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THE INFLUENCE OF VARYING LEVEL OF WHEAT PROTEINACEOUS INHIBITORS IN WHEAT-BASED ARTIFICIAL FEED ON DEVELOPMENT PARAMETERS OF SELECTED STORED PRODUCT INSECTS

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Abstract: The artificially made kernels from ground wheat grain, commercial wheat starch and wheat proteinaceous α-amylase inhibitors in different proportions were used as feed for adults of the granary weevil (*Sitophilus granarius* L.). In the case of larvae of the confused flour beetle (*Tribolium confusum* Duv.) and the Mediterranean flour moth *(Anagasta kuehniella* Zell.*)* the friable feed mixture were used. The survival of *S. granarius* adults has not been correlated with the soluble proteins extracted from wheat and amylolytic activity located in this protein fraction. On the other hand the weight of dust (the index of feeding intensity) produced during feeding has depended on the presence of α-amylase and trypsin inhibitors in wheat-based feed. *A. kuehniella* larvae have not developed at all on feed consisted of 50% wheat starch and 50% of crude α-amylase inhibitors from wheat. The same feed has caused 15.1 days of extension in development time of *T. confusum* larvae. It attests to specific native enzymatic apparatus existing in alimentary canals of three damaging grain species which can overcome some obstacles even if extremely highly active insect α-amylase inhibitors were present in feed. However, the sufficient nutrient should be available in feed compounds. Nevertheless, some reduction of insects population can be expected.

Key words: α-amylase inhibitors, artificial wheat-based feed, insect feeding intensity, larval development, *S. granarius, T. confusum, A. kuehniella*

I. INTRODUCTION

Resistance mechanisms of cereal grains to infestation by insect species are complex and depend on physico-chemical and biochemical properties of the grain and to the extend of subsequent biochemical and physical adaptation of stored product insects to these properties (Baker 1986; Warchalewski et al. 1989; Dobie 1991; Warchalewski and Nawrot 1993; Warchalewski et al. 1993). Stored seeds may have the high resistance to insect pests because of the lack of vital nutrients or the presence of compounds that adversely affect insect development (Taylor and Medici 1966; Medici and Taylor 1966; Yetter et al. 1979; Nawrot et al. 1985; Gatehouse et al. 1986; Dobie 1991; Baker et al. 1991; Huesing et al.

1991; Warchalewski and Nawrot 1993; Pueyo et al. 1995; Piasecka-Kwiatkowska 1999; Piasecka-Kwiatkowska and Warchalewski 2000a; 2000b). In general, insects tend to develop more slowly on resistant grain cultivars. Various studies of wheat grain resistance have been made, but there have been few attempts to determine the particular factors that govern resistance (Dobie 1991). Differences in feeding intensity of *S. granarius*, *T. confusum* and *A. kuehniella* on various wheat cultivars was reported by Warchalewski and Nawrot (1993). Only some grain properties like: grain hardness, non-protein nitrogen and intrinsic wheat proteinaceous inhibitors of insects α-amylase studied had a negative effect on some growth parameters. Earlier Nawrot et al. (1985) reported the failure of larvae of *T. confusum*, *T. granarium* and *A. kuehniella* to grow on artificially constructed wheat feed without low molecular mass compounds apparently lost during extraction and dialysis. Therefore, low molecular mass non-protein nitrogen compounds seems to play some role in development of these insects, however, their exact nature and mode of action are unknown. Baker (1988a) reported that α-amylase activity was depressed in grain weevils when fed on wheat grain and these lower enzyme activities may contribute to delayed development times on this cereal (Baker 1988b). Earlier Silano et al. (1975) speculated that proteinaceous inhibitors exist in wheat grain as naturally occurring insect resistance factor. They found that insect species normally attacking wheat grains and wheat products had higher amylase activities and were more susceptible to inhibition by wheat inhibitors than were the amylases of those insects that did not attack wheat. This speculation is also supported by results of Baker (1986) who reported that amylase/proteinase ratios in homogenates of isolated midguts of granivorous beetles, which feed primarily on cereals grains and cereal products, were extremely high. On the other hand, those insects species, which can feed and develop on feed of animal products or feed with the high protein content, had higher proteinase/amylase ratios than the granivorous coleopterans. The enzymology of insects digestive tract in relation to initial stages of digestion of large food polymers (starch or protein) reflects the biochemical adaptation of these stored-product insects to their preferred foods (Baker 1986). Nevertheless as a whole, so far published results indicate that amylase inhibitors in wheat could be somewhat involved in resistance of wheat grain to postharvest infestation. More extensive investigations are needed to clarify the role of wheat proteinaceous inhibitors in the increasing natural resistance to stored-grain insect pests infestation, particularly in terms of theirs extremely highly inhibition power towards α-amylases of infested insects. This model study was set out to get an answer to which extend the activity of proteinaceous inhibitors of wheat grain will effects insects digestive enzymes of three selected, economically important stored-product insects *in vitro* and *in vivo* when incorporated into artificial feed made in 100% from natural wheat grain components.

II. MATERIALS AND METHODS

Winter wheat variety LAMA (crude protein – 10.94 % d.wt. and 12.35% moisture content) grown on experimental plots of the Breeding Station Danko in Choryń, Poland was taken for preparation of insect feed. Common Hard Red Spring wheat (not pure source, 11.6% moisture content) variety Yecora Rojo was obtained from the Department of Agronomy and Range Science, UC Davis, USA for preparation of crude α-amylase inhibitors. Commercial available wheat starch (11.3% moisture content) was also used as a component of insect feed.

1. Basic analytical methods

Grain moisture content was determined according to the Polish standard method PN-65/R-74006.

Crude protein of wheat grain was determined according to Kjeldahl method with the use of the Swedish Tecator apparatus and the calculating index K = 5.7. Soluble protein content was determined by the Lowry et al. (1951) method at 750 nm.

Endogenous amylolytic activity (combined α- and β-amylase) was measured according to the colorimetric method of Bernfeld (1955) using 3,5-dinitro-salicylic acid at pH 5.5 and $\lambda = 487$ nm as was modified by Warchalewski and Tkachuk (1978). A unit of amylolytic activity is the amount of enzyme necessary to liberate dextrin or maltose equivalent to 1 mmol maltose during 1 min at 25°C from 0.9% soluble starch at pH 5.5.

To determine exogenous insect α-amylase inhibitory activity the modified Bernfeld (1955) method was used as described by Warchalewski (1978) and Warchalewski et al. (1989). The inhibitor activity was expressed as the number of α-amylase activity units by which the enzyme activity was diminished due to the inhibitor's action. The calculation was done according the following formula:

inhibitor activity = (amylase activity of insect enzyme + endogenous amylase activity of feedstuff components) – (remaining amylase activity of insect enzyme with inhibitor in feedstuff components).

Antiproteolytic activity was determined against bovine pancreas trypsin basing on the method of Nomoto and Narahashi (1959), after Warchalewski and Skupin (1973) modification.

3. Preparation of albumin extract from wheat grain LAMA variety

10 g of ground wheat grain (d.wt.) were used for three-step water extraction by shaking 1 h with 100 ml bidistillated water at 18°C. Suspensions were centrifuged 10 min, 20 000 × G at 40°C. After the third-step of extraction obtained supernatants were combined together with three independent replications by mixing of equal volumes of each combined supernatants. This average sample was taken for determination of soluble protein content, endogenous amylase activity, exogenous insects α-amylase inhibitory activity and antiproteolytic activity.

4. Preparation of crude α-amylase inhibitors

3000 g of wheat grain on dry weight basis were ground into whole flour in a MICRO SAMPLEMILL equipped with a # 3463 screen .020" slit. Obtained flour was extracted by shaking with deionized water (1:5 d.wt./v) for 2 h at 18°C. After shaking the suspension was left overnight at 4°C. Then clear liquid was decanted and the remaining portion of slurry was centrifuged 10 min 23 300 × G at 4°C. Collected supernatants were combined with earlier decanted liquid and freeze dried. Lyophilised crude α-amylase inhibitors was weighed (196.1 g) and put into vacuum-sealed polyethylene bag and stored in tightly closed jar prior to use.

4. Extraction of soluble proteins adsorbed on commercial wheat starch

10 g of wheat starch (d.wt.) was shaken 1h with 20 ml bidistillated water at 18°C. Suspension was centrifuged 10 min 15 000 × G at 18°C. Collected supernatant was filled up to 50 ml and use for determination of protein content, endogenous amylase activity, exogenous insects α-amylase inhibitory activity and antiproteolytic activity.

Preparation of crude insect α-amylases. In order to determine the inhibition effect of different feeds compounds incorporated into feed on insect α-amylase the own crude insect enzyme preparations were obtained. Taking into account the damage caused by insect-pests to the stored cereal grain and products the following insects were selected: *S. granarius* – adults reared on sound wheat grain, *T. confusum* – larvae reared on ground wheat grain and *A. kuehniella* larvae reared on wheat flour. Growing of insects was conducted under standard conditions in a thermostated cabinets at relative humidity 70% and 26°C temperature. After completion the growth larvae and imagines were washed with water, drained on filter paper and starved for 24 h or 48 h respectively in order to exclude the activity of native wheat α-amylase. In all cases 400 imagines or larvae were taken for preparation of crude enzymes following the procedure as described earlier by Warchalewski et al. (1989). The freezed α-amylase preparations prior to determinations were thaw and dissolved in 10 times diluted of stock buffor at pH 5.5.

Activity of insects α-amylase preparations were determined according to modified by Warchalewski and Tkachuk (1978) Bernfeld's method (1955). The specific activities of crude insects α-amylase preparations are given in Fig.1.

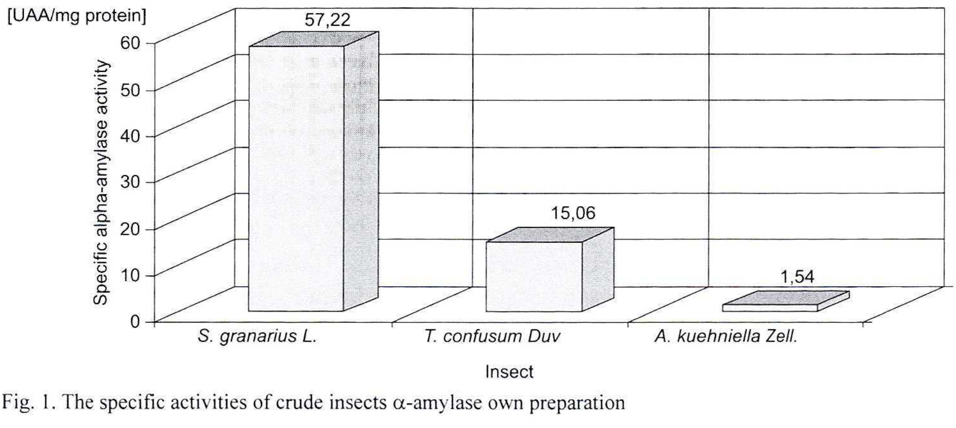


Fig. 1. The specific activities of crude insects α-amylase own preparation

5. Trials with insects

The test insects: *S. granarius*, *T. confusum* and *A. kuehniella* were reared in laboratory conditions. Considering the fact that progeny of the granary weevil (*S. granarius*) can only develop in sound grain the artificial kernels (granules 5 mm in diameter) were prepared as insect feedstuffs. Natural components which were used for preparation of feedstuffs were mixed with some drops of water and shaped in kernels. Starch played two functions: one as a main food component and the second as a binding factor. From 50 g of mixture was obtained 100 kernels. The following composition of feedstuffs were prepared from the mixture of components used in different proportions for forming wheat-based artificial kernels:

1. Sound wheat grain LAMA variety (control),
2. 50% wheat commercial starch + 50% crude α-amylase inhibitor,
3. 50% of ground wheat grain LAMA variety + 50% wheat commercial starch,
4. 50% of ground wheat grain LAMA variety + 50% crude α-amylase inhibitor,
5. 33.3% of ground wheat grain LAMA variety + 33.3% crude α-amylase inhibitor + 33.3% wheat commercial starch,
6. 45% of ground wheat grain LAMA variety + 45% crude α-amylase inhibitor +10% wheat commercial starch.

Twenty artificial kernels (10 grams) were placed into vessels in 5 replicates and infested with 10 beetles (5 pairs) 1–3 day old. Mortality of beetles and weight of dust produced were checked after 20, 33, 47 and 72 days. The results were compared with the same parameters obtained for sound wheat grain (control samples). The experiments were conducted in cabinets at 26°C and 70% relative humidity.

Larvae of the confused flour beetle *(T. confusum)* and the Mediterranean flour moth *(A. kuehniella)* were used for estimation the effect of natural wheat-based friable feed components used in mixtures at different proportions on larval development. The following mixtures of components were prepared as wheat-based feed:

1. 100% of ground wheat grain LAMA variety (control),
2. 50% wheat commercial starch + 50% crude α-amylase inhibitor,
3. 50% of ground wheat grain LAMA variety + 50% wheat commercial starch,
4. 50% of ground wheat grain LAMA variety + 50% crude α-amylase inhibitor.

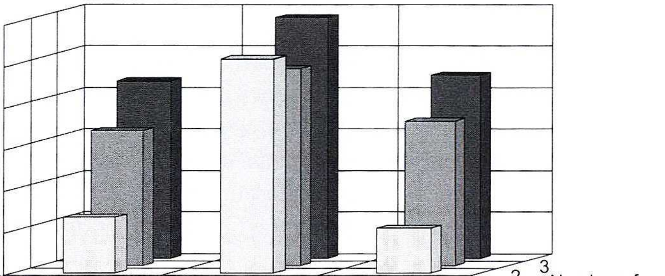
10 grams of all feedstuffs mixtures were put into plastic vials and next were placed inside vials 5 larvae of *T. confusum* or 3 larvae of *A. kuehniella* all 1 day old. Emerging adults were checked every day and larval development time and their mortality were established. All experiments were conducted at 5 replications in laboratory cabinets maintaining 26°C and 70% relative humidity.

Averages figures from 5 replications were analysed by t-Student's test at the level of $\alpha = 0{,}05$ and the least significance differences ($LSD_{0.05}$) were counted. Means followed by the same letter are not significantly different at $\alpha = 0.05$. The correlation analysis was also used for study interaction between biochemical properties of feedstuffs and insect development parameters.

II. RESULTS AND DISCUSSION

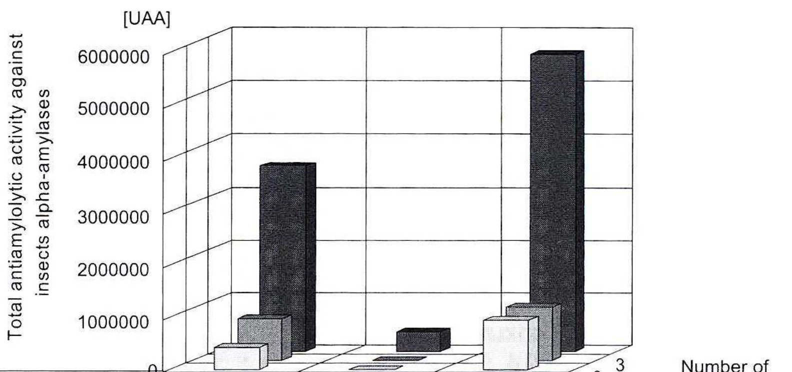
1. The effect of different feed composition on *in vitro* inhibition activity of α-amylases from *S. granarius*, *T. confusum* and *A. kuehniella*

The basic characteristic of natural wheat components incorporated into feedstuff prepared for feeding cereal insects are given on Fig. 2 and 3.



Number and wheat component	Soluble protein [mg]	Total amylolytic activity [UAA]	Total antiproteolytic activity [UPA]
1 – Wheat commercial starch	22	141276	12
2 – Wheat grain Lama variety	1762	55026	3032
3 – Crude alpha-amylase inhibitor	18825	622490	25812

Fig. 2. The basic characteristic of natural wheat components utilised for preparation of cereals feedstuffs for insects feeding trials. Results are given on 100 g dry weight basis



Number and wheat component	*S. granarius*	*A. kuehniella*	*T. confusum*
1 – Wheat commercial starch	418429	691	943040
2 – Wheat grain Lama variety	789583	670 *	1028851
3 – Crude alpha – amylase inhibitor	3522004	379910	5634622

*heat-treated albumins as described previously by Warchalewski et al. (1989).

Fig. 3. The antiamylolytic activities of natural wheat components utilised for preparation of cereals feedstuffs for insects feeding trials. Results are given on 100 g dry weight basis

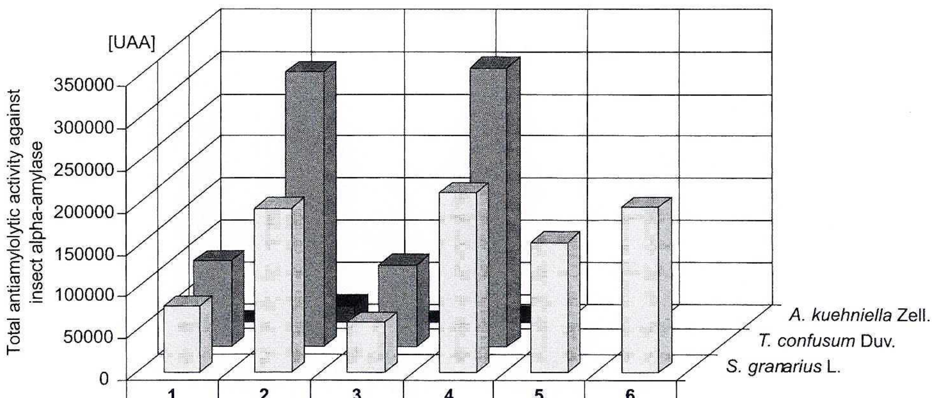
The total feed components inhibition activity against studied insects a-amylase were quite different and depended from the composition of feed mixtures used in feeding trials of *S. granarius*, *T. confusum* and *A. kuehniella* as can be seen in Fig. 4.

The highest inhibitory activity against insects α-amylases was found for *T. confusum* α-amylase in all feed diets. The same was reported earlier when the wheat, rye and triticale were analyed as a feed (Warchalewski et al. 1989; Piasecka-Kwiatkowska 1999). All food diets had a very low inhibitory activity against *A. kuehniella* α-amylase. However, in the case of *A. kuehniella* this digestive enzyme is not so important as for others tested insects. The ratio of amylase/proteinase digestive enzymes in *A. kuehniella* was 7.9 whereas in the case of *S. granarius* was over 822 times higher (Baker 1986). Also different antiproteolytic activity of feed might diminished digestive possibilities of insects proteolytic enzymes. Therefore, antiproteolytic activity of feed components were also taken under consideration. The antiproteolytic activity of feed used in this experiment are given on Fig. 5.

The feed No 2 and No 4 had the highest inhibitory activity against studied enzymes (Fig. 4 and 5).

2. *In vivo* effect of different feed on feeding intensity and progeny development of *S. granarius*

The results presented in Table 1 and 2 showed that the worst feed for adults of *S. granarius* L. was feed No 2 (50% wheat commercial starch + 50% crude α-amylase inhibitor) as well feedstuff No 5 (33.3% of ground wheat grain LAMA variety + 33.3% crude α-amylase inhibitor + 33.3% wheat commercial starch). The mortality of beetles on both



	1	2	3	4	5	6
S. granarius L.	78958	197022	60401	215579	156091	198206
T. confusum Duv.	102885	328883	98595	333174	n.a.	n.a.
A. kuehniella Zell.	67	19030	68	19029	n.a.	n.a.

1–6 numbers of wheat-based artificial feedstuff; n.a. – not applicable

Fig. 4. The antiamylolytic activity in each replication sample of feedstuffs mixtures (10 g) used for insects feeding trials

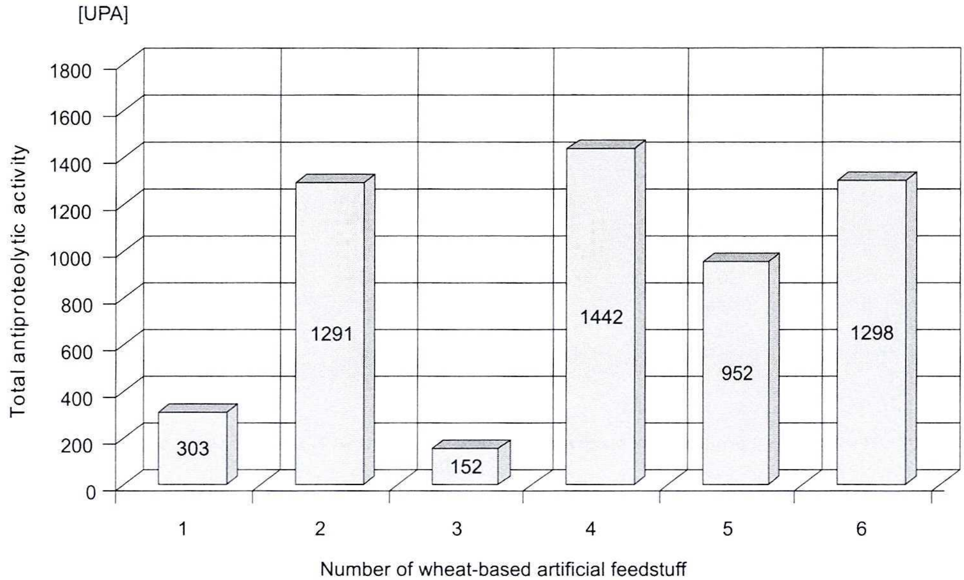


Fig. 5. The antiproteolytic activity in each replication sample of feedstuffs mixtures (10 g) used for insects feeding trials

feed was considerably higher and dust weight was less than those on feed No 3 and 6 (Tabs. 1, 2).

Despite of the high mortality of beetles on feed No 3 (50% ground wheat grain and 50% wheat starch), the development of progeny (the results are not presented in this paper) was possible like on feed No 1 (sound grain), presumable because of the very low antiamylolytic and antiproteolytic activities in both feed (Fig. 4 and 5). Following the successive days of experiments we noticed that the highest mortality of beetles was between 47 and 72 days but the beetles produced more and significantly different amount of dust during the first 20 days (Tabs. 1, 2).

The subsequent, increase of dust amounts in feed No 1 and 3 were connected with the intensity of larval feeding. The statistically difference of dust amounts in feed No 1 and 3 is probably caused by the lack of protective barrier (grain hardness) at the artificial kernels composed only from the 50% of ground wheat grain and 50% of wheat starch. It should be mention that the artificial kernels composed only from protein preparation (crude inhibitor) and wheat starch (feed No 2) were harder when compared with the feed No 3–6. Therefore, harder artificial kernels in the feed No 2 probably can have some contribution on development parameters of the grain weevils.

The correlation coefficients concerning the dust weight and mortality percent of the granary weevils calculated on account of different properties of feed used in feeding trials are presented in Table 3.

There were no statistically significant correlation at the level of significance $\alpha = 0.05$. It can be noticed a complete lack of correlation between the beetle mortality and feed prop-

Table 1

Mortality (%) of the grain weevils (*S. granarius* L.) fed on wheat-based artificial feedstuffs

Number of feedstuff	20 days	33 days	47 days	72 days	Σ
1	5 AB	4 AB	8 AB	45 B	62 AB
2	44 B	20 B	34 B	2 A	100 C
3	0 A	0 A	22 AB	60 BC	82 BC
4	20 AB	4 AB	4 A	40 B	68 AB
5	20 AB	0 A	2 A	74 C	96 C
6	0 A	0 A	18 AB	30 AB	48 A
$LSD_{0.05}$	42	16	26	35	27

Table 2

Dust weight (mg) produced by the granary weevils (*S. granarius* L.) fed on wheat-based artificial feedstuffs

Number of feedstuff	20 days	33 days	47 days	72 days	Σ
1	10.7 A	10.1 B	13.5 BC	33.8 B	68.1 B
2	22.0 BC	3.2 A	1.6 A	0.0 A	26.8 A
3	19.6 BC	16.5 C	23.5 D	38.3 B	97.8 C
4	16.6 AB	8.2 B	11.0 ABC	11.4 A	47.2 AB
5	26.1 C	8.3 B	6.5 AB	3.5 A	44.5 AB
6	22.6 BC	17.2 C	17.3 CD	15.2 A	72.3 BC
LSD0.05	8.7	4.4	9.5	17.6	29.5

erties. However a slightly negative correlation (statistically significant at the level $\alpha = 0.1$) between dust weight and feed properties were noticeable (Tab. 3). Therefore, the beetle mortality despite of significant differences in composition of feed do not depend on the amount of soluble proteins and amylolytic activity. On the other hand the weight of produced dust, as an indicator of feeding intensity, was inversely proportional to the presence of both α-amylase and trypsin inhibitors in feed. The amount of consumed and digested feed was enough for beetles to survival but they crushed the artificial kernels more intensively for searching its better parts particularly during the first 20 days and between 47 and 72 days of feeding.

3. *In vivo* effect of different feed on larval development of *T. confusum* and *A. kuehniella*

Quite similarly as for *S. granarius* the worst feed for larval development of these two pests was feed No 2 (Tab. 4).

The larvae of *A. kuehniella* did not develop at all and larvae of the *T. confusum* prolonged by about 48% their development time in comparison to those on ground wheat grain LAMA variety. Statistically significant extension of development time of *A. kuehniella* larvae was observed on feed No 2 (50% wheat commercial starch + 50% crude α-amylase in-

Table 3

The correlation coefficients between feedstuffs properties and the selected development parameters of the granary weevil (*S. granarius* L.) (n = 6)

Feedstuff properties	Development parameters	
	Mortality (%)	Weight of dust (mg)
Soluble protein [mg]	0.011	-0.717
Amylolytic activity [UAA]	0.200	-0.726
Antiamylolytic activity [UAA]	-0.025	-0.705
Antiproteolytic activity [UPA]	-0.021	-0.706

hibitor) and No 4 (50% of ground wheat grain LAMA variety + 50% crude α-amylase inhibitor). On the other hand moths reduction expressed in percent was not statistically significant with the exception of feed No 2. Wheat α-amylase inhibitors were shown to be very effective inhibitors of α-amylase activity of the larvae of confused flour beetle, when assessed *in vitro* (Fig. 3). However, when taken into consideration during the feeding trials the number of emerged beetles it seems that *T. confusum* is able to detoxify these compounds or that they overproduce their own α-amylase in response to enzyme inhibitors in feed. Moreover, larvae of the *T. confusum* on feed 3 and 4 growth faster than on control feed No 1 (Tab. 4). This phenomenon can be explained in the case of feed No 3 that more starch was available, the main nutrient for this granivorous beetle, a typical storage pest of wheat flour (Baker 1986), while in the feedstuff No 4 more proteins (albumins which include also inhibitors) were available. Wheat albumins are known to have a well balanced amino acids composition, which probable can speed up the development time of larvae of confused flour beetle. The correlation coefficients presented in Table 5 showed that the activity of enzyme inhibitor in feed do not influence on the development time, whereas it caused general weakness of larval condition and higher mortality and less percent of emerged adults.

In some wheat varieties resistance to insects *in vivo* was correlated with *in vitro* inhibition of α-amylase of these insect species (Applebaum and Konjin 1965; 1967; Yetter et al. 1979; Baker et al. 1991). Although these results and others (Warchalewski et al. 1989;

Table 4

Development time [days] and number of emerged adults of the Mediterranean flour moth (*A. kuehniella* Zell) and the confused flour beetle (*T. confusum* Duv.) on different wheat-based artificial feedstuffs

Number of feedstuff	Mediterranean flour moth		Confused flour beetle	
	Development time (days)	Number of moth (%)	Development time (days)	Number of beetle (%)
1	34.4 B	86.8 B	31.2 B	56.0 A
2	0.0 A	0.0 A	46.3 C	60.0 A
3	33.4 B	86.6 B	22.7 A	76.0 A
4	44.7 C	66.6 B	22.1 A	56.0 A
$LSD_{0.05}$	5.3	32.5	4.4	28.8

Table 5

The correlation coefficients between feedstuffs properties and the selected development parameters of the Mediterranean flour moth (*A. kuehniella* Zell) and the confused flour beetle (*T. confusum* Duv.) (n = 4)

Feedstuff properties	Development parameters			
	Mediterranean flour moth		Confused flour beetle	
	Development time (days)	Number of moth (%)	Development time (days)	Number of beetle (%)
Soluble protein (mg)	-0.272	-0.698	0.328	-0.556
Amylolytic activity (UAA)	-0.441	-0.812	0.427	-0.370
Inhibitory activity against Mediterranean flour moth α-amylase (UAA)	-0.343	-0.750	-	-
Inhibitory activity against Confused flour beetle α-amylase (UAA)	-	-	0.364	-0.498
Antiproteolytic activity (UPA)	-0.251	-0.682	0.315	-0.576

Warchalewski and Nawrot 1993; Warchalewski et al. 1993; Piasecka-Kwiatkowska 1999) would suggest that α-amylase inhibitors in wheat could be involved to the resistance of grain postharvest insect infestation, however, some other results are contradictory (Nawrot et al. 1985; Gatehouse et al. 1986; Warchalewski and Nawrot 1993).

In general, cereal α-amylase inhibitors may play a protective role against insects although it appears that some insects are able to detoxify these compounds or overflow their activity like in the case of *T. confusum*. This findings is in good agreement with Gatehouse et al. (1986) who stated that confused flour beetle is able to detoxify the wheat proteinaceous α-amylase inhibitors. Obtained results in this paper support also earlier suggestion of Gatehouse et al. (1986) that selecting wheat varieties for the high α-amylase inhibitory activity may not be a very reliable criterion in selecting wheat crop for natural insect resistance. However, adequately the high level of antiamylolytic activity in wheat grain can have some influence on reduction of insects population.

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WPŁYW RÓŻNEGO POZIOMU BIAŁKOWYCH INHIBITORÓW PSZENICY W SZTUCZNIE PRZYGOTOWANYCH POKARMACH NA PARAMETRY ROZWOJU POPULACJI WYBRANYCH OWADÓW MAGAZYNOWYCH

STRESZCZENIE

Sztuczne ziarna zrobione z handlowej skrobii pszennej i białkowych inhibitorów α-amylaz wymieszanych w różnych proporcjach oraz czyste ziarno pszenicy stanowiły pokarm wołka zbożowego (*Sitophilus granaris* L.). Dla larw trojszyka ulca (*Trbolium confusum* Duv.) i mklika mącznego (*Anagasta kuchniella* Zell.) te same produkty podawano w postaci sypkiej. Procent przeżywalności chrząszczy wołka zbożowego nie zależał od zawartości białka rozpuszczalnego ani od aktywności amylolitycznej pokarmu. Z drugiej strony ilość pyłu wytworzonego w czasie żerowania (wskaźnik intensywności żerowania) zależał od obecności inhibitorów α-amylazy i trypsyny. Larwy mklika mącznego nie kończyły rozwoju na pokarmie zawierającym 50% skrobii pszennej i 50 inhibitorów α-amylazy. Ten sam pokarm powodował przedłużenie rozwoju larw trojszyka ulca o 15,1 dnia. To świadczy o istnieniu wrodzonego, specyficznego aparatu enzymatycznego w przewodzie pokarmowym testowanych owadów, który może pokonać nawet ekstremalnie wysoką aktywność inhibitorów α-amylazy obecnych w pokarmie. Efektem działania inhibitorów może być tylko redukcja liczebności populacji.