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Research Article

Bio-treatment of Cheese Whey by Black Soldier Fly Larvae (*Hermetia illucens*) reared in Algeria

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Abstract

Whey, a by-product of cheese industry, poses environmental challenges when discharged untreated. This study aimed to mitigate whey pollution and transform it into value-added products using black soldier fly larvae (BSFL). By investigating whey effects on BSFL growth and performance, an innovative waste management strategy was explored. The five-old-day larvae were fed with four diets; dry spent coffee ground (SCG) as the control diet, while SCG wetted with acid, sweet and fermented whey were the test diets. The results showed that whey types increased significantly ($p < 0.05$) BSFL growth compared to the control, the dry weight (53.83±0.49 Vs 25.10±0.18 mg/larva), growth rate (1.59±0.06 Vs 0.44±0.01 mg/day) and survival rate (78.83±1.25 Vs 68.50±1.5 %) of larvae and reduced their development time (28.00±01 Vs 37.00±01 day). Furthermore, mixing SCG diet with whey enhanced positively their performance by increasing the waste reduction (26.73±0.81 Vs 16.87±0.37 %), waste reduction index (0.85±0.03 Vs 0.45±0.01 %/day), approximate digestibility (36.49±1.52 Vs 20.29±0.53 %) and bioconversion rate (17.94±0.47 Vs11.65±0.52 %). Adding whey to SCG diet increased protein content of BSFL (40.11 % vs. 34.18 %) and reduced fat (36.06 vs. 37.87 %) in dry mass. Whey also improved the composition of frass by increasing the phosphorus and total nitrogen levels. This approach demonstrates a sustainable solution for dairy industries waste, contributing to the circular economy and offering potential applications in animal feed and fertilizer production.

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1. Introduction

In the world, dairy industries are the main agri-food manufacturing that generates a large amount of wastewater and causes a lot of damage to the environment by generating different kinds of liquid effluents (Domingos et al., 2017). One of the most potential pollutants of them is cheese whey, a by-product of cheese industry, rich in organic matter (lactose, protein and lactic acid), mineral elements and a large amount of microorganisms (Sommella et al., 2016). Whey causes a great environmental impact if it is released untreated in nature and it is considered as an enormous economic lost (Papargyropoulou et al., 2014; Yadav et al., 2015). Furthermore, it is estimated that the production of 1 kg of cheese discharges approximately 10 litres of whey (Pires et al., 2021). Its global annual production reached a massive amount about 200 million tonnes in 2016 with a linear increase of 3 % per year (Domingos et al., 2018).

Algeria like several countries in the world experienced notable development in the dairy sector with the non-existence of an enhancement of whey which poses a problem and become a challenge for the dairy industry to reduce its dangerous impact on the environment (Acem et al., 2019; Pires et al., 2021). The treatment of this bio-waste is necessary in order to respond to a double problem; an environmental issue and an economic loss through developing a new creative way of rehabilitating whey as a value-added product (Papargyropoulou et al., 2014; Salomone et al., 2017). The management of whey became a challenge for dairy industry to reduce its hazardous impact on ecosystems (Barba, 2021; Bosco et al., 2018). Algeria has enacted several laws in order to protect the environment, which is reflected in (Journal officiel de la république algérienne démocratique et populaire n°77, Loi n° 01-19, 2001). It obliges factories and companies to manage their solid and liquid effluent wastes. The recovery and/or disposal of waste must be carried out in conditions that comply with environmental standards, in particular without: put in danger the health of people and animals and without posing a risk to water resources, soil or air, nor to fauna and flora (Journal officiel de la république algérienne démocratique et populaire n°77, Loi n° 01-19, 2001).

There are many techniques for cheese whey recovery but most of them are highly expensive (Papargyropoulou et al., 2014). Current whey management practices often face inefficiencies in disposal, resource use, and environmental impact.These limitations emphasize the urgent need for innovative solutions that can revolutionize whey management and maximize its potential impact. Implementing advanced technologies such as biorefining, anaerobic digestion, and whey valorization can not only mitigate waste but can also generate valuable products such as biofuels, functional ingredients, and bioplastics (de Albuquerque et al., 2020; Yadav et al., 2015). Adopting these innovative approaches holds the promise of transforming whey from a costly by-product into a valuable resource, benefiting both industries and the environment (Lappa et al., 2019; Pescuma et al., 2015; Zotta et al., 2020).

It is estimated that insect-based food waste treatment,particularly black soldier fly larvae (BSFL) *Hermetia illucens* (Linneus 1758) (Diptera: Stratiomyidae), is progressively more recognized as an environmentally friendly and low-cost method of installation and recycling. The treatment of bio-waste by these larvae has received considerable attention in latest years (Magee et al., 2021). According to the literature, BSL larvae have shown promise in managing various organic wastes including by-products (Hadj Saadoun et al., 2020), spent coffee ground (Permana & Ramadhani, 2018; Fisher et al., 2021), animal manures (Oonincx et al., 2015a; Rehman et al., 2017a), human faeces (Banks et al., 2014) and vegetable waste (Spranghers et al., 2017; Parra Paz et al., 2015). Moreover, the rapid breakdown of waste by the larvae and the resulting compost has strong potential as a sustainable alternative to traditional composting methods, with the added benefits such as suppressing plant pathogens present in soil (Dehliz et al., 2023; Widyastuti et al., 2021).

In the light of the above, the study aim was to assess the ability of *Hermetia illucens* to manage and bioconvert whey into useful products with low cost aligns well with the broader goals of environmental protection and economic sustainability. By other words, the effect of supplementing acid, sweet and fermented whey to spent coffee ground (SCG) diet on the growth and performance of BSF larvae as well as their potential in convert them into biomass rich in protein and fat for animal feed, and frass used as fertilizer.

2. Materials and methods

2.1. Animal materials and substrates

Black soldier fly larvae (*Hermetia illucens* L. 1758) were obtained from a colony of a small facility for the rearing of black soldier fly located in the city of Oued souf in south-eastern Algeria.

Four distinct substrates were utilized to feed Black Soldier Fly (BSF) larvae in the experiment. Firstly, chick starter feed was purchased from the FabGrain Company (Tiaret city, Algeria), used to feed the five days old larvae. Secondly, damaged fruits and vegetables were obtained from a local market in Tiaret city, were cut into small pieces of 0.5-1 cm and used for the preliminary preparation of larvae for experimentation. Thirdly, acid whey was obtained from Sidi Saada dairy (Relizane city, west of Algeria) while sweet whey was obtained from El Manbaa dairy (Saida city, west of Algeria). The fermented whey was obtained by incubating sweet whey at 37° C for 24 hours (Boudjema et al., 2009). The samples were transported under 4° C and must be used the day after their arriving to laboratory. Lastly, spent coffee ground was collected from Elbahdja Cafeteria located in Tiaret city, being completely dried at room temperature (28 ± 2° C) and kept in freezer (-25° C).

2.2. Experimental design

Preliminary preparation of larvae

10-12 days old BSF larvae were placed in a plastic box (60 x 40 x 12 cm) (Length x width x height) and kept in a small room (3 x 2 x 3 m) in laboratory under conditions (29 ± 2° C, 45 ± 11 % relative humidity and L12:D12 photoperiod). They were provided with damaged fruits and vegetables mixed with chick starter feed. When the majority of larvae transformed into pupa; characterized by a black colour of its cuticle, contrary to the larvae which have a white colour (May, 1960), they were placed in a new box (pupation container) containing carpentrywaste in the obscurity. The box was covered by a dark cage connected to a mesh (70 x 50 x 90 cm) serving as a transition space for pupae after they undergo metamorphosis (transforming of pupae into an adult fly). The mesh was exposed to a light source at a distance of about 60-70 cm and provided with water and an attractive containing fermented fruits and vegetables mixed with poultry feed in order to attract adult fly to lay their eggs in the eggie (this system was designed to facilitate the collection of BSF eggs). At each leg of the table, a container filled with water and drops of detergent was used as ant traps to safeguard BSF boxes and mesh from ant invasion (Dortmans et al., 2021).

Feeding of five-day-old-larvae (5-DOL)

After fly laying, the eggs < 24-hours-old were collected and placed over a strainer with four feet in a box using the method described by Dortmans et al. (2021) under the same previous conditions. After hatching, the new larvae fall directly onto the substrate and feed until they reach five days.

The feeding boxes containing the broiler chick starter feed (30 % dry matter, 70 % moisture) wetted with water as control (SCG), sweet whey (SCG+SW), acid whey (SCG+AW) and fermented sweet whey (SCG+FW).

BSF larvae rearing

The rearing of the black soldier fly (BSF) was done according to the method of Dortmans et al. (2021). Five days old larvae divided into four lots of 200 placed in plastic containers (11.5 x 9 x 4 cm) containing 30 % of dry spent coffee ground damped with 70 % of water as control diet, or sweet whey, acid whey and fermented sweet whey as test. Three replicates were made per treatment. The amount of added food was provided each five days with an average of 200 mg/larva/day (Manurung et al., 2016; Permana & Ramadhani, 2018).

BSF larvae traits

The BSF larvae were harvested after observing the first pre-pupa. From each container, all harvested larvae were counted and washed under running distilled water to remove residues, and their integuments were dried with paper towels. The dry mass of larvae was dried in an oven at 70° C to constant weight then kept in freezer at -25 °C.
To assess the efficiency of BSF larvae and reduction of substrates, many parameters were calculated:

- Survival rate (SR) was calculated to determine the feed effect following the equation below (Diener et al., 2009):
  Survival rate (SR) = (survival larvae numbers x 100)/ initial larvae numbers
- Growth rate of larvae (GR) was calculated by Diener's formula (Diener et al., 2009):
  Growth Tate (GR) = (initial dry weight –final dry weight) (mg) / time (days)
- Approximate digestibility was calculated according to Permana and Ramadhani (2018)
  Approximate digestibility (AD) = (Substrate-Residue) (g) x 100/ Residue (g)
- Waste Reduction Index (WRI) was measured according to Diener et al. (2009):
  Waste Reduction Index (WRI) = Waste Reduction /Time
  Waste Reduction (WR) = (Substrate-Residue) (g) x 100/Substrate (g)
- The ability of BSF Larvae to convert substrates into their biomass was calculated by Biomass Conversion Rate (BCR) and Feed Conversion Rate (FCR) with the formula below (Rehman et al., 2017b):

Biomass Conversion Rate (BCR) = [BSF Larvae biomass (g) / substrate consumed (g)] x 100
Feed Conversion Rate (FCR) = substrate consumed (g) /BSF Larvae biomass (g)

2.3. Chemical analysis of substrates, biomass and frass

Sweet, acid, fermented whey, dry spent coffee ground, dry harvested BSF larvae and frass were stored in freezer (-25° C) until their analyses. The biochemical composition of the BSF larvae, dry spent coffee ground and frass was analysed on dry mass basis, however sweet, fermented and acid whey were analysed on liquid basis.

Relative humidity was calculated using method of (AOAC, 2005, Method 930.15), ash was determined according to AOAC, 2005, Method 942.05 procedure, titratable acidity was found by Mathieu (1998), PH by PH/ORP meter (HI 2211, HANNA Instruments Inc , Woonsocket-USA), electrical conductivity by conductivity meter (EC 214, HANNA Instruments Inc , Woonsocket-USA), crude fat was calculated according to AOAC, 2005, Method 2003.05, crude fibre was determined according to AOAC, 2005, Method 978.10 procedure, lactose by method of (Dubois et al., 1956), phosphorus by method of (Pansu & Gautheyrou, 2006), Organic carbon was determined by (Nelson & Sommers, 1983). Total nitrogen was calculated by Kjeldahl Copper (AOAC, 2012, Method 2001.11), crude protein of BSF larvae was calculated by multiplying total nitrogen to 6.25 (Rumpold & Schlüter, 2013; Janssen et al., 2017). Whey proteins were calculated by method of Lowry et al. (1951).

2.4. Statistical analysis

The statistical analysis of all assays carried out in triplicate was performed with IBM SPSS statistic v 16.0 for Windows. Averages ± standards deviation were used to present data in this study. One-way ANOVA was used after pre-confirmation of the homogeneity of variance. If significant differences were noticed at $p < 0.05$ (when the results are too far, you go until 0.0001), than a Tukey's post-hoc test was performed to determine differences between the treatments.

3. Results

3.1. Biochemical composition of substrates

The nutritional content of the three types of whey is shown in (Table 1). Sweet whey had higher lactose and protein than acid and fermented whey, making it more nutritionally concentrated compared to acid whey. However fermented whey had the highest levels of lactic acid and therefore the lowest pH values. The differences in nutritional content can be attributed to the type of processing and the specific components removed during the production of each type of cheese (Božanić et al., 2014).

Table 1. Biochemical composition of acid, sweet and fermented whey

Nutritional Content	Acid whey	Sweet whey	Fermented sweet whey
Dry matter (g/l)	64.96±0.29	68.91±0.39	ND
Proteins (g/l)	6.21±0.32	6.92±0.18	ND
Lactose (g/l)	44.57±0.35	49.6±0.43	ND
Fat (g/l)	1.30±0.22	1.57±0.36	ND
Lactic acid (g/l)	3.23±0.16	1.08±0.08	4.46±0.15
Ash (g/l)	7.91±0.35	7.08±0.26	ND
PH	4,66±0.12	6,09±0.16	4.06±0.1

ND=Not detected.

The Table 2 recapitulates information about the nutritional content of spent coffee ground. The results showed a proteins rate of 14.22 % which expressed a low protein /carbohydrate ratio of ¼, along with a high crude fibre and crude fat levels. The pH of spent coffee grounds was slightly acidic.

Table 2. Spent coffee ground nutritional content on dry matter basis

Nutritional Content	Spent Coffee Ground
Ash	1.59±0.05
Crude proteins	14.22±0.32
Crude fat	13.29±0.57
Crude fibre	19.16±0.31
Total carbohydrate	61.58±0.85
Ratio proteins to carbohydrate	¼
PH	5.32±0.13

3.2 Effect of supplementation of different types of whey diet on dry weight mass, development time, survival rate, and growth rate of BSF larvae

This study showed that the addition of acid, sweet and fermented whey to spent coffee ground diet had high significant effect on the growth and the performance of black soldier fly larvae.

Dry weight mass

The use of different types of whey in diet with SCG had high significant effect on increasing of the final dry weight of black soldier fly larvae ($P < 0.0001$) (Figure 1). The BSF larvae in FW+SCG feeding were significantly the heaviest (53.83±0.49 mg/larva) compared to those fed with other diets. However, SCG-fed larva had significantly the lowest dry weight with 25.10±0.18 mg/larva only. There was no significant difference in final dry weight of larvae between larvae fed with SW+SCG and AW+SCG ($P > 0.05$). Overall, the dry weight of larva fed with different whey combinations (AW+SCG, SW+SCG and FW+SCG) was approximately double that of the dry weight larva fed only with SCG.

Development time

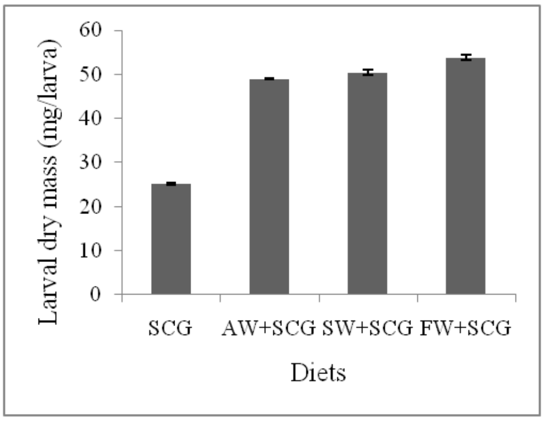
The results showed a significant difference of time needed for the development of larvae between the control diet and the other diets ($P < 0.0001$). The larvae nourished with AW+SCG, SW+SCG, and FW+SCG grew substantially faster than those of control diet SCG which had the longest development time by (37.00±01 days). The shortest development time was in BSF larvae fed at FW+SCG (28±01 days) (Figure 1).

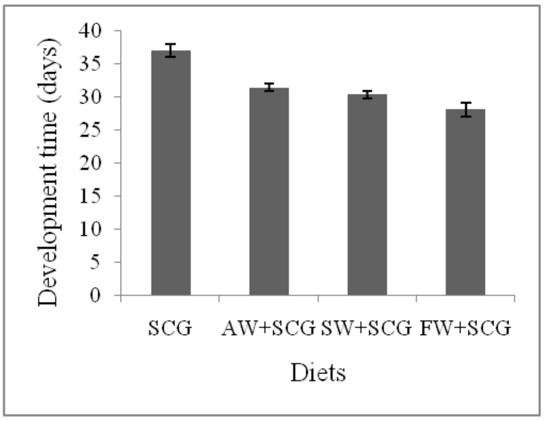
Survival rate

Concerning the survival rate of BSF larvae, results confirmed a significant difference between SCG diet and the SCG diet supplemented with whey ($P < 0.0001$). However, the survival rate in presence of different whey classes was approximately similar ranging from 77 to 78.83 % (Figure 1).

Growth rate

Identically, a significant lower growth rate ($P < 0.0001$) was observed with pure SCG diet against the three whey-enriched SCG diet. The most relevant growth rate ($p < 0.05$) was noted with FW+SCG association (Figure 1).





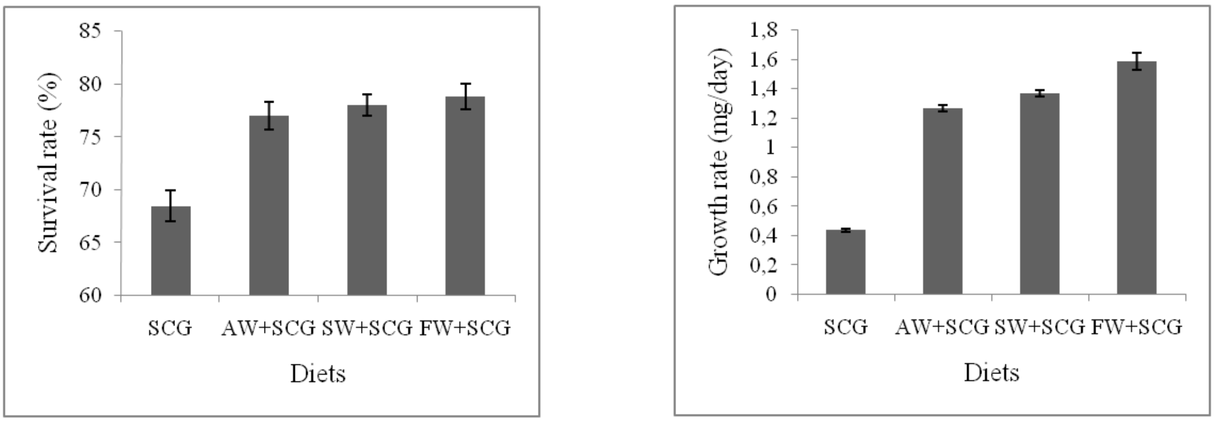


Figure 1. Larval Growth based on mean (±SD) larval weight, development time, survival rate and growth rate of BSF larvae fed with SCG; Spent Coffee Ground, AW+SCG; Acid Whey with Spent Coffee Ground, SW+SCG; Sweet Whey with Spent Coffee Ground, FW+SCG; Fermented whey with Spent Coffee Ground

3.3 Effect of supplementation of different types of whey diet on performance of BSF Larvae

Substrate reduction and waste reduction index

The supplementation of different types of whey to the spent coffee ground diet increased significantly the performance of BSF larvae (Figure 2). The substrate reduction (SR) refers to the ability of larvae to reduce organic matter of substrates while the waste reduction index (WRI) represents the SR by time. The percentage of substrate reduction was significantly different between larval diets ($P < 0.0001$). With regard to whey's category, all supplemented regimes were more efficient by giving a higher dry matter reduction ranging from 22.98±0.42 to 26.73±0.81 %. The latter representing the peak value was recorded for the FW+SCG diet. Furthermore, the same highly significant superiority ($P < 0.0001$) of whey diets (up to 0.85±0.01) over the control (0.45±0.01) was noted for the waste reduction index (WRI), without a statistical difference between all test treatments (Figure 2).

Biomass Conversion Rate, Approximate Digestibility and Feed Conversion Rate

Biomass Conversion Rate (BCR) defines the efficiency of larvae to convert the digestive feed into its biomass. Hence, BCR value has a relation with the Approximate Digestibility value (AD) that reveals the amount of food assimilated by larvae (Permana & Ramadhani, 2018). What is more, BCR and AD of larvae fed with whey treatments were significantly greater than those reared at the SCG diet ($P < 0.0001$), respectively. The FW+SCG diet had the most remarkable impact on both qualities; AD (36.49±1.52 %) and BCR (17.94±0.47). On the other hand, significant differences ($P < 0.0001$) in Feed Conversion Rate (FCR) were found among diets (Figure 2). The FCR value of the control diet averaged 8.59±0.38 was significantly the highest value.

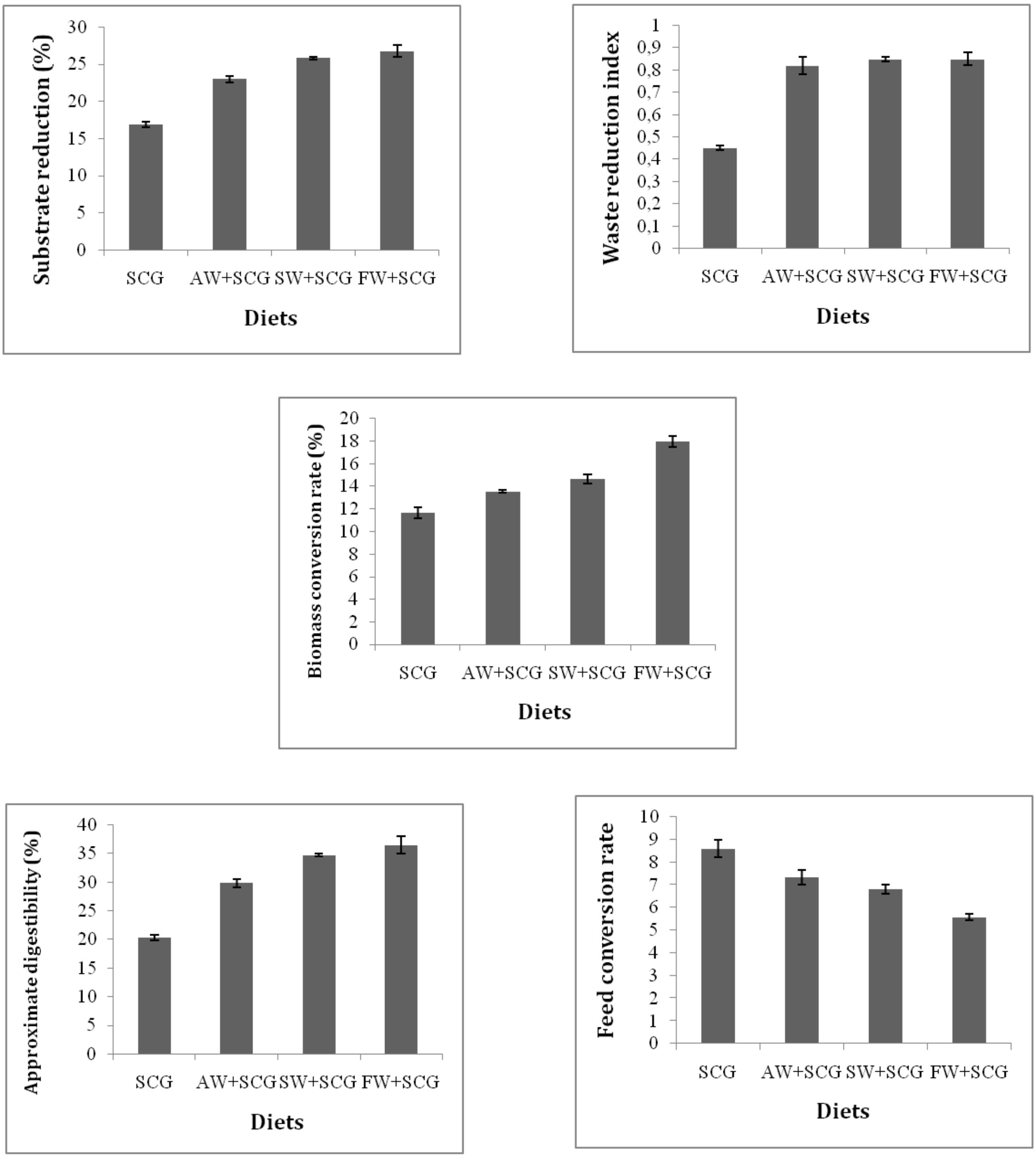


Figure 2. Larval performance based on mean (±SD) Substrate Reduction (SR), Waste Reduction Index (WRI), Approximate Digestibility (AD), Biomass Conversion Rate (BCR) and Feed Conversion Rate (FCR)

3.4 Nutritional content of BSF larvae

The protein content of BSF larvae was significantly affected in a positive manner by the integration of different types of whey to SCG diet ($P < 0.0001$) (Table 3). Overall, the types of whey added to SCG diet enhanced significantly, but with slight differences, the ability of BSF larvae to produce more protein ranged from 38.22±0.38 to 40.11±0.40 % than those fed at SCG (34.18±0.59

%). Inversely, the BSF larvae fed at SCG without whey produced significantly the highest fat content ($P < 0.0001$) reaching 37,87±0.24 % (Table 3). The obtained results for fat content produced by larvae was similar for all SGC whey mixtures. Larvae fed at FW+SCG had the maximum crude fibre content (11.21±0.43 %), where those fed at SCG had the minimum values (5.55±0.38 %). Numerically, the ash content of larvae fed at SCG with whey was around 07 %, however for those fed only at SCG, it was about 5.46±0.26 %.

Table 3. Mean (± SD) nutritional content percentage of BSF larvae in dry mass fed with SCG diet, AW+SCG, SW+SCG, and FW+SCG

Diets	Dry mass	Crude protein	Crude fat	Crude fibre	Ash
SCG	31.41±0.34	34.18±0.59	37.87±0.24	5.55±0.38	5.46±0.26
AW+SCG	35.74±0.57	39.15±0.25	35.18±0.31	11.21±0.43	7.03±0.30
SW+SCG	36.06±0.37	40.11±0.40	35.53±0.11	8.38±0.37	7.09±0.22
FW+SCG	37.16±0.22	38.22±0.38	36.06±0.26	9.92±0.37	6.92±0.08

SCG; Spent Coffee Ground, AW+SCG; Acid Whey with Spent Coffee Ground, SW+SCG; Sweet Whey with Spent Coffee Ground, FW+SCG; Fermented whey with Spent Coffee Ground

3.5. Chemical composition of frass

The addition of whey to SCG diet affected slightly the chemical composition of frass (Table 4). The dry mass of residue in SCG diet was the noticeable value (84.99±1.58 %), while the addition of whey fraction of various nature lowered it to an extent between 79.82±1.04 to 82.08± 1.88 %. This frass contained a high organic matter spanning from 97.76±0.23 to 96.34±0.52 %. Furthermore, Organic Carbon content of frass was approximately similar for all treatments with a top value of 6.46±0.15 % for AW+SCG diet. Likewise, there were no noticeable differences in carbon, total nitrogen and carbon to nitrogen ratio among residues of larvae fed at SCG alone or with whey. On the other hand, the supplementation of whey to SCG diet affected favourably the amount of ash content and available phosphorus of frass; their values were respectively up to (3.64±0.15 % and 66.37±0.24 ppm) for AW+SCGdiet, incomparison to those fed only at SCG (2.24±0.09 % and 37.12±0.23 ppm). In regard to Electrical conductivity, the results were also increased by the presence of whey in diet, ranging from 2.37±0.09 to 2.51±0.11 $mS.cm^{-1}$, and affording a shift of 1 $mS.cm^{-1}$ compared to SCG diet (1.59±0.09). Finally, the final PH of frass was somewhat neutral to basic.

Table 4. Chemical composition of frass in dry matter

Chemical Composition	SCG mean ±SD	AW+SCG mean ±SD	SW+SCG mean ±SD	FW+SCG mean ±SD
DM%	84.99±1.58	80.95±1.32	79.82±1.04	82.08±1.88
Ash%	2,24±0.09	3,64±0.27	3,12±0.17	2,94±0.25
OC %	6,42±0.22	6,46±0.15	5,99±0.30	6,23±0.15
TKN %	0,340±00.0	0,363±00.0	0,357±00.0	0,351±00.0
C:N ratio	18,44±0.68	17,77±0.19	16,93±0.59	17,79±0.30
AP (ppm)	37,12±0.23	66,37±0.24	60.02±0.40	59,41±0.22
EC mS cm−1	1,59±0.09	2,51±0.11	2,37±0.09	2,42±0.21
PH	7,1±0.04	7,60±0.04	7,40±0.11	7,31±0.08

DM; dry matter, OC; organic carbon, TKN; total Kjeldhal nitrogen, C: N Carbone to nitrogen ratio, AP; available phosphorus, EC; electrical conductivity, PH; potential of hydrogen

4. Discussion

4.1. Effect of whey supplementation to spent coffee ground diet on BSF larvae growth

This study showed that BSF larvae have the ability to manage and digest whey with SCG diets. Furthermore, the results showed clearly a huge significant positive effect of whey addition to the SCG diet on growth of BSF larvae.

The mixing of whey with SCG diet increased significantly the final dry weight of BSF larva (53.83±0.49 mg); it was approximately similar with those fed with poultry feed (55.6 mg) found by Gold et al. (2020). Even, the larvae fed only at SCG had the longest period for their growth by (37.00±01 days) comparably with that found by Permana and Ramadhani (2018) and Manurung et al. (2016) by around 38 days, nevertheless, the addition of whey on this control diet shortened the time needed for their development significantly by 9 days in the best cases. For the survival rate, its value of BSF larvae fed with SCG was far more than those fed with the same substrate in the recent study of Fischer et al. (2021) by (45.13 ± 10.2 %), and this gap was accentuated by mixing SCG with different types of whey (78.83±1.25 %) in a resembling way to the larvae fed at artificial feed in the work of Oonincx et al. (2015b) by (77 %). In addition, larvae growth rate was another parameter modulated by whey's inclusion to SCG diet. It was significantly heightened vis-à-vis those fed at SCG alone. The growth rate in this study was lower than that found by Gligorescu et al. (2020) (1.59±0.06 compared to 1.6 to 2.8 %). BSF larvae fed only on SCG grew slowly, had the lowest final dry weight, had the least survival rate, and growth rate. It may be caused by nutritional content of spent coffee ground that contains high amount of fibre, and low protein to carbohydrates rate. Indeed, it was previously confirmed that BSF larvae did not digest well fibre (Li et al., 2015; Permana & Ramadhani, 2018). Furthermore, unbalance ratio of protein to carbohydrate affected the growth, feed intake and prolong time development of larvae (Nation, 2008). It could be due to an another factor which is the negative impact of the hardness of SCG and their evaporation caused by the increase of room temperature which was ranging from 27 to 31° C, and as a result relative humidity decreased to reach 35 %. In addition, what was observed in this study that the drying of substrates obstructed the locomotion of larvae through the feed, and thus, it could reduce the nutrient availability and the growth of larvae. For this reason, a small volume of distilled water was added and shaking of containers was done every day to avoid the drying of substrates. Manurung et al. (2016); Oonincx et al. (2015a) and Rehman et al. (2017a) reported that the optimum temperature and relative humidity were 27-28° C and 60-70 %, respectively. According to Gobbi et al. (2013), high drying of feed caused by evaporation had a negative effect on growth of BSF larvae. Regard to different types of whey effect, growth of larvae was affected dramatically positive by the combination of whey with SCG diet. Hence, they grew fast, had a high final dry weight, the highest survival rate and growth rate. It may be caused by the richness of whey nutritional content. As mentioned above, whey contains an important amount of organic matter as lactose, protein and lactic acid. Therefore, whey's protein could enhance positively the nutritional value of SCG diet for larvae, that is why its effect was positively reflected on their growth parameters. In addition, a recent study showed that the supplementation of nitrogen to high-fibre diet increased significantly larval growth and consumption of substrate by BSF larvae (Palma et al., 2019).

Among the diets added with whey, this study showed that BSF larvae fed at SCG diet with fermented whey had the highest significant final dry weight, the most growth rate and short development duration. This could be affected by the quality and quantity of sweet whey fermentation products, such as lactic acid, and the likelihood of obtaining free soluble nutrients after fermentation including available amino acids, monosaccharides, perhaps minerals or even bacterial metabolites. A recent study by Gebiola et al. (2023) confirmed that the fermented substrates had a positive effect on growth of BSF larvae. They used bokashi-fermented as substrates for the rearing of

BSF larvae. Their results illustrated that bokashi-fermented diet increased final dry mass, the growth rate, and reduce the development time of larvae.

4.2. Effect of supplementation of different types of whey diet on performance of BSF Larvae

It is noticeable that the use of whey with SCG diet improved significantly the performance of BSF larvae. In fact, this was reflected positively on the reduction of dry matter by BSF larvae compared with the control diet (Figure 2). This study showed that the substrate reduction was lower than found in other studies, namely, the research of Miranda et al. (2019) in which it ranged from 30-48 % and that of Rehman et al. (2017a) where it reached 72 %, both using different types of manure. The evaporation of spent coffee ground may make it difficult for larvae to pass through feed. According to Gobbi et al. (2013), this incident happened quickly in small container and less quantity of substrates. Consequently, the access of larvae to feed was diminished. Gligorescuet al. (2018), Permana and Ramadhani (2018) and Myers et al. (2014), stated that decrease of moisture could influence negatively the performance of BSF larvae.

The AD (approximate digestibility) values were increased when the BSF larvae fed at SCG diets were added with different types of whey, especially with the fermented one, able to double almost the reference threshold of control regime (a difference of 16.20±0.99). On the other hand, Palma et al. (2019) found that the AD value was decreasing inversely to increasing of the amount of substrate. This study illustrated that the addition of whey to SCG diet augmented the assimilation of nutrient by larvae; it could be related to the rise of lactose, protein and lactic acid content enhancing the nutritional value of diet. In addition, AD values of larvae fed at FW+SCG was obviously higher than those fed at AW+SCG and SW+SCG. This it may be due to the excessive amount of lactic acid and other fermentation products present in fermented sweet whey in which more nutrients were assimilated by larvae. Not far from, BCR values of larvae fed at SCG diet in this study was higher than that of Permana and Ramadhani (2018) for the same substrate (11.65±0.52 against 2,71 to 5 %) or even than results of Manurung et al. (2016) for rice straw (6.11 to 10.85 %). Yet, it stilled significantly the lower most when compared with those enriched with whey ranging from (13.55±0.11 to 17.94±0.47 %). Although, these results were lower than those found by Diener et al. (2009) and Romano et al. (2021) by using chicken feed and sweet potato, spent coffee or dough as substrates, respectively. All in all, BSF larvae fed at SCG with whey converted more digested feed into their biomass. Therefore, it could be included that the major amount of lactose, protein and lactic acid offered by whey, passed into the larvae's intestines, was assimilated and transformed into biomass. Perhaps a surplus of these nutrients, logically more suitable than spent coffee ground nutrients, is conferred more quantity at a better quality.

Despite previous observations, FCR values of larvae fed only at SCG were exceptionally and significantly higher than those fed at SCG added with whey. It was comparable with those fed at fruit and vegetable waste found by Giannetto et al. (2019) by (9.29). For larvae fed at SCG with whey, FCR values were in agreement with (Rehman et al., 2017b) by (5.56 to 7.86). FCR values could be influenced by the substrate consumed by larvae. Therefore, while the BCR values were increasing, the FCR values were decreasing. This may be caused by the poor nourishing value of SCG diet compared to that added with whey. Thus, it is possible that whey could rectify the nutritional imbalanced of diets. Gligorescu et al. (2020) and Giannetto et al. (2019) examined the high FCR values with the low BCR value. They discussed that the high FCR values indicate an ease of digestion of the most amounts of substrates but with an excessive excretion caused by the low quality of their nutrients. Their results agreed with the findings in this study when larvae were fed only at SCG and proved the positive effect of adding whey to SCG diet for BSF larvae. Overall, the supplementation of whey to SCG diet may enhance the performance of BSF larvae.

4.3. Nutritional content of BSF larvae

BSF larvae can convert a variety of organic material into a protein and fat-rich biomass (Wang & Shelomi, 2017). The quantity and quality of substrates have an impact on body biomass of larvae (Nguyen et al., 2015). In this study, BSF larvae fed at SCG diet alone had significantly the lowest protein content compared to those fed with SCG and whey. Permana and Ramadhani (2018) found the similar protein content of BSF larvae fed at the same substrate. However, the addition of whey to SCG diet increased highly their protein content to reach about (40.11±0.40 %). This is align with the findings of Gold et al. (2020), Spranghers et al. (2017) and Nyakeri et al. (2017). For larvae fed at SCG, the diet was unbalanced owing to its carbohydrates proportion outweighing its protein fraction which might avoid larvae to digest and convert efficiently the nutrients into protein biomass. In addition, the supplementation of whey to SCG diet improved the larvae's protein content compared to those fed at crude SCG. Herein, the enhancement of nutritional content could be suggested to ameliorate the effectiveness of diet conversion into protein biomass. The concentration of nutrients (protein, lactose, lactic acid) in whey seems to bring a certain balance to the diet. In contrast to protein content, fat content of larvae fed only at SCG was the highest values by (37.87±0.24 %). These found values clearly concur with that found by Fischer et al. (2021) when larvae fed at spent coffee ground. Furthermore, Barragan-Fonseca et al. (2017) reported that larval lipid was ranging from 7 to 39 % and varied across different diets. The high values of fat content of larvae could be resulted by the unbalanced nutrient concentration of SCG diets, especially their high content of crude carbohydrates to protein ratio. Consequently, it led larvae to accumulate more fat than protein content into their biomass. Gold et al. (2018) suggested that the substrate carbohydrates influence the BSF larvae fat content. Moreover, according to Pimentel et al. (2017), larvae converted carbohydrates into lipid biomass and stored it as fat body. Basically, Barragan-Fonseca et al. (2017) discussed that larval body composition can be influenced by biotic and abiotic factors. This study was conducted in a period of high temperature (27-31° C) and low relative humidity (45±11 %). These conditions are unfavourable for BSF larvae growth and performance (Permana & Ramadhani, 2018). Hence, BSF larvae fed at low-protein and high-carbohydrate diets gained higher fat biomass in comparison to those fed at diets more balanced in carbohydrates and protein (Barragan-Fonseca, 2018).

4.4. Chemical composition of frass

The frass of *Hermetia illucens* is a mixture of undigested substrates, larvae skins, faeces, and microorganisms (Schmitt & de Vries, 2020; Yildirim-Aksoy et al., 2020). They further reported that the valuation of frass becomes more economical and ecological. In their study, Gärttlingand Schulz (2022) considered insect excrement as a potential alternative fertilizer to polluting agricultural chemicals. In addition, Yildirim-Aksoy et al. (2020) demonstrated that the use of BSF frass as feed at levels up to 300 g.kg^{-1} improved significantly the growth of catfish. In this research, the dry matter (DM) of frass in all treatments was lower than that found by Gao et al. (2019). It could be influenced by the abiotic factors of experiment including high temperature (29±2° C) and low humidity (45±11 %), in accordance with the come out of Permana and Ramadhani (2018) who demonstrated that SCG dries in unfavourable conditions. Furthermore, Gärttling and Schulz (2022) stated that DM content could be affected by rearing method and feeding substrate quality. This frass seemed to be an organic fertilizer by the high organic matter content 96 %. This value was higher than that reported by Gao et al. (2019) and Liu et al. (2019). It might be related to the poor ash amount of substrates and high percentage of dead larvae, for instance, the frass of larvae fed only on SCG diet had particularly the highest death rate of larvae and the lowest ash content. The values of organic carbon and total nitrogen were lower than found by Gao et al. (2019) and Menino et al. (2021). The chemical composition of frass was slightly affected by the addition of whey to SCG diet digested by BSF larvae. The different types of whey enhanced the quality and the quantity of mineral frass content by increasing its amount of ash and the available phosphorus-which was three fold that found by Praeg and Klammsteiner (2023) (66,37±0.24 vs 20.34±1.56 ppm). Generally, the supplementation of whey to SCG diet enhanced the quality of BSFL frass and made it a potential replacement for organic fertilizer capable of beneficially influencing soil quality.

5. Conclusion

This study attempted to address the significant environmental challenge of whey effluent rejection by exploring its potential as a spent coffee ground (SCG) diet additive, aiming to stimulate the performance, growth, and nutritional value of black soldier fly (BSF) larvae (*Hermetia illucens*) and enhance the chemical composition of the frass (residue). The results confirmed better BSF larvae development in the SCG diet enriched with sweet, acid, and fermented whey fractions, along with efficient digestion of their nutrients. Additionally, BSF larvae fed on whey-SCG showed an improved dry matter reduction quality and substrate conversion rate, yielding a straightforward favorable repercussion on protein and fat-rich biomass production, which is more useful as animal feed. Moreover, the resulting frass demonstrated high promise as a bio-fertilizer in sustainable agriculture. Everything considered, using whey-supplemented SCG diets for BSF larvae provides an eco-friendly solution for low-cost whey bio-treatment, thereby alleviating the burden of landfill pollution and greenhouse gas emissions associated with the decomposition of such organic waste. In perspective, complementary experiments are considered necessary to clarify and underline the use of black soldier fly for treating whey and/or other organic wastes in the tropical areas and under suitable growth condition.

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Author’s declaration and contributions

This study was supported by the Laboratory of Sciences, Food Technology and Sustainable Development (LSTADD), Faculty of Natural and Life Sciences, University Saad Dahlab Blida-1, BP 270 (9100), Algerian Ministry of Higher Education and Scientific Research. Tahar Hanafi conceived and designed the experiments. Tahar Hanafi, Badreddine Moussaoui and Bachir reghioui performed the experiments. Smain Megateli and Zoubida Meziane supervised the work. Laid Guemou analyzed the data.Tahar Hanafi and Badreddine Moussaoui wrote the manuscript.

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