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EFFECT OF SOME PHYTOPHAGOUS ARTHROPODS ON THE NUTRIENT CONTENT OF LEGUMINOUS PLANTS (FABACEAE)

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1. BACKGROUND OF THE RESEARCH, OBJECTIVE

In an economical animal husbandry, that meets the requirements of today's age, quality fodder and feeding have a key role. In Hungary during the 1970-80s, livestock production was high in European relations as well, but since 1990 the number of life stock has declined considerably. It follows, that protein feed requirement had previously reached 1.3-1.4 million tonnes per year, but today it is only 700-800 thousand tonnes per year. Due to the National Protein Program, the background of domestic protein feed is to be ensured by high-quality GMO free plants (soybean and peas), produced in Hungary. The advantage of producing leguminous plants is not only their high protein content (e. g. in the case of peas it is 22–28%, in the case of soy it is 38–41%). They are outstanding fore-crops, cohabiting with *Rhizobium* spp. bacteria, living on the roots (binding about 60 kg/ha N per year), enrich the soil in N. Although their ecological sensitivity is high, they moderately use water, sand organic resources of the soil. Taking into account the cultivation characteristics of our country, their specific yield is small, compared to other arable crops (pea: 2,48 t/ha; soy: 3,03t/ha; bean: 1,83 t/ha Central Statistics Office, 2016). At the same time their annual yield fluctuations are significant (pea: -9,49%, soy +49,26%, bean: -1,61%) for the past marketing years. In the knowledge of these, their cultivation requires extensive professional information.

In Hungary, the total area of production of protein crops (peas: 18.976 ha, 47.083 t; soy: 61.029 ha, 184.725 t; bean: 874 ha, 1600 t), does not cover the needs of domestic consumption. In the future, due to their growing production volume, it is necessary to pay more attention to modernization of their production, implementing of innovative practical elements in their production technology. Along with these requirements, due to the announced appropriations of the national protein program, the increase of acreage of leguminous, mainly of soybean areas, can be projected. As a result, import of protein items (primarily GMOs) are hoped to decrease.

In parallel, it should be mentioned, that legumes, especially soy, have an extensive arthropod malicious community. The objective mapping of the damage they cause, the effective defense against them, of course, clearly contribute to the economical production of these cultures. Overall, research on these pests and the sustainable approach to plant protection technologies, applied against them, will help to effectively implement the principles of the national protein program.

The leguminous arthropods produce a change in the qualitative and quantitative properties of their food crops. The overall aim of the dissertation was to study changes in the quality parameters of leguminous fodder plants damaged by certain arthropod species. My specific objectives were as follows.

1. In laboratory conditions we aimed to investigate the extent of change in the content of the soybean plants' plumage, damaged by seed corn maggot (*D. platura*).

2. We wondered, whether, besides the evident quantitative damage, caused by the Southern green shield bug (*N. viridula*) change as a result of some of the ingredients of the substance, namely the saccharides.

3. Our aim was to investigate the changes in the content of soya caused by two spotted spider mite (*T. urticae*), such as crude protein, crude fat, crude fiber, and raw ash content.

4. CT diagnostic methods were used to determine density and volume change compared to intact beans, damaged by *Acanthoscelides obtectus* (*A. obtectus*).

5. Finally, we wanted to reveal possible differences in feed utilization of items damaged by *Acanthoscelides obtectus*, feeding fish with them. The aim of our experiment was to investigate the possibility of using beans (*Phaseolus vulgaris*), damaged by (*Acanthoscelides obtectus* Say, 1831), as pelleted carp feed ingredient.

2. MATHERIAL AND METHOD

Analysis of the value change, caused on soybean by seed corn maggot [*D. platura* (L.)]

The soybean samples, damaged by seed corn maggot and the intact ones were collected from the Barcs arable area of the Drávacoop Zrt., (GPS coordinates: N 45°57'23.02538; E 17°23'35.25705) on 16th July, 2014. Detection of seed corn maggot was not difficult, because the maggots found in the stems were hatched in isolator, so it was easy to determine the pest. From the husks of the plants (of forced maturation in the presence of maggots) which were damaged, and it was visible by naked eye, intact and damaged seeds were collected by us by hand. Identification of the species was carried out with the naked eye or by the stereomicroscope following the cultivation of the rabbits found in the stalk. Legless larvae are dirty white, yellow, cylindrical and conical. The fully grown larvae are 0.50-0.63 cm tall and pointed with two black mouth rollers. The abdominal cavity is concave with two brown-black inserts at the back. The larvae lasts nearly 21 days.

Adults are brownish-gray flies that resemble home flies with three rear strips. They are about 0.48 to 0.64 cm long. The wings are pulled at rest over the abdomen.

We harvested both intact and damaged seeds from the pods of plants that were visibly damaged by the naked eye (in the presence of rabbits).

The samples, collected in this way, were placed in the Physiology and Biochemistry Laboratory of the Faculty of Agricultural and Environmental Sciences at Kaposvár University. The moisture and (MSZ 6496:2001) crude protein content (MSZ EN ISO 5983-2:2009) was tested on the 10-10 intact and damaged samples. The soybeans were cracked, a sample weighed 20 grams. The total amino acid content (MSZ EN ISO 13903:2005), the total fiber fraction (MTK-1990. II.8.2.), and the fatty acid composition of the samples was also determined (FAME-001:2001) (Hungarian standard 1977, 1978, 1981). Crude ash content was determined according to MSZ 5984:1992 (revoked standard).

In the laboratory of University of Pécs we also performed sugar mobilization tests with the intact and damaged items. The duration of the study was 7 days. In order to extract the water-soluble carbohydrate content of the 2 grams of the plant sample, three consecutive aqueous extraction (reflux systems) are sufficient, adjusted to the final uniform volume of 20 ml, the mixture can be used without chemical dilution for chemical determinations. By adjusting the Boehringer Glu / fru / suc UV body, a much higher sample number can be determined than the factory specification.

Glucose determination: 100µl sample + 330 µl Boehringer buffer +5 µl hexokinase enzyme, absorbance measurement at 340nm.

Fructose assay: previous mixture + 5 µl phosphofructose isomerase, absorbance at 340 nm.

Sucrose determination: 100 µl sample + 20 µl invertase enzyme + 5 µl hexokinase enzyme, absorbance at 340 nm.

Concentrations can be calculated from absorbance data based on the values of molar extinction coefficients. By adapting the sample collection and extrusion, the measured carbohydrate content of the samples is true. By changing the chemical protocol, you can save a lot of chemicals and money.

In the determination of the sugar content, the germinating seeds were individually chopped and refined at reflux in 3 x 10 cm^3 distilled water. The combined fractions were filtered, dried in vacuum distillation (40°C), and dissolved in distilled water. Glucose, fructose and sucrose was determined by Boehringer Mannheim GmbH (1996) test.

The results of the Weendei analysis and the effect of the damage on the content value change were evaluated, using the R program package, the single factor analysis of variance analysis ($P \leq 0,05$). For the statistical evaluation of the sugar mobilization, we used a two-sample t-test, using a Microsoft Excel program ($P \leq 0,05$).

Analysis of the value change, caused on soybean by Southern green shield bug [*Nezara viridula* (L.)]

The damaged and intact soy bean samples were collected from the Bicsérd arable land of the Fodder Research Institute of the Kaposvár University (GPS coordinates: N 46°04’08.68”, E 18°05’56.09”) in October, 2014. The soybean variety was Emese, a domestic breed. The husks, constituting the base of the samples, were manually removed from the plants. Damaged patterns showed a lot of sucking traces, which helped differentiate the seeds from the healthy items. We also succeeded in collecting 3-3 samples from damaged and intact items. This sample number was sufficient for 3 repetitions. Samples were scraped and a sample weighed 2 grams.

The low number of items is confirmed by the fact that, due to the strong variation in the degree of damage observed in each case, a strong damage event could not be predicted. Thus, due to the planned schedule of the study, the sample collection was relatively low.

The duration of the study was 7 days. In order to extract the water-soluble carbohydrate content of the 2 grams of the plant sample, three consecutive aqueous extraction (reflux systems) are sufficient, adjusted to the final uniform volume of 20 ml, the mixture can be used without chemical dilution for chemical determinations.

Determining the sugar content same, as in the case of comb-beakers.

In the laboratory of the University of Pécs we performed sugar mobilization tests with damaged and intact samples. For the statistical evaluation of the sugar mobilization, we used a two-sample t-test, using R program packet ($P \leq 0{,}05$).

Analysis of the value change, caused on soybean by two spotted spider mite (*Tetranychus urticae* Koch)

The damaged and intact soy bean samples were collected from the Bicsérd arable land of the Fodder Research Institute of the Kaposvár University (GPS coordinates: N 46°04'08.68", E 18°05'56.09") which were collected in September, 2015.

The husks, constituting the base of the samples, were manually removed by 7-7 replications of the well visibly damaged by mites and intact plants. The soybean variety was Emese. The seeds were removed from the husks, then the intact and damaged samples were taken to laboratory, following cleaning, sieving. The soybeans were scraped, a sample weighed 20 grams. On the isolated samples at the Physiology and Biochemistry Laboratory at the Faculty of Agricultural and Environmental Sciences of the Kaposvár University were conducted Weendei analyses. The samples were then subjected to protein composition tests at the laboratory of the Department of Molecular Biology and Genetic at the Department of Natural Sciences of Pécs University.

Protein isolation and SDS polyacrylamide gel electrophoresis (PAGE) were performed as follows. The protein isolation of the samples was performed on the basis of the protocol, developed by Wang *et al* (2006). After pulverization, the samples were triturated with liquid nitrogen, then in 2 ml Eppendorf tubes 10% TCA/acetone was added to them and were shaken vigorously for 30 seconds, after 3 minutes at 16.000 g of cooled (4°C) centrifugation the supernatant was discharged. The precipitate was washed with a mixture of 80% methanol (Szkarabeusz) and 0,1 M ammonium-acetate (Spectrum-3D). After another centrifugation (16.000 g, 4°C) the pellet was washed with acetone (Pancreac) then dried at 50 °C for 10 minutes. Subsequently, depending on the starting cell mass, the protein was extracted with 0,4-0,8 ml of 1:1 phenol, (pH 8,0; Sigma)/SDS buffer. The protein content of the phenolic phase was captured with 0,1 M ammonium-acetate methanol overnight. The next day, after 5 minutes of centrifugation (16.000 g, 4°C) the protein remained in white pellet form, which was washed with methanol and 80% acetone. The samples were stored at -80°C until use and was dissolved in sample buffer. Protein concentration was measured, using a Nanodrop 2000 UV-Vis scattering photometer at UV 280 nm.

Gel electrophoresis was performed, using the Biorad Miniprotean Tetra Cell device. SDS poly-acrylamide gels were compiled according to Laemmli, (1970) method. Separation was performed, using a 5% compression and 12% separating gel. Prior to applying the samples a 10 minute 150 V pre- run was used, while the samples were run at 120 Volts. The staining of the gels was carried out for 1.5 hours with Coomassie Brilliand Blue solution (0,1 v/v% Coomassie Brilliand Blue R-250; 40 v/v% methanol; 10 v/v% glacial acetic acid). The differentiation solution (10 v/v% acetic acid; 10 v/v% methanol) was replaced several times. FluorChemQ – Protein Simple device was used for documentation of gel images.

For the statistical evaluation of the Weendei analysis results, we used a single factor variance analysis ($P \leq 0,05$), using R program packet. The results were evaluated using a Tukey test.

Analysis of the damage, caused by (*Acanthoscelides obtectus* Say) by CT diagnostic methods

The damaged by *Acanthoscelides obtectus* (Say) and intact bean items were collected from the crop store of the Iregszemcse Fodder Research Institute at Kaposvár University (GPS: N46°41’18.79”, E 18°10’50.76”), in winter of 2016. Determination of the damaged samples was made on the basis of the selection of perforated seeds in perforated husks. The samples, collected in this way, were taken to the Physiology and Biochemistry Laboratory at the Faculty of Agricultural and Environmental Sciences of the Kaposvár University, which were subjected to Weendei analysis, after washing and purification. Determining the crude protein-and fatty acid composition (MSZ 1979), by examining 4-4 (20 g) damaged and intact samples as well as the crude ash content (MSZ 1992) and the total fiber fraction (EC 2009).

To determine the average number of larva per seed ($\bar{x}\pm SE$) and the loss of mass, caused by the *Acanthoscelides obtectus* (Say) larva on the bean items, we analyzed 30 randomly selected beans. This calculation was based on a study by Jermy (1952), according to which, the evolved *Acanthoscelides obtectus* (Say) imagoes usually leave the damaged seed by chewing a separate opening for flying out, and during their larvae post- embryonic development, they consume plant tissue, equivalent to 4,78 fold of their average imago body weight (5,3-5,7 mg).

In our study, we looked at the volume loss, and based on the outer shell of the core, we determined the enclosing volume. Then the volume of beans and air were separated within this volume. The calculation was done from this. First, it was found that the average radiosensitivity of damaged beans was reduced compared to healthy ones. Here we have seen the beans inside the two cotyledons are airy. Since the beans were from the same population, we compared the beans' bean material volumes and looked at the differences in volume.

The computerized tomography (CT) examination of the beans was performed by us at the Kaposvár University, Faculty of Agricultural and Environmental Sciences, Department of Diagnostics and Onco radiology, using a Siemens Somatom Definition Flash (Siemens Gmbh., Erlangen, Germany) type scanner. For the examination, there were randomly selected 54 healthy and 54 damaged beans. The two groups were placed in the 3×3×6 layout on the examination table and the following adjustments were made for the recording: tube voltage: 100 kV, dose: 300 mAs, thread rise:0,6, segment thickness: 0,6 mm, field of view: 55 mm. From the crude data, collected in the spiral mode, the cross sectional images were made every 0,1 mm for the entire test length using abdominal window and medium soft kernel (B30s). The images were archived in DICOM (Digital Imaging and Communications in Medicine) format.

From the overlapping cross-sectional images were made meta files with „mnc" extensions by using tools, developed by the McConnell Brain Imaging Center (MINC). From the meta files, the OpenIP (Kovács *et al.,* 2010) software was used, to extract the unique images of bean seeds. The volume and average radio density of the beans were calculated by using the threshold (-300HU) method. Three dimensional model was made with the threshold values, originating from the above evaluation, by which we could visualize (Fedorov *et al.*, 2012) the shape and size of holes of larva lines, generated during the damage. Three-dimensional reconstruction work was carried out using the 3D Sliecer 47.0 program.

The results from the content analysis (n≤50), and the measured data volume (mm3) of the seed tissue density (HU) and the volume of the chewed cavity, inside the seed (n>50) were subjected to Shapiro–Wilk and Kolmogorov-Smirnov test (Ghasemi and Zahediasl, 2012). The resulting volume and density data were analyzed by one-way analysis of variance (One way ANOVA) SPSS for Windows 11.5. program package and were evaluated by using the Tukey test (HSD) ($P \leq 0,05$).

Carp feeding experiment with items, damaged by (*Acanthoscelides obtectus* Say)

The experimental feeds containing intact and bean damaged by weevil beans have been compiled and prepared in the Fish Laboratory of the University of Kaposvár. As a first step, the chemical and amino acid composition of the used bean was determined at the Food, Animal Products and Feed Laboratory of the University of Kaposvár, according to the following methods: crude protein (MSZ EN ISO 5983-2:2009), crude fat (MSZ 6830-19:1979), crude fiber (152/2009/EK III/I), and crude ash (MSZ 5984:1992) and amino acid composition (MSZ EN ISO 13903:2005).

The beans, after being minced and humidified, were heated in a microwave oven (750 W, 25 minutes) to eliminate the antinutritive effect (Sharma and Seghal, 1992). Then followed the mixing with other ingredients and then 2 mm pellet feed was formed, using appropriate equipment. The pellet was dried in a drying oven at 35 ºC to air dry state.

The feeding experiment was set up in the Fish Laboratory of the University of Kaposvár, Aquaculture and Fishery Management Department. The experimental stock (N =180). was composed by one-summer carps with initial body weight of 67,9 ± 11,2 g. The fish, belonging to the groups fed by control and the two experimental feeds, respectively were stocked in individually aerated, 300 liters tanks working in a recirculation system in triplicate (N=3x3x20=180).

Fish were fedtwice a day until satiation. The weight of fish was measured weekly during the experiment and the body length of fish was measured at the end of the experiment. Condition factor was calculated as follows: $CF = W / L^{-3} \times 100$, where W – live weight (g); L – standard length (cm).

The intended determination of the apparent digestibility of the experimental feeds with bean failed, since fish consumed so small amount of them, that made it impossible to collect the amount of feces needed for analysis.

Initially, the outliers were excluded from the primary dataset, then a normality test was carried out on the remaining data (Shapiro-Wilk test). One-way ANOVA was used to detect the effect of different feeds on growth and CF then the treatment means were compared with Tukey’s test. Comparing the amino acid composition of intact and damaged bean items was done by Spearman correlation analysis.

The statistical evaluation was carried out by using the IBM SPSS Statistics Version 25 (1989, 2017) program package.

3 RESULTS

Investigations about seed corn maggots

Soybeans, damaged by the seed corn maggots, were noticeably compulsion ripe, a phenomenon, which has been supported by statistical surveys (P=0,003). Unlike our expectations, however, the difference in crude protein content was not statistically justified (P=0,455). In the damaged items, on average 0,6 % less crude protein values were measured. The crude fat content in the damaged items showed a significant difference, compared to the intact items (P=0,006). Further, the statistical analysis also confirmed the significant difference between the crude fiber (P=0,001) and the crude ash (P=0,029).

During the examination, related to sugar, it has been shown, that the sucrose content of the damaged plants is greater, after the fourth day, than the one of the healthy soybean samples.

The fructose and glucose content after the sixth day of the test showed a marked deviation in the damaged samples.

The fructose content on the 6^{th} day of the study showed a similar tendency in the intact and damaged items. The fructose value in the first days of the study was lower in the damaged seeds, and it was of higher one after the 6^{th} day. No statistically significant deviation was found for either fructose (P=0,165) or glucose (P=0,282). The sucrose content (P=0,359) decreased after the third day both in the intact soybean and in the one, damaged by seed corn maggots. Figures 7-9 clearly show, that there is no significant difference between the sugar-mobilization of intact samples and the ones, damaged by the pests.

The data show a difference (3-6%) for most amino acids. Damaged items have a higher proportion of glycine, alanine, valine, isoleucine, leucine. In contrast, cysteine is the same as arginine and methionine amino acids in intact batches. Cysteine and

Methionine are the two sulfur-containing amino acids that play an important role in the construction of proteins. It is surprising that two amino acids were reduced only in the damaged sample relative to the intact batch (methionine, arginine). In the case of methionine, the greatest change was observed in the negative direction (-2.13%) compared to the intact lot. In the case of cysteine, there was no difference between intact and damaged batches. In the positive direction, the greatest difference was in the case of the triosine amino acid (7.76%) in the damaged sample compared to intact. On average, the difference for amino acids was 3.68%. This can affect the physiological processes of the plant, such as protein synthesis.

Three generations of Nezara viridula can be developed in Hungary. So far only the damage, caused by the first generation has been reported (germicidal pest), in these soybean crops it was likely, that the damage of the second generation occurred in the middle of summer.

Soybeans, damaged by the maggots (*D. platura*) causes the soy to yield less per hectare, and the predicted deterioration in the value of the contents will be expected to result in a loss of value in the feed word.

Due to global climate change, more and more aggressive pressures of adventitious pests can be seen in many agricultural areas in the world, from which Hungary's soy production is no exception.

The increased CO2 concentration and global temperature increase fundamentally changed the physiological processes and ecological characteristics of the most diverse organisms. The plants also change, depending on the changed circumstances, which will further affect the pest characteristics of their arthropod pests.

Due to global climate change, more and more aggressive pressures of adventitious pests can be seen in many agricultural areas in the world, from which Hungary's soy production is no exception.

Investigation, related to *Nezara viridula*

In the sugar composition studies, it has been shown, that the sucrose content of plants, damaged by Nezara viridula, is less than in intact soybean samples at the time of the first study. The deviation proved to be significant (P=0,043).

In the case of fructose among the mono saccharides, there was no marked difference between the damaged and intact items. Characteristic, significant differences were observed in the case of glucose and sucrose (P= 3,179e-08, P= 0,043).

The glucose stagnated until the fifth day of the study, and then increased in both the intact and damaged by Nezara viridula soy. There is a significant difference in the case of glucose (P=3,179e-08). In the case of fructose, only minor differences were observed with respect to the intact soybean items, but the statistical difference was not justified (P= 0,467).

According to our results, glucose and sucrose content significantly differed from similar parameters in healthy batches. The bug that is injected with the saliva that it consumes into the crop that it is consumed begins to pre-digest, and the animal is subsequently sucked into a fluid that becomes fluid. There are different digestive enzymes in the saliva. The changes in the crop can be explained by the effect of the digestive enzymes injected by the bug into the crop.

The results of the sugar composition test indicated the loss of intrinsic value due to migratory fly *(N. viridula*). This damage process can have many unexpected physiological consequences as carbohydrate metabolism is of central physiological importance as the synthesis and degradation of proteins, lipids, nucleotides is also linked to many metabolites. Thus, the confusion of sugar composition has a strong effect on almost all molecular and, hence, many physiological processes, which can ultimately be manifested in changes in plant size, composition, energy supply, and resistance. The change in carbohydrate content results in reduced seed germination, disease resistance, and frost resistance. On the other hand, some parameters of

carbohydrate metabolism are quick and sensitive indicators for biotic and abiotic effects.
However, for biotic factors, the heat treatment should occur in the narrow band where the degree of degradation of the inhibitors is satisfactory, but the denaturation of the protein does not impair its biological value. Excessive heat treatment reduces the amount and digestibility of all amino acids, especially lysine, arginine and cysteine.

Investigations, related to two spotted spider mite

The results of organic analysis of soybean samples confirmed the fact of impairment of the quality, due to the damage, caused by two spotted spider mites. The crude protein content has been altered in a verified manner by the effect of damage (P=0,049). However, the change of the crude fat (P=0,643) and crude fiber content (P=0,069) as a result of the damage, was not confirmed by the statistical analysis. As a result of the damage, caused by the mite, change in the crude ash content did not show any significant difference either (P=0,859).
There was no significant change in the content of soy as a result of mite damage. Hildebrand et al. (1986b) also reported that the level of infection of growing mites is a slight increase in total soluble sugar and starch, and causes a slight decrease in the total nitrogen and phosphorus levels of soybean leaves. The mite infection has no effect on the composition of the seeds. Growing mite damage significantly reduced leaf chlorophyll content (55.26%) and caused a more drastic loss of leaf carotenoid content (79.3%).
The gel electrophoresis photograph shows the quantitative rearrangement of proteins due to damage. In the damaged batches, the presence of new proteins not observed in intact batches was recorded.
The method showed the presence of 48 kDa proteins in the damaged sample, while protein killing below 30 kDa was more pronounced in the sample as a result of the

normal activity of the spider mite. 69 kDa proteins are found in the damaged sample at a lower rate than in intact batches.

Our test results to confirm changes in protein composition and content of soybean samples are also supported by the results of earlier studies with other species.

The gel electrophoresis assay well reflected the quantitative rearrangement of proteins due to damage. Of course, changes in the content values can be traced back to other disorders. Thus, the loss of protein caused by sulfur deficiency can reach 40%, which is due to the inhibition of glycine biosynthesis.

Protein and nucleic acid composition of soy can be severely damaged by oxidative stress. There is also evidence of changes in protein content and oil composition caused by water stress. The protein structure of the soy produced for feed has clearly negative consequences for the digestion of farm animals. For example, the small intestine of piglets fed after isolation has a reduced digestive and adsorption capacity. Thus, inadequate feeding can cause diarrhea in their case. The background of this phenomenon is the rearrangement of amino acid residues, which may lead to the dominance of "non-essential" amino acids such as glutamine, which may be the cause of animal health disorders.

In human nutrition, various soy-based proteins play a role in the development of the appropriate hormonal condition and menstrual cycle. This is illustrated, for example, by the correlation between high soy consumption and low incidence of breast cancer.

Analyzing the damage, caused by (*Acanthoscelides obtectus* Say) using CT imaging technology

The fact of the restructuring of content, caused by the *Acanthoscelides obtectus* (Say) was clearly demonstrated by the Weendei analysis. Interestingly, in the case of the examined parameters, the damaged items show higher values, than the similar content ingredients of the intact samples. This is particularly apparent in the data of the crude protein of the damaged items. The Shapiro–Wilk test confirmed the normal distribution of the data in the study. In contrast, variance analysis showed different statistical correlations, according to which, the content of crude protein (P=0,042), crude fat (P=0,038) and the crude fiber (P=0,002) of the damaged items is significantly different from the similar values of the intact items, but this relationship was not detectable in the case of crude ash (P=0,519).

The average number of larvae, calculated by seeds, was 8.93±1.83. The average organoleptic loss, due to this nutritional deficiency, was about 234.76±8.54 mg/seed, which means a significant mass loss of the whole seed, namely 49.42%.

The density data from the CT diagnostic procedure show, that the damaged items have lower radio density (HU) values, than the intact ones. The average difference between the density values of these items was 75,834 HU (41,93%). So the larvae have gnawed the denser ingredients of the affected bean crop.

From the volume results, it can be seen that, due to the damage, the affected samples also suffered a significant volume loss. The average volume of the intact seeds was 1129.857±33.7185 mm^3. On average, the volume of a damaged seed is 296.162 mm^3 less, than that of an intact one. The value of the average volume loss was 26,21%.

The Kolmogorov-Smirnov test verified the normal distribution of the data in the study ($P>0.05$). Variance analysis confirmed the significant decrease in volume and density of the crops, tested (P= 0,000).

The visualized illustration of the damaged bean grains produced by 3D-dimensional reconstruction shows that the babysits chewed the core of the core, the cotyledon, and used it, and the pericarp remained almost intact. In most of the samples examined, the cotyledon containing the reserve nutrient, located in the central zone of the core, was completely destroyed. Thus, the reserve nutrients required for the developing embryo are completely absent for the damaged seeds.

The density and loss of volume of the bean samples in the fruit-damaged crop was clearly demonstrated. Weendei analysis has shown that quality parameters such as fat, fiber, and protein have changed. Quantitative increase in the protein content of damaged samples can be misleading, which can be attributed to the presence of glandular elements.

Our study has shown that insect damage is often difficult to detect externally and can be assisted by imaging techniques used in various veterinary and human health diagnostics.

The destruction of the bitter components of the bean, according to our experiment, is associated with the consumption of high protein high density dense scavenger (cotyledon).

For the qualitative and quantitative evaluation intact bones by bean weevil (*A. obtectus)*, the image reconstruction technique used by CT is excellent. The use of imaging diagnostic methods greatly contributes to the expansion of the physiological and pest knowledge of hidden lifestyle pests. The use of CT in the evaluation of plant protection pests is conducive to the implementation of effective chemical protection and thus to the practical realization of integrated plant protection (IPM).

Feeding carp by bean items damaged by (*Acanthoscelides obtectus Say)*

Laboratory tests showed higher protein content of the damaged than the intact bean items. This phenomenon is also known in case of soybean, damaged by Alydidae and Pentatomidae pests, where protein content can rise by up to 13%.

However, the Spearman correlation coefficient ($r = 0,939$, $p < 0,001$) calculated on the amino acid composition showed almost complete compliance of the items used in our experiment.

Evaluating the growth of fish, a significant treatment effect was. The control group, consumig commercial carpfeed, produced dynamic growth, in contrast with feeds containing damaged and undamaged bean. The compound feed containing the damaged bean was utilized so badly, that the fish even lost weight at the end of the experiment.

At the end of the experiment significant differences were found in the mean weight, length and condition factor of the different groups. The best results in all respects were achieved by the control group and the worst one were obtained on the feed, containing beans damaged by weevil.

The result show that such significant replacement of fishmeal by bean decreases the carp performance. It is not surprising because most fishes react negatively to the high proportion of plant-derived proteins in the feed. In the case of trout, replacement of fish meal with horse-bean, the 15% proportion caused the best growth (also as compared to control) the higher content of horse-bean has been gradually deteriorating performance. Increased horse-bean protein concentrate deteriorated the growth of Atlantic salmon. Replacement of soy with *Canavalia ensiformis* above the 11% ratio significantly deteriorates the performance of African cat fish (*Clarias gariepinus*).

It is likely, that the limiting effect of low level of amino acids (lysine, methionine, cystine) may be the cause of the phenomenon, since the values, measured in the beans are below the values, necessary for carps (Hungarian Fodder Codex, 1990), also from the measurable amounts in the soy. However, the fact, that the higher protein-bearing damaged beans have resulted in a worse growth, is already thought thought-provoking. A possible explanation may be that during the compilation of experimental feeds to produce the same raw protein content, the feed, containing intact bean contained more fish-meal, which in turn could provide essential amino acids in a larger proportion.

4. CONCLUSIONS

The overall aim of the dissertation was to study changes in the quality parameters of leguminous fodder plants damaged by certain arthropod species. In view of the objectives set, the following conclusions can be drawn from the available literature.

As a result of the damage caused by **seed corn maggot**, a forced sleep occurred. As a result of the damage, there was also a change in the content of crude fat, crude fiber and raw ash. Protein composition test data showed a difference of 3.68% for amino acids. As a result of the damage, the yield of the soy will decrease and the quality of the seed will also change.

The pestilence of the **green stink bug** could be proved by changes in glucose and sucrose content. The stress caused by arthropod pests has changed the sugars (glucose, sucrose) found in the seed of the plant. Changes in carbohydrate content are related to seed germination, plant tolerance to plant diseases, and frost tolerance.

Based on the results of the study, it can be assumed that the future coincidence of the present climatic extremes and agrotechnical effects can be expected due to the damage of the comb-shaped flower fly and the migratory fly as a result of the quality change of the produced soy products. Thus, from our experimental results, we conclude that pest control measures against pests can become a legitimate technological element in soy production.

The damage of **the two spotted spider** mite in the soy seed does not cause a significant change based on the results of the Weendei analysis. However, proteins of different weights appear in the protein composition, others disappear. Moth damage the leaf mainly to the photosynthetic surface of the plant and thereby reduce its size. In the case of feed soybean meal damage caused by the spider mite damage, rearrangement may cause digestive disorders following the feeding of farm animals.

Damage to babies was clearly confirmed by CT diagnostic method. Both density and volume loss occurred in the plant seed. The use of imaging techniques in plant protection can facilitate the design and implementation of effective chemical control.

Feeding **bean weevil** damaged and healthy beans with one-year carp in high ratio resulted reduced performance of carp. Beans are a well-suited protein source in fish feed, but can only be incorporated in a specific amount. Therefore, it is necessary to continue the experimental work in order to determine the amount of beans that can replace certain amount of fishmeal without deteriorating fish. It would also be important to determine the apparent digestibility and the effect of the bean-containing feeds on carp meat quality.

5. NEW RESEARCH RESULTS

1. Laboratory analytical methods were the first to prove the phenomenon of compression in soy plants as a result of the damage of the seed corn maggot [*Delia platura* (Meigen)] and the change in the values of crude fat, raw ash and crude fiber compared to intact seeds.
2. We proved the sugar composition changes in the soybean as a result of the damage of the green stink bug [*Nezara viridula* (L.)]. The laboratory test showed that the glucose and sucrose content of the seeds damaged by the bug was significantly changed compared to healthy batches, while the fructose content did not change significantly compared to the intact batches.
3. We first identified the rearrangement of the protein structure of soya beans damaged by two spotted spider mite (*Tetranychus urticae* Koch). Gel Electrophoresis (SDS PAGE) demonstrated the loss of high molecular weight proteins (69 kDa and above) from damaged batches, while lower molecular weight proteins (30 kDa and below) appeared.
4. Using the computerized tomography (CT) diagnostics of bean weevil, we proved the significant loss of density and volume of the beans (*Acanthoscelides obtectus* Say) damaged by babies (up to 26% volume and 40% loss of density).
5. Based on the results of the experiment with one-year-oldcarp (*Cyprinus carpio* L.), it can be concluded that the experimental feed containing the damaged bean significantly reduced the growth rate of the fish, compared to fish consuming intact beans and control feed. This is mainly due to reduced feed intake. Beans, whether they are intact or damaged, can only be recommended as pellet ingredients in much lower proportions than what were used in our experiment.

6. SUGGESTIONS (THEORETICAL AND PRACTICAL USE)

On the basis of our investigations, it can be assumed that, in the future coincidence of the present climate extremes and unfavorable agronomic effects, the insect damage can cause significant problems, regarding the expected quantitative and qualitative parameters of leguminous fodder plants. Based on our experimental results, we conclude, that plant protection interventions against pests are justified in soy production and may become more and more commonly used as a technological element in the future.

Increased CO_2 concentration and global temperature increase have fundamentally altered the physiological processes and ecological characteristics of a wide variety of organisms. depending on the changed circumstances, plants naturally vary, which will further affect the damage properties of arthropods. The greatest plant protection challenge of today is the timely detection of these altered agro biological conditions and the implementation of effective, sustainable defense. A positive result of these technological elements, among others, is ensuring of a full value, healthy feed.

Furthermore, we consider it necessary to continue the experimental work, in which we intend to determine the amount of bean, which, used in fish feed, does not impede growth and feed utilization. It would also be important, to determine the apparent digestibility and to examine the effect bean-based feed on carp meet quality.

The better understanding of hydro-biological processes in the ponds, and the increase of natural yields through agronomic interventions, continues to be a guarantee of successful fish production in ponds. At the same time, with regard to the production targets, the choice of suitable feeding stuff - which can be cereal, protein rich plant originated by-product or extruded compound feed – has a decisive influence on the profitability. When using reduced value by-products, it is recommended to perform pre- feeding tests.

7.SCIENTIFIC PUBLICATIONS FROM THE TOPIC OF THE DISSERTATION; INFORMATIVE PUBLICATIONS; LECTURES (CONFERENCE, SYMPOSIUM, CONSULTATION, MEETINGS)

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