429****	-.065	.283
HGB								1	**.875****	.209	**.401***	.182	-.056	-.144	-.152	-.162	.080	.103	-.051	-.239	.183
HCT									1	**.454****	**.336***	**-.315***	-.037	-.110	-.033	-.058	.102	-.009	.067	-.133	.175
MCV										1	**.798****	**-.529****	-.301	.141	**.326***	**.351***	-.277	**-.417****	**.623****	-.097	-.123
MCH											1	.085	**-.374***	.120	.212	.261	-.356*	**-.320***	**.549****	-.243	-.136
MCHC												1	-.033	-.064	-.227	-.201	-.036	.235	-.245	-.208	.024
PLT													1	**-.403****	**-.320***	**-.502****	**.797****	-.022	**-.350***	.141	.249
PDW														1	**.854****	**.909****	**-.580****	.091	.253	**.329***	.094
MPV															1	**.958****	**-.321***	.001	**.374***	.301	.048
P-LCR																1	**-.532****	.016	**.425****	.303	-.005
PCT																	1	.090	-.297	-.063	.188
RDW-CV																		1	.310	-.149	.303
RDW-SD																			1	-.173	-.010
N-RBC																				1	.037
IgG																					1

Table 3.18: Pearson Correlational (R^2) analysis for female hematological parameters

	WBC	NEU	LY	MO	BA	EO	RBC	HGB	HCT	MCV	MCH	MCHC	PLT	PDW	MPV	P-LCR	PCT	RDW-CV	RDW-SD	n-RBC	IgG
WBC	1	**.523****	**.965****	**.568****	**.391***	.284	**.558****	**.396***	**.410****	-.188	**-.334***	-.173	.037	.191	.156	.144	.097	**.543****	.000	.165	.158
NEU		1	**.512****	-.202	-.127	**.314***	**.325***	**.414****	**.331***	.016	.044	.046	.286	-.135	-.194	-.201	.184	.043	-.129	-.185	.262
LY			1	**.392***	.281	**.359***	**.486****	**.332***	**.351***	-.180	**-.316***	-.161	.052	.225	.207	.192	.135	**.486****	-.029	.168	.228
MO				1	**.649****	-.177	**.409****	.201	.247	-.197	**-.347***	-.182	-.198	.177	.146	.149	-.129	**.554****	.135	.267	-.179
BA					1	-.154	.184	.197	.226	.084	-.002	-.154	-.244	.143	.112	.102	-.199	**.371***	.249	.037	.008
EO						1	.088	.048	.123	.038	-.093	-.166	.111	.163	.175	.154	.170	.024	.065	-.217	.082
RBC							1	**.785****	**.761****	-.308	**-.511****	-.235	.028	.227	.096	.111	.071	**.420****	**-.324***	.011	.060
HGB								1	**.914****	.221	.129	-.168	-.103	.232	.122	.129	-.042	.150	-.077	-.054	.151
HCT									1	.381*	.055	-.552**	-.185	.267	.162	.161	-.099	.144	.106	-.087	.082
MCV										1	**.809****	-.478**	-.310	.066	.098	.072	-.246	**-.372***	**.630****	-.132	.030
MCH											1	.126	-.186	-.046	.011	-.005	-.171	**-.451****	**.434****	-.074	.103
MCHC												1	.246	-.151	-.122	-.103	.173	-.043	**-.414****	.096	.084
PLT													1	-.254	-.226	-.262	.876**	.138	-.164	.022	-.115
PDW														1	.972**	.976**	.228	**.318***	.192	.108	-.030
MPV															1	.991**	.266	.261	.230	.175	-.025
PLCR																1	.227	.274	.216	.161	-.036
PCT																	1	.260	-.043	.119	-.141
RDW-CV																		1	**.336***	.066	.076
RDW-SD																			1	-.157	-.005
N-RBC																				1	-.102
IgG																					1

Table 3.19: Pearson correlational (r^2) analysis for male and female serum oxidative parameters

Parameters	MDA_M	MDA_F	GSH_M	GSH_F	NOx_M	NOx_F	TOS_M	TOS_F	TAS_M	TAS_F	OSI_M	OSI_F
MDA_M	1	-.041	**.450****	.004	.194	.139	-.099	.296	.123	.123	.035	.151
MDA_F		1	-.122	.018	.034	.168	-.047	-.036	.231	.267	-.249	-.268
GSH_M			1	.042	.170	-.084	-.120	.139	.108	-.029	-.108	.159
GSH_F				1	.045	-.178	.119	.286	-.133	-.089	.162	**.330***
NOx_M					1	.016	.042	.001	.069	.234	.030	-.160
NOx_F						1	-.076	**.379***	.191	**.367***	-.102	.047
TOS_M							1	-.085	**.403***	.110	-.061	-.141
TOS_F								1	-.180	**.346***	.192	**.603****
TAS_M									1	-.048	**-.833****	-.159
TAS_F										1	.157	**-.498****
OSI_M											1	.095
OSI_F												1

** Correlation is significant at the 0.01 level (2-tailed). * Correlation is significant at the 0.05 level (2-tailed). * MDA_M (Malondialdehyde, male) * MDA_F (Malondialdehyde, female) *GSH_M (Reduced glutathione, male) *GSH_F (Reduced glutathione, female) *NOx_M (Nitric oxide, male) *NOx_F (Nitric oxide, Female) *TOS_M, TOS_F (Total oxidative status) *TAS_M, TAS_F (Total antioxidative status) *OSI_M, OSI_F (Oxidative status index)

Table 3.20: Pearson Correlational (R^2) analysis for male tissues oxidative parameters

Parameters	SPSS	MDA (Heart)	MDA (Liver)	MDA (Kidney)	GSH (Heart)	GSH (Liver)	GSH (Kidney)
MDA (Heart)	Pearson Correlation (R^2)	1	-.179	-.199	-.107	-.183	.076
MDA (Liver)	Pearson Correlation (R^2)		1	**.454****	-.101	.075	-.227
MDA (Kidney)	Pearson Correlation (R^2)			1	.025	.230	**-.553****
GSH (Heart)	Pearson Correlation (R^2)				1	-.035	.210
GSH (Liver)	Pearson Correlation (R^2)					1	-.188
GSH (Kidney)	Pearson Correlation (R^2)						1

*Correlation is significant at the 0.05 level (2-tailed). ** Correlation is significant at the 0.01 level (2-tailed). *MDA (Malondialdehyde), GSH (Reduced glutathione)

Table 3.21: Pearson Correlational (R^2) analysis for female tissues oxidative parameters

Parameters	SPSS	MDA (Heart)	MDA (Liver)	MDA (Kidney)	GSH (Heart)	GSH (Liver)	GSH (Kidney)
MDA (Heart)	Pearson Correlation (R^2)	1	.016	-.282	-.077	.129	**-.329***
MDA (Liver)	Pearson Correlation (R^2)		1	.016	-.014	-.095	-.079
MDA (Kidney)	Pearson Correlation (R^2)			1	**.366***	**-.394***	.130
GSH (Heart)	Pearson Correlation (R^2)				1	-.129	.090
GSH (Liver)	Pearson Correlation (R^2)					1	-.041
GSH (Kidney)	Pearson Correlation (R^2)						1

4. DISCUSSION

This section discusses all the experimental parameters in this study, including the mean body weight, average daily feed consumption, hematological parameters, biochemical serum parameters, oxidative parameters in serum and tissues, total oxidative status and total antioxidative status, and histopathological findings.

4.1. Mean Body Weight

In this study, adult rats were used to evaluate the effect of BSF larvae as a replacement to soybean meal on mean body weight. The statistical analysis showed no significant difference in all groups for initial and final weight gain and difference in initial and final weights of the rats among all groups ($p > 0.05$), as shown in Table 3.1. So, the BSF larvae were good regarding weight gain because there was no statistically significant difference in weight gain in adult rats compared to the soybean meal. However, in this study, the treatment diets showed similar weight gain results as the control diet. Similar to this study, weaned rabbits were fed with BSF larvae meal, and yellow mealworm and soybean fat were substituted partially and 100%. The results showed no significant difference in performance and weight gain compared to the control (Gasco et al., 2019b). Similarly, in another study, fish meal was replaced by the BSF larvae meal for feeding Nile tilapia fish. There was no statistically significant difference in all experimental groups compared to the control (Tippayadara et al., 2021). Similar to this study, it was seen that the substitution of fishmeal with 15% BSF larvae meal and 15% yellow mealworm in Siberian sturgeon caused no significant effect on weight gain and production parameters (Józefiak et al., 2019).

In a study on piglets, soybean meal was replaced as 5 and 10%, and similar to this study, it was seen that there was no significant difference in the growth performance of the piglets as compared to the control (Biasato et al., 2019). Contrary to this study, BSF larvae fat fed to piglets has shown that body weight gain was increased linearly (Heugten et al., 2019). In a study on sheep, soybean meal was substituted with BSF larvae meal, and coffee husk was replaced with cocoa pod husk in the diet. It was seen that there was no significant difference in live weight gain in all experimental groups as compared to the control (Rahman et al., 2021).

Contrary to this study, in a study on guinea fowl, it was seen that the body weight increased significantly in all experimental groups compared to the control (Wallace et al., 2017). Similar to the results found in this study, it was seen that BSF feeding to weaned piglets showed not any significant difference in average daily weight gain as compared to the control in all experimental groups (Driemeyer, 2016). Similarly, a study on Atlantic salmon showed that fishmeal replacement with BSF larvae meal did not significantly affect the daily weight gain and average daily feed consumption (Belghit et al., 2019). In another study on mirror carp, it was seen that the BSF inclusion as a replacement for fishmeal did not significantly affect the average weight gain and feed efficiency in all experimental groups compared to the control (Xu et al., 2020b).

4.2. Average Daily Feed Consumption

Average daily feed consumption was statistically analysed for all experimental groups for all nine weeks of the study. The results showed that the rats were neither overfeeding nor under-feeding because the feed intake in all groups during the trial was very similar to the control group. There was no statistically significant difference among all experimental groups, indicating that the average feed intake was very similar to the control group for all experimental groups ($p > 0.05$), as shown in Table 3.2. It could be postulated that the physical parameters of the experimental diets containing BSF larvae were very similar to the control diet containing soybean. The smell, taste, and flavour of the BSF larvae-containing diets did not hold back the rats from eating less as compared to the control because the feed intake of all experimental groups was very similar to the control group. This result is in agreement with the previous findings of Tippayadara et al. 2021; Józefiak et al., 2019; Gasco et al., 2019.

In a study on Nile Tilapia fish, fish meal was substituted by BSF larvae meal at different levels, and it was seen that there was no significant difference in growth performance and weight gain among fishmeal-fed and BSF larvae-fed fish groups (Tippayadara et al., 2021). Fish meal was replaced with BSF larvae meal in African catfish, and its effect on growth performance showed no significant difference between 25 and 50% replacement. In contrast, 100% replacement showed a considerable decrease in the production and performance parameters compared to the control (Adeoye et al., 2020).

In a study on sheep, soybean meal was substituted with BSF larvae meal, and coffee husk was replaced with cocoa pod husk in the diet. It was seen that there was no significant difference in weight gain, feed intake, and growth performance in all experimental groups as compared to the control (Rahman et al., 2021). In a study on dogs, 5% BSF larvae meal and 10% fermented oat were evaluated as compared to the commercial diets, and the results showed that there was no significant difference in body weight gain and feed intake of all experimental groups as compared to the control (Seo et al., 2021). In a European sea bass fish study, fish meal was replaced with BSF larvae meal, and it was seen that, similar to this study, there was no significant difference in weight gain and feed efficiency (Abdel-Tawwab et al., 2020). In a study, BSF larvae meal and fat were used for feeding dogs, and it was seen that the body weight gain and feed consumption were not significantly different in all experimental groups as compared to the control (Freel et al., 2021).

In a study on piglets, the fishmeal was replaced with BSF larvae meal, and it was seen that there was no significant difference in live weight gain and feed intake in all groups as compared to the control (Chia et al., 2019). Similar to this study, in another study on piglets, BSF larvae fat has shown no significant difference in average daily feed intake (Heugten et al., 2019). Similar to this study, in a study on weaned piglets, it was seen that there was not any significant difference in average daily feed intake as compared to the control in all experimental groups (Driemeyer, 2016). Similar results were found in a study on rabbits where BSF and yellow mealworm was supplemented as a replacement to soybean meal, and it was seen that there was not any significant difference in average daily feed intake as compared to the control in all experimental groups (Gasco et al., 2019a). In a study on Jain carp, the substitution of fishmeal with BSF larvae has shown no significant difference in growth performance, live weight gain, and nutrient utilization in all experimental groups compared to the control (Li et al., 2017).

4.3. Hematological Parameters

The hematological parameters, including WBC, NEU, LY, MO, BA, EO, RBC, HGB, HCT, MCV, MCH, MCHC, PLT, PDW, MPV, P-LCR, PCT, RDW-CV, RDW-SD, and n-RBC were measured for male and female rats as a combined and gender-specific effect. It was seen that some of the parameters were statistically non-significant, while some of the parameters were significant for all groups in the study, as shown in Table 3.3.

MCV is the parameter that indicates the size of the red blood cells. The statistical analysis showed that MCV increased linearly for the 5% group compared to all groups. However, it decreased significantly linearly in the 10%, 15%, and 20% groups compared to the control group. The MCV value shows the type of anemia; microcytic, macrocytic, or normocytic anemia (Maner and Moosavi, 2019). The MCHC indicates the hemoglobin content related to the size of the red blood cells. It was increased significantly as a combined and linear effect for all groups compared to the control. MCHC is an essential indicator of increased blood sugar levels. In diabetic conditions, the MCHC, MCV, RBC, and WBC levels decrease (Nasirian et al., 2017).

The PDW indicates platelet functionality and represents platelet size changeability. It was increased significantly for the 15 and 20% groups compared to the control as linear and quadratic effects. The MPV is the parameter that indicates the average size of the platelets, and it increased significantly in the 5, 15, and 20% groups compared to the control for combined and linear effects. However, the levels of BA, EO, RBC, and HGB in male and female rats were within the normal range, as reported by (Matsuzawa et al., 1993).

Basophils are the cells that increase in the body during infection. In this study, as the basophils are within normal range and the non-significant difference is seen in all groups, it indicated that BSF larvae did not cause any infection in rats. The P-LCR represents the number of platelets more significant in size than 12 fL in the blood. It also increased significantly for all groups except fed with 10% BSF larvae as a combined and linear effect. It was highest in the 20% group.

All of the other parameters remained non-significant in all groups. In a study on broilers, soybean fat was replaced by BSF fat totally and partially, and it was seen that there was no significant difference among all of the hematological parameters in all treatment groups as compared to the control (Schiavone et al., 2017a). Similar to our study, the hematological parameters were within the normal ranges as described by (Lumeij 2008; Vigneshwar et al., 2021). In a study on piglets, soybean meal was replaced as 5 and 10%, and similar to this study, it was seen that there was no significant change for most of the hematological parameters. However, unlike this study, it was seen that the monocytes were increased in 10% BSF larvae compared to the control, and neutrophils were increased in 5% BSF group compared to the control. Still, they were within physiological ranges (Biasato et al., 2019).

In a study, the effect of BSF larvae was seen on the hematological profile of laying hens, and BSF was offered in three different ways; fresh, dry, and methanol extract. Similar to our study, it was seen that fresh and dried BSF diets showed no significant difference in hematological traits. Still, the BSF methanol extract group showed that the hematological parameters were significantly increased compared to all other experimental groups but were within the normal ranges (Irawan et al., 2020). In a study on Nile Tilapia fish, fish meal was substituted by BSF larvae meal at different levels. There was no significant difference in all hematological parameters among fishmeal-fed and BSF larvae-fed fish groups (Tippayadara et al., 2021). Fish meal was replaced with BSF larvae meal in African catfish, and its effect on hematological parameters showed no significant difference for all experimental groups compared to the control (Adeoye et al., 2020). In a study on sheep, soybean meal was substituted with BSF larvae meal, and coffee husk was replaced with cocoa pod husk in the diet. It was seen that there was no significant difference in hematological parameters in all experimental groups as compared to the control (Rahman et al., 2021). In a study on dogs, 5% BSF larvae meal and 10% fermented oat were evaluated as compared to the commercial diets, and the results showed that there was no significant difference in hematological parameters except basophils and white blood cells of all experimental groups as compared to the control (Seo et al., 2021).

Similar to the study reported by Abdel-Tawwab et al., 2020, it was seen that in this study, WBC, LY, MO, NEU, HCT, and MCH were not significantly different as compared to the control. In another study on Dusky kob juveniles, all of the haematological parameters were not significantly different and remained within the normal ranges (Madibana et al., 2020). In a study on European sea bass fish, fish meal was replaced with BSF larvae meal, and it was seen that there was no significant difference in RBC, HCT, MCV, HGB, MCH, and MCHC in all experimental groups (Abdel-Tawwab et al., 2020). However, in this study, MCHC was significantly different as a linear and combined effect compared to other groups. HGB in female rats as a quadratic effect was significantly different. MCV and MCHC in male rats were also significantly different as a combined and linear effect. In a study, BSF larvae meal and fat were used for the feeding of dogs, and it was seen that all of the hematological parameters were not significantly different in all experimental groups as compared to the control (Freel et al., 2021).

In a study on piglets, the fishmeal was replaced with BSF larvae meal, and it was seen that, similar to this study, there was no significant difference in WBC and RBC of piglets at the end of the trial in all groups as compared to the control. However, the pigs being fed with 25, 75, and 100% BSF diets showed decreased platelet count compared to 0 and 50% BSF groups (Chia et al., 2019). Similarly, in this study, platelet count was significantly decreased in male rats fed with 20% BSF larvae meal compared to all other groups. BSF larvae fat fed to piglets has shown that WBC, RBC, HGB, HCT, NEU, LY, MO, EO, and BA were not different significantly, but platelets count was increased linearly (Heugten et al., 2019).

Compared to this study, HGB in female rats as the quadratic effect was significantly different compared to the control. EO was significantly increased in 5 and 20% BSF groups compared to all other groups cubically. Similarly, in a study on beagles, it was seen that there was not any significant effect on hematological parameters (Hong et al., 2020). Similar to this study, in a study on weaned piglets, it was seen that there was not any significant difference in hematological parameters as compared to the control in all experimental groups, except that the values of hematocrit and hemoglobin were

increasing with increasing levels of BSF inclusion (Driemeyer, 2016). Similar results were found in a study on rabbits where BSF and yellow mealworm was supplemented as a replacement for soybean meal. There was no significant difference in hematological parameters compared to the control in all experimental groups (Gasco et al., 2019b). In reviews on BSF implications in poultry (Iqbal et al., 2019; Shah and Çetingul 2022a), antimicrobial and immunological effects of BSF in rabbits, rats, and reptiles (Shah and Çetingül 2022b), BSF importance in aquaculture (Çetingül and Shah 2022), it was reported in several types of research that there had not been any adverse effect of BSF utilization for feeding of animals as 50-70% replacement to soybean and fishmeal. The level of WBC in all groups was within the normal range (1.96-8.25, 10^3/µl), as reported by (Giknis and Clifford, 2008). The level of NEU in all groups was within the normal range, as reported by (Vigneshwar et al., 2021). All groups' LY, BA, RBC, HCT, MCV, MCH, MCHC, PLT, PDW, and MPV levels were within the normal range (0.50-4.5, 10^3/µl), as reported by (Schleh et al., 2013). The level of MO in all groups was within the normal range, as reported by (Dantas et al., 2006). The level of MO in all groups was within the normal range, as reported by (Dantas et al., 2006). The level of eosinophils in male and female rats was within the normal range, as reported by (Matsuzawa et al., 1993). The levels of HGB, P-LCR, PCT, RDW-CV, RDW-SD, and n-RBC in male and female rats were within the normal range, as reported by (Schleh et al., 2013).

4.4. Biochemical Parameters

All the biochemical parameters in serum were analysed for males and females combined in and gender-specific way. The statistical analysis of male and female rats as a combined effect showed that creatinine, total protein, AST, ALT, calcium, phosphorous, non-HDL, and IgG remained significantly unchanged for all experimental groups ($p > 0.05$), as shown in Table 3.4. Similarly, feeding beagle dogs with housefly larvae meal caused no significant difference in serum biochemistry profile in experimental groups compared to the control (Hong et al., 2020). Similarly, in a study, BSF larvae were fed to the piglets, and the results showed no significant difference in serum biochemical parameters (Driemeyer, 2016).

The uric acid level was significantly decreased compared to the control group for combined and linear effects ($p < 0.05$). Uric acid shows the protein quality; if there is less uric acid in serum, it is considered generally good in terms of protein quality. Researchers documented serum uric acid content as an analytic and predictive dynamic of several multifactorial ailments. Moreover, the uric acid level in all groups was within the normal range (1.7-3.0 mg/dl), as reported by (Nugrahaningsih et al., 2021). Moreover, ALP, total cholesterol, LDL, and HDL cholesterol levels were also significantly changed compared to the control group ($P < 0.05$).

AST (SGOT) is the enzyme that mediates the conversion of food particles into energy. If its level gets high in the serum, it indicates liver disorders because it comes from the hepatocytes to the serum. Moreover, the normal range of AST (SGOT) is 75-142 IU/L, as reported by (Giknis and Clifford, 2008), and it was seen that in this study, the level of AST was within the normal range. BSF oil was offered for feeding in a study on piglets, and its effects were evaluated as compared to soybean oil on biochemical serum parameters. The results showed no significant difference in the levels of total protein, ALT, ALP, AST, IgG, calcium, phosphorous, and creatinine (Heugten et al., 2019). In another study, fish meal was replaced with BSF larvae meal for goldfish feeding, and the results showed that BSF larvae meal feeding caused a drop in the levels of AST, ALT, total cholesterol, and LDL cholesterol with linear and quadratic effects ($P < 0.05$) (Khieokhajonkhet et al., 2022). However, in this study, the levels of total cholesterol, AST, and ALT were non-significant, and levels of total cholesterol, LDL, and HDL cholesterol were increased as linear and combined effect as compared to the control group in all experimental groups. Creatinine shows kidney function and health. Muscles make creatinine as a by-product of energy bioreactions. If kidneys are healthy, creatinine is excreted in the urine. If the creatinine level exceeds the normal range, it indicates poor function of the kidneys and kidney injuries or inflammations. The statistical analysis of the creatinine shows that creatinine level was non-significant for all groups ($p > 0.05$) in male rats. At the same time, there was a significant cubic effect in female rats ($p < 0.05$). In female rats, the creatinine level significantly decreased in the 5% group compared to the control group, while it increased significantly compared to the control for the 10% group.

However, the 15 and 20% groups were similar to the control. However, the normal range of creatinine for Wistar rats is 0.4-0.8 mg/dl, and it was seen that the level of creatinine was within the normal range (Kohn and Clifford, 2002). However, some other studies show that the creatinine levels were non-significant in all experimental groups compared to the control, as reported by Heugten et al., (2019), where piglets were fed with BSF oil as a replacement for soybean oil. Total protein in serum shows the total amount of albumin and globulin in the blood. Albumin is more than globulin in blood for healthy individuals and maintains the oncotic pressure in plasma. The statistical analysis showed no significant effect on this parameter for all groups in male and female rats ($p > 0.05$). Moreover, the average level of total protein in rats is 4.5- 7.5 g/dl (Zaias et al., 2009), and it was seen that complete protein was within the normal range in this study. Similar to this study, in a study on piglets where BSF oil was fed as compared to soybean oil and levels of total protein, albumin and globulin were unchanged (Heugten et al., 2019).

ALT (SGPT) is the enzyme that aids the liver in producing energy from food, and its levels in the body get high in case of liver disease, liver injuries, or inflammation. The statistical analysis indicates that ALT levels were significantly higher in the 10% group compared to the control as a linear effect ($p < 0.05$). However, ALT levels in the 5% and 15% groups were similar to the control. Moreover, in the 20% group, the ALT level was less than 10% but more than the ALT level in the control group. However, AST (SGOT) was statistically non-significant in all groups as male and female combined effect ($p > 0.05$). However, in males, AST levels significantly decreased linearly compared to the control in all experimental groups. Moreover, the normal range of ALT (SGPT) is 63-175 IU/L and for AST (SGOT) is 75-142 IU/L, as reported by (Giknis and Clifford, 2008), and both parameters were within the normal ranges in this study. In a study, European seabass was fed with BSF larvae meal as a replacement for fishmeal, and the results showed that there was not any significant effect on total protein, globulin, albumin, ALT, and AST levels in all experimental groups as compared to the control (Abdel-Tawwab et al., 2020). Similar to this study, in another study of cats, feeding of BSF larvae showed a significant increase in ALT in all experimental groups compared to the control group, but the ALT levels were within the normal ranges

(Pezzali and Shoveller, 2021). ALP is the enzyme produced from the liver mainly and in small quantities produced by the gall bladder, kidney, pancreas, intestines, and bones. If the liver is damaged, ALP starts to release in the blood, and its level gets high. In this study, it was seen that ALP was significantly increased in 5, 10, 15, and 20% groups as compared to the control as a combined, linear, and cubic effect in both male and female rats ($p < 0.05$). However, the levels of ALP were within the acceptable range of 80-350 IU/L in serum, as reported by (Miller and Shortliffe, 2021; Hasan et al., 2018; Owu et al., 1998). Similar to this study, in a study on dogs, it was seen that feeding of BSF larvae meal and fermented oats caused a significant effect on levels of ALP in all experimental groups compared to the control. However, the values of ALP were within the normal ranges (Seo et al., 2021).

Total cholesterol indicates the level of cholesterol is high in the body and is an indicator of blood pressure and heart diseases. The statistical analysis of cholesterol total suggests that it increased significantly in the 5, 10, 15, and 20% groups compared to the control as a linear and combined effect ($p < 0.05$). However, as reported by (Ihedioha et al., 2013), the typical range of cholesterol total is 80-130 mg/dl and was within the normal range for all groups in this study. The cholesterol levels were increased linearly in all experimental groups compared to the control in a study where BSF oil was fed to the piglets to replace soybean oil (Heugten et al., 2019). In a study on dogs, fermented oat and BSF larvae meal were provided for feeding, and it was seen that after twelve weeks of the feeding trial, the cholesterol level in serum was decreased compared to the control (Seo et al., 2021).

LDL-cholesterol indicates the bad cholesterol in the body. A high level of LDL cholesterol in the body causes the formation of plaques in blood vessels and atherosclerosis. It causes heart attacks, high blood pressure, and thrombosis in the body. The statistical analysis showed that the LDL levels increased significantly compared to the control linearly and as a combined effect ($p < 0.05$). However, the typical range of LDL cholesterol is 10-30 mg/dl, as reported by (Ihedioha et al., 2013), and it was seen that in this study, all LDL was within the normal range for all groups.

HDL cholesterol indicates the level of good cholesterol in the body. The statistical analysis of this parameter shows that it was significantly increased for the 10, 15, and 20% groups compared to the control and the maximum for the 15% group. However, the typical range of HDL cholesterol is 40-60 mg/dl, as reported by (Ihedioha et al., 2013), and it was seen that in this study, HDL level was within the normal range for all groups. Compared to the other studies, it was seen that in a study on piglets in Kenya, fishmeal was replaced by BSF larvae meal. The results showed no significant difference in total serum cholesterol, LDL cholesterol, HDL cholesterol, and triglycerides levels in experimental groups compared to the control (Chia et al., 2019). In a study on cats, BSF larvae feeding has shown that the cholesterol level decreased over time (Pezzali and Shoveller, 2021). In a study on mirror carp fish, it was seen that BSF larvae feeding caused improvement in the biochemical serum profile and cholesterol levels (Xu et al., 2021). In a study on yellow catfish, a 30% substitution of fishmeal with BSF larvae meal showed a significant increase in the level of total cholesterol and nitric oxide in serum compared to all other experimental groups (Hu et al., 2017). In a study on Jain carp, the replacement of fishmeal with BSF larvae meal has shown that the total serum cholesterol levels decreased in all experimental groups compared to the control (Li et al., 2017). Levels of calcium in the blood indicate many disorders. Very high or deficient calcium in serum represents bone, kidney, thyroid, and parathyroid diseases. The calcium level was non-significant for all groups in male and female rats ($p > 0.05$). Moreover, the normal range of calcium in the serum of albino rats is 5-10 mg/dl, as reported by (Anthony and Parsons, 1957; Boguszewska-Czubara et al., 2011), and it was seen within the normal range for male and female rats.

In a study on cats, it was seen that BSF larvae feeding caused a decrease in levels of calcium in all experimental groups compared to the control (Pezzali and Shoveller, 2021). The level of phosphorous in the blood indicates kidney disorders, and if there is too high phosphorous in the blood, it means kidney stone formation risks. The statistical analysis shows that the phosphorus level was non-significant for male rats ($p > 0.05$). In contrast, it was statistically significant for female rats as a linear effect and increased linearly for all experimental groups compared to the control ($p < 0.05$).

Moreover, the normal phosphorous range is 2.5-5 mg/dl, as reported by (Marcy, 1944; Anthony and Parsons, 1957), and it was seen within the normal range for male and female rats. In a study on dogs, it was seen that feeding of BSF larvae meal and fermented oats had caused significant effect on levels of phosphorous in all experimental groups compared to the control (Seo et al., 2021). IgG level in blood represents the presence of infections. The statistical analysis showed that it was non-significant for all experimental groups ($p > 0.05$). Moreover, the IgG levels in male and female rats were within the acceptable ranges (Salauze et al., 1994; Hedger and Hettiarachchi, 1994). Compared to other studies, feeding BSF larvae to dogs has caused no significant difference in the IgG level in all experimental groups compared to the control (Seo et al., 2021). In a study on piglets, it was seen that feeding 50% and 100% BSF larvae caused a linear increase in the level of IgG in serum (Ko et al., 2020). In a study, feeding of BSF larvae pulp to mirror carp showed a significant decrease in the total lipid profile in the body in all experimental groups compared to the control (Xu et al., 2020a).

4.5. Oxidative Stress Parameters

TOS represents how much oxidative stress exists in the individual's body. The statistical analysis showed that it was statistically non-significant for all groups when the combined analysis was performed ($p > 0.05$). However, as a gender-specific effect, TOS was statistically significant in male rats compared to the control ($p < 0.05$) as a combined, linear, and quadratic effect. Moreover, it was seen that for the 15% group, it increased to a maximum extent in male rats. Similarly, the statistical analysis of female rats shows that TOS increased significantly at 15% and 20% compared to the control ($p < 0.05$). Conversely, it was significantly decreased in the 5 and 10% groups compared to the control ($p < 0.05$). However, the values of TOS were within acceptable physiological ranges. TAS represents how much the body resists oxidative stress, and the statistical analysis shows that it was also statistically non-significant for all groups as a combined effect ($p > 0.05$). However, as a gender-specific effect, it was seen that TAS was significantly increased in male rats in all experimental groups compared to the control as a quadratic effect ($p < 0.05$). The TAS level was higher in the male 15% group compared to the other experimental groups and control ($p < 0.05$).

Similarly, for female rats, TAS was increased significantly for all experimental groups compared to the control linearly ($p < 0.05$). It was higher in the 20% group than in the other experimental and control groups ($p < 0.05$). The levels of OSI were non-significant for all groups compared to the control ($p > 0.05$). However, as a gender-specific effect, it was significantly different in female rats as a cubic effect and decreased in 5, 10, and 20% groups as compared to the control. The levels of MDA in serum reduced glutathione GSH in serum, and NOx was also measured. The statistical analysis shows that these parameters were also statistically non-significant for all groups compared to the control as combined and gender-specific effect ($p > 0.05$). Moreover, the values of the oxidative stress parameters in serum were within the normal ranges, as reported by (Ahrami et al., 2016; Saka and Aouacheri, 2017).

The MDA and GSH in the liver, kidney, and heart were analysed. It was seen that the level of MDA in the liver of male rats was significantly decreased compared to the control for the increasing concentration of BSF larvae % as a combined, linear, and quadratic effect ($p < 0.05$). Similarly, the statistical analysis showed that the level of MDA in the liver was significantly decreased for the 5% BSF female group compared to the control as cubic effect ($p < 0.05$). The levels of MDA in the kidneys of male rats decreased significantly for all experimental groups compared to the control ($p < 0.05$) as a combined, linear, and quadratic effect. However, the levels of MDA in the kidney of female rats were non-significant for all groups ($p > 0.05$). The levels of MDA in the heart indicated that it was statistically non-significant for all male and female rats compared to the control ($p > 0.05$).

The statistical analysis showed that the level of GSH in the liver was non-significant for male rats in all experimental groups compared to the control ($p > 0.05$). However, it was seen that the level of GSH in liver was significantly increased in female rats linearly with an increasing percentage of BSF larvae in all experimental groups compared to the control ($p < 0.05$). The levels of GSH in the kidney of male rats were significantly increased for all experimental groups as compared to the control ($p < 0.05$) as a combined, linear, and quadratic effect. It was seen that the levels of GSH in the kidney were significantly increased with increasing inclusions of BSF larvae ($p < 0.05$).

However, the levels of GSH in the kidney of female rats decreased considerably in the 5% BSF group compared to the control as cubic effect ($p < 0.05$). The level of GSH in the heart of male rats was also statistically non-significant compared to the control ($p > 0.05$). However, GSH levels in the female rats' hearts decreased significantly compared to the control ($p < 0.05$) as the combined and linear effect. Regarding the healthy ranges of oxidative biomarkers, the levels of MDA in the liver and kidney of male rats were higher compared to the female rats, and the levels of GSH in female rats were higher than that of the male rats. In conclusion, the levels of MDA and GSH in the liver and kidneys presented themselves in a healthy range. It showed that increasing the levels of BSF larvae improved the antioxidative stress conditions compared to the control, and the levels of MDA and GSH in the heart presented themselves in a healthy range, as reported by (Bahrami et al., 2016; Saka and Aouacheri, 2017).

Comparing the results of oxidative parameters in this study with other studies, it was seen that the replacement of fishmeal with BSF larvae meal for feeding African catfish showed that there was no significant difference in the levels of MDA in all experimental groups as compared to the control (Fawole et al., 2020). In another study, the maximum inclusion of BSF larvae meal for feeding Siberian sturgeon fish showed no significant difference in MDA levels in the liver and kidney in all experimental groups as compared to the control (Caimi et al., 2020). In a study on dogs, housefly larvae feeding showed a decrease in levels of MDA in serum compared to the control (Hong et al., 2020). The antioxidant activities of BSF larvae protein derivatives and hydrolysates were analysed in a study compared to the fishmeal and chicken meal. The results showed that BSF peptides better protected the cellular and oxidative damage caused by myeloperoxidase and neutrophil activities (Mouithys-Mickalad et al., 2020).

In a study on rainbow trout, it was seen that the feeding of BSF larvae meal caused no significant effect on MDA levels in the liver and kidney. Still, other oxidative enzymatic activities were changed (Elia et al., 2018). In a study on piglets, feeding yellow meal worms showed no significant effect on MDA and GSH levels in tissues of all experimental groups (Ringseis et al., 2021). In a study on European seabass fish, it was seen that feeding of BSF larvae as 35 and 50% replacement to fishmeal caused a

significant increase in serum MDA concentration compared to the control (Abdel-Latif et al., 2021). In a study on mirror carp fish, the comparative effect of BSF larvae oil, silkworm oil and yellow mealworm oil was evaluated. The results showed that the levels of MDA in the liver decreased significantly compared to silkworm and yellow mealworm oil (Xu et al., 2020b). In a study on yellow catfish, a 30% substitution of fishmeal with BSF larvae meal showed a significant decrease in superoxide radical formation in serum compared to all other experimental groups (Hu et al., 2017). In a study on Jain carp, the substitution of fishmeal with BSF larvae showed a significant increase in antioxidative status because of increased catalase enzyme activity in all experimental groups compared to the control (Li et al., 2017). The feeding of BSF larvae showed a significant linear increase in catalase activity and consequently decreased serum MDA levels compared to the control (Xu et al., 2020b).

4.6. Histopathology

The histopathology of the duodenum, liver, kidney, heart, ovaries, uterus, and testis was performed for male and female rats, and this section is elaborated in detail. Changes in the structure of the intestinal lumen and mucosa are often used to analyse gut functionality, for instance, during physiological development or in response to specific food and feed ingredients (Skrzypek et al., 2005). Villus length is one of the parameters for determining the absorption of nutrients from the intestines into the bloodstream or bioavailability (Awad et al., 2008). For instance, in a study on broilers, the supplementation of fiber and betaine caused an increase in the length of the villus (Santos et al., 2019). BSF larvae and pupae have higher percentages of chitin in their composition; according to a study, it varies from 8-24%. Most chitin is present in shedding and cocoons (Soetemans et al., 2020).

In this study, it was seen that there was a significant increase in the villus length with an increasing percentage of the BSF larvae inclusion as a replacement to soybean meal (Table 3.32), and it was because of the chitin content in the BSF larvae and the lauric acid which has antimicrobial activities. Similar to this study, replacing soybean oil with BSF larvae oil at 0, 25, 50, 75, and 100% in broilers has caused a significant increase in the villus length (Chen et al., 2022).

Contrary to this study, in a study on Japanese Eel, fishmeal was replaced with defatted BSF larvae at levels 0, 15, 30, 45, 60, and 75%. It was seen that the villus length was significantly decreased in the 75% replacement group, and the density of goblet cells was also considerably lower in the 45, 60, and 75% groups (Kuo et al., 2022). In a study on broilers, soybean oil was replaced at 0, 25, 50, 75, and 100% BSF oil, and it was seen that the villus height was not significantly changed. However, the crypt depth and villus height/crypt depth ratio were changed considerably compared to the control (Chen et al., 2022). Similar to this study, in research on native chicken, the replacement of fishmeal with maggot meal has caused a significant impact on the villus length (Auza et al., 2021). Similarly, replacing soybean oil with BSF larvae oil in broilers has caused a considerable increase in villus length, but there was no difference in digestibility (Kim et al., 2021).

This study showed no histopathological changes in male rats' liver, kidney, heart, and duodenum, as shown in Figure 3.19 and Figure 3.21. Moreover, in female rats, hyaline cylinders were present in all treatment groups (Figure 3.20, B2-B5), and oedema formations were observed in the ovarian interstitial cells (Figure 3.20, D3-D5). No other histopathological findings were found in the liver, uterus, heart, and duodenum. Similar to this study, it was seen that defatted BSF larvae meal inclusion in the ducks' diets did not cause any significant change in the histopathological findings (Gariglio et al., 2019). Similar to this study, in research on Atlantic salmon, the replacement of fishmeal with BSF larvae meal has shown no adverse effects on liver and muscle health and an improved distal intestinal histology (Weththasinghe et al., 2021). Similar to this study, supplementation of BSF larvae fat in broilers has caused no significant effect on the gut morphometric traits and histopathological findings in the organs (Dabbou et al., 2021). Similar to this study, 50% and 100% replacement of soybean oil with BSF larvae oil in broilers has caused no significant effect on gut morphometric traits. Some histopathological changes in the liver, spleen, thymus, and bursa of Fabricius were seen in all experimental groups, but these histopathological findings were not significant (Schiavone et al., 2018).

In a study on piglets, including partially defatted BSF larvae meal caused no significant effect on gut morphometry and histopathological findings (Biasato et al., 2019). Similar to this study, in research on broilers, the inclusion of yellow mealworm did not cause any significant effect on histopathological findings in the liver, kidney, heart, and testis (Biasato et al., 2018). Similar to this study, in research on rabbits, it was seen that the inclusion of BSF larvae fat did not cause any effect on gut morphometric traits, and tissue histopathology was unaffected (Gasco et al., 2019). Similarly, in a study on broilers, BSF larvae have caused no significant effect on intestinal morphometric traits and organ histology (Hartinger et al., 2021). Similar to this study, in research on Atlantic salmon, BSF larvae inclusion did not cause any significant effect on gut histology (Leeper et al., 2022).

5. CONCLUSION

To sum up, the study recommended that BSF has a great potential as a nutrient enriched alternative feedstuff for rats' diet and the study served as a model for monogastric animals. Interpreting to the consequences of its application in rats, up to 20% replacement of soybean with BSF larvae have a lot of scope for feeding to monogastric animals without causing any negative impact on blood chemistry, tissue and serum oxidative parameters, and histopathology. Moreover, the partial replacement of conventional feedstuffs with BSF larvae is highly encouraged. Even though numerous studies on the effects of BSF larvae as a feedstuff in poultry, pigs and a few studies in rabbits have been reporting no negative effects of partial replacement of BSF larvae on poultry and pigs, this study is the first one where BSF larvae are used in rats for feeding. This shows that BSF larvae can substitute soybean meal alternatively.

Furthermore, considering the benefits and prospects of BSF larvae for better sustainability, it is highly recommended to feed it to poultry, fish, pigs, cats, dogs, quails, ducks, reptiles, birds, and laboratory animals and the EU allows the use of BSF larvae for their feeding to these animals. Briefly, concerning global food and feed security, alternative feed resources are of prime importance and BSF larvae are more economical, environmentally friendly, sustainable, and have health promoting nutritional profile in terms of presence of chitin, lauric acid, antimicrobial peptides and essential amino acids. Compared to the soybean meal, BSF larvae are totally free of genetically modified organism (GMO) and BSFL promotes more healthy and organic livestock production. There should be more promotion and validation of the beneficial aspects of BSF larvae to maintain food and feed supply chain related to monogastric animals.

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