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Effects of brewer's spent grain on the growth and nutrition of the giant mealworm beetle, *Zophobas atratus*

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Abstract

The giant mealworm beetle, *Zophobas atratus* (Coleoptera: Tenebrionidae), is a tropical beetle. As this beetle can be used as an ingredient in animal feed, the effects of brewer's spent grain (BSG) on the development and nutritional value of *Z. atratus* were investigated. As results, there were no significant differences on the larval survival rate, body weight, and duration at different content of BSG, mixed with conventional feed, wheat bran (WB). Based on these results, BSG can be mixed with wheat bran as a *Z. atratus* food ingredient without any problems. The nutritional value was compared between 100% wheat bran control and 50% BSG experimental groups. The moisture, crude protein, carbohydrates, and amino acids were 1.1-1.4 times higher in the 50% BSG group. In the BSG group, the linoleic acid content was 1.6 times higher than that in the control group. In the wheat bran group, the oleic acid content was 38.4%, which was 1.3 times higher than that in the BSG group. As minerals, the control and BSG groups showed high potassium and phosphorus contents. In terms of hazardous materials, four heavy metals (lead, mercury, arsenic, and cadmium) and two microorganisms (*Escherichia coli* and *Salmonella* spp.) were not detected. There were no significant differences in developmental characteristics between the wheat bran and BSG mixed groups, and the nutritional values were better in the BSG mixed group. Therefore, BSG can be used as alternative food source for rearing *Z. atratus*.

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Introduction

Insects have a short life cycle with high reproductive potential and are highly efficient in converting plant proteins to animal proteins. The protein content of insects is 44–70%, which is higher than that of the general feed for livestock and human food. In addition to

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proteins, insect bodies contain fatty acids, minerals, and vitamins, and thus more than 1,500 insect species have been consumed as human feed with high nutritional values in 113 countries (Ramos-Elorduy, 1997). The giant mealworm beetle, *Zophobas atratus* is known as a super worm or a super mealworm with high protein content. It is a tropical species that has spread from its native habitat in Central and South America, but it has not been identified when this beetle was first reared by humans (Park *et al*., 2013). In Belgium, it has been reared as an edible insect since 2013. In Korea, *Z. atratus* was imported as a major protein source for arthropods, birds, amphibians, and small mammals (Jabir *et al*., 2012; Park *et al*., 2013). Moreover, mealworm inculding *Z. atratus* can feed on agricultural byproducts such as wheat straw, tangerine shell, spent mushroom substrate, brewer's spent grain, and distillers dried grain indicating that it can be reared in an environmentally friendly way (Zanuncio *et al.,* 2000; He *et al.,* 2006; Huang *et al.,* 2011). Based on these advantages, many studies have been conducted on how to utilize *Z. atratus* as a mass-product (Tschinkel, 1981; Quennedey *et al.,* 1995; Jabir *et al.,* 2012; Kim *et al*., 2015; Yoon *et al*., 2018).

Considering both economic and environmental aspects, various efforts have been made to utilize agricultural byproducts (Mussatto *et al.,* 2006). Among agricultural byproducts, brewer's spent grain (BSG), which constitutes approximately 85% of the total byproducts of the brewing industry, is rich in cellulosic and non-cellulosic polysaccharides, and thus has strong potential to be recycled. This low-cost agricultural byproduct with high economic potential is valuable for animal nutrition (Aliyu and Bala, 2011). The major nutritional factors of spent grain are crude fat, crude proteins, roughage from fungal fermentation in yeast, and various minerals; thus, it is suitable for animal feed, and many studies have been conducted to investigate its possible usages (Song, 2005; Ganesan *et al.,* 2007; Choi *et al.,* 2008; Kim *et al.,* 2016). Thus BSG has been used as livestock feed (Szponar *et al*., 2003; Aliyu and Bala, 2011).

Recently, there has been an increasing interest on producing *Z. atratus* as human food and animal feed in mass-rearing facilities, and thus a standard mass-rearing protocol is necessary to guide *Z. atratus* producers. In addition, it is important to develop alternative feed with high nutritional value and low cost for *Z. atratus* to reduce its production cost and to produce a high quality product. Therefore, the present study analyzed the effects of these alternative feeds on larval survival, weight, and duration, and the nutritional value of the agricultural byproducts BSG, mixed with different amounts of conventional feed, i.e., wheat bran (WB). Based on this study, we want to suggest the best alternative feed for *Z. atratus* in a mass production system.

Materials & methods

Experimental insects and rearing

The insect, *Z. atratus* used in the experiment was seventh or eighth instar larvae (60 days after hatching) of third generations. This insects were reared year-round in 26±2°C under approximately 65% relative humidity and 12L:12D lighting conditions at the Division of Applied Entomology, Department of Agricultural Biology, National Institute of Agricultural Sciences. The larvae of *Z. atratus* were kept in a plastic box (27 × 36 × 8 cm (width × depth × height)). As a major feed sources, approximately 0.8 cm of wheat bran (WB) or brewer's spent grain (BSG) was thinly spread on the bottom of the box, and fresh cabbage leaves and carrots were supplied twice per week as a water source.

Development characteristics of *Z. atratus* larvae with BSG

Preparation of BSG feed

The BSG used in the experiment was purchased at a OB beer brewery (Icheon, Korea). The BSG was a byproduct of beer brewing and was dried at 70°C for 24 h using a hot air circulating drier (FC-2D-1, Universal Scientific Co. LTD., Kimpo, Korea) and treated with a high speed pulverizer (HJM-10100, Han Sung Pulverizing Machinery Co. LTD., Gwangju-si, Korea). After this treatment, their resulting dried powders were used BSG feed.

Growth of *Z. atratus* larvae on the different BSG contents

Z. atratus larvae were fed 100% WB on the standard rearing protocols. To test the effects of BSG on the growth of *Z. atratus* larvae, different amounts of BSG were mixed with WB as 90% WB + 10% BSG, 70% WB + 30% BSG, 50% WB + 50% BSG, 30% WB + 70% BSG. And then, the larval survival rate, body weight, and developmental period were investigated at different contents of BSG. The standard feeding ration was set at 10 g of 100% wheat bran, and BSG was added as previously described by weight. Each experimental group consisted of 10 individuals of seventh or eighth instar larvae (60 d after hatching) in a plastic container (10 × 4 cm (diameter × height)). All the experimental groups were replicated three times. As a water source, 5 g of fresh cabbage leaves were supplied twice per week.

Nutritional analysis of *Z. atratus*

Preparation of *Z. atratus* for analysis

All the *Z. atratus* larvae were kept in a plastic box (27 × 36 × 8 cm (width × depth × height)) for four months. The control group was fed with 100% wheat bran, and the experimental group was fed with 50% wheat bran and 50% BSG. For the analysis, all larvae were fasted for one day and were frozen at –70°C in a cryogenic freezer (CLN-70UW, NIHON freezer, Tokyo, Japan) for 24 h. The frozen larvae were dried at 70°C for 17±1 hours to remove moisture and pulverized using a multifunction grinder (KSP-35, Koreamedi, Daegu, Korea).

Analysis of general composition

The general composition of the *Z. atratus* larvae was analyzed based on the criteria of the Association of Official Analytical Chemists (AOAC) (AOAC, 2003). The moisture content was analyzed based on an atmospheric pressure drying method at 105°C. The crude ash content was analyzed by dry ashing at 550°C. The crude protein content was determined by nitrogen determination using the micro-Kjeldahl method. The crude fat content was analyzed using the ether extraction method. Finally, the carbohydrate content was calculated from the analyzed contents of moisture, ash, crude protein, and crude fat.

Analysis of amino acid composition

Amino acids were analyzed using the ninhydrin method (AOAC, 2003). For hydrolysis, 50 mg of each sample was placed into a bottle with 40 mL of 6N-hydrochloric acid and nitrogen gas at 110°C for 24 hours. The hydrochloric acid was concentrated under reduced pressure at 50°C, diluted with 50 mL of 0.2 N sodium citrate buffer (pH 2.2), and filtered using filter paper (0.45 μm, Pall Life Sciences, California, USA). The filtered samples were analyzed using an amino acid analyzer (L-8900 High-Speed Amino Acid Analyzer, Hitachi, Tokyo, Japan).

Analysis of fatty acid composition

The fatty acid composition was analyzed based on Folch *et al*. (AOAC, 2003). To extract the fatty acid, 50 g samples were mixed with 250 mL of a chloroform:methanol (2:1) solution using a homogenizer at 3,000 rpm. The moisture was removed with anhydrous sodium sulfate, and the filtered sample was concentrated at 50-55°C. After adding 1 mL of tricosanoic acid, 1 mL of a 0.5 N sodium hydroxide was added to the sample. After heating at 100°C for 20 min, the mixture was allowed to cool for 30 min. After adding 2 mL of boron trifluoride, the mixture was heated for 20 min and allowed to cool for 30 min. After adding 1 mL of heptane and 8 mL of sodium chloride, the supernatant was removed and analyzed using gas chromatography (US/HP 6890, Agilent Technologies, Seoul, Korea) to estimate the amounts of fatty acids.

Analysis of mineral composition

To analyze the trace elements and heavy metals in *Z. atratus*, 50 mg of hot air-dried powder sample was pretreated, and it was placed in an electric furnace at 600°C for 2 h or more. This sample was dissolved overnight using a hydrochloric acid solution (1:1). The dissolved solution was filtered using No. 6 filter paper (Whatman International Co., Maidstone, UK). To measure the trace elements and heavy metals, the sample was analyzed using an inductively coupled plasma optical emission spectrometer (ICP-OES, Horiba, Kyoto, Japan) (AOAC, 2003).

Analysis of harmful microorganisms

The presence of harmful microorganisms (*Escherichia coli* O157:H7*, Salmonella* spp.) was investigated based on the provisions of the Food Code to ensure the safety of the larvae of *Z. atratus* (AOAC, 2003). To detect *E. coli* O157:H7, 25 g of sample was added to 255 mL of modified EC broth (Novobiocin Supplement, Thermo Fisher Scientific, Basingstoke, UK), and it was incubated for 24±2 h at 35-37°C. The incubated medium was inoculated into MacConkey sorbitol agar medium (Sorbitol MacConkey Agar, Thermo Fisher Scientific, Basingstoke, UK) supplemented with cefixime (0.05 mg/L) and potassium tellurite (2.5 mg/L) and cultured at 35-37°C for 18 h. The purple colonies that were presumed to be *E. coli* were inoculated into agar medium and cultured for analysis with Gram staining and the O/H serotype test. To check for *Salmonella* spp., 25 g of sample with 255 mL of peptone water was incubated at 35-37°C for 24±2 h. After incubation, 0.1 mL of the cultured sample was inoculated into 10 mL of Rappaport-Vassiliadis medium and cultured at 42±1°C for 24±2 h. After inoculation on XLD agar medium, the colonies that were black or red in the center were inoculated onto normal agar medium at 36±1°C for 24 h and transferred to TSI (triple sugar iron) agar medium (Thermo Fisher Scientific, Basingstoke, UK). When a colony was suspected to be a *Salmonella* sp., it was confirmed using the Gram stain method and O/H serum aggregation test.

Statistical analysis

A one-way ANOVA test was carried out to verify the larval survival rate, weight, development period at different BGS contents. For all statistical analyses, the statistics package SPSS PASW 22.0 for Windows (IBM, Chicage, USA) was used.

Results & Discussion

Development of *Z. atratus* larvae with BSG

Survival rate

The survival rate of the *Z. atratus* larvae fed BSG was compared to that of those fed 100% wheat bran. This experiment was conducted until all larvae became pupae. Therefore, the duration of this experiment was 16 wk for all groups (10, 30, 50, 70% BSG added to the wheat bran and 100% wheat bran). The control group with 100% wheat bran and the 10% BSG group showed 100% survival rate until the 16th wk, but the 30, 50, and 70% BSG groups showed 83.3-90.0% survival rate at the 16th wk (Fig. 1). The survival rates of larvae were not affected by the different contents of BGS (one-way ANOVA test: $F(4,10)=0.833$, $P=0.534$ at 2 wk; $F(4,10)=1.700$, $P=0.226$ at 4th wk; $F(4,10)=1.700$, $P=0.226$ at 6th wk; $F(4,10)=3.300$, $P=0.057$ at 10th wk; $F(4,10)=3.300$, $P=0.057$ at 12th wk; $F(4,10)=2.000$, $P=0.171$ at 14th wk; $F(4,10)=1.559$, $P=0.259$ at 16th wk).

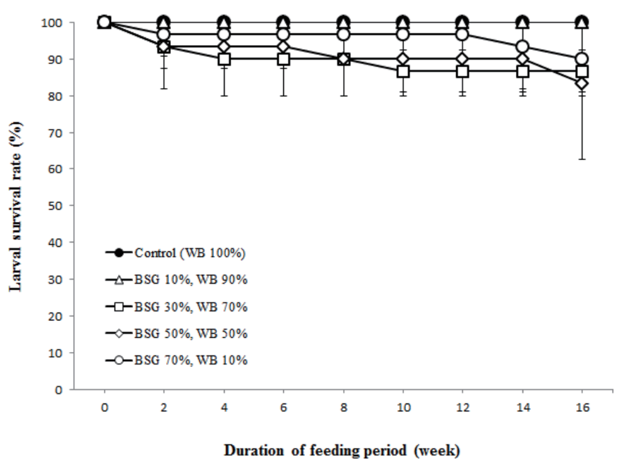

Fig. 1. Survival rate of *Zophobas atratus* larvae with different contents of Brewer's spent grain (BSG) as an alternative feed. Values are means ± S.D. One-way ANOVA test, P>0.05. Each experimental group consisted of 10 individuals of 7th or 8th instar larvae. All the experimental groups were replicated three times.

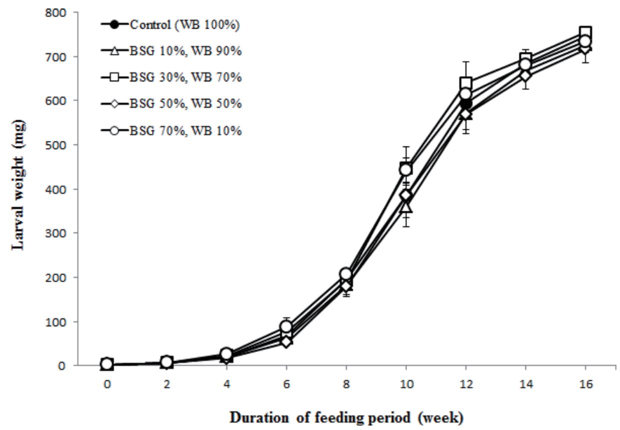

Fig. 2. Average larval weight of *Zophobas atratus* larvae with different contents of Brewer's spent grain (BSG) as an alternative feed. Values are means ± S.D. One-way ANOVA test, P>0.05.

Body weight of *Z. atratus* larvae

At the 4th and 6th wk after feeding experiments, the body weight of the 70% BSG group, 89.0 mg, was 1.3 times higher than that of the control group, 67.3 mg, and that of the 30% BSG group was 77.2 mg. The body weight of the 10% BSG group, 62.8 mg, was similar as that of the control group, but that of the 50% BSG group, 53.6 mg, was lower than that of the control group. During the 8th and 10th wk, the 70% BSG group showed a body weight of 442.2 mg during the 8th wk, and the 30% BSG group showed a body weight of 447.9 mg. The body weight of the 50% BSG group was 386.4 mg, which was similar to that of the control group, 385.7 mg. However, the body weight of the 10% BSG group, 361.0 mg, was lower than that of the control group. Between the 12th and 16th w, the body weight of all BSG groups (717.1-753.9 mg) did not differ from that of the control group (754.4 mg during the 16th wk) ($F(4,10)=2.066$, $P=0.161$ at 12th wk; $F(4,10)=1.219$, $P=0.362$ at 14th wk; $F(4,10)=1.628$, $P=0.242$ at 16th wk) (Fig. 2).

Developmental period of the larvae

The developmental period of the larvae was measured as the period from the 7 to 8th instar (approximately 60 d after

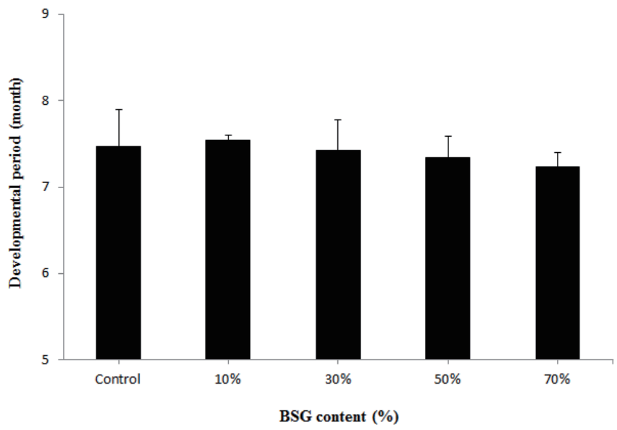

Fig. 3. Mean developmental periods of *Zophobas atratus* larvae with different contents of Brewer's spent grain (BSG) as an alternative feed. Values are means ± S.D. One-way ANOVA test, P>0.05.

hatching) to pupation, and it was checked every week. This period for the 10% BSG group was 2.3 d longer than that of the control group. However, the development periods for the 30, 50, and 70% BSG groups were shorter than that of the control group. Even though it was not statistically significant (F(4,9)=0.396, P=0.807), the development period of the 70% BSG group was 6.9 d shorter than that of the control group (Fig. 3).

Based on the developmental characteristics of *Z. atratus* larvae, BSG can be mixed with wheat bran as a *Z. atratus* food ingredient without any problems. As an alternative to rice bran as a food source, 30% BSG was fed to *Catla catla* (Ham.) and *Labeo rohita* (Ham.), which belong to Cypriniformes, and weight gain was observed (Kaur and Saxena, 2004). Kim *et al.* (2017a) that larval and pupal weight, larval development and survival, and pupation rate of *T. molitor* were higher in larvae fed on 50% BSG or 50% DDG mixed with 50% WB groups than in larvae fed on 100% WB.

Nutritional analysis of *Z. atratus* larvae based on the feed ingredients

Analysis of general components

When the general components of *Z. atratus* were compared based on the feed ingredients, the 50% BSG group showed 6.7% moisture, 47.1% crude protein, and 7.4% carbohydrates, but the control group with 100% wheat bran showed 5.3%, 40.4%, and 6.1%, respectively (Table 1). For crude protein, 45.6% in the 50% BSG group and 36.3% in the control group were found, and the crude ash and crude fiber were 4.8-4.9% and 2.5%, respectively,

Table 1. Composition of the nutrients in *Zophobas atratus* larvae fed wheat bran (WB) and brewer's spent grain (BSG)

Components (%, w/w)	Standard feed	Alternative feed
	WB 100%	BSG 50%, WB 50%
Moisture	5.3	6.7
Crude protein	40.4	47.1
Crude fat	45.6	36.3
Crude ash	4.9	4.8
Crude fiber	2.5	2.5
Carbohydrates†	6.1	7.4

†Crude carbohydrates = 100 - (moisture + crude protein + crude fat + crude ash)

so these values were similar between the two groups. Protein contents have been reported as 8.5-14.7% for eggs, 16.1-35.1% for meats, 7.1-56.0% for fishes, and 7.9-26.1% for beans (Baek *et al.*, 2017). Among edible insects, the protein content has been reported as being 70.4% for grasshoppers, 56.8% for silkworms, 50.3% for mealworms, 64.3% for crickets, 57.8% for white spotted flower chafers, and 39.3% for rhinoceros beetles (Baek *et al.*, 2017). When compared to these food ingredients, *Z. atratus* may be a strong candidate high protein source.

Analysis of amino acids

The amino acid content was compared between the control and 50% BSG groups for 17 amino acids (Fig. 4 and 5). In general, the amino acid content was 1.1-1.4 times higher in the 50% BSG group than in the control group. The essential and nonessential amino acid contents for the 50% BSG group were 1.2 times higher than those for the rhinoceros beetle because the essential amino acid contents for edible insects were 13.2-17.8% for mealworms, white spotted flower chafers, and rhinoceros beetle larvae, and the nonessential amino acids contents were 21.6-30.4% (Baek *et al.*, 2017; Kim *et al.*, 2016). Essential amino acids cannot be synthesized in an organism, or only trace amounts can be synthesized, so they should be acquired from the diet (Choi, 1997; Kim *et al.*, 2017a). In the 50% BSG group, the essential amino acids of valine, leucine, and lysine were 1.2 times higher than those of control group. In particular, leucine, which alleviates hyperglycemia, was the most abundant essential amino acid as 3.3%, in the 50% BSG group. The methionine, threonine, isoleucine, phenylalanine, and histidine contents were relatively low, at 0.3-1.8%. The methionine content was the lowest in the control group (0.3%) and 50% BSG group

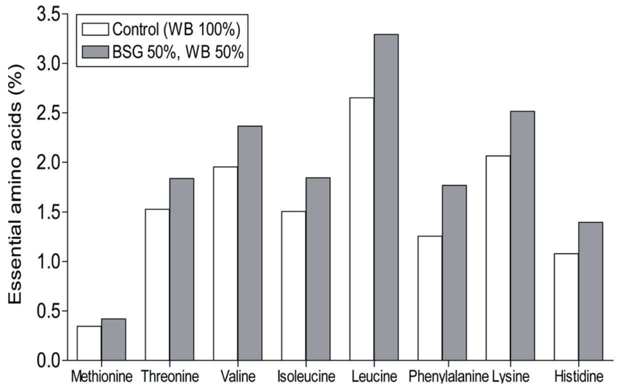

Fig. 4. Essential amino acid content of *Zophobas atratus* larvae fed wheat bran (WB) and brewer's spent grain (BSG).

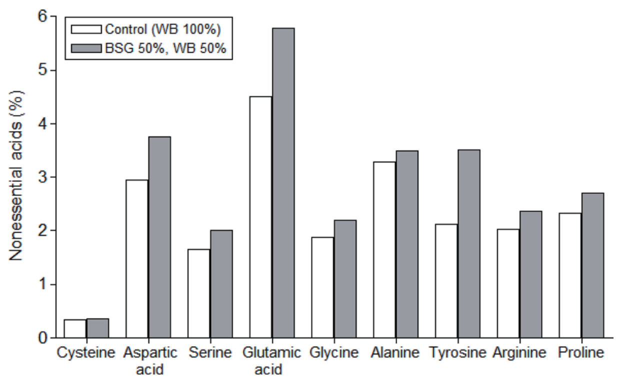

Fig. 5. Nonessential amino acid content of *Zophobas atratus* larvae fed wheat bran (WB) and brewer's spent grain (BSG).

(0.4%), and phenylalanine was 1.4 times higher in the 50% BSG group (1.8%) than in the control group (1.3%) (Fig. 4). Nonessential amino acids can be synthesized by organism, so they are not required in the diet (Choi, 1997; Kim *et al.*, 2008; Kim *et al.*, 2017b). Among the nonessential amino acids (cysteine, aspartic acid, serine, glutamic acid, glycine, alanine, tyrosine, arginine, and proline), glutamic acid, which can be used as a neurotransmitter and regulates sensors with GABA, was the most abundant nonessential amino acid, at 5.8% for the 50% BSG group and 4.5% for the control group; it was 1.3 times higher in the 50% BSG group than in the control group. Aspartic acid, which promotes relief from liver fatigue, was 1.3 times higher in the 50% BSG group (3.8%) than in the control group (2.9%), and the content of alanine, which activates liver function to prevent hangovers and plays a role in detoxification, was 3.5% for the 50% BSG group and 3.3% for the control group (Kim *et al.*, 2017b). Serine, glycine, arginine, and proline were 1.2 times higher in the 50% BSG group than in the control group. Tyrosine, which promotes signaling in neuronal cells and produces important brain chemicals to regulate mood, was 1.7 times higher in the 50% BSG group than in the control group (Young, 2007). In both the 50% BSG and control groups, cysteine was detected at a low concentration (Fig. 5). Like previously recorded in edible insects, *Z. atratus* in the 50% BSG and control groups have high amino acid content, so they have a chance of a good food ingredient.

Analysis of fatty acids

The fatty acid analysis of *Z. atratus* was divided into two categories: saturated fatty acids and unsaturated fatty acids (Fig. 6). Among all the fatty acids, unsaturated fatty acids were 60.7% in the control group and 62.2% in the 50% BSG group, and saturated fatty acids were 39.3% in the control group and 37.8% in the 50% BSG group, so the unsaturated fatty acid content was 1.5-1.6 times higher than the saturated fatty acid content. Among the three fatty acids, palmitic acid (C16:0)) was the most abundant fatty acid, and it was 32.1% in the control group and 30.1% in the 50% BSG group. Among the 10 unsaturated fatty acids, three polyunsaturated fatty acids–eicosapentaenoic acid (EPA) (C20:5n3), docosahexaenoic acid (DHA)(C22:6n3), and docosatetraenoic acid (C22:4n6)—were not detected. Among the seven detected unsaturated fatty acids, oleic acid (C18:1n9) was the most abundant unsaturated fatty acid, at 38.4% for the control group and 29.2% for the 50% BSG group, so it was 1.3 times higher in the control group than in the 50% BSG group. As a monounsaturated fatty acid, oleic acid (C18:1n9) is widely found in animal fat and vegetable oil. Oleic acid can decrease

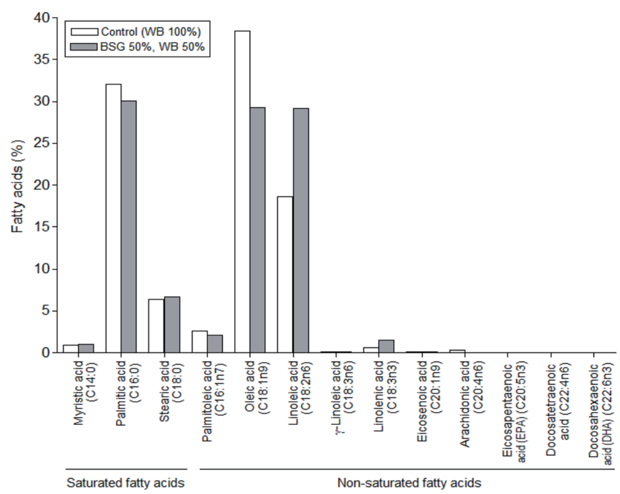

Fig. 6. Fatty acid composition of *Zophobas atratus* larvae fed wheat bran (WB) and brewer's spent grain (BSG).

LDL cholesterol, which induces arteriosclerosis, and increase HDL cholesterol, which protects the liver. In addition, it is known to help in the growth and development of infants and prevent cancer and decreased memory from aging (Kim *et al.*, 2017b; Nam and Lee, 2007; Natali *et al.*, 2007). The next most abundant fatty acid, linoleic acid (C18:2n6), was 18.6% in the control group and 29.2% in the 50% BSG group. Linoleic acid and linolenic acid are constituents of the biomembrane and act to lower the cholesterol content of the blood, so it is important to acquire them from the diet (Kim *et al.*, 2017a; Kim *et al.*, 2017b; Lee *et al.*, 2011). Moreover, a mixed solution of linoleic acid and linolenic acid was reported to show anticancer and antioxidant effects and to reduce blood cholesterol and body fat (Angerer and von Schacky, 2000; Harper and Jacobson, 2001; Lim *et al.*, 1997; Yoo *et al.*, 2013). The saturated fatty acid content of *Z. atratus* was 39.3% for the control group and 37.8% for the 50% BSG group, so it was 1.6-1.8 times higher than that for the edible insects of mealworms (23.2%) and white spotted flower chafers (21.5%), and similar to that of rhinoceros beetle larvae (39.2%). The unsaturated fatty acid content of *Z. atratus* was 60.7% for the control group and 62.2% for the 50% BSG group, so it was 1.2-1.3 times lower than that of mealworms (76.8 5) and white spotted flower chafers (80.8%) and similar to that of rhinoceros beetle larvae (59.6%) (Baek *et al.*, 2017; Kim *et al.*, 2016). Unsaturated fatty acids are high in vegetable oil and fish oil, and their content is 84.1% in olive oil, 62.5% in avocado oil, 65.6% in eggs, 56.5% in beef, 70.6% in mackerel, and 69.3% in duck meat (Cho and Lee, 2014; Hong *et al.*, 2012; Indriyani *et al.*, 2016; Koo *et al.*, 2002; Seo *et al.*, 2010). Unsaturated fatty acids cannot be synthesized by organisms, so they should be acquired from the daily diet. In both the control and 50% BSG groups, *Z. atratus* showed a high content of unsaturated fatty acids, so this beetle showed its potential as an alternative to meat and fish.

Analysis of minerals

When the mineral contents were compared between the 50% BSG and control group in 100 g of dried sample, potassium (781.1 and 746.7 mg, respectively), phosphorous (570.2 and 553.7 mg), sodium (116.9 and 112.1 mg), magnesium (112.5 and 107.9 mg), calcium (41.9 and 55.9 mg) were detected in the 50% BSG and control groups and are shown in order according to the amount detected (Fig. 7). There were almost no differences in the contents of potassium, phosphorous, sodium, and magnesium between the 50% BSG and control groups, but calcium was 1.3 times higher in the 50% BSG group than in the control group. As trace minerals, the amount of zinc was 8.4 mg, the amount of copper was 1.4 mg, and the amount of manganese was 1.2 mg in both groups. The amounts of phosphorous and potassium were similar in both groups. It has been reported that phosphorous is involved in skeleton and tooth elements and energy metabolism, and potassium is involved in muscle contraction and relaxation as well as in the prevention and alleviation of high blood pressure (Baek *et al.*, 2017; Ophir *et al.*, 1983; Seol *et al.*, 1990). Among diverse minerals, phosphorous (424.7-593.2 mg) and potassium (865.2-1597.0 mg) have been detected in high amounts in three edible insects (mealworms, white spotted flower chafers, and rhinoceros beetle larvae) (Baek *et al.*, 2017; Kim *et al.*, 2017b). Minerals have diverse functions in hormone and enzyme activities, skeleton and tooth synthesis, and physiological control, including water and pH balance. Based on our results, *Z. atratus* is a strong food ingredient candidate because of its high content of minerals.

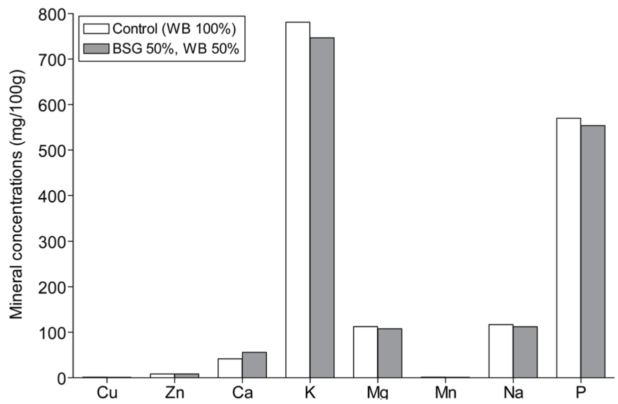

Fig. 7. Mineral contents of *Zophobas atratus* larvae fed wheat bran (WB) and brewer's spent grain (BSG).

Hazardous material analysis

In the hazardous material analysis, heavy metals—lead (Pb), mercury (Hg), arsenic (As), and cadmium (Cd)—and microorganisms—*E. coli* and *Salmonella* spp.—were checked, and the results are summarized (Table 2 and 3). Heavy metals were not detected in the control and 50% BSG groups, and *E. coli* and *Salmonella* spp., which are listed as hazardous microorganisms on the food ordinance regulations, were not detected. Based on these results, *Z. atratus* is safe as a food source (Table 2 and 3).

When the nutritional content and developmental characteristics

Table 2. Heavy metals in *Zophobas atratus* larvae fed wheat bran (WB) and brewer's spent grain (BSG)

Hazardous substance	Content	Standard feed	Alternative feed
		WB 100%	BSG 50%, WB 50%
Heavy metals (mg/kg)	Lead (Pb)	0.00	0.00
	Mercury (Hg)	0.00	0.00
	Arsenic (As)	0.00	0.00
	Cadmium (Cd)	0.00	0.00

Table 3. Pathogenic microbes in *Zophobas atratus* larvae fed wheat bran (WB) and brewer's spent grain (BSG)

Hazardous substance	Content	Standard feed	Alternative feed
		WB 100%	BSG 50%, WB 50%
Pathogenic microbes	*Escherichia coli* O157:H7	ND†	ND
	Salmonella spp.	ND	ND

†ND, not detected

of *Z. atratus* were compared, the 50% BSG group was determined to be the most effective group. Based on this study, BSG has strong potential as an alternative feed ingredient for *Z. atratus* rearing. Therefore, BSG is considered to be worthy of being used as an alternative feed for *Z. atratus* larvae.

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