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Effects on protein levels on *Gallaria mellonella* (Lepidoptera: Pyralidae) adults at different age of changing the start time of the light

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Abstract
Effects of protein levels *Gallaria mellonella* adults at different age of changing the start time of the light were investigated in this study. Insects were reared at, six different photoperiod regimes eighteen hours light, six hours dark (18L; 6D) first level, (18L; 6D) second level, twelve hours light, twelve hours dark (12L; 12D) first level, (12L; 12D) second level, six hours light, eighteen hours dark (6L; 18D) first level, (6L; 18D) second level). Illumination was done with 40 W fluorescent bulbs in different photoperiod regi-mes. It was made second level photoperiod regimes for 18L;6D, 6L; 18D, 12L; 12D under the condition different light on time. Insects were fed with honey free comb. Calculating the amount of protein for analysis, Lowry test was used. Protein level in 100 mg of adults increased in the first days of adult of females in connection with their age but decreased afterwards. No difference was observed in males. Amount of protein in some age groups, no statistically significant differences, and while significant differences were found in some age groups. Generally, Under the same photoperiod regimes, there were increase amounts of protein in the first level photoperiod since significant variations in protein levels can be observed in connection with sex and start of the light. This shows the importance of change the start time of light.

Keywords: *Galleria mellonella,* photoperiod, protein, start time of the light

1. Introduction
Protein, lipid and carbohydrate metabolism plays role in many vital activities of insects. Many other factors such as sex, age; developmental stages, diapause, nutrient quality and level seasonal conditions, temperature, host type in some species, sexual activity, use of insecticide are effective on the levels of these substances. [1,2,3,4,5,6,7,8,9,10,11]
Proteins are the last option among the substances to be used as fuel. Proteins are effective especially on metamorphosis, growth, cocoon and cuticula formation and flying. Protein level varies especially before or during metamorphosis in the developmental stage [5,8,11,12].
G. mellonella is a harmful species for combs and its larvae are known as causing serious damages in beekeeping [13,14,15,16,]
There are not studies effects on protein levels on *Gallaria mellonella* adults at different age of changing the start time of the light. Calculating the amount of protein for analysis, Lowry test [17] was used. It is important to know the biological characteristics of this insect for biological control and laboratory studies in this insect. Changing the start time of light is very important in many vital activities of insects. There are very few studies on insects bright start time [19,20,21,22].

2. Materials ve Methods
Large wax moth *Galleria mellonella* (Linnaeus, 1758) (Lepidoptera: Pyralidae) were used in expe-riments. Studies were carried out under the laboratory conditions at 28 ± 2°C temperature and % 65 ± 5 relative humidity. Insects were reared at, six different photoperiod regimes eighteen hours light, six hours dark (18L; 6D) first level,(light phase began in the morning (18L; 6D) second level, (light phase began in the evening) (twelve hours light, twelve hours dark (12L; 12D) first level, (light phase began in the morning (12L; 12D) second level,(light phase began in the evening)six hours light, eighteen hours dark (6L; 18D) first level,

(light phase began in the morning) (6L; 18D) second level) (light phase began in the evening). Illumination was done with 40 W fluorescent bulbs in different photoperiod regi-mes. It was made second level photoperiod regimes for 18L;6D, 6L; 18D, 12L; 12D under the condition different light on time. Insects were fed with honey free comb. Calculating the amount of protein for analysis, Lowry test [18] was used. İnsects were let to proliferate in jars at predefined constant temperature and humidity in different photoperiod regimes. One day old aduts were weighed and stocked on the first day of their adult life. Ten adults matured on the same day from five and fifteen days old groups put into a jar and were fed on comb without honey. Five of them were weighed five days later and another five of them 15 days later, respectively.Processed for he mentioned three age groups were repeated three times with samples taken from the the population at different times. For each analysis, 15 adult insects from a certain age group were weighed and stocked in 1.5 ml Eppendorf tubes at -50 °C until they had been analyzed.

Biochemical analysis: For protein analysis, one, five and fifteen days old adults stocked at -50°C were used.

Protein analysis: First of all a standard protein graph was created for specifying protein values that will be obtained through protein analysis. About 0.1% bovine serum albumin was used. The standard stock solution in 1 mg mL^{-1} concentration was prepared. Then standard protein solutions in 10, 20, 25, 30, 40, 50, 75 and 100 µg mL^{-1} concentrations were prepared through serial dilutions of the standard stock solution. Lowry method was applied to these solutions and the absorbance values

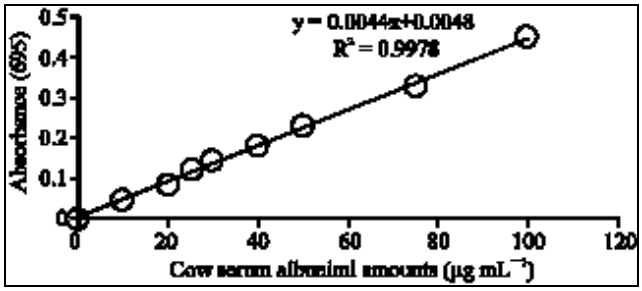

Fig : Standard protein graph

monitored by a spectrophotometer at 695 nm wavelength against blank. These processes were repeated three times for each concentration of the standard solution. Standard protein graph was created by using the absorbance values obtained.

Total protein analysis in the stocked samples was based on the method developed by Lowry [17] The samples weighed and kept at -50 °C were transferred into larger tubes for homogenization after they had been kept at room temperature for a while. Each tube had one insect in it and 5 mL work buffer was added to the tubes. Each insect was homogenized at 8,000 rpm for 7 min. The homogenate was centrifuged at room temperature at 3,500 rpm for 15 min. About 100 µL of the supernatant generated in the tube at the end of the centrifuging was processed with Lowry method and monitored with a spectrophotometer at 695 nm wavelength against blank. The monitored absorbance values were evaluated by using the standard protein graph.

One Way Analysis of Variance (ANOVA) was used for the comparison of more than two groups. Ave-rages were assessed by using Student-Newman-Kuel (SNK) Test, when the test results were signifi-cant. Independent Two Samples t-test was emplo-yed for the comparison of two groups and a = 0.05 confidence limit was taken as basis.

Under the same photoperiod regimes, there were increase amounts of protein in the first level photoperiod. Under All photoperiod regimes in females, the amounts of protein increased as the adult aging and Amount of protein in some age groups, no statistically significant differences, and while significant differences were found in some age groups.

3. Results and Dissussion

Result of age and sex related protein level analysis on adult *G. mellonella* kept in 18L:6D (first level) is given in Table 1.

Table 1: Protein level of adult *G. mellonella* in 18L:6 D(first level) photoperiod.

Adult age(days)	Protein level(mg/100mg insect)(Ort±SH)*		
	Femele	male	P**
1	4.71±0.10 a	4.54±0.09 a	P>0.05
5	6.26±0.08 b	6.09±0.07 b	P>0.05
15	5.93±0.11 c	6.38±0.1 c	P<0.05

*Average of 3 repeated processes each with 5 beings, **Significance level between the protein levels of males and females from the same age group, Average differences in the same column with the same letter(s) are not significant ($p<0.05$)

Result of age and sex related protein level analysis on adult *G. mellonella* kept in 18L:6D (second level) is given in Table 2.

Table 2: Protein level of adult *G. mellonella* in 18L:6 D(second level) photoperiod.

Adult age(days)	Protein level(mg/100mg insect)(Ort±SH)*		
	female	male	P**
1	5.04±0.07 a	4.46±0.06 a	P<0.05
5	6.51±0.1 b	5.16±0.03 b	P<0.05
15	6.06±0.06 c	5.47±0.05 c	P<0.05

Result of age and sex related protein level analysis on adult *G. mellonella* kept in 12L:12D(first level) is given in Table 3.

Table 3: Protein level of adult *G. mellonella* in 12L:12D D(first level) photoperiod.

Adult age(days)	Protein level(mg/100mg insect)(Ort±SH)*		
	female	male	P**
1	5.42±0.07 a	4.63±0.08 a	P<0.05
5	6.58±0.07 b	5.30±0.08 b	P<0.05
15	6.08±0.11 c	6.17±0.07 c	P>0.05

Result of age and sex related protein level analysis on adult *G. mellonella* kept in 12L:12D(second level) is given in Table 4.

Table 4: Protein level of adult *G. mellonella* in 12L:12D D(second level) photoperiod.

Adult age(days)	Protein level(mg/100mg insect)(Ort±SH)*		
	female	male	P**
1	4.78±0.1 a	4.33±0.08 a	P<0.05
5	5.53±0.06 b	5.28±0.08 b	P<0.05
15	5.07±0.07 c	5.76±0.07 c	P<0.05

Result of age and sex related protein level analysis on adult *G. mellonella* kept in 6L:18D (first level) is given in Table 5.

Table 5: Protein level of adult *G. mellonella* in 6L:18D D(first level) photoperiod.

Adult age(days)	Protein level(mg/100mg insect)(Ort±SH)*		
	female	male	P**
1	4.44±0.09a	4.14±0.10 a	P<0.05
5	5.80±0.08 b	5.47±0.06 b	P<0.05
15	5.20±0.03 c	5.82±0.07 c	P<0.05

Result of age and sex related protein level analysis on adult *G. mellonella* kept in 6L:18D(second level) is given in Table 6.

Table 6: Protein level of adult *G. mellonella* in 6L:18D D(second level) photoperiod.

Adult age(days)	Protein level(mg/100mg insect)(Ort±SH)*		
	female	male	P**
1	4.11±0.06 a	3.64±0.07 a	P<0.05
5	5.54±0.07 b	4.38±0.06 b	P<0.05
15	4.77±0.08 c	5.31±0.07 c	P<0.05

In females, the protein level was increased in the first five days of adult life but then decreased afterwards. In males, the protein level kept increasing after the fifth day of adult life.

Under the same photoperiod regimes, there were increase amounts of protein in the first level photoperiod. Under All photoperiod regimes in females, the amounts of protein increased as the adult aging and Amount of protein in some age groups, no statistically significant differences, and while significant differences were found in some age groups.

Age and protein level relations, variations in protein level accompanied by aging and different protein necessities of females and males were studied by various researches. [18] In this study, variations in protein levels of adults in accordance with the adult age are observed. For this variation it was observed that in females increase in the beginning was followed by a decrease, whereas the increase kept going in males. Protein levels of females first increased in the beginning of adult life for high rate of egg production. However, as the reproduction potential declined by aging in the following days of life, the protein demand necessary for the egg production was also declined. Protein levels of adults were decreased when this condition was united with the decreased anabolic reactions. Since the protein used by males for sperm production is not consumed in large quantities as by females, the decrease observed in the protein level of males was less than the decrease observed for females. The results obtained from this study of the age related protein level variation are in agreement with the results of other researches. In a research with *Spodoptera littoralis* (Lepidoptera: Noctuidae) the effects of host-plant, photoperiod, day time, developmental stage and sex on the protein band number and concentration were studied and it was found that the protein band number of the last instar larvae change [11].

Age-related changes in hemolymph free amino acids and proteins were examined, when Colorado Potato Beetle-*Leptinotarsa decemlineata* was reared under both short-day (8:16) (L:D) and long-day (17:7) (L;D) conditions. Under a short-day photoperiod, the total free amino acid concentration in the hemolymph increased gradually up to 20 days of adult life, but the long-day beetles showed marked increases during the first 10 days and then decreased afterwards. Proline, glutamine and valine were the most abundant free amino acids in both sexes [8].

In a study with *Bactrocera tryoni* (Diptera: Tephritidae) it was revealed that for egg production, protein consumption of sexually matured adults are higher than non-sexually matured females [12]. High protein levels of the females began to decrease especially after the second week. The increase in the protein level until becoming sexually matured and then the decrease in the protein level with the start of egg production and age related protein issues, which were observed in this study with *G. mellonella*, are in line with other studies with different insects.

4. Conclusion

Protein levels derived from adults were found different in males and females as well as in different photoperiod. Protein levels were found diffrerent in start time of light in same photoperiod. Generally, under the same photoperiod regimes, there were increase amounts of protein in the first level photoperiod. These differences might be due to photoperiod induced endocrine imbalance changing the protein level. it is also important that it is time to start time of the light not only photoperiod [19,20,21,22,23]. There are no studies on the bright start time in same photoperiods on *G. mellonella.* In this study, age and sex related variations in the insect metabolism in six different photoperiods and the effects of these variations on protein levels were observed.

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