MEALWORM LARVAE WERE SUBJECTED TO THE EFFECTS OF CEREAL AMYLASE/TRYPSIN INHIBITORS ON THEIR DEVELOPMENTAL TRAITS AS WELL AS THE AMOUNT OF DIGESTIVE ENZYMES

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ABSTRACT:

For Tenebriomolitor L. larvae, the goal of this study was to determine whether or not cereal -amylase/trypsin inhibitor (ATI) treatments had any influence on their development characteristics or their production of selective digestive enzymes. The larvae were fed three distinct types of foods containing a range of alpha-amylase/trypsin inhibitors: wheat meals, sorghum meals, and rice meals. The foods included a variety of alpha-amylase/trypsin inhibitors. The alpha-amylase/trypsin inhibitors were administered in different amounts and with different compositions. "A total of five feeding periods (five hours, five days, and ten days) were used to examine the developmental and biochemical characteristics of the larvae, and the relative abundances of -amylase and selected proteases in the larvae were determined using liquid chromatography-tandem mass spectrometry. The weight of the participants increased by a range of 21 percent to 42 percent after five days of eating, depending on their initial weight." As a result of the Kjeldahl technique, we were able to discover that the total protein concentrations of the larvae varied between 44.0 and 49.9 grammes per 100 grammes of body weight, depending on the species.

Keywords: Growth Behavior; Amylase/Trypsin Inhibitors; Digestive Enzymes,

INTRODUCTION

This beetle insect is known as the yellow mealworm, and it feeds mostly on grains and grain products [1, 2]. "Tenebriomolitor, also known as the yellow mealworm (Coleoptera: Tenebrionidae), is a type of beetle insect that feeds predominantly on grains and grain products [1, 2]." The development of a beetle takes approx. Four to five hunderd eggs on average. The eggs hatch into larvae that change colour from whitish to yellowish brown and can grow to 2.5 cm in length. The larvae then develop into pupae, which can last up to eight months under favourable conditions, and finally the eventual development into beetles that can survive for approximately 96 days [2, 3]. According to the fresh weight of T. molitor larvae [4], water makes up around 56 percent of the weight, while protein makes up 18 percent and fat makes up 22 percent. However, both the protein and fat levels are nearly twice as high as the dried weight [4]. "According to recent studies, the proximal composition of T. molitor is 55.6–69.8 percent moisture, 8.9–21.9 percent fat, 13.7–27.6 percent crude protein, 0.9–1.5 percent ash, and 3.1–4.8 percent other components, whereas the dry weight composition is 34.5–38.3 percent moisture, 45.6–49.1 percent fat, 13.7–26.6 percent crude protein, 0.9–1.5 percent ash, and 3.1–4.8 percent other components." Mealworm larvae contain macronutrients such as calcium (0.21 percent), phosphorus (1.06 percent [7]. Cereal proteins are the most nutritionally significant proteins in T. molitor because they are found in the greatest amounts and thus provide the most nutritional value.

Prolamins are the most common kind of storage protein found in cereals, accounting for as much as 50% of the total protein content of the seeds when it comes to storage protein storage. According to certain investigations, T. molitor may thus possess digestive enzymes that are capable of choosing and cleaving peptide bonds that include these amino acid residues with high precision and efficiency. A hitherto undiscovered complicated enzyme system for protein digestion was revealed in their digestive tracts, which was previously unknown (gut). The digestive enzymes detected in the intestines of T. molitor larvae include various digestive enzymes obtained either from the insect itself or from the insect's intestinal microbiota, which are both advantageous to the insect's health. As previously mentioned, protein digestion in these animals is a compartmentalized process in which cysteine peptidases play an important role. This is due to the pH gradient that regulates enzyme activity in these animals. "As a consequence of the significant pH gradient prevalent in the gastrointestinal tract, various kinds of peptidases may be found in different regions of the digestive system. With an anterior pH of 5.6 and a posterior pH of 7.9, there are pH gradients present in the larval midgut, resulting in varied degrees of digestive enzyme activity in various parts of the larval midgut. Anterior pH of 5.6 and posterior pH of 7.9 are detected in the larval midgut, indicating the presence of pH gradients. Despite the fact that cystine peptidases and carbohydrases are primarily found in the anterior midgut, serine peptidases are found mostly in the posterior midgut due to their neutral or alkaline pH optimalities. Cysteine peptidases and carbohydrases are primarily found in the anterior midgut due to their acidic pH optimalities. It is thought to be one of the most significant protein-cleaving enzymes since it may be found throughout the digestive system and is responsible for a variety of functions."Chimotrypsin is a serine endoproteinase enzyme that belongs to the serine endoproteinase superfamily, which also includes other chymotrypsin-like enzymes.

MATERIALS AND METHODS

At the time of collecting, the larvae were around 4 weeks old on average. A kind donation from the University of Altnba s in Turkey provided the wheat cultivars "Siyazan and Esperya of the species Triticumaestivum L. (originating in Central Anatolia, Turkey) for use in this study."Damougari and S35 sorghum cultivars (Sorghum bicolor L. Moench) were obtained from the Institute of Research and Agronomic Development (IRAD) in Maroua, Cameroon, and were planted in the field. Both the sorghum and wheat samples were selected based on the quantity of ATI contained in them, which was determined by mass spectrometry using the same protocols as in our previous research, as previously stated. ATI levels were low in wheat Siyazan and sorghum Damougari samples, but they were high in wheat Esperya and sorghum S35 samples, indicating that ATI levels were high in these crops. A third type of cereal, whole rice flour (Item Nr. 2618, Bauckhof&Bauck GmbH, Rosche, Germany), was used in addition to the first two types of cereals in order to more accurately examine the outcomes of the feeding procedure. This was done in order to more accurately examine the outcomes of the feeding procedure. Table 1 lists the most important characteristics of the samples that were selected.

Sample	**Cultivar**	**Protein Content * (g/100 g)**	**ATI Content ****	
			(PA/mg Flour)	(PA/µg Protein)
Wheat	Siyazan	15.6	79431 ±1218	931 ± 14
	Esperya	13.9	1,460,159 ±95,183	25,231 ± 1645
Sorghum	Damougari	8.5	14,903 ± 223	935 ± 14
	S35	7.9	147,671 ± 2509	11,017 ± 187
Rice	n.a	7.9	473,317 ± 5349	17,174 ± 193

* "total protein content of samples was evaluated according to the Kjeldahl method (N × 6.25); ** ATI amounts are given in terms of relative content, as described in our previous works [30,39]. Tables S1–S3 (Supplementary Data) present the optimized conditions of the HPLC-MS/MS method used for the quantification of wheat, sorghum and rice ATIs. n.a.: not available"

Table 2. Protein content from sodium phosphate and Ambi/urea extracts, and total protein content, determined according to the Kjeldahl method, of larvae after 5 h, 5 days and 10 days (n = 3).

Feeding Samples								
			I.L	Rice	Esperya	Siyazan	Damougari	S35
		t = 0	9.42 ± 0.19					
	Na3PO4	5 H		7.80 ± 0.09 a	8.22 ± 0.04 b	7.90 ± 0.16 a,b	8.20 ± 0.22 a,b	8.80 ± 0.10 c
Protein content (mg/mL)	buffer	5 Days		7.94 ± 0.18 a	8.82 ± 0.25 b	8.97 ± 0.28 b	8.40 ± 0.23 c	8.05 ± 0.19 a,c
		10 Days		8.50 ± 0.08 a	9.02 ± 0.08 b	9.33 ± 0.04 b	8.06 ± 0.07 c	8.49 ± 0.30 a
		t = 0	34.93 ± 0.44					
	Ambi/urea	5 H		26.73 ± 0.72 a,b	25.21 ± 0.69 a	27.05 ± 0.16 b	29.45 ± 0.11 c	26.56 ± 0.71 a,b
	buffer	5 Days		37.47 ± 1.38 a	26.91 ± 1.01 c	32.74 ± 0.21 b	34.75 ± 0.38 d	33.97 ± 0.75 b,d
		10 Days		22.39 ± 0.74 a	19.95 ± 0.34 b,c	19.78 ± 0.16 b	30.41 ± 0.21 d	27.48 ± 0.75 e
		t = 0	53.4					
	Total Protein (g/100 g)	5 Hours		48.6	49.7	49.8	49.9	48.7
		5 Days		45.1	46.8	46.0	48.1	44.8
		10 Days		44.0	47.5	45.7	45.6	43.9

"I.L.: initial larvae before starting with the feeding experiments. t = 0 represents the initial larvae at the starting conditions. Rows within the group labeled with a different letter were significantly different at p = 0.05 (Tukey test)."

Effect and impact of Feeding Cereals on Larvae Protein Content and Amylase Activity

Protein Content

The findings of the investigation, which are connected to the protein composition of the extracted proteins, are summarised in Table 2. A total of 7.80 0.09 to 9.42 0.19 mg/mL of protein was found in the sodium phosphate extracts; in contrast, 19.78 0.16 to 37.47 1.38 mg/mL of protein was found in the Ambi/urea extracts, while the sodium phosphate extracts had a protein concentration of 7.80 0.09 to 9.42 0.19 mg/mL. When compared to all of the samples tested, sodium phosphate extracts yielded the highest protein concentrations (7.80 0.09 to 9.42 0.19 mg/mL), which was the case for all of the samples tested (7.80 0.09 to 9.42 0.19 mg/mL). It was discovered that the protein concentrations in sodium phosphate solutions of the larvae fed with wheat flour Siyazan and Esperya were significantly higher (p 0.05) than the protein concentrations in sodium phosphate solutions of the larvae fed with rice and sorghum flours in both the 5-day and 10-day groups. There was no difference between the larvae that were fed wheat flour that had low and high concentrations of ATIs and the larvae that were given the same wheat flour in the same experimental groups (Table 2). "It turns out that the findings obtained with the Ambi/urea extracts are not applicable in this situation. The protein levels extracted using the Ambi/urea buffer after 5 and 10 days of feeding were considerably greater in larvae fed with rice and sorghum meals (p 0.05) than in larvae fed with maize or soybean meals after 5 and 10 days of feeding, as shown in Table 2. Between days 1 and 5 of the feeding regimen, a significant fall in protein concentrations was seen, which was followed by a significant rise in protein concentrations after day 10. As a final step, the total protein concentrations of larvae were assessed using the Kjeldahl technique, with values ranging from 44.0 to 49.9 grammes of protein per 100 grammes of body weight depending on the results of the experiment. The values varied from 44.0 to 49.9 grammes of protein per 100 grammes of protein, depending on the protein source. Our findings revealed that the overall protein value of the larvae dropped with time in all instances, independent of what kind of meal they were given throughout their feeding period. After just 5 hours of feeding, protein levels dropped significantly, from around 53.4 percent per 100 grammes of body weight at the beginning of the testing to an average of 49 grammes per 100 grammes after only 5 hours of eating. Protein concentration decreased during the experiment and reached an average of 46 and 45 percent after days 5 and 10, respectively, five and ten days into the experiment."

RESULTS AND DISCUSSION

When it comes to the average weight of the larvae throughout the duration of the feeding tests, only the 5-day group exhibited statistically significant differences from the other groups in comparison to the other groups. A possible explanation for this conclusion might be that different persons eat differing quantities of food at different times. A substantial difference was found in the food consumption of larvae fed with Damougari sorghum flour and larvae fed with wheat flour when the two groups were compared. The larvae fed with Damougari sorghum flour had much lower weights than the larvae given wheat flour. "As an added bonus, this was found when comparing the wheat-fed larvae to the rice-fed larvae. When compared to a rice flour diet, a five-day feeding regimen consisting mostly of wheat flour resulted in significantly higher weight gain in the larvae than the rice flour diet. It's possible that the observed results are due to the fact that the protein levels in the wheat samples were about two times higher than those in the rice samples. The death rate of larvae was also documented, and differences between larvae were only detected after a 10-day period of monitoring was completed. The findings were the same when larvae were fed rice flour as when they were given corn meal. Although the observed patterns do not rule out the potential that cereal ATIs have an influence on the mortality rate of larvae, it would be interesting to take into account the amount of flour that is being utilised in the experiments. Not only can gluten-free flours such as wheat, sorghum, and whole rice differ in terms of their protein content, but they may also differ in terms of their relative ATI contents. It has previously been proven that protein concentrations have an effect on the mortality and weight of T. molitor larvae [12].

In this study, it was shown that ATIs had an effect on larval development, with ATI concentrations between 0.19 and 0.53 leading to a significant reduction in larval survival, as evidenced by the findings. The results of this research, on the other hand, do not definitively establish that the ATI concentration has a possible detrimental effect on the weight gain of the larvae. The protein content of extracts prepared with sodium phosphate buffer and extracts prepared with ammonium bicarbonate/urea was determined in both cases. The protein concentrations obtained from the ammonium bicarbonate/urea extracts ranged from 20 to 37 mg/mL, which was significantly higher than the protein concentrations obtained from the sodium phosphate buffer extracts (8 to 9 mg/mL), which was a significant improvement over the values obtained from the sodium phosphate buffer extracts (8 to 9 mg/mL). “According to a comparison between the two methodologies, protein concentrations differed across experimental groups.” Protein concentrations, for example, were found to be significantly higher in extracts of larvae fed low-ATI wheat flour (Siyazan) over a period of 10 days (7.90 0.016 mg/mL), "while values were determined to be 9.33 0.004 mg/mL in the 5 h group when using sodium phosphate buffer," according to the study. When the ammonium bicarbonate/urea extracts from the 10-day group (19.78 0.16 mg/mL) were compared to the 5-hour group (27.05 0.16 mg/mL), it was discovered that the 10-day group had significantly lower protein concentrations (19.78 0.16 mg/mL)." The total protein values of the larvae, as evaluated by the Kjeldahl technique, were observed to vary within a rather narrow range throughout the course of the trials despite the fact that wheat flours contain nearly double the amount of protein found in sorghum and rice flours. As a result, the data demonstrate that the amount of protein contained in the meals had no direct impact on the amount of protein detected in their larval counterparts (protein content). They revealed that T. molitor larvae fed meals containing up to three times the quantity of protein in the meal did not differ significantly in their protein levels from one another.” As previously stated by Behmer et al. [24], insects are endowed with natural regulatory mechanisms that allow them to maintain a healthy protein balance.

CONCLUSION

T. molitor larvae were subjected to this research, which looked at the effects of five different cereal-based ATIs on their growth characteristics as well as their digestive enzyme levels. It was revealed that the larvae were given diets made from wheat, sorghum, and rice that had varied amounts of inhibitors, ranging from low to high, depending on the species of larvae. The larvae were fed on a daily basis for a total of ten days, and the findings of the feeding trials were utilised to evaluate their developmental features. According to the findings, “the lowest dietary ATI concentration was found in Damougari sorghum flour and the highest in wheat meal, with the lowest weight gain occurring in Damougari sorghum flour and the most weight gain occurring in Damougari sorghum meal. When sorghum meal was fed to larvae, it resulted in a substantial decrease in pupation when compared to when wheat or rice flours were fed to the larvae in a similar vein, the researchers found. According to our results, after 10 days of feeding with all meal samples, a statistically significant rise in the mortality rate of the larvae was seen. When the protein composition of the feed was taken into consideration, it was discovered that the patterns of specific activities varied depending on the type of feed that was provided to the larvae, and that these patterns were very similar to the patterns of amylase activities in the feed when these patterns were taken into consideration. "The newly designed HPLC-MS/MS technology allowed for the detection of larval digestive enzyme biomarkers and, as a consequence, the determination of their relative levels using the newly developed HPLC-MS/MS technology," the researchers write. Between the first and fifth days after feeding the larvae with wheat, sorghum, and rice meals, there was a significant decrease in the relative content of -amylase, CLIP domain-containing serine protease, modular serine protease zymogen, and C1 family cathepsin B24, whereas the amounts of dipeptidyl peptidase I and chymotrypsin were significantly increased between the first and fifth days. Throughout the course of the feeding session, the amount of trypsin in the circulation remained constant. In conclusion, the findings of this study imply that the feeding strategy may have an impact on the larval development process, and on the basis of the findings, wheat may be recommended as a larval food source." If meal worms are being assessed as a potential alternative protein source for food and feed production, feeding practises may also be beneficial in changing the nutritional content of the worms.

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