Effects of Brewer's spent grain (BSG) on larval growth of mealworms, *Tenebrio molitor* (Coleoptera: Tenebrionidae)

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Abstract

Mealworms, *Tenebrio molitor* (Coleoptera: Tenebrionidae), are widely used as food source for animal rearing as well as human diet. Conventionally, mealworms raised on wheat bran. In this study, we investigated the effects of brewer's spent grain (BSG) on the growth of mealworm larvae to reduce the rearing cost. We prepared five different diets with various BSG content, 0, 10, 30, 50, and 70% of heated air dried BSG with wheat bran. We compared survivorship, larval weight, developmental duration, pupation rate, and pupal weight of five different groups of *T. molitor* raised on these five diets. The larval survivorships were almost similar with the control group except a group with 70% of BSG. For the larval weight, groups with 30% and 50% of BSG gained more weight than that with control treatment, 0% of BSG. For the developmental period, the groups with 30% of BSG took less time compared to the control group. In all treatment groups, more than 90% of pupation rate were observed. Especially, the group with 30% of BSG showed highest pupation rate among the five groups. Wheat bran with 30~50% of BSG was the optimal diet for successive insect rearing among the five diet treatments. Based on this study, we concluded that adding BSG to wheat bran helps to improve the quality of *T. molitor* and to reduce the rearing period.

© 2016 The Korean Society of Sericultural Sciences Int. J. Indust. Entomol. 32(1), 41-48 (2016)

Received: 24 Mar 2016 Accepted: 25 Mar 2016

Keywords: *Tenebrio molitor*,

larvae,

brewer's spent grain (BSG)

Introduction

Insects are a promising candidate for animal feed ingredients because it can be efficiently utilized as good nutrients by livestock. Insects convert vegetable proteins that obtained from their food into animal protein inside of their body before accumulating nutrients. This process is $4 \sim 9$ times efficient compared to other livestock. Insects including

mealworms, black soldier flies, grasshoppers and crickets are identified as a promising candidate for an essential protein ingredient for animal feed because they are rich in essential amino acids (Choi *et al.*, 2015). Especially, mealworms are known as a feed ingredient with fats, proteins, various amino acids, unsaturated fatty acids, and minerals (Kim *et al.*, 2014; Huang *et al.*, 2006; Huang *et al.*, 2007; Huang *et al.*, 2011; Ye *et al.*, 1997; Yoo *et al.*, 2013).

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Rearing mealworms is more environmental friendly because of low feed cost (He *et al.*, 2006; Huang *et al.*, 2005; Huang *et al.*, 2011; Kim *et al.*, 2014; Tian and Xu, 2003; Wu *et al.*, 2008; Zanuncio *et al.*, 2000) as well as alternative food sources such as wheat straw, tangerine shell, and spent mushroom substrate that have not been used (Kim *et al.*, 2014; Li *et al.*, 2012). With these advantages, many researches are focusing on improving mealworm industry (Kim *et al.*, 2014; Li *et al.*, 2012).

The brewing industry generates relatively large amounts of by-products and wastes; spent grain, spent hops and yeast being the most common. However, they can be readily recycled and reused because they are also organic matter. The brewing industry tends to be more environmental friendly as consumers prefer environmental friendly products (Ishiwaki et al., 2000, Mussatto et al., 2006). Spent grain is the most abundant brewing by-product, corresponding to around 85% of total by-products generated (Aliyu and Bala, 2011; Mussatto et al., 2006; Tang et al., 2009). Thus, Brewer's spent grain (BSG) is a readily available, high volume low cost by-product of brewing and is a potentially valuable resource for industrial exploitation (Robertson et al., 2010). Thus, increased endogenous metabolism as well as high proteolytic activity in BSG affects its composition within a very short time (Aliyu and Bala, 2011; Ikurior, 1995). BSG are known as its high nutritional value including cellulose, hemicelluloses, lignin, proteins as well as various minerals, vitamins, and amino acids (Aliyu and Bala, 2011; Essien JP and Udotong, 2008; Mussatto et al., 2006; Tang et al., 2009). Therefore, Several attempts have been made to utilize BSG in animal feeds, production of value-added compounds (xylitol, lactic acid, among others), microorganisms cultivation, or simply as raw material for extraction of compounds such as sugars, proteins, acids and antioxidants (Aliyu and Bala, 2011).

The standard mass-rearing techniques for mealworms are required as insect farming industry is rapidly growing as well as it is about to be industrialized. In this study, we investigated the effects of BSG on larval growth of mealworms such as larval survivorship, larval weight, larval period, pupation rate, pupal weight. Based on this study, we can conclude that BSG will be used as a promising alternative mealworm food source mixed with wheat bran.

Materials and Methods

Experimental Animals

All life stages of T. molitor have been kept in insect rearing facilities at National Institute of Agricultural Science for more than five generation at 25 ± 3 °C with $50\sim60\%$ RH and 14L:10D light condition. The room temperature was controlled by automated thermostat and monitored by thermometers, and the light condition was set for the optimal growth rate of T. molitor based on previous studies. Larvae of T. molitor were maintained in the plastic box ($27 \times 36 \times 8$ (length x width x height)) filled with 0.8 cm of wheat bran as a food source and fresh cabbage leaves or carrots as a water source that was replace every 2 wk

Feed with Different Contents of brewer's spent grain (BSG)

Larvae of *T. molitor* were fed wheat bran based on the standard rearing protocols. To test the effects of BSG on the growth of *T. molitor* larvae, different contents of BSG were mixed with wheat bran. BSG was dried at 80°C for 24 h. The dried BSG was ground by Hi-Jet Milling Machine (HJM-10100, Hansung Pulverizing Machinery CO. LTD., Gwangju-si, Gyounggi-do, Republic of Korea). This dried powder was mixed with wheat bran with different content by its weight.

Larval Growth of T. molitor with Different Diets

Seventh or eighth larvae that were 60 d after hatching were tested their growth rate with different diet. For each group, 30 larvae were tested in a plastic container (10 x 4 cm (diameter x height)) with three biological replications. All the larvae were fed wheat bran with different contents of BSG. We put 5 grams of cabbage leaves as a water source twice per week. As a control diet, we used wheat bran without any BSG based on the standard rearing condition. Each group of larvae fed on different contents of BSG was compared its survivorship, average larval weight, duration for each development stage, pupation rate, and pupal weight.

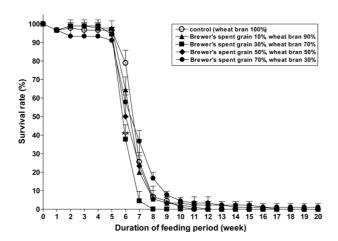


Fig. 1. Survivorship of *Tenebrio molitor* larvae with different contents of Brewer's Spent Grain (BSG) as a feed supplement. 1 way ANOVA, Tukey's multiple comparison test, **, p < 0.01

Results and Discussion

Larval Survivorship

The larval survivorship of each group maintained on wheat bran with different contents of BSG was compared to the control group that were maintain on 100% of wheat bran. The survivorship was checked until pupation. Therefore, the control group had been checked for 10 wk, the 10% BSG group for 11 wk, the 30% BSG group for 7 wk, the 50% BSG group for 16 wk, and the 70% BSG group for 20 wk.

We checked the survivorship for the first 4 wk because insect producers conventionally selling *T. molitor* 5 wk after seventh or eighth instar larvae. The survivorships for the control group and the 30% and 50% BSG groups were the highest (98.89 \pm 1.92 (mean \pm S.D.) for the first 4 wk, but that for 70% of BSG was the lowest (93.33 \pm 8.82 (mean \pm S.D.) (Fig. 1). At the fifth week, pupae were observed from all the groups except the control group that required 6 wk for the pupation.

Larval Weight

The maximum weight gain rate was calculated by the percentage of the weight gain for each experimental groups compared to the maximum larval weight of control group (Table 1). When compared average larval weights with different diet groups to that of the control group, the group with 30% BSG was the highest from first week to seventh

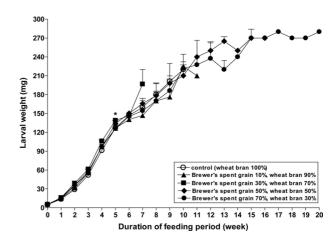


Fig. 2. Average larval weight of *Tenebrio molitor* larvae with different contents of Brewer's Spent Grain (BSG) as a feed supplement.

1 way ANOVA, Tukey's multiple comparison test, *, p<0.05

week except the fourth week. Especially, the maximum weight gain rate for the 30% BSG was the highest at the second week as 40%. It was not checked after 7 wk when pupation was observed. The group with 50% BSG showed the highest maximum weight gain rate at fourth, eighth, and ninth weeks. Especially, this group showed 20% higher than that of the control group. However, the groups with 10% and 70% BSG showed low weight gain rate especially the group with 10% of BSG showed no weight gain after fifth week. The group with 70% brewers group showed low weight gain rate after third week as well as no weight gain at seventh and ninth weeks.

Developmental Duration

The duration for pupation from seventh or eighth instar larvae ca. 60 d after hatching was measured by checking the status of *T. molitor* every week. This duration was not significantly different except the group with 30% BSG that required shorter time to pupate (Fig. 3, Table 2).

Pupation Rate

The pupation rates for each group were more than 90%, so it was not significantly different for all the groups except that for 30% BSG with the highest pupation rate (Fig. 4, Table 2).

Table 1. Larval weight changes with different contents of Brewer's Spent Grain (BSG) as a feed supplement.

Weeks		Larvae (n=90)			
	Feeds	Average Larval Weight Maximum Larval Weight Percentage of Maximum			
		(mg)	(mg)	Larval Weight Gain (%)	
1	control (100% Wheat Bran)	13.71±0.47	13.72		
	BSG 10%, Wheat Bran 90%	14.56±1.28	15.69	14	
	BSG 30%, Wheat Bran 70%	15.99±0.74	16.83	23	
	BSG 50%, Wheat Bran 50%	14.64±1.41	16.17	18	
	BSG 70%, Wheat Bran 30%	15.74±0.11	15.87	16	
_	control (100% Wheat Bran)	29.02±0.99	30.07		
	BSG 10%, Wheat Bran 90%	31.57±1.24	32.62	12	
2	BSG 30%, Wheat Bran 70%	39.04±1.41	40.52	40	
	BSG 50%, Wheat Bran 50%	35.59±3.89	38.00	31	
	BSG 70%, Wheat Bran 30%	36.53±1.16	35.96	30	
	control (100% Wheat Bran)	51.95±1.55	52.07		
	BSG 10%, Wheat Bran 90%	55.79±4.85	60.69	17	
3	BSG 30%, Wheat Bran 70%	61.43±3.42	65.33	26	
	BSG 50%, Wheat Bran 50%	55.34±6.24	62.33	20	
	BSG 70%, Wheat Bran 30%	57.82±2.70	60.00	15	
_	control (100% Wheat Bran)	91.15±2.22	92.76		
	BSG 10%, Wheat Bran 90%	97.51±5.44	103.45	13	
4	BSG 30%, Wheat Bran 70%	106.04±2.46	108.67	19	
	BSG 50%, Wheat Bran 50%	97.32±10.75	109.33	20	
	BSG 70%, Wheat Bran 30%	97.16±4.01	100.69	10	
5	control (100% Wheat Bran)	127.13±2.68	127.93		
	BSG 10%, Wheat Bran 90%	126.58±0.47	127.00	0	
	BSG 30%, Wheat Bran 70%	139.01±2.20	141.38	11	
	BSG 50%, Wheat Bran 50%	133.82±5.08	139.31	10	
	BSG 70%, Wheat Bran 30%	125.91±5.38	131.43	3	
6	control (100% Wheat Bran)	146.84±4.19	151.36		
	BSG 10%, Wheat Bran 90%	140.42±5.10	145.50	0	
	BSG 30%, Wheat Bran 70%	146.38±5.81	153.00	4	
	BSG 50%, Wheat Bran 50%	150.22±1.45	151.76	3	
	BSG 70%, Wheat Bran 30%	145.37±3.90	149.44	2	
7	control (100% Wheat Bran)	159.81±10.89	161.11		
	BSG 10%, Wheat Bran 90%	146.94±13.34	160.00	0	
	BSG 30%, Wheat Bran 70%	196.67±33.00	220.00	38	
	BSG 50%, Wheat Bran 50%	165.70±8.42	171.11	7	
	BSG 70%, Wheat Bran 30%	154.33±4.04	158.00	0	
8	control (100% Wheat Bran)	180.00±17.32	180.00		
	BSG 10%, Wheat Bran 90%	170.00±14.14	180.00	0	
	BSG 30%, Wheat Bran 70%	-	-	-	
	BSG 50%, Wheat Bran 50%	178.33±20.21	200.00	11	
	BSG 70%, Wheat Bran 30%	170.17±28.26	188.00	4	
9	control (100% Wheat Bran)	201.17±28.26	202.00		
	BSG 10%, Wheat Bran 90%	176.25±33.59	200.00	0	
	BSG 30%, Wheat Bran 70%	<u>-</u>	-	_	
	BSG 50%, Wheat Bran 50%	198.00±7.00	203.00	11	
	BSG 70%, Wheat Bran 30%	186.28±11.11	199.00	0	
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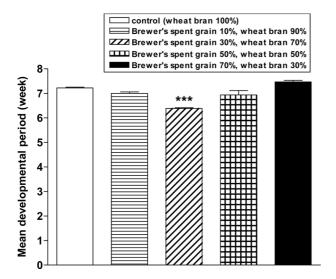


Fig. 3. Mean developmental periods of *Tenebrio molitor* larvae with different contents of Brewer's Spent Grain (BSG) as a feed supplement.

1 way ANOVA, Tukey's multiple comparison test, ***, p<0.001

Pupal Weight

There was no significant difference for pupal weight between the control group and the group with 10% BSG. However, the group with 30% BSG showed less pupal weight and that with 50% BSG showed more pupal weight compared to the control group (Table 2). In addition, the pupal weight was compared by time (Table 3). At the sixth week, the group with 50% BSG showed 32% heavier pupal weight than that of the control group. However, all the groups showed less than 10% heavier pupal weight than that of the control group at the seventh week. The group with 30% BSG showed 32% heavier than the control group at the eighth week. At the ninth week, the group with 50% BSG showed 14% and that with 70% BSG showed 13% heavier than the control group. However, the groups with 10% and 30% BSG were not check any more

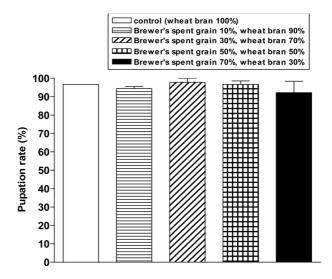


Fig. 4. Average pupation rate of *Tenebrio molitor* larvae with different contents of Brewer's Spent Grain (BSG) as a feed supplement.

1 way ANOVA, Tukey's multiple comparison test, ns

after the ninth week.

Mealworms are generally maintained on rice bran, wheat bran, bean flour, and peanut flour as a food source and various vegetable leaves and fruit skins as a nutritional supplement (Kim *et al.*, 2014). It was confirmed that an agricultural byproduct, citrus pulp, mixed with wheat bran can be a good feed for raising horses because the growth rate and quality of horse were not decreased (Chae *et al.*, 2013; Kim *et al.*, 2014). As it used as a food source for cattle and swine rearing, spent mushroom substrates that containing wheat bran can be used as a food source for mealworms. With its economic advantages, the quality of mealworm may be expected to improve with ingesting mushroom ingredient because it may improve the immune system of mealworm and reduce insect diseases (Kim *et al.*, 2013). Moreover, it was expected to reduce the production price and to improve the quality of meat by feeding cattle on

Table 2. Averages of larval duration, pupal weight, and pupation rate with different contents of Brewer's Spent Grain (BSG) as a feed supplement.

Feeds	Larval Duration (wk.)	Pupal Weight (mg)	Pupation Rate (%)
control (100% Wheat Bran)	7.22±0.05	143.39±0.88	96.67±3.33
BSG 10%, Wheat Bran 90%	7.00±0.09	139.30±7.55	94.44±8.82
BSG 30%, Wheat Bran 70%	6.38±0.06**	131.30±6.17	97.78±3.33
BSG 50%, Wheat Bran 50%	6.94±0.30	151.20±6.25	96.67±7.70
BSG 70%, Wheat Bran 30%	7.46±0.11	155.73±10.44	92.22±1.92

Table 3. Pupal weight gain with different contents of Brewer's Spent Grain (BSG) as a feed supplement.

Weeks	Feeds	Pupae (n=90)		
		Average Pupal Weight (mg)	Maximum Pupal Weight (mg)	Percentage of Maximum Pupal Weight Gain (%)
_	control (100% Wheat Bran)	103.33±13.33	103.33	
	BSG 10%, Wheat Bran 90%	113.04±4.38	116.67	13
6	BSG 30%, Wheat Bran 70%	124.08±3.80	128.42	24
	BSG 50%, Wheat Bran 50%	126.37±9.09	136.67	32
	BSG 70%, Wheat Bran 30%	117.92±3.61	120.00	16
7	control (100% Wheat Bran)	124.10±4.00	124.29	
	BSG 10%, Wheat Bran 90%	125.71±3.87	130.00	5
	BSG 30%, Wheat Bran 70%	130.56±3.53	133.57	8
	BSG 50%, Wheat Bran 50%	135.65±0.63	136.25	10
_	BSG 70%, Wheat Bran 30%	129.86±1.46	131.25	6
	control (100% Wheat Bran)	151.11±4.00	152.86	
	BSG 10%, Wheat Bran 90%	149.64±3.87	162.50	8
8	BSG 30%, Wheat Bran 70%	183.33±3.53	200.00	32
_	BSG 50%, Wheat Bran 50%	158.57±0.63	168.57	12
	BSG 70%, Wheat Bran 30%	144.68±1.46	148.33	0
	control (100% Wheat Bran)	148.00±35.36	149.42	
9	BSG 10%, Wheat Bran 90%	-	-	-
	BSG 30%, Wheat Bran 70%	-	-	-
	BSG 50%, Wheat Bran 50%	145.50±31.82	168.00	14
	BSG 70%, Wheat Bran 30%	158.06±8.36	167.67	13

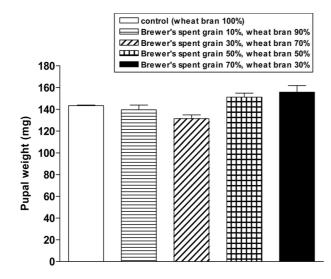


Fig. 5. Average pupal weight of *Tenebrio molitor* larvae with different contents of Brewer's Spent Grain (BSG) as a feed supplement.

1 way ANOVA, Tukey's multiple comparison test, ns

the dry fermented spent mushroom substrates from king oyster mushrooms that can be improved storage period and palability (Moon *et al.*, 2012).

Wheat bran with 30% and 50% of BSG maximized the larval survivorship, but that with 70% of BSG showed the lowest larval survivorship when compared to the control group. Wheat bran with 30% BSG showed the highest larval weight gain from the first to seventh week except the fourth week when compared the larval weight increase for the first 9 wk. During the eighth to ninth weeks, wheat bran with 50% of BSG made the highest larval weight gain. However, wheat bran with 10% and 70% of BSG showed relatively low larval weight gain. The larval duration for wheat bran with 30% of BSG was significantly shorter than that for other diets. The pupation rates were more than 90% for all groups. The pupal weight was not different between the wheat bran with 10% of BSG and the control feed. However, wheat bran with 30%

was lighter than control group, and those with 50% and 70% were heavier. Wheat bran with 30% showed less pupal weight because of shorter larval period.

BSG has been used as a feed supplement (Szponar *et al.*, 2003). As dietary fiber has positive effects on non-infectious diseases, BSG and its products improve the quality of insect as a food supplements. Moreover, feed mixed with BSG is good for improving digestion efficiency as well as alleviating constipation and diarrhea (Prentice *et al.*, 1978: Tang *et al.*, 2009). When 30% of BSG was used as a substituted for rice bran, *Catla catla* (Ham.) and *Labeo rohita* (Ham.) showed significant body weight gain (Kaur and Saxena, 2004).

BSG have been used various human foods including breads and snack to increase fiber content because it is a byproduct for human diets. BSG contains a large amount of dietary fibers, so it has diverse advantages to prevent diseases including cancer, gastrointestinal disorders, diabetes and heart disease (Stojceska *et al.*, 2008). Moreover, BSG has been used in diverse areas: making building bricks, adsorbing and immobilizing heavy metals, growing microorganisms and enzyme, producing bioethanol and lactic acid, extracting hydroxycinnamic acids, and manufacturing xylitol and pullulan (Aliyu and Bala, 2011).

BSG has positive effects on larval growth of mealworm because it is an agricultural by-product with lots of potential with high nutrition contents for feed formulations. In this study, we confirmed that wheat bran with 30% and 50% BSG had good potential as a feed supplement for rearing mealworms. In Korea, mealworms have been used as feed for various pets. Therefore, the farmers rearing mealworms in mass-rearing facilities have been growing. To improve this industry, we need to find better quality food sources and to develop automated mass-rearing system. In this study, we confirmed the potential of BSG as a feed supplement to improve the quality of mealworm with low cost. Therefore, we expected that this study will help insect farmers to increase benefit as well as improve insect production industry.

Acknowledgments

This research was a part of the project titled "Development of alternative fish meal resources using edible insects", funded by the Ministry of Oceans and Fisheries, Korea.

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