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EVALUATION OF SUPPLEMENTATION OF DEFATTED BLACK SOLDIER FLY (*HERMETIA ILLUCENS*) LARVAE MEAL IN BEAGLE DOGS

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Abstract

The objective of this experiment was to test the effects of supplementation of defatted black soldier fly (*Hermetia illucens*) larvae (BSFL) meal in beagle dogs. A total of nine healthy female beagles (initial body weight 12.1 ± 1.76 kg) were fed grain-based diets with three levels of BSFL meal (0, 1% or 2%) in a 42-day feeding trial. At the end of week 6 of the experiment, all dogs were intraperitoneally challenged with *Escherichia coli* lipopolysaccharide (LPS) at 100 µg/kg of body weight. Albumin concentration was linearly increased with increasing BSFL meal level (P<0.05). A linear increase (P<0.05) in calcium concentration was observed when increasing dietary BSFL meal. Although dietary treatments did not affect the digestibility of ether extract, the digestibility of dry matter and crude protein were linearly increased with increasing the level of BSFL meal. The concentration of tumor necrosis factor-α was linearly decreased but glutathione peroxidase (GPx) concentration was linearly increased when increasing the level of BSFL meal at 6 h after challenge (P<0.05). In addition, there were quadratic increases in concentrations of GPx and superoxide dismutase with increasing dietary BSFL meal level at 3 h after challenge (P<0.05). These findings from the present study demonstrate that BSFL meal can be supplemented in the diet to convert beneficial effects to beagle dogs, indicated as improved digestibility of dry matter and crude protein and anti-inflammatory and anti-oxidative capacity.

Key words: blood profile, digestibility, dogs, *Hermetia illucens*

Insects have been proposed as a promising, high quality, and efficient alternative protein feedstuff for animal feeds (Charlton et al., 2015). Insects are such an alternative protein source because they can sustainably by reared on organic side streams

and they have a favorable feed conversion efficiency (Veldkamp et al., 2012). The production of insects specifically with the intention of being fed to livestock has been the subject of evaluations for several decades (Veldkamp and Bosch, 2015; Cullere et al., 2016; Khan et al., 2016).

The black soldier fly (*Hermetia illucens*) can grow on a wide range of decomposing organic materials, such as fruits, vegetables to kitchen wastes, and livestock manure (Martínez-Sánchez et al., 2011). Therefore, being potentially interesting in reducing environmental criticisms by transforming waste into valuable biomass, black soldier fly is a high-quality animal protein feedstuff (Nguyen et al., 2015). Previous studies have suggested that black soldier fly larvae (BSFL) could be used as feed ingredient for pigs (Józefiak et al., 2016), poultry (Marono et al., 2017; Mwaniki et al., 2018; Secci et al., 2018), and fish species (St-Hilaire et al., 2007; Renna et al., 2017). Apart from the growing farm animals population, the population of pet animals (dogs and cats) is also large and growing, therefore, the availability of high quality and sustainable protein sources for pet food production is increasing in importance (McCusker et al., 2014; Bosch et al., 2016; Leriche et al., 2017). Bosch et al. (2014; 2016) indicated that the use of insects as protein sources in dog food is drawing attention. Kröger et al. (2017) and Kierończyk et al. (2018) studied the application of BSFL meal in dogs. To our best knowledge, however, the study of the inclusion of BSFL meal into dog diet is still limited. Therefore, the aim of the present experiment was to determine the effects of inclusion of 0, 1%, and 2% BSFL meal in beagle dogs.

Material and methods

All the animal procedures were reviewed and approved by the Animal Care and Use Committee of Dankook University (DK-1-1712).

Source of BSFL meal

The defatted BSFL meal used in the present study was provided by Foodyworm Inc. (Seoul, Republic of Korea). The nutrient contents of BSFL meal is presented in Tables 1 and 2.

Table 1. Chemical composition and amino acid concentration of black soldier fly larvae meal

Item	%
1	2
Moisture	7.93
Crude protein	53.64
Crude fat	13.43
Crude ash	11.02
Amino acids	
aspartic acid	4.85
threonine	2.15

Table 1 – contd.

1	2
serine	2.35
glutamic acid	6.11
proline	2.89
glycine	2.69
alanine	3.62
valine	3.68
isoleucine	2.06
leucine	3.61
tyrosine	3.08
phenylalanine	2.19
histidine	1.60
lysine	3.42
arginine	2.73
cysteine	0.70
methionine	1.33
tryptophan	0.65

Table 2. Fatty acid components of black soldier fly larvae meal

Item	%
Saturated fatty acid	
C8:0	0.01
C10:0	1.19
C12:0	29.61
C14:0	5.57
C15:0	0.12
C16:0	15.14
C17:0	0.26
C18:0	3.96
C20:0	0.06
Unsaturated fatty acid	
C14:1	0.20
C15:1	0.15
C16:1	3.42
C17:1	0.19
C18:1	20.49
C18:2*n6*	13.07
C18:3*n6*	0.06
C18:3*n3*	2.43
C18:4*n3*	0.16
C20:1*n9*	0.61

Experimental design, animals, and housing

A total of 9 female beagle dogs, in good general health, aged 15–18 months, with initial body weight (BW) of 12.1 ± 1.76 kg were randomly allotted to one of three dietary treatments with three replications per treatment and one beagle dog per replication (cage), according to initial BW. The dietary treatments included commercial basal diets with 0, 1%, or 2% of BSFL meal. One month before the experiment, all the dogs were fed the same commercial pelleted diet as the basal diet used in the present study for the adaption. The commercial basal diet was formulated to meet nutrient requirements in accordance with the Association of American Feed Control Officials (AAFCO, 2009) nutrient guide for dogs. The nutrient level of the basal diet is shown in Table 3. Experimental dogs were individually fed twice daily (08:00 h and 16:00 h). Beagles were housed in cages (100 cm × 210 cm) that were equipped with a feeder, a water bucket, and slatted plastic flooring in an environmentally controlled room. Dogs were allowed free access to drinking water throughout the experiment. Room temperature and relative humidity were maintained at 20 ± 3°C and 50 ± 10%, respectively. At the end of week 6 of the experiment, all dogs were intraperitoneally injected with *Escherichia coli* lipopolysaccharide (LPS, *E. coli* serotype 055: B5) at 100 μg/kg of BW.

Table 3. The analyzed nutrient level of basal diet (as-fed basis)

Item	%
Dry matter	90.59
Crude protein	32.01
Crude fat	19.97
Crude fiber	2.20
Crude ash	8.79
Calcium	1.96
Total phosphorus	1.26

Sampling and measurements

The apparent total tract digestibility (ATTD) was performed using the total collection method (AAFCO, 2009). To estimate the ATTD of crude protein (CP), dry matter (DM), and ether extract (EE), during the last 3 days of the experiment, feces were collected at least two times daily and weighed. Fecal samples from the same dog were pooled and mixed, after which fecal samples were kept at –20°C until required for analysis. For chemical analysis, fecal samples were oven-dried at 55°C for 72 h and ground to pass through a 1.0-mm screen (Lei and Kim, 2018; Liu et al., 2018). Dietary and fecal samples were analyzed for DM (method 930.15), CP (method 984.13), and EE (method 920.39) using the AOAC (2007) method. The ATTD of DM, EE, and CP was calculated as follows:

$$\textit{ATTD of nutrient (\%)} = [(\textit{nutrient intake, g} - \textit{nutrient excretion, g})/\textit{nutrient intake, g}] \times 100$$

At the end of week 6, blood samples were collected via jugular vein from each dog in non-heparinized tubes. Blood samples were centrifuged at 1,500 × g for 20 minutes to get serum and then frozen at –20°C until further analysis (Kruger et al., 2016). The concentrations of alanine transaminase (ALT), albumin, aspartate transaminase (AST), bilirubin, blood urea nitrogen (BUN), globulin, glucose, and protein were analyzed using commercially specific available enzyme-linked immunosorbent assay (ELISA) kits (Quantikine, R&D Systems, Minneapolis, MN, USA). Serum high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), total cholesterol (TC), and triglyceride (TG) concentrations in serum were determined enzymatically using reagent kits (Wako Pure Chemical Industries Ltd., Tokyo, Japan). The amounts of calcium (Ca), chlorine (Cl), magnesium (Mg), phosphorus (P), potassium (K), and sodium (Na) in serum were determined by flame atomic absorption spectrophotometry (AA-6300, Shimadzu Corp., Tokyo, Japan).

Before challenge and 3 and 6 h after challenge, blood was collected via jugular vein from each dog into non-heparinized tubes. Then, blood samples were centrifuged at 1,500 × g for 20 minutes to get serum and then frozen at –20°C until analysis (Kruger et al., 2016). The serum tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) concentrations were assessed using specific commercially available ELISA kits (Quantikine, R&D Systems, Minneapolis, MN, USA) according to the manufacturer's instructions. The concentrations of superoxide dismutase (SOD) and glutathione peroxidase (GPx) in serum were determined using commercial kits (Cell Biolabs, Inc. San Diego, CA, USA) following the instructions.

Statistical analysis

All data were analyzed as a randomized complete block design using the general linear model procedures of SAS (version 9.2, Institute Inc., Cary, NC, USA). The individual beagle was considered as the experimental unit. Both linear and quadratic polynomial contrasts were performed to determine the effects of a different level (0, 1%, and 2%) of BSFL in the diet. Variability in data was expressed as the pooled standard error of the mean and a probability less than 0.05 was considered statistically significant.

Results

The protein, glucose, globulin, BUN, bilirubin, AST, and ALT concentrations in serum were not affected by dietary treatments ($P>0.05$; Table 4). However, albumin concentration was linearly increased with increasing BSFL meal level ($P<0.05$). No differences in blood cholesterol, triglyceride, HDL-C, and LDL-C were observed among treatments ($P>0.05$; Table 5). The concentrations of tested minerals in blood did not differ among dietary treatments with the exception of calcium ($P>0.05$; Table 6). With the increasing level of BSFL meal, a linear increase ($P<0.05$) in calcium concentration was observed. Although the digestibility of EE was not influenced by dietary treatments, increasing the level of BSFL meal linearly increased the ATTD

of DM and CP (Table 7). Before the challenge, the IL-6, TNF-α, SOD, and GPx concentrations in serum did not differ among treatments (P>0.05; Table 8). However, the concentration of TNF-α was linearly decreased while GPx concentration was linearly increased when increasing the level of BSFL meal at 6 h after challenge (P<0.05). In addition, there were quadratic increases in concentrations of GPx and SOD with increasing dietary BSFL meal level at 3 h after challenge (P<0.05).

Table 4. Effects of black soldier fly larvae (BSFL) meal on selected serum parameters in beagle dogs

Item	BSFL meal (%)			SEM[1]	P-value	
	0	1	2		linear	quadratic
Protein (mg/mL)	64.84	63.42	62.21	0.102	0.270	0.664
Albumin (mg/mL)	28.11	32.12	36.8	0.134	0.017	0.609
Glucose (mg/mL)	0.83	0.80	0.77	0.023	0.456	0.933
Globulin (mg/mL)	35.01	31.32	25.94	0.191	0.062	0.867
Blood urea nitrogen (mg/dL)	0.06	0.06	0.08	0.007	0.290	0.496
Bilirubin (μg/mL)	0.98	1.02	0.97	0.091	0.898	0.876
Aspartate transaminase (U/mL)	0.03	0.03	0.03	0.002	0.336	0.464
Alanine transaminase (U/mL)	0.03	0.06	0.03	0.008	0.801	0.187

[1]SEM, standard error of the mean.

Table 5. Effects of black soldier fly larvae (BSFL) meal on blood lipid profiles in beagle dogs

tem (mg/mL)	BSFL meal (%)			SEM[1]	P-value	
	0	1	2		linear	quadratic
Cholesterol	1.79	1.54	1.84	0.079	0.828	0.176
Triglyceride	0.69	0.71	0.76	0.069	0.792	0.954
High-density lipoprotein cholesterol	1.32	1.26	1.31	0.134	0.871	0.583
Low-density lipoprotein cholesterol	0.17	0.12	0.13	0.017	0.415	0.475

[1]SEM, standard error of the mean.

Table 6. Effects of black soldier fly larvae (BSFL) meal on mineral profiles in beagle dogs

Item	BSFL meal (%)			SEM[1]	P-value	
	0	1	2		linear	quadratic
Calcium (mg/mL)	0.09	0.12	0.14	0.001	0.020	0.660
Phosphorus (mg/mL)	0.04	0.04	0.05	0.002	0.055	1.000
Sodium (mmol/mL)	0.15	0.15	0.15	0.001	0.070	0.656
Potassium (μmol/mL)	5.21	4.89	5.22	0.072	0.622	0.071
Chloride (mmol/mL)	0.11	0.11	0.11	0.001	0.386	0.151
Magnesium (mg/mL)	0.02	0.02	0.02	0.001	0.108	0.809

[1]SEM, standard error of the mean.

Table 7. Effects of black soldier fly larvae (BSFL) meal on nutrient digestibility in beagle dogs

Item (%)	BSFL meal (%)			SEM[1]	P-value	
	0	1	2		linear	quadratic
Dry matter	71.97	74.55	75.21	2.964	0.017	0.992
Nitrogen	73.16	77.06	78.51	2.640	0.039	0.825
Ether extract	78.80	78.97	79.22	3.523	0.934	0.994

[1]SEM, standard error of the mean.

Table 8. Effects of black soldier fly larvae (BSFL) meal on blood profile in beagle dogs challenged with lipopolysaccharide

Item	BSFL meal (%)			SEM[1]	P-value	
	0	1	2		linear	quadratic
Interleukin-6 (pg/mL)						
before injection	15.72	17.84	15.20	2.264	0.091	0.290
after 3 h	66.35	67.09	63.94	7.351	0.314	0.312
after 6 h	65.60	67.21	55.94	6.422	0.213	0.291
Tumor necrosis factor-α (pg/mL)						
before injection	4.55	5.53	5.65	1.412	0.563	0.550
after 3 h	17.77	18.47	18.47	2.974	0.342	0.537
after 6 h	13.64	12.63	7.48	3.025	0.038	0.779
Superoxide dismutase (U/mL)						
before injection	1.78	1.94	2.32	0.781	0.239	0.692
after 3 h	1.06	3.61	2.81	1.124	0.441	0.036
after 6 h	1.05	1.67	0.89	0.390	0.798	0.196
Glutathione peroxidase (nmol/min/mL)						
before injection	58.96	54.76	55.01	5.261	0.849	0.894
after 3 h	3.11	6.72	3.64	0.754	0.989	0.034
after 6 h	42.12	50.94	53.05	4.642	0.014	0.371

Discussion

Black soldier fly represents one of the most promising insect species that can be used as a protein source for livestock and fish (Biancarosa et al., 2018). This study evaluated the application of BSFL meal in beagle dogs. In this study, the protein, glucose, globulin, BUN, bilirubin, AST, and ALT concentrations in serum were not influenced by treatments, whereas albumin concentration was linearly increased when increasing BSFL meal level. The increased concentration of albumin in the serum of the dogs fed higher level of BSFL meal might have resulted from the increased flow of protein to the intestine (Min et al., 2003). The blood lipid profiles (concentrations of cholesterol, HDL-C, LDL-C, and triglyceride) were not influenced by treatments

indicating that inclusion of BSFL meal had no harmful effects on lipid metabolism in beagle dogs. Similarly, in fish and broilers, Li et al. (2016) and Dabbou et al. (2018) observed that inclusion of BSFL oil did not influence serum cholesterol, triglyceride, HDL-C, and LDL-C contents in serum. In this study, the concentrations of phosphorus, sodium, potassium, chloride, and magnesium in blood were not influenced by treatments, whereas a linear increase in calcium concentration was observed when increasing the dietary BSFL meal. Dabbou et al. (2018) indicated that inclusion of defatted BSFL meal increased phosphorus content in serum of broilers, but the concentrations of calcium, magnesium, and iron did not differ from dietary treatments. Schiavone et al. (2017) suggested that BSFL oil had no effects on serum phosphorus, magnesium, and iron concentrations.

In this experiment, increasing the level of BSFL meal linearly increased the digestibility of DM and N, but the digestibility of EE was not affected by treatments. This indicates that providing BSFL meal has a positive effect on DM and CP digestibility. However, Cutrignelli et al. (2018) completely replaced soybean meal with BSFL meal and found that laying hens fed diet with BSFL meal showed lower apparent ileal digestibility of DM and CP compared with hens offered diet without BSFL meal, but lipid digestibility was not affected by treatment. The authors suggested that the reductions in DM and CP digestibility were related to the chitin in the BSFL meal which could negatively affect the nutrient digestibility (Longvah et al., 2011). In broiler quails, Cullere et al. (2016) found that the digestibility of DM, and CP were not affected by the inclusion of BSFL meal, whereas the digestibility of EE was reduced by supplementation of BSFL meal. In addition, in weaned pigs, Spranghers et al. (2018) observed that inclusion of 4% or 8% BSFL meal had no effects on apparent ileal and total tract digestibility of DM and CP. Further studies are warranted to test higher levels of BSFL meal on nutrient digestibility in beagle dogs.

Cytokines play an important role in the immune and inflammatory response. Previous studies have indicated that over-production of TNF-α (pro-inflammatory cytokine) has negative effects on intestinal integrity and epithelial function (Waititu et al., 2016; Yu et al., 2017; Xu et al., 2018 a, b). In the present study, the concentration of TNF-α was linearly decreased when increasing the level of BSFL meal at 6 h after challenge. The down-regulation of TNF-α may indicate that inflammation induced by LPS was alleviated. The concentration of GPx was linearly increased when increasing the level of BSFL meal at 6 h after challenge. In addition, there were quadratic increases in concentrations of GPx and SOD with increasing dietary BSFL meal level at 3 h after challenge. The GPx and SOD are major anti-oxidative enzymes in serum (Štukelj et al., 2013). The increased concentrations of GPx and SOD may suggest that BSFL meal improved the anti-oxidative capacity. Li et al. (2016) suggested that the improved anti-oxidative property indicated as increased catalase activity in serum from fish by the inclusion of BSFL meal could be attributed to the chitin and its derivatives in BSFL meal. In the present study, the improved anti-oxidative capacity may be caused by the chitin and its derivatives in BSFL meal, although the chitin in BSFL meal and experimental diets was not specifically analyzed (Khoushab and Yamabhai, 2010; Ngo and Kim, 2014).

In conclusion, these findings from this study demonstrate that BSFL meal can be supplemented in the diet to convert beneficial effects to beagle dogs indicated as improved ATTD of DM and CP and anti-inflammatory and anti-oxidative capacity.

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