ENTOMOLOGY

Effects of diet composition on growth performance and feed conversion efficiency in *Alphitobius diaperinus* larvae

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Abstract

Alphitobius diaperinus has been recommended for mass-production as feed in a rearing facility because of its small size and short biological cycle. This study evaluated the effects of wheat bran and casein or their blend as insect diets on growth performance and feed conversion efficiency of *A. diaperinus* larvae in the laboratory. Casein and wheat bran were the protein and carbohydrate sources of choice, respectively, for diet preparation. Five experimental diet treatments to be tested were designed as follows: control (100% casein), T1 (75% casein +25% wheat bran), T2 (50% casein +50% wheat bran), T3 (25% casein +70% wheat bran), and T4 (100% wheat bran). A total of 150 new hatched larvae were randomly allotted to one of the five dietary treatments, with three replicates (10 hatched larvae per replicate). The standard colonies were composed of 10 hatched larvae, without distinction of sex, reared in a plastic box (14×8×5 cm) provided with aeration holes on the top. The evaluation of *A. diaperinus* larvae included growth performance and feed efficiency. Using casein and wheat bran blends for diet had a positive effect on weight gain and feed conversion ratio of *A. diaperinus* larvae, including an increase in average larval survival and average larval weight. Using casein and wheat blends (75% casein +25% wheat bran or 25% casein +70% wheat bran) as insect-rearing diet will allow effective utilization of the feed for poultry when using the edible portion of mealworms before reaching the pupae stage.

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Introduction

Insects are a nutritionally interesting material because of containing many nutrients that are essential to humans (Ekpo, 2011). The interest in the use of insect as food has been reported in several studies (Ekpo & Onigbinde, 2005; van Huis, 2013). For example, three species of edible larvae belonging to *Tenebrionidae* are currently produced: *Tenebrio molitor* L. (the Yellow mealworm), *Zophobas atratus* Fab. (the Giant mealworm), and *Alphitobius diaperinus* Panzer (the Lesser mealworm) (Van Broekhoven *et al*., 2015). Extensive literature is available regarding dietary effects on the promotion of growth and chemical composition of *Tenebrio molitor*, more than that for the other two species (Van Broekhoven *et al*., 2015). According to previous studies (Ricciardi & Baviera, 2016), *Alphitobius diaperinus* (*A. diaperinus*) has been recommended for mass-production as feed in a rearing facility because of its small size and short biological cycle. Furthermore, compared to *T. molitor* or *Z. atratus*, *A. diaperinus* can be more easily used to produce protein flour for human consumption or diets for farm animals (Ricciardi & Baviera, 2016).

In poultry farms where feedstuff is stored or in poultry sheds, *A. diaperinus*, known as the lesser mealworm or darkling beetle, is a cosmopolitan species commonly gathered at appropriate places based on temperature and humidity (Salin *et al*., 2000; Chernaki-Leffer *et al*., 2007; Sallet *et al*., 2013). They can cause husbandry, sanitary, and economic losses to the poultry industry (Hazeleger *et al*., 2008; Gazoni *et al*., 2012). For example, *A. dia-*

perinus is a potential carrier of avian viruses (Newcastle disease and Gumboro disease) making poultry prone to viral infections that cause immuno-suppression. As a result, methods have been developed, such as synthetic chemical insecticides (pyrethroids and organophosphates) to control *A. diaperinus* (Gazoni *et al*., 2012). However, chemical control is rather difficult due to the resistance of pest population to these compounds (Lambkin *et al*., 2010). Thus, one possibility for effective control is the use of poultry feed additives based on lesser mealworm larvae during the biological cycle of *A. diaperinus* that corresponds to the period of immaturity before reaching pupae stage. From a nutritional point of view, casein has been widely used in artificial rearing diets because proteins or amino acids for insect optimal growth are required in high concentrations. The role of carbohydrates and proteins in increasing the productivity of lesser mealworm larvae must be clearly understood before its mass production as poultry feed. Therefore, this study aimed to investigate the effects of wheat bran and casein or their blends as insect diets on the growth performance and feed conversion efficiency of *A. diaperinus* larvae in the laboratory.

Materials and Methods

Insect preparation

New larvae (0 day) hatched from eggs of *A. diaperinus* were obtained from the National Institute of Agricultural Sciences (Wanju, South Korea). This study was conducted at the Research center for Insect Production, Joongbu University, Geumsan, South Korea from January to April, 2018. During the experiment (65 days), larvae of *A. diaperinus* were reared in an incubator chamber under controlled conditions up to the pupae stage of development. Rearing conditions consisted in a temperature of 22~24°C and 55~65% RH.

Diet preparation

Selected protein and carbohydrate sources in this study were casein and wheat bran, respectively. The five diet treatments included the Control (100% casein), T1 (75% casein +25% wheat bran), T2 (50% casein +50% wheat bran), T3 (25% casein +70% wheat bran), and T4 (100% wheat bran). Chemical analysis of these diets was carried out according to the methods of the Association of Official Analytical Chemists (AOAC, 2005). Chemical composition of the experimental diets is shown in Table 1.

Growth performance and feed efficiency

A total of 150 new hatched larvae were randomly allotted to one of the five dietary treatments described, with three replicates (10 hatched larvae per replicate). The standard colonies were composed by 10 hatched larvae -without distinction of sex- reared in a plastic box (14×8×5 cm) provided with aeration holes on the top. Larvae were allowed to feed freely until diet depletion. Each box was observed daily and moistened with radish (1 g). For growth performance (weight gain, feed intake, and feed conversion ratio), all larvae and feeders from each box were weighed at the beginning (day 0) and at the end of the experiment (day 65). Additionally, every larva in each rearing box was weighed using small tweezers and recorded at 5-days intervals to determine average larval survival and average larval weight. Larval survival percentages from each rearing box were calculated by dividing the number of larvae that died in the period by the initial number of larvae placed and multiplied by 100. Average larval survival percentages from each treatment were calculated as the sum of larval survival percentages from each rearing box divided by the number of replicates. Likewise, average larval weight was calculated as the same way of average larval survival percentages do. In terms of feed conversion efficiency for each diet, feed conversion ratio (FCR) was calculated as follows: FCR=weight of ingested diets/weight gained (Van Broekhoven *et al*., 2015)

Statistical analysis

Data obtained for weight, feed intake and feed conversion ratio for *A. diaperinus* larvae before reaching the pupae stage (pre-pupae) were subjected to one-way ANOVA, and differences among treatments were compared with Tukey´s test at the 5% significance level using the general linear model (GLM) procedures of SAS Institute (SAS, 2002). Also, average larval survival and average larval weight was compared in relative to each treatment as measured over the days of the flush cycle.

Results

The results obtained for growth performance and feed conversion efficiency of *A. diaperinus* larvae fed diets based on casein and wheat bran or their blends are shown in Table 2. Final body weight, weight gain and feed conversion ratio of *A. diaperinus* larvae were affected by diet composition (wheat bran or their blends, $P<0.05$). However, there was no statistical difference in initial body weight and feed intake among dietary groups ($P>0.05$)

Average larval survival and average larval weight of *A. diaperi-*

Table 1. Chemical composition of casein and wheat bran or their blends as experimental diet.

Parameters (%)	Treatment*				
	Control	T1	T2	T3	T4
Dry matter	91.9	91.6	91.1	90.4	90.3
Crude protein	86.9	74.5	53.6	38.3	15.2
Ether extract	1.23	1.51	1.67	3.01	3.66
Crude ash	2.99	3.28	3.85	4.12	4.39
Neutral detergent fiber	0.00	8.32	16.1	31.6	39.3
Acid detergent fiber	0.00	1.54	3.80	7.90	10.3
Hemicellulose	0.00	6.78	12.3	23.7	29.0

*Control: 100% casein; T1: 75% casein +25% wheat bran; T2: 50% casein +50% wheat bran; T3: 25% casein +70% wheat bran; T4: 100% wheat bran.

nus as a function of time is shown in Tables 3 and 4 and in Figure 1. Over the period from day 5 through day 35, average larval survival from five dietary groups varied from 73.3% to 93.3%, and then reached 100% by day 65 (Table 3). For average larval weight (Table 4), it was similar across all groups until day 15. As time increased, average larval weight of *A. diaperinus* increased in all groups.

Table 2. Growth performance and feed conversion efficiency of *Alphitobius diaperinus* larvae fed diets with casein and wheat bran or their blends.

Parameters	Treatment*					P-value
	Control	T1	T2	T3	T4	
Initial body weight (g)	0.0005±0.00013**	0.0006±0.00017	0.0005±0.00012	0.0005±0.00014	0.0005±0.00013	0.9032
Final body weight (g)	0.0111±0.0030[b]	0.0214±0.006[a]	0.0207±0.0059[a]	0.0214±0.0058[a]	0.0195±0.0050[a]	P<0.0001
Weight gain (g)	0.0106±0.0029[b]	0.0208±0.0068[a]	0.0202±0.0057[b]	0.0209±0.0069[a]	0.0190±0.0054[a]	P<0.0001
Feed intake (g)	0.1502±0.042	0.1596±0.044	0.1622±0.045	0.1720±0.048	0.1681±0.168	0.6935
Feed conversion ratio(g feed/g gain)	14.15±3.96[a]	7.69±2.15[b]	8.01±2.24[b]	8.24±2.30[b]	8.89±2.49[b]	0.0014

[a-b]Means in the same rows with no common superscript are significantly different (P<0.05).
*Control: 100% casein; T1: 75% casein +25% wheat bran; T2: 50% casein +50% wheat bran; T3: 25% casein +70% wheat bran; T4: 100% wheat bran. **Values are expressed as means ± standard errors.

Table 3. Average larval survival rates (%) and larval survival number of *Alphitobius diaperinus* as a function of time.

Day	Treatment*				
	Control**	T1	T2	T3	T4
0	100.0 (30/30)	100.0 (30/30)	100.0 (30/30)	100.0 (30/30)	100.0 (30/30)
5	93.3 (28/30)	80.0 (24/30)	100.0 (30/30)	80.0 (24/30)	80.0 (24/30)
10	73.3 (22/30)	93.3 (28/30)	73.3 (22/30)	93.3 (28/30)	80.0 (24/30)
15	86.7 (26/30)	93.3 (28/30)	86.7 (26/30)	80.0 (24/30)	86.7 (26/30)
20	86.7 (26/30)	86.7 (26/30)	86.7 (26/30)	100.0 (30/30)	100.0 (30/30)
25	93.3 (28/30)	86.7 (26/30)	100.0 (30/30)	93.3 (28/30)	100.0 (30/30)
30	86.7 (26/30)	86.7 (26/30)	86.7 (26/30)	100.0 (30/30)	100.0 (30/30)
35	100.0 (30/30)	100.0 (30/30)	100.0 (30/30)	80.0 (24/30)	93.3 (28/30)
40	100.0 (30/30)	100.0 (30/30)	100.0 (30/30)	100.0 (30/30)	100.0 (30/30)
45	100.0 (30/30)	100.0 (30/30)	100.0 (30/30)	100.0 (30/30)	100.0 (30/30)
50	100.0 (30/30)	100.0 (30/30)	100.0 (30/30)	100.0 (30/30)	100.0 (30/30)
55	100.0 (30/30)	100.0 (30/30)	93.3 (28/30)	100.0 (30/30)	100.0 (30/30)
60	100.0 (30/30)	100.0 (30/30)	100.0 (30/30)	100.0 (30/30)	100.0 (30/30)
65	100.0 (30/30)	100.0 (30/30)	100.0 (30/30)	100.0 (30/30)	100.0 (30/30)

*Control: 100% casein; T1: 75% casein +25% wheat bran; T2: 50% casein +50% wheat bran; T3: 25% casein +70% wheat bran; T4: 100% wheat bran. **Values are expressed as average larval survival rates (the sum of larval survival number from replicates of treatment /the total number of larval from replicates of treatment)

Table 4. Average larval weight (g) of *Alphitobius diaperinus* as a function of time.

Day	Treatment*				
	Control	T1	T2	T3	T4
0	0.0005	0.0006	0.0005	0.0005	0.0005
5	0.0006	0.0007	0.0007	0.0009	0.0007
10	0.0007	0.0010	0.0009	0.0011	0.0008
15	0.0009	0.0014	0.0010	0.0013	0.0013
20	0.0013	0.0017	0.0018	0.0023	0.0025
25	0.0019	0.0023	0.0034	0.0033	0.0033
30	0.0020	0.0024	0.0038	0.0039	0.0036
35	0.0023	0.0055	0.0065	0.0062	0.0053
40	0.0027	0.0084	0.0099	0.0096	0.0079
45	0.0033	0.0126	0.0139	0.0133	0.0111
50	0.0039	0.0165	0.0175	0.0170	0.0145
55	0.0062	0.0201	0.0197	0.0203	0.0175
60	0.0095	0.0207	0.0201	0.0208	0.0181
65	0.0111	0.0214	0.0207	0.0214	0.0195

*Control: 100% casein; T1: 75% casein +25% wheat bran; T2: 50% casein +50% wheat bran; T3: 25% casein +70% wheat bran; T4: 100% wheat bran

Discussion and Conclusions

Initial average body weight of all *A. diaperinus* larvae at the beginning of experiment (day 0) were similar in all groups. At the end of the experiment (day 65), T1 (75% casein +25% wheat bran) and T3 (25% casein +70% wheat bran) were higher in final body weight than control (100% casein), T2 (50% casein +50% wheat bran), and T4 (100% wheat bran) larvae. In the current study, the increase in larvae weight gain in all dietary groups occurred in the following order: T3 > T1 > T2 > T4 > Control. Consequently, the observed effect on weight gain was heavily dependent on diet blend composition and blend nutrition value. In other words, certain blends allowed the possibility of an effective utilization of *A. diaperinus* larvae as poultry feed additives (*i.e.,* when supplied as the edible portion of mealworms − late larvae or prepupae − before reaching pupae) for improving growth performance of larvae. In a study using different cereal flours (wheat, barley, corn, and rice flour) on lesser mealworm, Hosen *et al*. (2004) showed differences in growth and development of *A. diaperinus*. They explained the difference in growth and development of *A. diaperinus* based on the chemical composition of each cereal flour and metabolic function. In addition, Van Broekhoven *et al*. (2015) reported that, as is the case with protein source, larval performance is affected by starch source, rather than by the absolute amount of starch.

Groups that gained the highest feed conversion ratios (FCRs) in this study were T1, followed by T2, T3, T4 and Controls. The extremely high FCRs observed for *A. diaperinus* larvae in T1 groups would be explained by the differences between increase in weight gain and the lower amounts of diet consumed. Conversely,

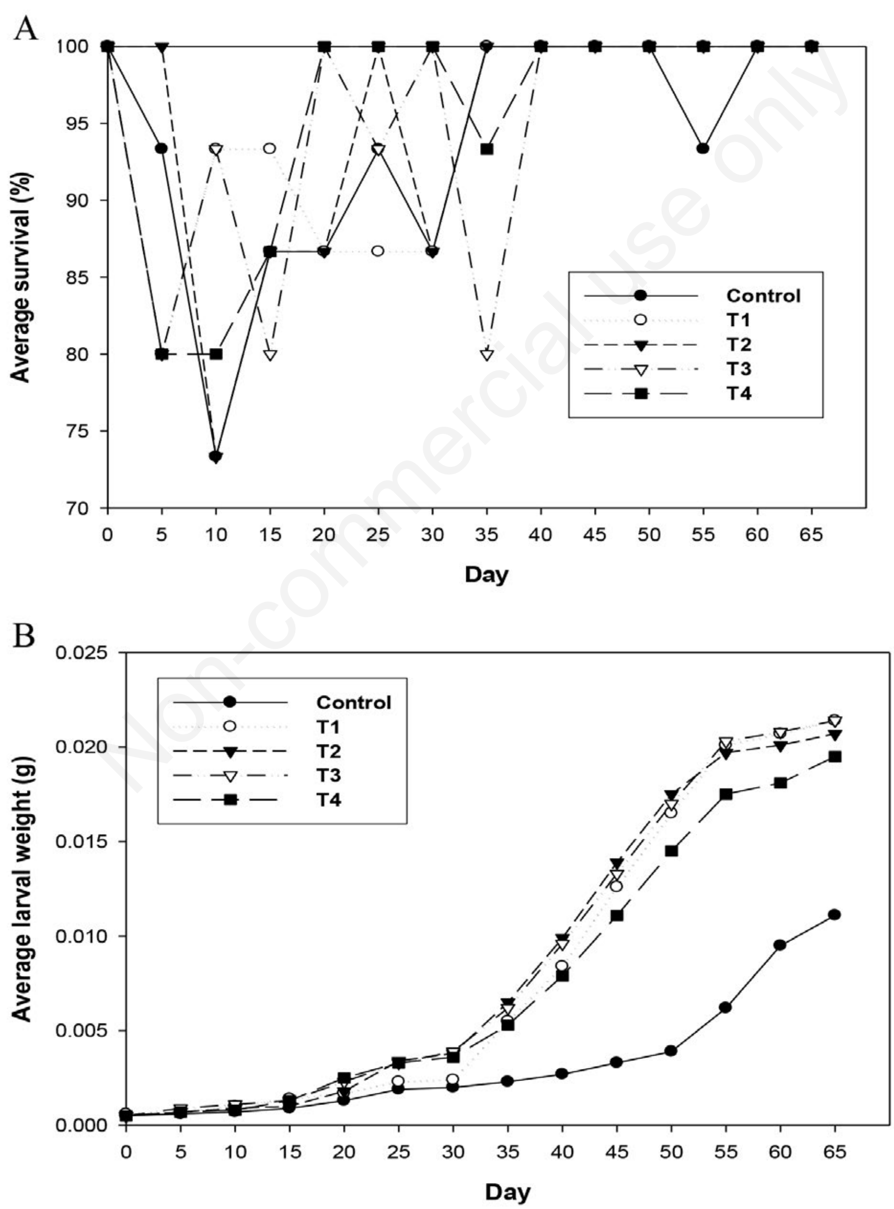

Figure 1. Average larval survival (A) and average larval weight (B) of *Alphitobius diaperinus* as a function of time. Treatment means Control: 100% casein; T1: 75% casein +25% wheat bran; T2: 50% casein +50% wheat bran; T3: 25% casein +70% wheat bran; T4: 100% wheat bran.

Van Broekhoven *et al*. (2015) reported no differences in FCR in *A. diaperinus* fed an HPLS (high protein, low starch) diet, in comparison to the control diet or a HPHS (high protein, high starch) diet. The results from the present study suggest that a mixture of casein and wheat bran improves FCRs by *A. diaperinus*. To our knowledge, this is the first report of FCR values for *A. diaperinus*.

Especially during the period from day 5 to day 35, the groups with lower average larval survival rates were observed among controls (100% casein) and in T4 (100% wheat bran) as single groups. Conversely, T1, T2, and T3 blend groups showed the higher average larval survival rates with partially similar trends over the period from day 5 to day 35. These findings are somewhat consistent with those of van Broekhoven *et al*. (2015), who observed that survival of *A. diaperinus* larvae was lower for the control diet than for experimental diets. In general, the main factor directly associated with the biological cycle of the mealworm is ambient temperature. The highest survival rates were recorded at a temperature of 30°C, which is considered as the most suitable temperature for development of immature phases (Chernaki-Leffer *et al*., 2001; Sallet *et al*., 2013). Further, low temperatures that can contribute to effectively control or reduce the population of this insect species were reported under 16.5°C at which no development of immature phases (Chernaki-Leffer *et al*., 2001; Sallet *et al*., 2013) seems to occur. Other studies showed that the variation in the duration of larval stages, from 35 to 70 days with up to 13 stages, depended on the temperature (Sallet *et al*., 2013). Thus, the reason for the higher average larval survival among insect groups fed the blends tested here resulted in an interaction between the ambient temperature of 22~24°C used and the diet blends.

Overall, *A. diaperinus* larvae in groups T1 and T3 showed the highest larval weight, followed by T2, T4, and Controls. This tendency resulted in higher weight gains observed in larvae feeding on dietary blends than larvae feeding on either protein or carbohydrate (Table 1). Our observation is also supported by Anderson (2000) regarding insect growth rate (Table 2 and 4) can be altered by the nutritional quality of diet (Table 1). At present, the exact mechanisms on the increase of the general survival or weight as dietary blends are unknown.

Results from this study suggested that weight gain and feed conversion ratio in growth performance of *A. diaperinus* larvae was greatly improved by using casein and wheat bran blends rather than by using either one separately. Blends of protein and carbohydrate increased average larval survival and average larval weight of *A. diaperinus* as a function of time. Among all the diets utilized in this study, the best results were observed in larvae fed T1 (75% casein + 25% wheat bran) and T3 (25% casein + 70% wheat bran). Thus, determining the optimum dietary blend composition will enable effective utilization of the feed in poultry when supplied the edible portion of mealworms (late larvae or prepupae) before reaching the pupae stage.

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