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The feeding efficiency of grain bran for the Hermetia illucens larvae

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Abstract. In the paper is presented the result of one experimental study on the growing of the black soldier fly Hermetia Illucens (Diptera: Stratiomyidae) on wheat and rice bran (from 7 days old to the prepupal stage of larvae). Feeding ration was given for larvae every four days in the following amounts: 45, 90 and 120 mg/larvae/day. For each experimental group (100 larvae, n=3) was calculated the indices related to its growth, mortality and feeding efficiency of wheat and rice brans. In general, the results of study showed a trend, in which larger ration of wheat and rice brans contributed to higher biomass and faster growth of the H. Illucens larvae. However, for wheat bran feed the diet 90 mg/l/d was the optimal and met both conditions: the nutrient requirement to gain of a big larvae biomass and provide a high degree of fed substrate degradation. The protein content of the prepupals in this experimental study was consisted from 36-43% and confirmed the opportunity to usage of dry prepupa meal in the aquaculture feed industry.

1 Introduction

Fish protein currently accounts for about 31% of the total animal protein supply in the Asian region, according to FAO [1]. The rapid expansion of aquaculture is not only increasing the demand for fishmeal from wild fish stocks, but also putting pressure on natural fish populations and leading to rising prices for this feed source. It takes more than 6 kg of wild fish to produce 1 kg of farmed fish, especially when carnivorous fish are farmed [2]. In this context, alternative protein sources, ideally from animal origins, may prove to be a very attractive alternative for farmers who currently depend on fishmeal for their product. Research indicates that insects are a complete substitute for fishmeal, fish oil and protein mixes. Diptera have a similar protein profile to fishmeal. They contain high levels of essential amino acids [3]. It was interesting to note that the fatty acid composition of insects can be altered by dietary substrate modulation and thus adapted to the feeding preferences of fish species.

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The black soldier fly is a two-winged insect *Hermetia illucens* (Diptera: Stratiomyidae) that is cultivated in large quantities in South East Asia, Indonesia, the Netherlands, China and the USA. There are examples of the black soldier fly being used to decompose waste [3-11]. The larvae of these flies have the potential to convert large amounts of organic waste, including waste products from birds and pigs (manure), into high-protein biomass. Furthermore, the end-product of black soldier fly production, zoohumus, is widely used as a natural organic fertiliser [4].

The replacement of fish flour in poultry and fish feed with living or dried H. illucens larvae leads to a better utilisation and deposition of nitrogen and, as a result, to a higher body weight increase in domestic animals [5-16].

Grain and grain-fruit-vegetable mixtures are considered to be the most preferred substrates for the establishment of soldier fly larval biomass in the world. According to our unpublished data, the most convenient and cheapest food substrate for the larvae of H. illucens in Uzbekistan is grain wheat bran, which is accessible, nutritious and easy to prepare as a feed [6]. As an alternative to wheat bran, we also used rice bran in our experiment. The aim of the study was to compare growth rates, development time and dry biomass of black soldier fly with different diets of two types of bran. During the experiment, we wanted to get answers to the following questions: what is the optimal amount of wheat and rice bran needed per day to both: be well digested as a food and achieve maximal dry weight of the larvae in the fastest possible development time; and how does the rate of decomposition of bran substrates relate to the development time of black soldier fly larvae?

2 Materials and methods

Adult H. illucens flies were maintained under a 14-h light/10-h dark photoperiod. After hatching from eggs, larvae were kept on minimal experimental diet (45 mg/larva/day, hereafter referred to as mg/l/d) for up to 7 days and then experiments with different diets were started.

In each experiment (n=3, three replicates), one hundred of 7-day-old larvae were used. At the initiation of the experiment, they were placed in plastic boxes, covered with a black cloth and fed with wheat/rice bran for: 45, 90 and 120 mg at day per larva. The intake of wheat/rice bran was calculated according to the daily diet and fed to the larvae every four days.

The bran was given to the larvae until more than 50% of the larvae in the box had passed the prepupa stage, recognisable by their dark colour.

The food remaining at the end of the experiment was used in the calculations as a residue, which was weighed and dried in a chamber at T=60°C for 6 hours after the experiment. At the end of the experiment, the larvae were measured (wet biomass) and then dried in an oven at 60°C for 18 h to determine their dry biomass. The nutritional composition of the both: larvae (pre-pupae) and wheat/rice bran was analysed using a feed analyser (manufactured in China).

Data analysis. The growth of the larvae was calculated from the increase in biomass and expressed as a growth rate (GR) according to the following equation:

$$GR(\%) = \frac{\textbf{final weight} - \textbf{initial weight}}{\textbf{time}} \textbf{ x 100\%} \quad (1)$$

Where "time" was the number of days required for 50% of the larvae to reach the prepupal phase. Higher GR means faster larval growth.

Survival rate (SR) was determined using the following equation:

$$SR(\%) = \frac{\textbf{Number of larvae survival}}{\textbf{Initial number of larvae}} \text{ x } 100\% \quad (2)$$

The ability of the larvae to convert food into their biomass (ECD) was measured using the following equations [6]:

$$ECD(\%) = \frac{\textbf{ECI}}{AD} \textbf{ x } 100\% \quad (3)$$

$$ECI(\%) = \frac{\textbf{B}}{(T - R)} \textbf{ x } 100\% \quad (4)$$

$$AD(\%) = \frac{\textbf{(T} - \textbf{R)}}{R} \textbf{ x } 100\% \quad (5)$$

Where: *ECI* = Feed digesting efficiency; *AD* = Estimated digestibility; *B* = Final dry biomass, mg; *T* = Total amount of feed given in mg; *R*= Dry feed residue, mg.

The substrate decomposition index was used to account for the period of time required for larvae to reduce the amount of feedd substrate (WRI) [7]:

$$WRI = \frac{\frac{T - R}{T}}{t} \textbf{ x } 100 \quad (6)$$

Where D =(T-R)/T, a t - number of days the larvae fed on the substrate.

Statistical analysis. Data from each experiment (n=3) were statistically analysed using one-way ANOVA followed by Tuckey's post hoc test. Statistical analysis was performed to determine the difference between each treatment group (separately for both: wheat bran diets and rice bran diets) in developmental time (days), GR, SR, ECD and WRI ($p<0.05$). All analyses were performed using the Statistica software.

3 Results and Discussion

Nutritional content of wheat/rice bran and its effect on larval growth

Nutrient analysis of feed substrates is necessary because it influences the growth and survival of H. illucens larvae [8]. Protein and carbohydrates are required by the larvae for biomass production, but excess carbohydrates in the diet reduce the efficiency of larval conversion. Excess carbohydrates are not converted into protein by H. illucens larvae, but are converted into lipids, which are used by the black soldier fly as a food reserve for the next stage of development (from prepupal to adult stages) [8].

The nutritional content of the wheat and rice brans used in the experiment is shown in Table 1.

The nutrient content was determined on the basis of the dry weight of the bran. We can see that wheat and rice bran contain similar amounts of protein (12 to 15%) and different amounts of fat (rice bran contains two and a half times as much).

Table 1. The nutritional content of the wheat and rice brans.

Nutritional Content	Wheat bran	Rice bran
Moisture	6.22	4.62
Crude Protein	14.83	12.35
Crude Fat	6.75	15.85
Crude Ash	4.15	9.67
Phosphorus	0.92	1.72
Calcium	1.35	1.90
Salt content	0.26	0.00

The growth rate of larvae is influenced by the nutritional balance of the diet and the content of dietary components such as crude protein and lipids. Protein is essential for larvae to grow and thrive. Lipids are essential for developing and storing food for adult H. illucens. A well-balanced diet when feeding the larvae will allow them to meet their nutritional requirements in order to enter the next prepupal stage in a shorter period of time. The growth of black soldier fly larvae is positively affected by a higher proportion of food [9].

Table 2 shows the growth rates and survival rates of black soldier fly larvae reared on wheat and rice bran.

Table 2. Developmental period (egg to pupa), growth rates (GR) and survival rates (SR) of larvae.

Feed ration (mg/larvae/day)	Days of development		GR growth rate (mg/day)		SR (%)	
	mean	SD	mean	SD	mean	SD
45_{wheat}	19.7a	1.30	0.30a	0.07	98.68a	1.53
90_{wheat}	16.9ab	1.20	0.47ab	0.11	98.67a	2.31
120_{wheat}	15.7b	1.50	0.6b	0,06	97.72a	1.55
45_{rice}	56.0A	3.20	0.09A	0,01	86.00A	1.00
90_{rice}	42.0B	2.50	0.12A	0,01	90.00B	1.00
120_{rice}	28.0C	1.20	0.37B	0,07	99.00C	1.70

abc - significant differences within wheat feed means with different letters differ ($p<0.05$). ABC - significant differences within rice feed means with different letters differ ($p<0.05$).

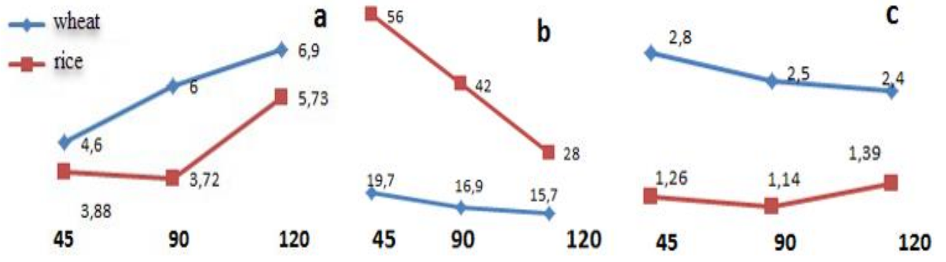

Fig. 1. Dry biomass B (a), development time t (b) and decomposition index WRI (c) for H. illucens larvae fed wheat and rice bran 45, 90 and 120 mg/l/d.

Compared to a low concentration diet, a diet containing wheat and rice bran fed at a higher daily ration has a more balanced composition. In trials, the concentration of bran had an effect on growth, larval development time and prepupal weight (Table 2, Figure 1). Larvae fed daily with 120 mg/l/d wheat and rice bran exhibited the fastest development time of 15.7 ± 1.5 and 28 ± 1.0 days, the highest growth rate of 0.6 ± 0.06 and 0.37 ± 0.07

mg/day and the highest prepupal dry weight of 6.92 ± 0.6 and 5.73 ± 0.7 g, respectively. Larvae fed 90 mg bran a day had prepupal dry weights of 6.0 ± 0.3 and 3.72 ± 0.17 g, and developed in 16.9 ± 1.2 and 42 ± 2.5 days for wheat and rice bran, accordingly (Table 2, Fig. 1). A feed at a dose of 45 mg/l/d of wheat and rice bran resulted in a decrease in prepupal dry weight to 4.6 ± 0.4 and 3.88 ± 0.5 mg and an increase in development time to 19.7 ± 1.3 and 56 ± 3.2 days, respectively. In all diets fed by wheat bran (45, 90, 120 mg/l/d), the larvae completed the development and reached the prepupal phase and showed a low mortality rate of 1.7-2.3% (Table 2). In experiments with rice bran, all larvae completed the development stage and entered the prepupal phase, but only larvae fed 120 mg/larva/day had low mortality rates of 1-2%, similar to larvae from the wheat bran groups; larvae fed 45 and 90 mg/l/d had 10% or higher mortalities.

Black soldier fly larvae feeding daily on waste coffee grounds at a maximum feeding rate of 200 mg/l/d developed in 25.3 days; at a minimum feeding of coffee grounds at 12.5 mg/l/d, larval mortality was very high - about 80% [10]. The cause of this, as these authors suggest, was that evaporation in the plastic cup, in which larvae placed occurred more rapidly with a small amount of food. It has been noted that rapid evaporation of moisture from feed leads to high mortality of larvae [11]. In our trials with rice bran (90 and 45 mg/l/d), we added water to the substrate as it evaporated, leading to a maximum mortality of 10%.

Feed efficiency conversion (ECD) and the substrate reduction index (WRI). The ECD index indicates the ability of larvae to convert ingested feed into biomass. In the case of wheat bran, the higher ECD value for the minimum diet (45 mg/l/d) shows that the amount of feed being digested increases with a reduced diet. The lower ECD values for the maximum diet with wheat bran (Table 3) suggest that a high feed leads to an increase in the rate of passage of nutrients through the larval intestines, while the amount of food consumed decreases. In this case, the food digested by the larvae was not converted into biomass, but used for metabolism [12].

Table 3. ECD and WRI levels of H. illucens fed wheat and rice bran.

Feed rations (mg/l/d)	Feed efficiency (ECD)		Feed reduction index (WRI)	
	mean	SD	mean	SD
45_{wheat}	43.93a	13.27	2.57a	0.20
90_{wheat}	22.90ab	9.04	2.78a	0.40
120_{wheat}	15.87b	4.91	3.07a	0.25
45_{rice}	8.19A	1.15	1.26A	0.07
90_{rice}	7.22A	2.84	1.41A	0.29
120_{rice}	16.05B	1.85	1.39A	0.08

ab - significant differences within wheat feed means with different letters differ ($p<0.05$). ABC - significant differences within rice feed means with different letters differ ($p<0.05$).

In the dietary intake of rice bran, lower diets (mg/l/d) did not increase ECD, but lower ECD was found for the 45 and 90 mg/l/d rations. In comparison, the highest rice bran diet - 120 mg/l/d - led to a bigger feed conversion ratio.

Previous studies by others have reported that chicken feed for black flies has an ECD value of 24.4-38.0% [13-14], and coffee grounds waste - 3.5-5.0% [13]. In the current study, ECD values ranged from 15.9-43.9% for feeding wheat bran and from 8.2-16.1% for feeding rice bran.

The WRI index measures the ability of larvae to degrade organic matter. A higher WRI value means that larvae have a higher ability to decompose organic material. Feed levels of

45, 90 and 120 mg/l/d led to food reduction for wheat bran of 2.57 ± 0.20, 2.78 ± 0.40 and 3.07 ± 0.25% for the three diets, respectively. For rice bran, these values were 1.26±0.07, 1.41±0.29 and 1.39±0.04 for the 45, 90 and 120 mg/l/d diets, respectively (Table 3, Figure 1).

There was no statistically significant difference in WRI values between the 45, 90 and 120 mg/L/d diets for the both: wheat bran and rice bran.

The WRI values for chicken diet and spent coffee grounds fed to H. illucens larvae ranged from 1.1-3.8 [14] and 0.83-4.0 [15], respectively. In our experiment, WRI was higher for wheat bran-fed larvae, ranging from 2.57 to 3.07, than for rice-fed larvae, ranging from 1.26 to 1.39. In both cases, digestibility index values increased with dietary intake.

Nutrient composition of larvae and optimal feeding rations. H. illucens larvae are known to accumulate up to 40-50% protein when reaching prepupal stage. The duration of the development of the black soldier fly larvae has a significant influence on the biochemical composition of the larvae. Dried prepupae grown on feed wheat grain [16] displayed higher protein content and lower body fat content compared to larvae of earlier age (larvae), 42.8 and 31.9% and 37.6 and 38.3%, respectively.

The protein content of the dry biomass of H. illucens prepupae was high (36-43%) and the fat content lower (11-21%) compared to the above data (Table 4). Although wheat and rice bran initially contain low amounts of protein.

Table 4. Biochemical composition of H. illucens prepupae fed wheat and rice bran.

Nutritional Content	120 mg/l/d		90 mg/l/d		45 mg/l/d	
	wheat	rice	wheat	rice	wheat	rice
Moisture	11.60	2.66	10.10	2.78	8.37	1.15
Crude Protein	41.30	36.25	38.90	36.42	40.90	42.90
Crude Fat	11.43	16.00	11.73	15.60	11.89	21.45
Crude Fiber	4.46	3.38	4.73	3.78	4.59	3.31
Crude Ash	22.80	16.92	20.35	15.29	20.38	22.90
Phosphorus	1.01	3.80	0.99	3.31	1.07	5.18
Calcium	4.00	5.35	4.24	4.49	4.26	7.80
Sodium	0.38	0.41	0.32	0.47	0.37	0.55

Feeding H. illucens with wheat bran resulted in a 2.5-fold increase in protein synthesis and a 3.5-fold gain in protein biomass compared to the dietary substrate.

4 Conclusion

The growth and development of H. illucens from the larval to the prepupa stage was influenced by daily feed intake. In general, study results showed a tendency that higher concentrations of wheat and rice bran supported faster growth of H. illucens larvae.

For larvae maintained on wheat bran, ECD efficiency was highest in the 45 mg/l/d diet and was not statistically different from the 90 mg/l/d group. However, ECD index was statistically higher for 90 mg/day than for 120 mg/day. Meanwhile, prepupae dry biomass was 6 and 6.9 g and development time of larvae to prepupa was 16.9 and 15.7 days for the 90 and 120 mg/l/d diets, respectively. It was assumed that a diet of 90 mg wheat bran per l/d would be more effective than a diet of 120 mg/l/d, as menshioned above indicators were not significantly different between these two groups; and the WRI index of fwas the same value for these dietary diets. Therefore, a diet of 90 mg/l/d wheat bran satisfied both the nutritional needs of the larvae and guaranteed a high level substrate degradation. With a

diet of 45 mg/l/d wheat bran, which we fed to the soldier flys, the increase in dry biomass was lower compared to the other diets.

For rice bran, based on the parameters of development time, ECD and WRI values and dry biomass of the prepupa, a diet of 120 mg/l/d was more effective. Diets of 90 and even more so 45 mg/l/d were insufficient. Dry biomass in the maximum rice bran diet was 1.5 times higher than in other diets: 5.73g versus 3.88 and 3.72g; and larval development was nearly 2 times shorter compared to other diets: 28 days versus 42 and 52 days.

The high protein content (36-43%) of dried H. illucens prepupa fed with cereal bran increases its potential as flour for producing fish fodders.

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