Effects of dietary inclusion of black soldier fly (*Hermetia illucens*) larvae meal on growth performance and carcass yield of broilers

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ABSTRACT

Black soldier fly larvae meal (BSFLM) has been proven as a potential low-cost protein source that can replace soy bean meal in poultry diets. A study was conducted to determine the feed intake, growth performance and carcass characteristics of broilers fed a diet varying in inclusion levels of BSFLM. Day-old ROSS 308 broiler chicks ($n = 60$) reared on a starter commercial diet for the first three weeks were randomly allocated to one of the three broiler grower dietary treatments using a completely randomised design. The broiler grower diets were the control diet, which contained no BSFLM (T1), T2 contained 5% BSFLM and T3 contained 10% BSFLM replacing soybean meal. There was a significant difference ($p < 0.05$) in the feed intake, where the control had the highest intake followed by 5% BSFLM inclusion. There was a significant difference ($p < 0.05$) in the final live weight where the 5% BSFLM had the highest among the treatments and the control was the lowest. The inclusion of BSFLM had a significant ($p < 0.05$) effect on the carcass weight and thighs weight with the highest mean for the 5% BSFLM inclusion and the lowest with the control treatment. There was no significant ($p > 0.05$) difference in the slaughter weight, wings, drumstick and breast muscles among the treatments. The study concludes that the inclusion of BSFLM at 5% had a positive effect on the growth performance, carcass yield and characteristics of broiler chickens.

1 Introduction

The increase in the world population has resulted in a global increase in the consumption of food patterns as well as changes in lifestyles and changes in food preferences which have increased the demand for animal protein (Boland *et al.*, 2013). Sánchez-Muros *et al.* (2014) reported that there is a preference for protein from animal sources over plant protein sources because protein from animal sources constitutes a good balance of essential amino acids and an excellent content of vitamins. Chicken meat has both nutritional (i.e., it contains a significant amount of high-quality and easily digestible protein, and a low portion of saturated fat and a high proportion of polyunsaturated fatty acids (PUFA), and several health benefits as reviewed by Kralik *et al.* (2017). The awareness of such benefits associated with chicken meat and increased knowledge of chicken production has made the broiler meat popular (Vernooij *et al.*, 2018).

The increasing demand for animal protein has directly influenced the demand for livestock feed and automatically placing pressure on the already scarce natural resources (Van Huis, 2013). This resulted in an increase in the price of feedstuffs due to their high demand and scarcity. Typically, feed costs account for over 70% of the total cost of poultry production (Raza *et al.*, 2019), reducing the profitability of farmers. The costs of conventional

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feed protein ingredient resources such as soybean meal and fishmeal are very high and their availability is expected to be limited in the future (Makkar *et al.*, 2014). Major conventional ingredients in poultry feed may be impacted by global warming and climate change, as well as increasing costs in both feed and energy, affecting food security (Nkukwana, 2012).

The utilization of non-conventional feed such as insect-based feed as an alternative protein source has gained momentum worldwide in recent years (Van Huis, 2013). The black soldier fly (*Hermetia illucens*) larvae (BSFL) have emerged as one of the promising insects that can be used as feed components in poultry diets (Khan, 2018; Barragan-Fonseca *et al.*, 2017; Dörper *et al.*, 2021). The BSFL can convert low-quality organic waste into high levels of high-quality protein (37- 49%) and fat (7- 39%) (Dörper *et al.*, 2021), although higher levels of crude protein (CP) and fat have also been reported at 37% to 63% (Barragan-Fonseca *et al.*, 2017) and 15% to 49% (Dabbou *et al.*, 2018), respectively. Therefore, BSFL has both nutritional (essential amino acid profile, CP content ($\leq 53\%$)), crude fat content ($\leq 58\%$) and calcium content ($\leq 7\%$) and health benefits to poultry (De Souza Vilela *et al.*, 2021). Moreover, rearing black soldier larvae on decomposing organic waste is important in the reduction of the environmental waste load and is more environmentally friendly, efficient and promotes sustainable farming (Van Huis, 2013; Diener *et al.*, 2011; Spranghers *et al.*, 2017). On the other hand, the BSFL larvae also contain chitin (54 g/kg to 106 g/kg DM), the main component of the exoskeleton of insects which is an indigestible substance, and it can negatively affect nutrient utilization and digestibility particularly protein digestibility by chitin-protein matrixes (Dörper *et al.*, 2021).

Recent studies have shown promising results and have indicated that insects could be a possible replacement for protein-rich feedstuffs or ingredients in the diets of poultry and can improve the sustainability of the poultry supply chain (Makkar *et al.*, 2014; El-Hack *et al.*, 2020; Vauterin *et al.*, 2021). Although there are several studies on the use of BSFLM in commercial feeds of poultry, the results have been inconsistent. There is also a lack of information on the effects of inclusion levels of BSFLM in broilers produced in Namibia. It is well known that the nutritional composition of BSFL is influenced primarily by the composition of the organic substrate (Gobbi *et al.*, 2013; Liland *et al.*, 2017). Therefore, the objective of this study was to investigate the effect of the inclusion of black soldier fly larvae meal (BSFLM) as a protein source on the feed intake, growth performance and carcass yield of broilers.

2 Materials and methods

2.1 Description of the study site

The study was conducted at the University of Namibia's Neudamm Campus, which is situated at a latitude of $22°27'2''$S, longitude $17°21'38''$E and altitude of 1856 m above sea level. The average day temperatures lie at $30°C$ in January to $20°C$ in July, while the night lie between $17°C$ in January and $7°C$ in June. Average annual rainfall ranges between 350 – 400 mm. Most of the rainfall is received from January to May (Mendelsohn, 2002)

2.2 Experimental design and diets

A Completely Randomized Design (CRD) with three treatments replicated 5 times was used for allocating treatments to experimental units. The three experimental treatments were BSFLM 0% inclusion level (T1, the control), BSFLM 5% inclusion level (T2) and BSFLM 10% inclusion level (T3). Twenty chicks were housed in 5 pens (experimental units) with 4 chicks allocated to each treatment and each treatment was replicated 5 times. A grower diet containing yellow maize, wheat bran, soybean meal and premix was formulated as the control diet. Treatments 2 and 3 were formulated by partially replacing soybean meal with the inclusion of BSFLM meal as a protein source at different inclusion levels (5 and 10%).

The BSFL meal was supplied by Superfly Bio Converters, Northern Industry, Windhoek, Namibia. The BSFL were reared on a wet brewer grain. Black soldier larvae were milled through a 3 mm sieve with a milling machine prior to inclusion in diets. Other ingredients used in the formulation of experimental diets were procured from Animal Fedco Namibia, while the commercial diet starter was sourced from Agra, Namibia. A commercial starter feed was fed to the chicks for the first 3 weeks ($0 - 21^{\text{st}}$ days), while the formulated diets were fed from day 22 until the end of the experiment. The chemical composition of BSFLM, SBM and dietary treatments used in this study are presented in Table 1 and Table 2, respectively.

2.3 Experimental birds and management

A total of 72 one-day-old chicks were procured from Animal Fedco Namibia. All the birds were vaccinated upon hatching against Newcastle disease, infectious bronchitis, fowl infectious laryngotracheitis, Marek's disease and coccidiosis. In the first three weeks, chicks were kept in floor pens with infrared bulbs for brooding purposes and fed a commercial broiler starter. The floor was covered with paper boxes instead of wood shavings as young chicks tend to eat wood shavings. The birds were given stress Pac (complex forte, vitamin and amino acid supplement) dissolved in water to control stress among chicks for the first 2 weeks.

After the first three weeks, 60 birds were randomly selected and assigned to their experimental diets and pens (1.6 m $\times$ 1.5 m). The birds were housed in a total of 15 pens, with 5 pens per treatment and each pen had 4 birds. The floor was covered with wood shavings as a source of bedding to absorb moisture, reduce the contact between the birds and manure, and in the process provide insulation from the cold floor. Wood shavings were replaced on weekly basis to prevent the smell from building up and an environment that is conducive for disease outbreaks. The feeders and drinkers were cleaned daily before the addition of new feed and water. The experiment was conducted over a period of 49 days, and it consisted of a starter period ($0 - 21^{\text{st}}$ days), and a combined grower and finisher period (22 – 49 days). Feed and water were provided *ad libitum*.

2.4 Measurements of feed intake and growth performance

Feed intake was obtained by subtracting the leftover feed from the total quantity of feed provided daily. The birds were weighed at the beginning of the experiment to obtain their initial body weights, and subsequently on weekly basis to determine the growth performance. Body weight (BW) was calculated as final body weight minus initial body weight. Feed conversion ratio (FCR) was measured by dividing the average mass of the consumed feed by the average mass of the broiler. The mortality and health status of birds were monitored daily.

2.5 Slaughtering procedures and carcass dissection

A day before slaughtering, the birds were deprived of feed for them to empty their crops while water was available *ad libitum*. One bird per pen was randomly selected for slaughter and carcass evaluation. During slaughter, birds were weighed before being sacrificed by cervical dislocation. The birds were fully bled, scalded, plucked and washed. The head, neck, and feet were removed. Subsequently, the carcasses were manually eviscerated, cutting off the neck and through the respiratory system, and the oesophagus was removed, before they were placed in a chiller ($4°C$) overnight for dripping and cooling. Visceral organs (liver, spleen, heart, and bursa) and gizzards were removed by hand through an opening around the vent and sternum. Carcasses were then weighed individually and expressed as percentages of live bodyweight. The drumsticks, thighs, wings and breast muscles were cut from the joints of the carcasses and weighed.

2.6 Chemical analysis of the BSFM, SBM and experimental diets

The BSFLM, SBM and experimental diets were ground to pass through a 1 mm sieve and stored in airtight plastic containers. Samples were analyzed for dry matter (#930.15), crude fat (#920.39), and crude protein

(CP) (#954.01) following the procedures of the Association of Official Analytic Chemists (AOAC, 2005). Total nitrogen was converted by the factor of 6.25 to crude protein. Feed samples were analyzed for neutral detergent fiber (NDF) and acid detergent fiber (ADF) according to the procedures of Van Soest *et al.* (1991) using ANKOM fiber analyzer. Calcium and phosphorus were analyzed using Inductively Coupled Plasma-Optical Emission Spectrometer (ICP-OES) (icap 6000 series).

2.7 Statistical analysis

The feed intake and live weight data were analysed using the general linear mixed effects model which is an alternative of the repeated measures analysis of variance (ANOVA) to control for correlated errors emanating from the data measured from the same experimental units (pens) repeatedly. The data were analysed in SPSS version 27, and significant differences were tested at 5% level of significance. The mathematical model for the Generalised linear model used is given in equation (1).

$$Y_{ijk} = \mu + \tau_i + W_j + (\tau \times W)_{ij} + cage(\tau)_{ki} + e_{ijk}, \tag{1}$$

where

Y_{ijk}	is the observation (weight or feed intake) taken from the k^{th} experimental unit of the i^{th} treatment in the j^{th} week
μ	is the overall mean for the experiment
τ_i	is the i^{th} treatment effect ($i = 1, 2, 3$ BSFLM inclusion levels)
W_i	is the effect of the j^{th} week ($j = 1, 2, 3, 4$ weeks)
$(\tau \times W)_{ij}$	is the i^{th} treatment and j^{th} week interaction
$cage(\tau)_{ki}$	is the effect of the k^{th} cage nested within the i^{th} treatment
e_{ijk}	random error term

The data on carcass components yield were analysed using the one-way ANOVA model equation (2)

$$Y_{ij} = \mu + \tau_i + \varepsilon_{ij}, \tag{2}$$

where

Y_{ij}	is the observation (e.g. thigh, breast weight, of the j^{th} replicate of the i^{th} treatment (inclusion level)
μ	is the overall mean for the experiment
τ_i	is the effect treatment ($i = 1, 2, 3$ BSFLM inclusion level)
ε_{ij}	is the random error of the j^{th} replicate of the i^{th} treatment Significant different means were detected using the Duncan Multiple Range test at 5% level of significance

3 Results

3.1 Broiler growth performances

The average weekly feed intake of the birds during the experiment are presented in Table 3. There was a significant difference ($p < 0.05$) in feed intake during week 4, where birds fed a diet without BSFLM (control diet) had a higher feed intake (2134.20 g) than the birds fed 5% BSFLM inclusion level (1401.00 g). There was no significant ($p > 0.05$) difference in the feed intake between birds fed a control diet and 10% BSFLM inclusion level and no difference between birds fed 5% BSFLM inclusion level and 10% BSFLM inclusion level in week four. There was no significant ($p > 0.05$) difference in feed intake at week 5, 6, 7 and 8 among the treatments.

The overall feed intake and growth performance of the birds are presented in Table 4. The feed intake during the grower and finisher period was greater ($p < 0.05$) for birds on the control diet as compared to that of birds on the BSFLM diets. There was no difference ($p > 0.05$) in the feed intake between 5% and 10% BSFLM inclusion level. The average daily intake was similarly higher ($p < 0.05$) for birds on the control diet compared to those on BSFLM diets which were similar ($p > 0.05$). There was no significant ($p > 0.05$) difference in the final live weight of the birds on the three diets. Similarly, the feed conversion ratios of the birds did not differ ($p > 0.05$) among the treatments.

The weekly live weight of the birds during the finisher growth phase are presented in Figure 1. There was no significant ($p > 0.05$) difference in live weight at week 3, 4 and 5 among the three treatments. There was a significant difference ($p > 0.05$) in live weight during week 6 where 5% BSFLM inclusion treatment had higher live weight compared to control and 10% BSFLM inclusion (1459.3 vs 1310.1 and 1353.5 g, respectively). The live weight of the birds differed significantly ($p < 0.05$) among the three treatments at week 7. The 5% BSFLM inclusion diet had birds with the highest live weight (1745.4 g) followed by those on 10% BSFLM inclusion (1559.2 g) and the least was the birds on the control treatment (1380.8 g).

3.2 Carcass characteristics and components yield

The effects of dietary treatments on the slaughter weight, carcass weight, wings, drumstick, thigh and breast muscles are presented in Table 5. There was no significant difference ($p > 0.05$) in slaughter weight among the treatments. There was a significant difference ($p < 0.05$) in carcass weight between the control and 5% BSFLM, where the 5% BSFLM was higher (1262.00 g) than the control (972.40 g). The 10% BSFLM did not differ ($p > 0.05$) with the control and 5% BSFLM treatments in the carcass weight. There was no significant ($p > 0.05$) difference in the weight of wings, drumstick and breast muscles among the three treatments. However, there was a significant difference ($p < 0.05$) in thigh weight among treatments. The 5% and 10% BSFLM treatments had greater thigh weights compared to the control treatment, 214.80 g and 185.40 g vs 168.20 g, respectively.

4 Discussion

4.1 Chemical composition of BSFLM

The DM content (92.8%) of BSFLM determined in the present study was similar to that reported by Spranghers *et al.* (2017) but lower than the DM reported by De Marco *et al.* (2015) which was found to be 95.7%. Protein is the most abundant nutrient in BSFLM. The crude protein content from this study was found to be higher (49% CP) than the protein content reported by Makkar *et al.* (2014) which ranged between 41.1% - 43.6%. However, the CP content from the current study was lower than the CP content reported by Nguyen *et al.* (2015) of 57.9% for BSL reared on fish waste. Fat content is found to have the most variation in BSL which ranges from 7% to 39% DM (Barragan-Fonseca *et al.*, 2017). The crude fats content reported in this study (28%) is within the expected value reported in the literature.

Phosphorus is one of the crucial minerals needed for bone development. Phosphorus from the BSFLM used in the study was 1.25%, which was higher than the 0.88% reported by Dierenfeld and Kin (2009), however, it was lower than that reported by Barragan-Fonseca *et al.* (2017). Calcium is another crucial mineral for bone growth. Calcium from the BSFLM used in the study was 1.67% which was lower than 5.36% (Dierenfeld and Kin, 2009). The differences in the chemical composition of the BSL can be accredited to the different types of substrates on which the BSF was reared on to produce larvae and the age at which the larvae were harvested for use (Barragan-Fonseca *et al.*, 2017; Liland *et al.*, 2017; Galassi *et al.*, 2021). In comparison to SBM used in this study, BSFLM had a higher crude fat, calcium, and phosphorus, but a similar CP content to SBM. Although the nutritive value of commercial SBM depends on numerous factors, such as seed variety, environmental conditions during growing, harvesting and storage of the beans and the procedure used for oil extraction, the CP content of SBM has been reported to range from 41 to 50% (Ibáñez *et al.*, 2020).

4.2 Growth performance

The inclusion of black soldier larvae meal had a negative effect on the feed intake. The decline in feed intake with the inclusion of BSFLM agrees with Attivi *et al.* (2020) who observed a decrease in feed intake at 6% and 8% inclusion levels of BSFLM. This could be attributed to the high fat content of BSFLM. The decrease in feed intake is supported by Veldkamp *et al.* (2005), who noted that feed intake decreases linearly as dietary energy increases with high energy in diets. There was a significant difference in the feed intake, with the highest intake in T1 followed by T3 and T2 the lowest. This could be because the birds being fed with BSFLM (T2 and T3) took time to fully adapt to the diets, while the birds fed T1 did not need to adapt to a diet that is comparable to a commercial broiler grower.

The inclusion of BSFLM in the diet of chickens had a positive effect on the live weight and average daily gain of chickens in the study. Dabbou *et al.* (2018) also made similar observations of a positive effect of BSFLM on improving live weight. These findings were contrary to the findings by Onsongo (2017) who observed that BSFLM inclusion did not affect body weight. However, a higher inclusion level may reduce the efficiency of the diet because of the high chitin content which is fibre found on the exoskeleton of the BSFL. In high content, the chitin might reduce the utilization of nutrients and digestibility (Hossain and Blair, 2007; Schiavone *et al.*, 2018; El-Hack *et al.*, 2020), hence a decrease in final live weight was observed.

4.3 Carcass characteristic and cut yield

According to Fernandes *et al.* (2013), the improvement in carcass characteristics of broiler breeds in recent decades has been pronounced, resulting in substantial genetic gains. Of particular importance is the yield of the breast and legs, since they are the most expensive cuts as they are well paid for on the market (Fernandes *et al.*, 2013). The dressing percentage was highest in 5% BSFLM carcasses and lowest in the control treatment. This implies that the inclusion of BSFLM had a positive effect on the carcass yield of the birds. Hence, the inclusion of BSFLM in broiler diets improves the carcass yield of broiler chickens. Supplementation of broiler feeds with high protein alternatives such as BSFLM is a practical method of decreasing the ever-increasing costs of broiler feeding. Since the breast is one of the most expensive and valued carcass components in the processing industry, birds given the 5% and 10% BSFLM presented a higher yield of this cut which may improve the meat yield (Attivi *et al.*, 2020). There was a significant difference in the weight of thighs. This observation was in line with that of Schiavone *et al.* (2018) who observed increased thigh weight at 0, 5%, 10% and 15% inclusion level of BSFLM in broiler diets.

5 Conclusion

The study revealed that BSFLM is a rich source of protein and other essential nutrients such as fat. The addition of BSFLM improved the growth performance, carcass characteristics and meat yield in broilers. Therefore, it can be concluded that BSFLM could be included in broiler diets up to 5%, in place of soybean meal, without compromising effects on broiler growth performance and carcass characteristics in broiler production.

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Table 1: Chemical composition of black soldier fly larva meal and soybean meal used in the experiment.

Nutrient (% of DM)	BSFLM	SBM
Dry matter	92	93.4
Crude fat	28	2.3
Crude protein	49	49.9
Calcium	1.67	0.23
Phosphorus	1.25	0.65

BSFLM = Black soldier fly larva meal, SBM = Soybean meal

Table 2: Chemical composition (%DM) of the experimental diets.

Nutrient	T1 (0% BSFLM)	T2 (5% BSFLM)	T3 (10% BSFLM)
DM	92.59	90.95	93.06
Ash	3.10	3.07	3.96
CP	20.09	20.05	20.82
Crude fat	3.40	6.35	6.27
NDF	23.56	26.21	28.34
ADF	8.95	9.52	10.57
Ca	0.10	0.16	0.26
P	0.43	0.44	0.58

DM=dry matter, CP =Crude protein, NDF=Neutral detergent Fiber, ADF=acid detergent fiber, Ca=Calcium, P=Phosphorus.

Table 3: Feed intake (g) per week of broilers fed the treatment diets.

TREATMENT	Week 4	Week 5	Week 6	Week 7	Week 8
T1 (0% BSFLM)	2134.20^{a}	1608.40	1933.80	1653.00	2524.60
T2 (5% BSFLM)	1401.00^{b}	1294.60	1572.40	1163.20	1882.00
T3 (10% BSFLM)	1740.00^{ab}	1505.40	1323.40	1492.60	1737.20
SEM	120.972	114.113	188.225	130.512	160.059
P-value	0.031	0.555	0.444	0.317	0.093

^{abcd}Means with different superscripts in a column differed significantly at $P < 0.05$
BSFLM 0% – control diet, BSFLM 5% – 5% inclusion of BSFLM, BSFLM 10% – 10% inclusion of BSFLM.

Table 4: The growth performance of broilers during the finisher phase (21- 49 d).

Parameter	Treatment diet			SEM	Sig. Level
	T1(0%BSFLM)	T2(5%BSFLM)	T3(10%BSFLM)		
Initial body weight (d21)/g	610.85	575.35	580.05	27.88	NS
Final Body Weight (d49)/g	1380.75^{b}	1745.35^{a}	1559.23^{a}	77.11	*
Feed Intake	2102.02^{a}	1462.64^{b}	1599.72^{b}	158.72	*
Ave. Daily Intake (g/day)	73.84^{a}	53.99^{b}	57.47^{b}	2.89	*
Ave. Daily Gain (g/day)	41.53	41.72	37.45	2.56	NS
Feed Conversion Ratio (g:g)	1.95	1.45	1.83	0.17	NS

^{ab}Means in the same row with different superscripts differ significantly ($P < 0.05$); * = $P < 0.05$,
NS = not significant ($P > 0.05$); SEM = Standard error of the mean.

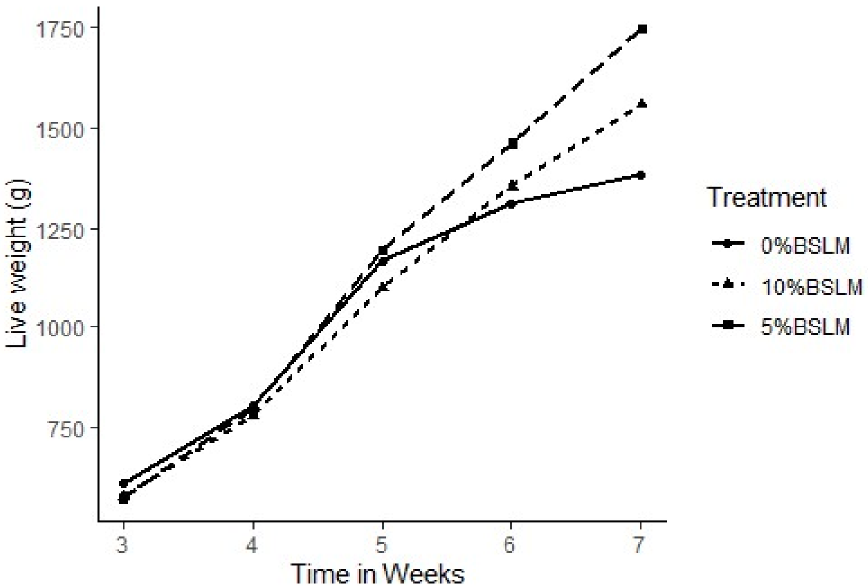

Figure 1: Live weight gain of the broiler birds fed the treatment finisher diets.

Table 5: Effects of diet on carcass characteristics and cuts yield.

Yield	Treatment diet			SEM	Sig. Level
	T1(0%BSFLM)	**T2(5%BSFLM)**	**T3(10%BSFLM)**		
Carcass characteristics					
Slaughter weight (g)	1453.6	1791	1553	117	NS
Carcass Weight (g)	972.40^{b}	1262.00^{a}	1076.80^{ab}	70.51	*
Dressing %	70.31	70.53	70.92	7.63	NS
Organ yield					
Wings (g)	125.2	147.4	131.8	14.45	NS
Drumstick (g)	161.6	196	166.8	14.45	NS
Thighs (g)	168.20^{b}	214.80^{a}	185.40^{a}	10.86	*
Breast muscles (g)	318.2	430	343.2	35.305	NS

^{ab}Means in the same row with different superscripts differ significantly ($P < 0.05$); * $= P < 0.05$, NS = not significant ($P > 0.05$); SEM = Standard error of the mean.