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# An in Vivo Study for the Use of *Lupinus Albus* (Fabaceae) in *Drosophila Melanogaster* Diet

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**Abstract**

Based on the oral chronic toxicity studies in humans, the daily intake of *Lupinus albus* L. (termiye, white lupine) with diet is recommended to be about 0.02%. The study was designed to investigate the use of lupine shells in nutrition. Dried *L. albus* shell was added to the artificial diet of the model organism (*Drosophila melanogaster*) and then lipid peroxidation, antioxidant enzyme activity, total oxidation, and total antioxidant activity were determined in the tissues obtained from the third larval phase. Changes that occurred in the larval midgut cells were examined microscopically. As the amount of shell consumption increased, malondialdehyde concentration (0.33 - 0.09 ± 0.71 nmol/ mg protein) and glutathione S transferase activity (19.91 - 14.06 ± 0.04 nmol/ mg protein/ dk) decreased statistically compared to the control. In addition to this, larval total oxidation level and total antioxidant activity also decreased ($P < 0.05$). No damage was detected in the larval midgut epithelial cells.

**Keywords:** *Drosophila melanogaster*, Lipid peroxidation, *Lupinus albus*, Oxidative status, Total antioxidant activity

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## 1. INTRODUCTION

In recent years, many researchers have tried to develop environmentally friendly technologies by using herbal sustainable resources and the safety of the herbal applications used in the traditional treatments of humans and animals has been evaluated[1,2]. According to the free radical theory, plant components are also highly toxic like environmental pollutants and chemicals because they cause oxidative damage in cells, lipids, nucleic acids, and proteins by providing reactive oxygen species (ROS)[3]. For this reason, researchers have focused on plant products that suppress ROS, have low toxicity, environmentally friendly, biodegradable, but do not cause resistance in the organisms.

In Turkey, known as termica in many regions and simply consumed, Lupin (*Lupinus albus* L., Fabaceae: Leguminosae) is used as gluten-free raw material in the food industry and feed stuff in animal nutrition (monogastric animals, poultry and pigs)[4]. Moreover, Lupin is a functional food with high sugar (5.82%), protein (32.2%), fiber (16.2%), and fat (5.95%) content. Its fat content contains saturated (13.5%), monounsaturated (55.4%), and polyunsaturated (31.1%) fatty acids as oil[5]. Toxic secondary metabolites such as alkaloids found in Lupin species have antifungal and insect deterrent activity. However, the total amount of alkaloid in *L. albus* is just 0.186 g which is eliminated to remove the bitter taste for commercial sale, therefore it is known to be safe for use as feed stuff[4-6].

Model organisms are vital for making successful predictions about the living organisms and the environment[7]. Although the digestive system in humans has similarities with digestive system of the model organisms, it is much more complex. However, *Drosophila* intestine is similar to the human gastrointestinal system in both structure and function[8]. The digestion in *Drosophila* larva stage starts in the mouth and continues throughpharynx, esophagus, anterior midgut, middle midgut, posterior midgut end hindgut, and ampulla. In addition, *Drosophila melanogaster* has great advantages such as high reproducibility and no ethical concern, and therefore is frequently used in research as a model organism for nutrition. The effect of ROS on the metabolism can be explained by the balance of oxidant-antioxidant mechanisms. Oxidative stress in tissues can be estimated by the amount of lipid peroxidation products such as malondialdehyde (MDA) and total oxidation level (TOS). One of the antioxidant enzymes, Glutathione S transferase (GST) and total antioxidant activity (TAS) is, are used for explaining the antioxidant balance against oxidation.

In recent years, shells, fats, and extracts of plants have been usedas performance enhancer in animal nutrition[9]. This study aimed to investigate the possible use of lupin shell (LK) in nutrition. Larvae of *D. melanogaster* were fed with a diet containing dried *L. albus* shell and the oxidative stress levels of tisses and changes in the intestinal epithelial cells were observed

## 2. MATERIALS AND METHODS

*D. melanogaster* (Oregon) were housed (60-70% humidity and 25 ± 2ºC) at the culture laboratory in the Department of Gastronomy, Necmettin Erbakan University and fed with an artificial diet[10]). Commercially available *L. albus* shells were removed, dried in the oven at 60°C for 24 hours, and ground to the powder. In oral chronic toxicity studies performed in humans, it is recommended to use a daily average consumption of 0.02% per day[11], therefore the dried-ground LK at a ratio of 0.02-0.1% was added to the diet of the larvae. After the preliminary feeding studies of the consumed shell ratio, the experimental setup was established. The hatched new larvae were transferred to flasks (100 pcs) using a fine-tipped brush. The larvae were monitored daily until reaching the third stage, and the larvae were collected by washing in 20% isotonic solution. For biochemical analysis, larval tissues (100 pcs) were extracted in homogenization buffer (pH 7.4, + 4°C) by ultrasonic homogenizer.

### 2.1. Biochemical analysis

MDA, TOS level and GST and TAS activity were determined in order to detect total oxidative stress level. In addition, for each concentration, the larval midgut dissections (Olympus SZ61) were stained with orsein, the changes in the epithelial cells were visualized under the microscopic examination and were photographed with the Cameram program (Olympus C3 X 33; at least 25 times). The amount of MDA was determined by the method of Jain and Levine[12] and Glutathione S transferase (EC 2.5.1.18) by Habig et al.[13]. By using the kits (Rel Assay Diagnostics), TOS (μmol $H_2O_2$ E/L) and TAS (mmol Trolox Eq/L) were measured in Biochrom Libra S22 and oxidative stress index (OSI = TOS / TAS) was determined according to the standard formula[14].

### 2.2. Statistic analysis

The experiments were repeated four times. In the evaluation of the data, one-way analysis of variance (ANOVA, F test) was performed by statistical package program and LSD test was performed to determine the significance of the difference between means. The significance of the means was evaluated at the 0.05 probability level and the degrees of freedom were given. All chemicals were also purchased from Sigma Chemical Co (St. Louis, MO).

## 3. RESULTS

In the diet fed with LK (0.02%), the amount of MDA increased by 0.33 ± 1.25 nmol/mg protein, and the increased concentration of feeding caused statistically similar results (0.1 and 0.09 ± 1.12 nmol/mg protein). Figure 1 shows GST activities of insect that antioxidant resistance to lipid peroxidation occurs in those fed with 0.02% LK. It was determined that GST activity decreased against low peroxidation in insects fed with high concentration LK (14.06 ± 0.02; $P < 0.05$). When TOS values formed in larval stage of insect were examined, the change observed in the amount of MDAwas similar to that of the larva fed with 0.02% LK and while the TOS level was 60.00 ± 1.08, its level in the control group and at the highest feeding concentration was 20.00 ± 1.65 (Figure 2, $F_{11} = 67.007$ μmol/L; $P < 0.05$). The activity of Larval TAS was determined to decrease from 0.5 to 0.25 ± 0.173 ($F_{11} = 1.186$ mmol/L; $P = 0.349$). While the OSI index of the individuals fed with control food was in the 40 s, the reduction in the LK-fed from 120 (0.02%) to 80 (0.1%) indicates that increase in the stress level can be reduced, but not to the minimum level ($P = 0.456$). Deformations were not observed in the epithelial tissues of larval midgut under microscopic examination (Figure 3).

## 4. DISCUSSION

Each species has to meet its energy needs in order to survive, which makes it necessary to consume foods / nutrients necessary for the living. It is essential to know that the organism is not damaged when using substitute products in nutrition. For example, the addition of Lupin to the chicken diets for five weeks (20%) did not change the growth performance[15]; it was digested by sheep[16], it can be used up to 30% in rainbow trout diet[17]; it was used for feeding chicken and ducks; it did not significantly affect[18] the blood parameters (50 and 100%); it did not cause a significant change in monocytes[19]; the addition of up to 20% of the diet to the chicken health did not have any negative effects[20]. In human studies, it is known that Lupin meals taken daily with 12 mg (0.02%) oral diet as a food are digestible and did not cause a significant change in blood parameters[11].

Although the diet content used in nutrition is important for living, nondigestible nutrients are either directly excreted from the body or increase oxidation, adversely affecting survival. Especially in larvae, it has to be fed continuously for pupulation to occur, but there is no feeding during the pupal period[21] which shows that food consumed in the body accumulates. For example, the use of Lupin in feeds at 15-30% level in different organisms reduced the intake of feed although it increased feed intake with the use of Lupin in the feeding of *Helip aspersa*, a lung snail[22]. In birds, Lupin digestibility decreases when given at 300 g/kg[23]. The small amount of

LK used in this study accumulated in the midgut of the larva, increasing the amount of MDA and TOS, and the intact epithelial tissue in the feeding with increasing concentration suggests that the non-digestible LK was discarded or taken into the body. Lupin added to the chick diet lowered the intestinal viscosity[24]. Digestive system of *D. melanogaster*[25], which is a valid model for research of mammalian digestion has a strong barrier role to respond directly to environmental pathogens such as pathogenic infections, toxic substances, and pro-oxidants that pollute food[26]. This barrier limits contact with potentially harmful substances such as toxins and pathogens, thus giving them selective properties. This selectivity is supported by a strong mucosal immune system. *Drosophila* immune defense, which is devoid of an adaptive immune system, is based only on the natural immune system[26]. In addition, the peritrophic membrane around the intestine helps the antioxidant system[27]. The fact that fruit flies have a unique antioxidant system similar to that of mammals is preferred by researchers[28]. There are many enzymes that detoxify toxins in *Drosophila*. The increase in superoxide dismutase, catalase, GSTactivities against the increase in larval MDA is seen as the natural defense mechanism of the body[29]. Foods such as green tea and broccoli added to the diet in *Drosophila* reduce total lipid peroxidation and increase the antioxidant activity[30]. In the study, the feeding of larvae with 0.02% LK was thought to be related to increased oxidation (MDA and TOS) balance between antioxidant defense by increasing GST and TAS activity. The absence of deformation in the midgut epithelium also suggests that antioxidant concentration can be increased in tissues[31]. It is stated that Lupin added at 35% level or above to the diet has been shown to have a negative effect on various parts of the gastrointestinal tract[32]. Oxidative stress occurs due to increased free radicals and antioxidants with a scavenging effect against them, and degradation of oxidative balance[33]. Although the use of LK caused stress at low concentration, it was determined that the stress was reduced by an antioxidant mechanism. Similarly, in diabetic rats, it is known that Lupin reduces the amount of MDA[34]. Up to 30% Lupin can be used in the diet of rainbow trout; It is said to reduce triglyceride, cholesterol, alkaline phosphatase and lactate dehydrogenase[17]. However, flies gain resistance to free radical stimulants, thus attenuating the toxic effects of ROS[35]. Although there is the idea that insect has gained resistance in feeding with increased LK, low MDA and TOS amount and deformation are not observed. Lupeol [36], the most prominent minor component in the lipid portion of the lecane, is a triterpene alcohol that plays a role in the regeneration of the epidermal tissue[37]. This information is thought to be renewable midgut epithelium with the use of 0.1% LK.

It can be asserted that the OSI index obtained by using 0.1% LK reduces lipid peroxidation levels and the oxidation is tried to be balanced with the antioxidant system. It is stated that Lupin (50% of food), which is consumed together with the other nutrients, has a high feed potential in the feeding of poultry with its macro and micro element content, and it will affect the health of the intestine positively[38,39].

*L. albus* (100 g) contains 1.42 phytic acid, 0.9 saponin, 0.01 tannin, 0.01 trypsin inhibitors, 0.8 raffinose[6]. It is known that the amount of alkaloits of Lupin, which is cooked at 100 ° C or kept at 30 ° C for 45 hours, decreases by 43%, some of the proteins are degraded and the bitterness is reduced by 50%[40]. Although Lupin shows toxic effects on poultry, they stated that Lupin 150 g/kg can be used for the removal of the bitterness[20]. Lupin can be used upto 150 g/kg of diet in order to reduce the bitterness of diet. Saponin and tannins have a lethal effect on insects. However, the reduction by cooking explains why oxidation is reduced in insects feeding with 0.1%. It was determined that the amount of alkaloids in LK mixed with food and the thermal drying process did not cause any oxidation in the insect and did not adversely affect the model organism morphologically and biochemically.

## 5. CONCLUSION

It is thought that the use of *L. albus* shell in the model organism, an insect, in the amount of 0.02 or 0.1%, does not cause any damage to the tissue

in terms of biochemical oxidation. It can be used as feed additive as a functional product after further research investigate its potential benefits or disadvantages. This will also contribute to the elucidation of the mechanism of action of natural products in the nutritional oxidative stress. We plan to determine the effect of the rate of Lupine accumulation in the adipose tissue of *D. melanogaster* fed with lupine and how this accumulation causes the saturated and unsaturated fatty acid reserves in future studies.

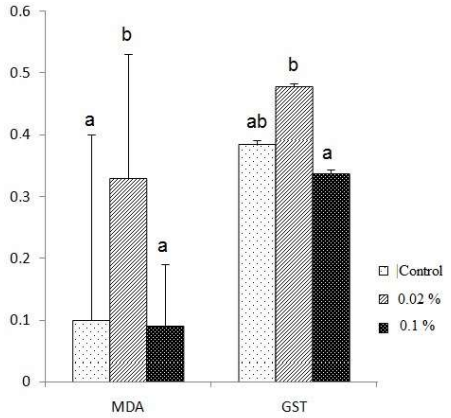


Figure 1 The amount of MDA and the change in GST activity in the feeding of the *Drosophila melanogaster* larvae with Lupin shell (0.02% and 0.1% LK). The average of four replicates, 100 larvae per replicate was used

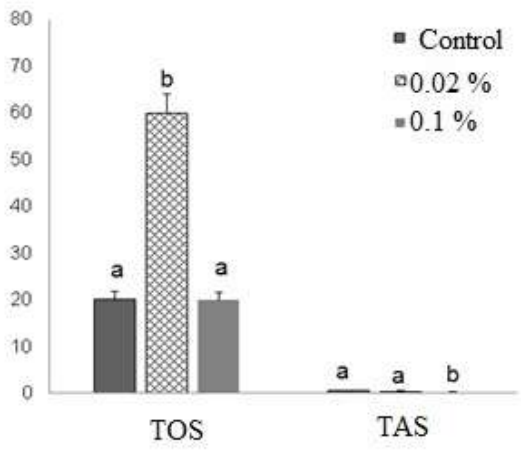


Figure 2 Total oxidation (TOS) and total antioxidant capacity (TAS) changes in feeding of *Drosophila melanogaster* larvae with Lupin shell (0.02% and 0.1% LK). The average of four replicates, 100 larvae per replicate was used

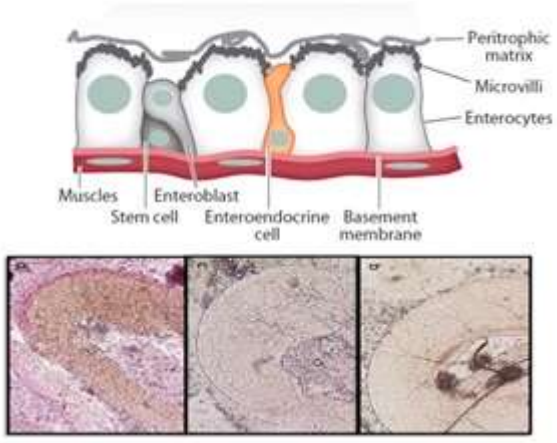


Figure 3 *Drosophila melanogaster* larvae in the digestive system a. midgut epithelial structure (Lemaitre & Miguel-Aliaga, 2013), b. The control group of the midgut epithelium, c. The group midgut epithelium fed with 0.02% Lupin shell (LK), d. The group midgut epithelium (X 100) fed with 0.1% LK

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***Authors' Contribution***

E.G: designing the study, performing the analyses and writing the first draft of the manuscript.

H.F.N: managing the analyses of the study.

M.N and Z.B: writing the first and final draft of the manuscript.

### *The Declaration of Ethics Committee Approval*

The authors declare that this document does not require an ethics committee approval or any special permission.

### *The Declaration of Research and Publication Ethics*

The authors of the paper declare that they comply with the scientific, ethical and quotation rules of SAUJS in all processes of the paper and that they do not make any falsification on the data collected. In addition, they declare that Sakarya University Journal of Science and its editorial board have no responsibility for any ethical violations that may be encountered, and that this study has not been evaluated in any academic publication environment other than Sakarya University Journal of Science.

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