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The impact of the protein-carbohydrate ratio in animal feed and the initial insect population density on the development of the red flour beetle, Tribolium castaneum

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ABSTRACT

Progeny numbers and life parameters of *Tribolium castaneum* reared on a range of different animal feeds which varied in their protein-carbohydrate ratios (corn starch; corn feed flour; wheat bran; soybean meal; corn gluten; soy protein concentrate and soy protein isolate), four initial (population) densities (1, 2, 5 and 10 insect pairs) were evaluated. Adult insects were kept for seven days on a range of different diets to feed and oviposit before they were removed. After removing the adults, the emerging progeny were examined. The study found *T. castaneum* offspring could not develop on a carbohydrate-rich diet (corn starch) and on protein-rich diets (corn gluten, soy protein concentrate and soy protein isolate). Soy isolate showed a high, possibly insecticidal effect on parents. The fastest total development (egg to adult) (23.25–23.88 days, depending on the initial density), the highest offspring number (111.63–324.13) and the highest offspring body mass (1.38–1.73 mg) were recorded in wheat bran, while the slowest egg to adult development (35.13–37.88 days), the lowest offspring number (25–29) and the lowest offspring mass (1.04–1.48 mg) were recorded in soybean meal. The higher initial densities caused the eclosion period to be prolonged, reductions in female productivity and in offspring body mass on all diets. Initial density interacted differently with diet type in terms of offspring number, so as the density rose, the number of offspring in wheat bran and corn feed flour increased, while in soybean meal it remained low at all initial densities. This research gives us a better insight into the development of *T. castaneum* in animal feed that can help us to improve existing pest management in a way of finding a better solution to store products based on their susceptibility to this storage pest.

1. Introduction

In South-East Europe animal feed is typically made from various raw plant materials (wheat, corn, soybeans, etc.) that are ground to obtain an optimal particle size (Vukmirović et al., 2017) and then added to mixtures to provide a targeted ratio of protein and carbohydrates suited to the physiological needs of the animals, their species and age (Crump et al., 2002, Balthrop et al., 2011). Animal feed is predominately rich in carbohydrates and contains smaller amounts of plant proteins, especially soy proteins, unlike food for humans where proteins are mostly of animal origin (Visser et al., 2014; Mukherjee et al., 2016). This gives animal feed a different nutritional value and a different risk of insect infestation during storage compared to food for human consumption (Đukić et al., 2016).

One of the most damaging insect species invading grain commodities stored in silos, warehouses and storage facilities around the world is the red flour beetle, *Tribolium castaneum* (Herbst). This insect is a secondary pest of stored products, preferring to feed on flour and other milled products for human and animal consumption. Both adults and larvae of *T. castaneum* feed and cause extensive damage by reducing the quantity and quality of stored products (Rees 2004; Hagstrum and Subramanyam, 2006; Mahroof and Hagstrum 2012; Nayak and Daglish, 2018).

Development duration and productivity of *T. castaneum* strongly depend on the nutritional value of stored products (Borzoui and Naseri, 2016; Arthur et al., 2019; Skourti et al., 2020). The nutritional value of a

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diet is usually understood as the ratio of proteins to carbohydrates in it (Wong and Lee, 2011; Karimi-Pormehr et al., 2018). Changes in the protein-carbohydrate ratio, for example between different cereals cultivars (Borzoui and Naseri, 2016; Karimi-Pormehr et al., 2018; Namin et al., 2018) or due to the addition of small additive quantities in diets (Lale et al., 2000; Scharf et al., 2015; Sial et al., 2017), could strongly affect the duration of insect development and survival of immature stages. It was also found that diets with an optimal nutritional value attract storage insects more than unsuitable diets, which increases the susceptibility of these diets to attacks by stored-product insects (Edde and Phillips, 2006; Halliday et al., 2019; Đukić et al., 2020). Most studies have been focused on the determination of *T. castaneum* development parameters in diets used for human consumption (Arthur et al., 2019; Astuti et al., 2020; Gerken and Campbell, 2020; Fardisi et al., [2013, 2016, 2017]), so there is very limited knowledge on its development in diets intended for animals and the susceptibility of these diets to infestation by this pest. Stored-product insects have been found to reproduce successfully on distilled dried grains with solubles and on some types of animal feed (Fardisi et al., 2013; Đukić et al., 2016), but have also been recorded as having a low reproductive capacity or complete inability to reproduce on some diets intended for animal feed (Đukić et al., 2016). Previous research has partially determined the relationship between the development pattern of *T. castaneum* and the protein-carbohydrate ratio and initial insect population density in animal feed (Đukić et al., 2016), but it did not clearly define the susceptibility thresholds of animal feed to this pest attack, which indicated the need for more detailed research of this poorly studied area. It was determined that the other secondary pests such as the Indianmeal moth, *Plodia interpunctella* (Hübner) had lower fecundity and fertility in diets with high or low amounts of proteins (Borzoui et al., 2018). Sawtoothed grain beetle, *Oryzaephilus surinamensis* (L.) had the longest larval development in legumes rich in proteins (Awadalla et al., 2021) and it's determined that the best protein-carbohydrate ratio for this species is around 15%:82% P:C (Astuti et al., 2018). Considering this information, we hypothesize that *T. castaneum* could have similar protein and carbohydrates needs, taking into account that these species are secondary pests and have similar diet preferences.

Previous research determined that initial insect population density causes density dependence when vital rates depend on it (Pointer et al., 2021; Vries et al., 2020). It could have a negative or positive influence (Allee effects) on fecundity, offspring development, body mass and mortality (Longstaff, 1995; Halliday et al., 2015; Halliday et al., 2019; Vries et al., 2020). On the other hand, when it comes to the effect of density on the different *T. castaneum* developmental stages in animal feed and the potential interaction between initial density and the protein-carbohydrate ratio of diet, the data is scarce and incomplete.

The focus of this research was to determine the impact of animal feed with different protein-carbohydrate ratios (corn starch (0.8%/85%), corn feed flour (9%/71%), wheat bran (16%/53%), soybean meal (44%/30%), corn gluten (60%/23.5%), soy protein concentrate (66%/11.8%) and soy protein isolate (90%/1%)) and four initial adult insect densities (1, 2, 5, and 10 insect pairs) on the life history parameters and offspring output of *T. castaneum*. The findings help in determining whether and how diet type interacts with initial insect population density. The main goal was to determine the susceptibility threshold of different feed types to *T. castaneum* attacks through the protein-carbohydrate ratio. This will widen our knowledge and understanding of what makes one commodity highly susceptible to attack and another safe from it, which could potentially improve existing pest management of stored animal feed.

2. Materials and methods

2.1. Insects

We conducted experiments with a laboratory culture of *T. castaneum*. The rearing diet of this culture was wheat flour (95%) with brewer's yeast (5%). From the original culture, pupae were separated using sieves with 1 mm and 2 mm apertures (Haver & Boecker, Oelde, Germany) and sexed following the procedures described by Halstead (1963). Separated pupae were then transferred in 2-L glass jars which contained rearing diet and placed in a climatic chamber (Sutjeska, Serbia). Air temperature in the chamber was 30±1 °C, and relative humidity (r.h.) was 50 ± 5%. Male and female adults of 15–20 days old, were used in the experiment.

2.2. Feed commodity

All feeds were purchased from local, Serbian producers. Protein and carbohydrate ratio of all diets is presented in Table 1.

The diets were placed in the freezer for minimum of 15 days to eliminate potential insect infestation. Three days before the experiments, the diets were taken out of the freezer and placed in the laboratory (25 ± 1 °C and 50 ± 5% r. h).

2.3. Bioassay

For each diet, four 10 g samples were put into plastic vials (4 cm in diameter, 9 cm in height). Each quantity of 10 g was weighed with a compact balance KT-31 (Hyundai, Korea).

Insects were added inside each vial at the initial densities of 1, 2, 5 and 10 pairs. The vials were covered by a cotton cloth for ventilation and fixed with rubber bands. Vials with insects were then placed in a climatic chamber (Sutjeska, Serbia) at 30 ± 1 °C temperature and 50 ± 5% r. h. (measured by a data logger, Kestrel 4000, USA). The whole procedure was repeated twice, so that the total number of replications was 8 (4 × 2).

Insects were left for seven days on the tested diets to feed and oviposit before they were gently removed with soft paintbrush, and the diets with their offspring were returned to the vials and placed in the chamber.

We followed the procedures previously described by Đukić et al. (2021) in order to determine: the duration of egg, larval and pupal stages, total egg to adult cycle, first adult emergence, eclosion period, total number of offspring, offspring numbers per female and body mass of adult offspring.

2.4. Data analysis

Before analysis, all data were *log(x+1)* transformed to normalize the variance (Zar, 2010) and submitted to one-way ANOVA. The Tukey's HSD test at 0.05 level of significance was used to separate means.

The regression analysis for all nine response variables (life parameters) and two factors, initial density and diet type, were conducted. Linear ($y = a + bx$) and quadratic ($y = a + bx + cx^2$) were used as the most suitable forms of dependence. The coefficient R^2 was also calculated, which measures how well the regression line adapts to the original data, i.e. it shows the share of the explained variability in the total variability of the dependent variable.

Table 1
Protein-carbohydrates ratio of the tested diets (percents).

Diets	Proteins (%)	Carbohydrates (%)	Producer
Corn starch	0.8	85	Dr. Oetker d.o.o., Šimanovci
Corn feed flour	9	71	Mirotin Tisa d.o.o, Savino Selo
Wheat bran	16	53	Letina d.o.o, Novi Bečej
Soybean meal	44	30	Soja Protein, Bečej
Corn gluten	60	23.5	Jabuka A.D., Starch Industry, Pančevo
Soy protein concentrate	66	11.8	Soja Protein, Bečej
Soy protein isolate	90	1	Soja Protein, Bečej

We also used grouping analysis, which is a method intended for the classification of individual observation units based on their similarity, and the aim of the analysis is reflected in the formation of a certain number of highly internally homogeneous groups of observation units. Observation units grouping is based on different indicators (characteristics) that are measured individually for each observation unit and the outset is the choice of the appropriate distance measure, since it is necessary to determine how "similar" or "different" they are. There are several different measures of distance, and in this paper, the square of Euclidean distance was used, which is calculated using the following expression (Kovačić, 1994):

$$d_{ij}^2 = \sum_{k=1}^{p} (X_{ik} - X_{jk}),$$

where p is the number of indicators* (life parameters and offspring output), x_{ik} is the value of the observation unit, xi for the indicator Xk, and xjk is the value of the observation unit xj for the indicator Xk. Based on the chosen distance measure and the basic (n x p) data matrix (n objects classified on the basis of p indicators), a (n x n) distance matrix is formed. Elements of data matrix show the level of similarity or difference between all object pairs that are grouped. With the aim of the analysis presented in this paper, a hierarchical agglomerative grouping analysis was implemented on selected indicators based on the application of the average linking method.

The statistical analyses were done using statistical package IBM SPSS Statistics 17.0 (Ibm Corp. Released, 2017).

3. Results

3.1. Offspring development

Parents mortality was recorded only in the soy protein isolate, and it was 86.1 ± 1.6% for 1 pair; 84.5 ± 1.3% for 2 pairs; 85.7 ± 2.0% for 5 pairs and 84.8 ± 0.9% for 10 pairs. In the corn gluten, soy protein concentrate (protein-rich diets) and corn starch (carbohydrate-rich diet), only few larvae were found that had failed to reach the pupal stage, and they were excluded from further data processing.

Neither diet type nor initial density statistically significantly affected egg and pupal stage lengths (Table 2). However, diet type had a statistically significant effect on the length of the larval stage and the total length of development. The shortest larval stage (14.38–14.75 days) as well as the shortest total development (23.25–23.88 days) were recorded on wheat bran, and the longest larval stage (26.38–29 days) and total development (35.13–37.88 days) were recorded on soybean meal. Initial insect population density did not significantly affect the length of the larval and pupal stages, although in soybean meal the length of the larval stage was somewhat longer at the highest initial density (29 days) than in the lower densities (26.38–27.25 days).

There was a statistically significant variation on the first day of adult emergence in relation to diet type (Table 3). The earliest first day of eclosion occurred in wheat bran (16.25–17.25 day) and the latest in soybean meal (31.38–37 day). Initial density did not affect the first day of emergence in the case of corn feed flour and wheat bran, while in soybean meal the first day of emergence at the highest initial density (37 days) was statistically significantly later than at lower initial densities (31.38–32.25 days) (Table 3).

3.2. Offspring output

Diet type had a statistically significant effect on the eclosion period (Table 3). The shortest period of eclosion was recorded in wheat bran (14.38–24.38 days) and the longest in soybean meal (22.63–30.13 days). Initial density also had a significant effect on the eclosion length: as the initial density increased in all diets, the eclosion period lengthened (Table 3).

Table 2
Development duration (mean days ± SE) of *T. castaneum* maintained on seven diets at four initial densities.

Diets	Duration of egg stage (days)				Duration of larval stage (days)				Duration of pupa stage (days)				Total duration of development cycle (days)			
	Initial densities (number of insect pairs)															
	1	2	5	10	1	2	5	10	1	2	5	10	1	2	5	10
Corn starch	*4.25± 0.16Aa	4.38± 0.18Aa	4.50± 0.19Aa	4.38± 0.18Aa	-	-	-	-	-	-	-	-	-	-	-	-
Corn feed flour	4.50± 0.19Aa*	4.50± 0.19Aa	4.38± 0.18Aa	4.25± 0.16Aa	21.88± 0.55Ba	21.38± 0.50Ba	21.75± 0.37Ba	22.13± 0.48Ba	4.88± 0.13Aa	4.75± 0.16Aa	4.75± 0.16Aa	4.63± 0.18Aa	31.25± 0.62Ba	30.63± 0.50Ba	30.88± 0.58Ba	31.00± 0.46Ba
Wheat bran	4.38± 0.18Aa	4.25± 0.16Aa	4.25± 0.16Aa	4.13± 0.13Aa	14.75± 0.37Aa	14.50± 0.19Aa	14.38± 0.18Aa	14.75± 0.16Aa	4.75± 0.16Aa	4.75± 0.16Aa	4.63± 0.18Aa	4.38± 0.18Aa	23.88± 0.30Aa	23.50± 0.19Aa	23.25± 0.16Aa	23.25± 0.16Aa
Soybean meal	4.25± 0.16Aa	4.25± 0.16Aa	4.38± 0.18Aa	4.38± 0.18Aa	26.38± 1.40Ca	27.25± 1.46Ca	26.75± 0.82Ca	29.00± 0.68Ca	4.50± 0.19Aa	4.38± 0.18Aa	4.75± 0.16Aa	4.50± 0.19Aa	35.13± 1.32Ca	35.88± 1.52Ca	35.88± 0.93Ca	37.88± 0.79Ca
Corn gluten	4.50± 0.5Aa	4.50± 0.19Aa	4.13± 0.13Aa	4.25± 0.16Aa	-	-	-	-	-	-	-	-	-	-	-	-
Soy protein concentrate	4.38± 0.18Aa	4.13± 0.13Aa	4.25± 0.16Aa	4.13± 0.13Aa	-	-	-	-	-	-	-	-	-	-	-	-
Soy protein isolate	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

*Means within columns followed by the different uppercase letter and means within rows followed by the different lowercase letter are significantly different, Tuckey's HSD test at P < 0.05.

Table 3
Offspring parameters (mean ± SE) of *T. castaneum* maintained on tree diets at four initial densities.

Diets	Nnumber of pairs			
	1	2	5	10
	First day of adult emergence (days +SE)			
Corn feed flour	*24.38 ± 0.71Ba	23.75 ± 0.41Ba	23.88 ± 0.58Ba	23.88 ± 0.40Ba
Wheat bran	17.25 ± 0.49Aa	16.63 ± 0.18Aa	16.25 ± 0.16Aa	16.25 ± 0.16Aa
Soybean meal	31.38 ± 0.63Ca	31.63 ± 0.60Ca	32.25 ± 0.45Ca	37.00 ± 1.56Cb
	Eclosion duration (days +SE)			
Corn feed flour	17.50 ± 0.65Aa	21.63 ± 0.71Bb	26.88 ± 0.58Bc	28.75 ± 0.67Bc
Wheat bran	14.38 ± 0.46Aa	15.25 ± 0.25Aa	20.63 ± 0.32Ab	24.38 ± 0.26Ac
Soybean meal	22.63 ± 1.63Ba	25.63 ± 1.50 Cab	30.13 ± 2.26Bb	29.88 ± 1.65Bb
	Average total number of offspring (mean +SE)			
Corn feed flour	65.88 ± 2.08Ba	108.50 ± 5.40Bb	176.13 ± 11.68Bc	248.63 ± 4.27Bd
Wheat bran	111.63 ± 7.39Ca	180.25 ± 5.41Cb	257.63 ± 9.41Cc	324.13 ± 11.22Cd
Soybean meal	25.00 ± 2.81Aa	28.25 ± 7.33Aa	29.00 ± 7.60Aa	25.50 ± 6.48Aa
	Average number of offspring per female (mean +SE)			
Corn feed flour	65.88 ± 2.08Bd	54.25 ± 2.70Bc	35.23 ± 2.34Bb	24.86 ± 0.43Ba
Wheat bran	111.63 ± 7.39Cd	90.13 ± 2.70Cc	51.53 ± 1.88Cb	32.41 ± 1.12Ca
Soybean meal	22.63 ± 1.63Ac	14.13 ± 3.66Ab	5.80 ± 1.52Aab	2.55 ± 0.65Aa
	Average body mass of insect (mg + SE)			
Corn feed flour	1.62 ± 0.06 ABa	1.61 ± 0.06Ba	1.49 ± 0.06Ba	1.43 ± 0.06Ba
Wheat bran	1.73 ± 0.05Bb	1.62 ± 0.06Bb	1.51 ± 0.07 Bab	1.38 ± 0.05Ba
Soybean meal	1.48 ± 0.05Ac	1.35 ± 0.03Ac	1.20 ± 0.02Ab	1.04 ± 0.03Aa

* For each parameters separately, means followed by the different uppercase letter and means within rows followed by the different lowercase letter are significantly different, Tuckey's HSD test at $P < 0.05$.

Statistically, the lowest total number of offspring was determined in soybean meal (25-29 adult) and the highest in bran (111.63–324.13 adults). Initial density did not have any effect on the number of offspring in soybean meal, while the number of offspring increased statistically significantly with increasing initial density in corn feed flour (65.88–248.63 adults) and bran (111.63–324.13 adults) (Table 3).

The lowest productivity of females was recorded in soybean meal and, depending on the initial density, it ranged from 2.55 to 22.63 adults, while the highest productivity was recorded in bran (32.41–111.63 adults). Initial density had a statistically significant effect on female productivity on all diets. With increasing initial density, female productivity significantly decreased (Table 3).

Adult had a statistically significant lower body mass in soybean meal, which, depending on the initial density, ranged from 1.04 to 1.48 mg, compared to corn feed flour (1.43–1.62 mg) or in bran (1.38–1.73 mg). Initial density did not affect the mass of insects in corn flour feed, while in bran and soybean meal, the mass of offspring decreased with increasing initial density (Table 3).

3.3. Regression and cluster analyses

Regression analysis determined the form of dependence of the observed indicators depending on the initial density in corn feed flour, wheat bran and soy protein isolate (Tables 4–6).

Based on the results of the cluster analysis (Fig. 1), we can conclude that the observation units were grouped into three separate clusters. The first group consists of 27 observation units from corn feed flour and wheat bran and initial densities of 5 and 10 pairs. The second cluster consists of 31 observation units, all based on soybean meal (all initial densities). Thirty-eight observation units, based on corn feed flour and wheat bran and with initial densities of 1 and 2 pairs, were classified as the third group.

4. Discussion

On all diets, the eggs hatched successfully, except in the soy protein isolate where adult insects did not reproduce due to the strong, potentially insecticidal effect of the diet. We determined that approximately 85% of adult insects died after 7 days in soy protein isolate at all initial densities. Possible insecticidal effect was demonstrated, considering that 100 and 78% of *T. castaneum* adults were able to survive 7 and 14 days without any food, respectively (Daglish 2006). It is known that soybean consists of various antinutritive components, including trypsin protein inhibitors and lecithin (Mukherjee et al., 2016). These substances can significantly reduce (Sokoloff et al., 1966) or completely inhibit (Halliday et al., 2019) *T. castaneum* offspring production in soybean flour. They can also negatively affect the growth of its larvae, leading to larval development disorders (Oppert et al., 2003; Walski et al., 2014) and caused adult mortality (Kikuta, 2020). However, this is the first report of the high, potentially insecticidal effect of soybean protein isolate. This is probably due to the fact that soy protein isolate is much more concentrated and has higher amounts of protein (90%) and antinutritive components than the widely used soybean flour (~49%). Halliday et al. (2019) also determined that all *T. castaneum* beetles died in soybean flour after 6 weeks, however, another study determined that 100% of *T. castaneum* adults were died after 4 weeks without food (Daglish, 2006), so it is not clear whether insects died from starvation or from the soybean flour's insecticide effect. The soy protein concentrate in the current study was probably not concentrated enough (66% of proteins) to kill adults and prevent them laying eggs, but its nutritional value prevents larvae from developing. The inability of larvae to develop in

Table 4
Regression coefficients (mean ± SE) for linear ($y = a + bx$) and quadratic ($y = a + bx + cx^2$) dependence form of the 9 observed response variables depending on initial density (1, 2, 5 and 10) for corn feed flour diet.

Response variables	Regression coefficients± SE			R^2	ANOVA	
	a	b	c		F	p
Duration of egg stage	4.538 ± 0.016	−0.290 ± 0.030		0.991	105.800**	0.009
Duration of larval stage	21.772 ± 0.467	−0.089 ± 0.240	0.013 ± 0.021	0.776	0.755^{nsd}	0.631
Duration of pupa stage	4.869 ± 0.090	−0.033 ± 0.051	0.001 ± 0.004	0.913	24.920*	0.040
Total duration of development cycle	31.190 ± 0.530	−0.158 ± 0.272	0.014 ± 0.024	0.510	0.176^{nsd}	0.860
Eclosion period	14.565 ± 1.213	3.622 ± 0.622	−0.221 ± 0.054	0.995	49.286*	0.010
First day of emergence	24.365 ± 0.494	−0.194 ± 0.254	0.150 ± 0.220	0.658	0.383^{nsd}	0.753
Average total number of offspring	62.198 ± 14.646	19.463 ± 2.569		0.983	57.391*	0.017
Average number of offspring per female	75.706 ± 2.263	−11.325 ± 1.161	0.625 ± 0.101	0.999	186.145*	0.042
Average body mass	1.638 ± 0.025	−0.022 ± 0.040		0.962	25.160*	0.038

No significant diference-NSD for $p > 0.05$; * for $0.01 < p < 0.05$; ** for $p < 0.01$ df: for linear regression $\upsilon_{1=1}$, $\upsilon_{2=2}$, df: for quadratic regression $\upsilon_{1=2}$, $\upsilon_{2=1}$.

Table 5
Regression coefficients (mean ± SE) for linear (y = a + bx) and quadratic (y = a + bx + cx^2) dependence form of the 9 observed response variables depending on initial density (1, 2, 5 and 10) for wheat bran diet.

Response variables	Regression coefficients± SE			R^2	ANOVA	
	a	b	c		F	p
Duration of egg stage	4.369 ± 0.099	−0.033 ± 0.051	0.001 ± 0.004	0.913	2.492^{nsd}	0.409
Duration of larval stage	14.893 ± 0.108	−0.203 ± 0.055	0.019 ± 0.005	0.970	79.84*	0.024
Duration of pupa stage	4.872 ± 0.027	−0.020 ± 0.014	−0.002 ± 0.001	0.998	120.167*	0.044
Total duration of development cycle	24.044 ± 0.180	−0.255 ± 0.092	0.018 ± 0.008	0.966	7.079^{nsd}	0.257
Eclosion period	13.519 ± 0.938	1.142 ± 0.165		0.996	48.112*	0.020
First day of emergence	17.509 ± 0.314	−0.408 ± 0.161	0.028 ± 0.014	0.960	5.817 ^{nsd}	0.281
Average total number of offspring	73.129 ± 26.300	51.225 ± 13.494	−2.623 ± 1.179	0.993	34.077*	0.020
Average number of offspring per female	131.65 ± 2.950	−22.410 ± 1.516	1.250 ± 0.132	0.999	419.39*	0.035
Average body mass	1.723 ± 0.036	−0.036 ± 0.006		0.992	31.952*	0.030

No significant diference-NSD for p > 0.05; * for 0.01< p < 0.05; ** for p < 0.01 df: for linear regression $\upsilon_{1=1}$, $\upsilon_{2=2}$, df: for quadratic regression $\upsilon_{1=2}$, $\upsilon_{2=1}$.

Table 6
Regression coefficients (mean ± SE) for linear (y = a + bx) and quadratic (y = a + bx + cx^2) dependence form of the 9 observed response variables depending on initial density (1, 2, 5 and 10) for soybean meal diet.

Response variables	Regression coefficients± SE			R^2	ANOVA	
	a	b	c		F	p
Duration of egg stage	4.183 ± 0.045	0.053 ± 0.023	−0.003 ± 0.002	0.965	6.740 ^{nsd}	0.263
Duration of larval stage	26.881 ± 0.970	−0.171 ± 0.498	0.038 ± 0.044	0.936	3.515 ^{nsd}	0.353
Duration of pupa stage	4.275 ± 0.243	0.144 ± 0.124	−0.012 ± 0.011	0.759	0.679 ^{nsd}	0.651
Total duration of development cycle	34.936 ± 0.357	0.278 ± 0.063		0.970	19.769*	0.047
Eclosion period	19.896 ± 0.521	3.144 ± 0.267	−0.215 ± 0.023	0.998	133.959*	0.049
First day of emergence	31.685 ± 0.297	−0.277 ± 0.152	0.081 ± 0.013	0.999	223.714*	0.047
Average total number of offspring	23.807 ± 1.815	2.082 ± 0.931	−0.192 ± 0.081	0.922	2.849 ^{nsd}	0.386
Average number of offspring per female	27.419 ± 3.352	−6.483 ± 1.720	0.401 ± 0.150	0.988	19.736 ^{nsd}	0.157
Average body mass	1.469 ± 0.044	−0.045 ± 0.008		0.971	33.290*	0.029

No significant diference-NSD for p > 0.05; * for 0.01< p < 0.05; ** for p < 0.01 df: for linear regression $\upsilon_{1=1}$, $\upsilon_{2=2}$, df: for quadratic regression $\upsilon_{1=2}$, $\upsilon_{2=1}$.

corn gluten and corn starch the current study probably arises from the unbalanced nutritional value of this diet, i.e., the high amounts of proteins and small amounts of carbohydrates in corn gluten, or the other way around in the case of corn starch. Earlier studies have shown that an unbalanced diet with high amounts of proteins (Fabres et al., 2014; Đukić et al., 2016) or high amounts of carbohydrates (Wong and Lee, 2011; Astuti et al., 2020) causes a complete absence of *T. castaneum* offspring. The length of the egg and pupal stages in this study were the same in all diet types and at all initial densities, because these stages do not feed and largely depend on abiotic factors such as temperature and humidity (Fardisi et al., 2016; Skourti et al., 2019), which were constant in this experiment.

The development of *T. castaneum* offspring and offspring performance on diets that provide enough nutrients for their development also took place according to a pattern of carbohydrate ratios in the diets. The fastest development and the highest number of offspring with the highest body masses were recorded in wheat bran. Wheat bran, which is mostly used for livestock feed, is known as a high-value reserve of nutrients, rich in carbohydrates, quality proteins and B complex vitamins (Prückler et al., 2014; Balandrán-Quintana et al., 2015; Katileviciute et al., 2019). Previous studies also determined the optimality of this diet for *T. castaneum* development (Seifelnasr et al., 1982; Đukić et al., 2016) and its great attraction for this pest (Đukić et al., 2020). With 16% of proteins and 53% of carbohydrates, this diet represents the optimal animal feed for *T. castaneum*, making it highly susceptible to pest attacks. Diets such as compound feed with a similar protein-carbohydrate ratio were also very suitable for this pest's development (Đukić et al., 2016). However, if the percentage of protein is below 10%, as in the case of corn feed flour, there is a slight prolongation of the development cycle and a decrease in the number of offspring compared to wheat bran. Skourti et al. (2020) recorded similar results comparing *T. castaneum* life parameters in cracked corn and wheat. In this study, development was not possible in corn starch with less than 1% of protein. This was confirmed by previous studies on food for human consumption where, in diets with less than 5% of proteins, *T. castaneum* offspring failed to develop (Wong and Lee, 2011; Gerken and Campbell, 2020; Astuti et al., 2020). The longest development cycle, the lowest number and lowest body mass of offspring were recorded in high-protein soybean meal (44% proteins and 30% carbohydrates). Clearly, this pest is more sensitive to higher amounts of proteins than to higher amounts of carbohydrates in animal feed.

Initial density affected the eclosion period in all diets. A longer eclosion period at higher initial densities due to a higher number of offspring were recorded in previous studies (Longstaff, 1995; Đukić et al., 2016). Offspring numbers logically correlate with the parents density in the case of wheat bran and corn feed flour, but the productivity of females decreased with increasing initial insect population density in all diets. This behavior is assumed to be a reproductive strategy of females in order to avoid overcrowded diets and a lack of food for their offspring (Longstaff, 1995; Assie et al., 2008; Đukić et al., 2021; Pointer et al., 2021). It is interesting that in soybean meal even at the initial density of 10 pairs, the number of offspring remains low because the nutritional value of this animal feed simply cannot support more offspring: a similar interaction of initial density and diet type was recorded in patent flour (Đukić et al., 2021). This interaction is also visible in the cluster analysis where the development parameters in soybean meal at all initial densities are collected into one cluster, while wheat bran and corn feed flour coexist at higher initial densities (5 and 10 pairs) in one and at lower (1 and 2 pairs) in another cluster. With higher competition and lack of accessible food, offspring body mass decreased at higher initial densities, as was determined in earlier studies (Sokal and Karten 1964; Đukić et al., 2016).

Our study showed that animal feed with 60% and above of protein is not susceptible to attacks by this pest. Soy protein isolate, with a high percentage of protein (90%), has potentially insecticidal properties and the possibility to improve control programs of *T. castaneum*, but this requires more detailed research. On the other hand, animal feeds with a protein percentage below 1% are also not susceptible to *T. castaneum* attack because insect development is not possible in them. Diets with a protein percentage above 40% are moderately susceptible to attack

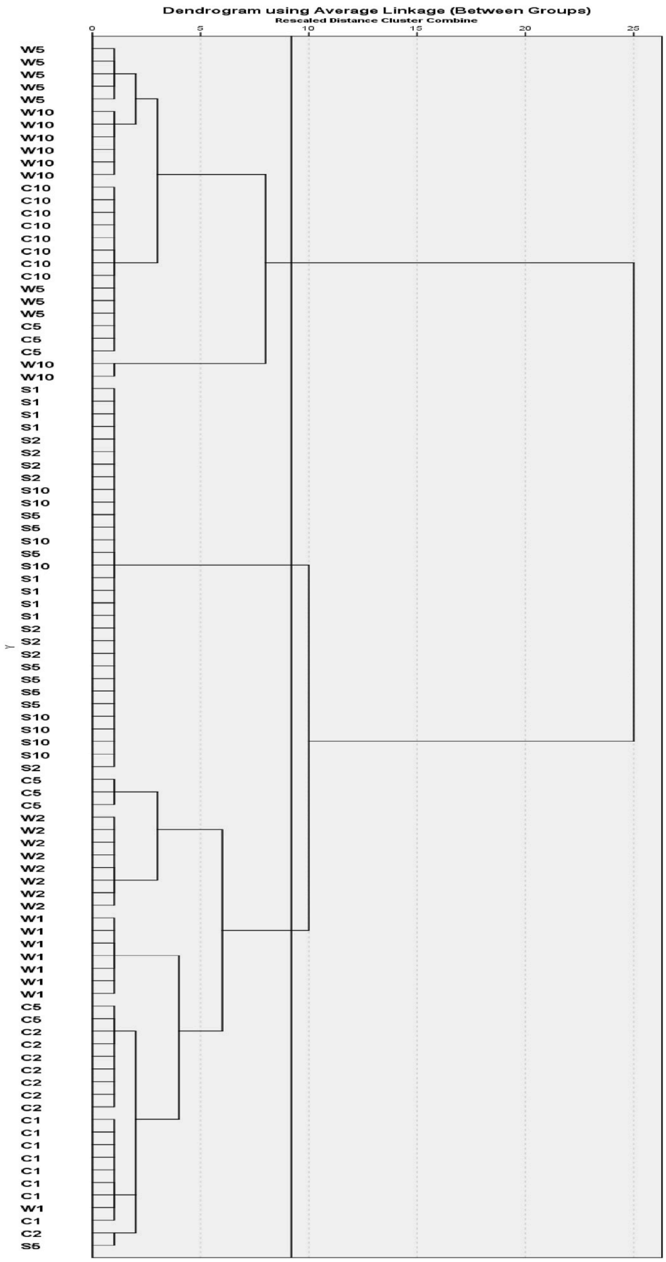

Fig. 1. Dendrogram: The result of cluster analysis of all observed replicates on three diet types (C, W, S) and different initial densities (1, 2, 5, 10) based on 9 observed indicators (duration of egg, larval and pupal stages, total egg to adult cycle, first adult emergence, eclosion period, total number of offspring, offspring numbers per female and body mass of adult offspring). C – corn feed flour, W – wheat bran, S – soybean meal; 1,2,5,10 – number of pairs (initial density).

because, although it is possible for *T. castaneum* to develop in them, development is significantly slowed down and the number of offspring is several times lower than in susceptible diets. The feeds that are most susceptible to *T. castaneum* attack are those with a protein percentage between 9 and 16%, such as wheat bran, and various compound feeds, and these diets need the highest degree of protection.

This study helps us to understand the life cycle of *T. castaneum* in animal feed. It established that the fecundity and development of this pest largely depend on the feed protein-carbohydrate ratio and that different protein-carbohydrate ratios afford different levels of animal feed susceptibility to insect infestation. The study also provides insight into the complex interaction of feed and the initial density of *T. castaneum* and showed that an interaction between initial density and diet type exists, but this relation is complex and needs more detailed research. The results have led us to the conclusion that high-protein animal feed, especially soy protein isolate, could be used in storage facilities as a barrier around feed that is highly susceptible to infestation by *T. castaneum*, as long as the other feeds are not already infested. This would significantly improve existing pest management, making it safer and more eco-friendly.

In addition to feed nutritional value and initial insect population density, other factors such as temperature and relative humidity can affect the dynamics of stored-product insects' growth and development (Mahroof et al., 2003; Arthur et al., 2019). Likewise, stored insect species co-occurrence in storage and competition can influence the population growth of some insect species (Kavallieratos et al., 2017; Sakka and Athanassiou, 2018) including *Tribolium* species (Sokoloff et al., 1966; Bullock et al., 2020). During feed storage, different scenarios are possible and several factors could affect population growth at the same time. All those factors should have been considered if we want to apply the results of a laboratory study such as this in real-world conditions.

Authors contribution

Nikola Đukić: Visualization, Investigation, Writing – original draft, preparation. Andja Radonjić: Conceptualization, Writing – review & editing. Blaženka Popović: Formal analysis. Petar Kljajić: Conceptualization, Methodology, Writing – review & editing. Marijana Pražić-Golić: Writing – review & editing. Goran Andrić: Conceptualization, Methodology, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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