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From The Editor;

Dear Readers and Authors,

As “International Journal of Science Letters (IJSL)”, we are pleased and honored to present the first issue of 2022. IJSL, is an international double peer-reviewed open access academic journal published on the basis of research- development and code of practice.

The aims of this journal are to contribute in theoretical and practical applications in relevant researchers of Life Sciences, Biology, Biotechnology, Bioengineering, Agricultural Sciences, Food Biotechnology and Genetics institutions and organizations in Turkey, and to publish solution based papers depending on the principle of impartiality and scientific ethics principles, focusing on innovative and added value work, discussing the current and future.

With these thoughts, we are especially thankful to academicians honoring with the articles, valuable scientists involved in editorial boards and reviewers for their contributions to the evaluation processes with through their opinions/ideas/contributions/criticisms in this issue of International Journal of Science Letters.

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Research Article

Usage of encapsulated *Hypericum scabrum* in ayran and determination of antioxidant, phenolic and sensory properties

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Abstract

Hypericum scabrum (HS) widely used in traditional medicine due to its bioactive compounds was extracted by using ethanol-water (3:7, v/v). The extract was encapsulated with maltodextrin and gum arabic in a spray dryer in order to protect the phenolic compounds in its structure. Different amounts of microcapsules were added to our traditional drink, i.e. ayran (drinking yoghurt). The total phenolic content (TPC) and DPPH radical scavenging activity of the microcapsules in extract of HS and ayran samples were determined. The amount of total phenolic compounds in the microcapsule provided a superior effect than the extract. The ayran samples were supplemented with 2%, 3%, 4%, 5% and 6% of *Hypericum scabrum* microcapsules and it is observed that total phenolic content and DPPH radical scavenging activity indicated an increase with concentration. TPC and DPPH activity were determined as 268.86 mg GAE100 mL^{-1} and 78.05% for 6% supplemented samples. As a result of the sensory analysis, ayran samples supplemented with 4% of HS microcapsule gained the highest scores by the panelists and received more appreciation than the control group. It is concluded that HS4 (ayran produced by 4% HS supplemented microcapsule) sample was determined as the best sample according to the sensory analyses while the HS6 (ayran produced by 6% HS supplemented microcapsule) sample had the highest value in terms of DPPH scavenging activity and TPC results. The overall results of the present study revealed that 4% HS supplemented ayran can be produced with its enhanced health beneficial and desirable properties.

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1. Introduction

In recent years, natural products have gained interest due to the their diverse pharmacological properties and minimum side effects (Aras et al., 2018; Farhan et al., 2019; Farooqi and Ahmad, 2019; Farooqi et al., 2019; Qureshi et al., 2019). Some medicinal plants include diverse of natural antioxidants, such as phenolic acids, flavonoids, and tannins, which exhibit stronger antioxidant activities. It is known that these compounds are of important in terms of preventing the beginning or progressing of many diseases (Kızıl et al., 2011). Studies have shown that antioxidant activities of natural products are significantly effective on different diseases. Studies are focused on natural antioxidants in order to prevent the negative effects of the synthetic antioxidants in human life. It has been proved that compounds such as polyphenols, anthraquinones, phenolic terpenes and flavonoids are responsible for the antioxidant activity of plants (Uysal et al., 2018; Zengin et al., 2018a, b; Lazarova et al., 2019).

The Hypericum genus belongs to the Hypericaceae family and it has been used as a traditional medicine for a long time due to its biological activities. In addition, it is widely used especially in the field of health for its bioactive phytochemicals (Llorent-Martinez et al., 2018; Keser et al., 2020). It consists of approximately 500 flowering plant species. Although there are lots of studies on *Hypericum perforatum* (HP) belonging to genus Hypericum, studies on *Hypericum scabrum* also known as 'Yeastherb, Kepir herb'are insufficient. This plant has also antiseptic, anthelmintic, antimicrobial and antifungal properties (Unal, 2008; Ebrahimzadeh, 2013; Pirbalouti, 2014). Moreover, secondary metabolites of Hypericum species are hypericin, flavonoids, tannins, phenolic acids, hyperoside, quercitrine, isooctrine, routine and chlorogenic acid (Barnes et al., 2001; Dall'Agnol et al., 2003).

Chemical preservation techniques are highly effective in the prevention of antimicrobial abilities, enzyme inhibitiors and antioxidant activities of the extracts as well (Hasanuddin, 2019). Encapsulation is a method that protects the plant extract from undesirable reactions such as temperature, oxygen and heat during processing and storage (Koç et al., 2015; Rosa et al., 2019). There are several methods for encapsulation of materials such as spray, cooling/freezing, extrusion coating, spray drying, liposome binding, coacervation and centrifugation, fluid bed coating (Madene et al., 2006). Among these methods, spray drying is the most preferred method due to the easy use of equipment, constant production, wide selection of carrier materials, good retention of volatile compounds and low cost (Calvo et al., 2012; Mahdavi et al., 2016). The

choice of coating material in the encapsulation method is one of the most important factors affecting the success of the process. The coating material should protect the core material against external effects, prevent any reaction, and have a high emulsion stability anda structure that allows film formation (Madene et al., 2006). In the food industry, maltodextrin, whey protein isolate, and gum arabic are used for coating materials, respectively (Chew et al., 2018; Korma et al., 2019). Maltodextrin have some alternatives such as low cost, neutral taste, low viscosity at high solids concentration, and providing good protection against oxidation (Barros Fernandes et al., 2014; Korma et al., 2019). Coating materials are often used in combinations in order to obtain enhanced properties. It is combined with maltodextrin, gum arabic and other coating materials to provide the desired properties in encapsulation (Korma et al., 2019).

In this study, the bioactive compounds of the HS plant were encapsulated with the combination of maltodextrin and gum arabic coating materials. In addition, the usage of HS microcapsules in the production of ayran was investigated and the results were evaluated in terms of TPC, DPPH, and sensory analyses, respectively. Although there are studies on the chemical structure, volatile compounds, and medicinal use of the HS, to the best of our knowledge, this is the first report for encapsulation of HS and use of HS microcapsules in foods.

2. Materials and Methods

2.1. Material

Hypericum scabrum (Figure 1) was collected from Amasya region in 2018 between June and August. Identification of the plant was performed by Dr. Cengiz Yıldırım and specimen was deposited at the Herbary of Ondokuz Mayıs University, (OMUB 0527). The aerial parts of the plant were dried in the shade at room temperature and then grounded to a fine powder. A combination of maltodextrin (DE 16.5-19.5, Sigma, St. Louis, MO, USA) and gum arabic (Merck, Darmstadt, Germany) were used as coating material for the encapsulation of HS extract. Ayran products were produced in OTAT Provisions Industry and Trade LLC, Samsun/Havza according to the process of the factory and supplied from the factory.

Figure 1. *Hypericum scabrum*

2.2. Extraction Procedure

Ultrasonic assisted extraction (UAE) technique was used to extract the bioactive compounds of HS. Extraction of the plant was performed at 30°C for 40 min with ethanol-water (3:7, v/v) solvent mixture, keeping the material to liquor (M:L) ratio as 1:30 (v/v) based on the previous results (Seyrekoğlu and Temiz, 2019).

2.3. Encapsulation of the Extract

Encapsulation of HS extract was carried out at 180°C in a spray dryer (B-290, Buchi Corporation, Flawil, Switzerland). The aspirator speed of the spray dryer was set as 100% (35 $m3h^{-1}$), the air flow rate was 50% (601 Lh^{-1}), and the feed rate was 30% (9 mL min^{-1}) as well. Spray drying was applied using 10% of coating concentration and same ratio of coating material and core, respectively. Maltodextrin and gum arabic were used as coating materials in equal proportions. Drying process was given in Figure 2. TPC and DPPH radical scavenging activity of the samples were determined for HS microcapsules. Analyses of the microcapsules were performed according to the method of Robert et al. (2010) with slight modifications. For this purpose, 10 mL of ethanol-acetic acid-water (50:8:42, v/v/v) mixture was added on 1 g of microcapsule sample and vortexed for 2 mi. It was centrifuged at 1000 rpm for 5 min and then filtered through 0.45 μm porous fitler paper (Robert et al., 2010).

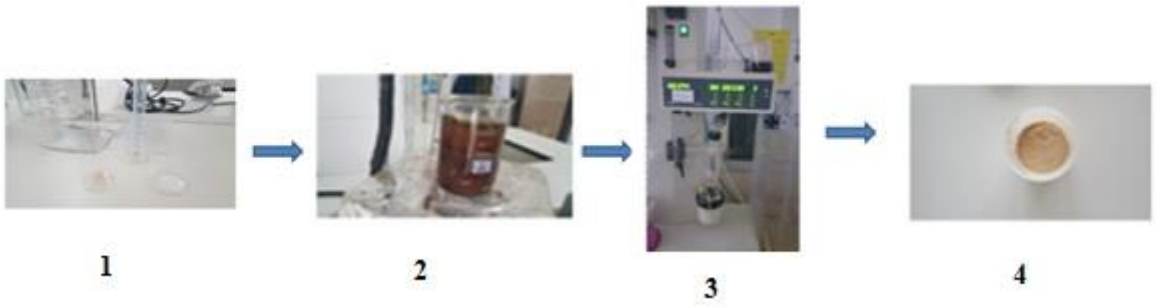

Figure 2. Production of *Hypericum scabrum* microcapsule; 1, Preparing of coating materials; 2, Mixing of *Hypericum scabrum* extract and coating materials; 3, Drying process; 4, *Hypericum scabrum* microcapsule

2.4. Microcapsule Supplemented Ayran Production

The HS microcapsules were used during the ayran production process of OTAT Food Industry and Trade CLL. Different amounts of microcapsules (1%, 2%, 3%, 4%, and 6%) were added to the drinking cup of ayran after the ayran production process (Figure 3). Produced microcapsule supplemented ayran samples were subjected to TPC and DPPH analyses, respectively.

Acidified ethanol (30 mL) was added to 20 mL of ayran sample and kept at 4°C for 16 h in order to extract the bioactive compounds from microcapsule supplemented with ayran. Afterwards, the sample was centrifuged at 6000 rpm for 3 min. The experiments were carried out according to the method of Singleton and Rossi (1965) and as it specified in the method, the best result has been obtained by applying centrifugation at the specified time and rpm since there is turbidity and proper reading is not performed. The solution was filtered through Whatman No: 1 filter paper. TPC and DPPH were analysed in the filtrate (Singleton and Rossi, 1965; Koca et al., 2008).

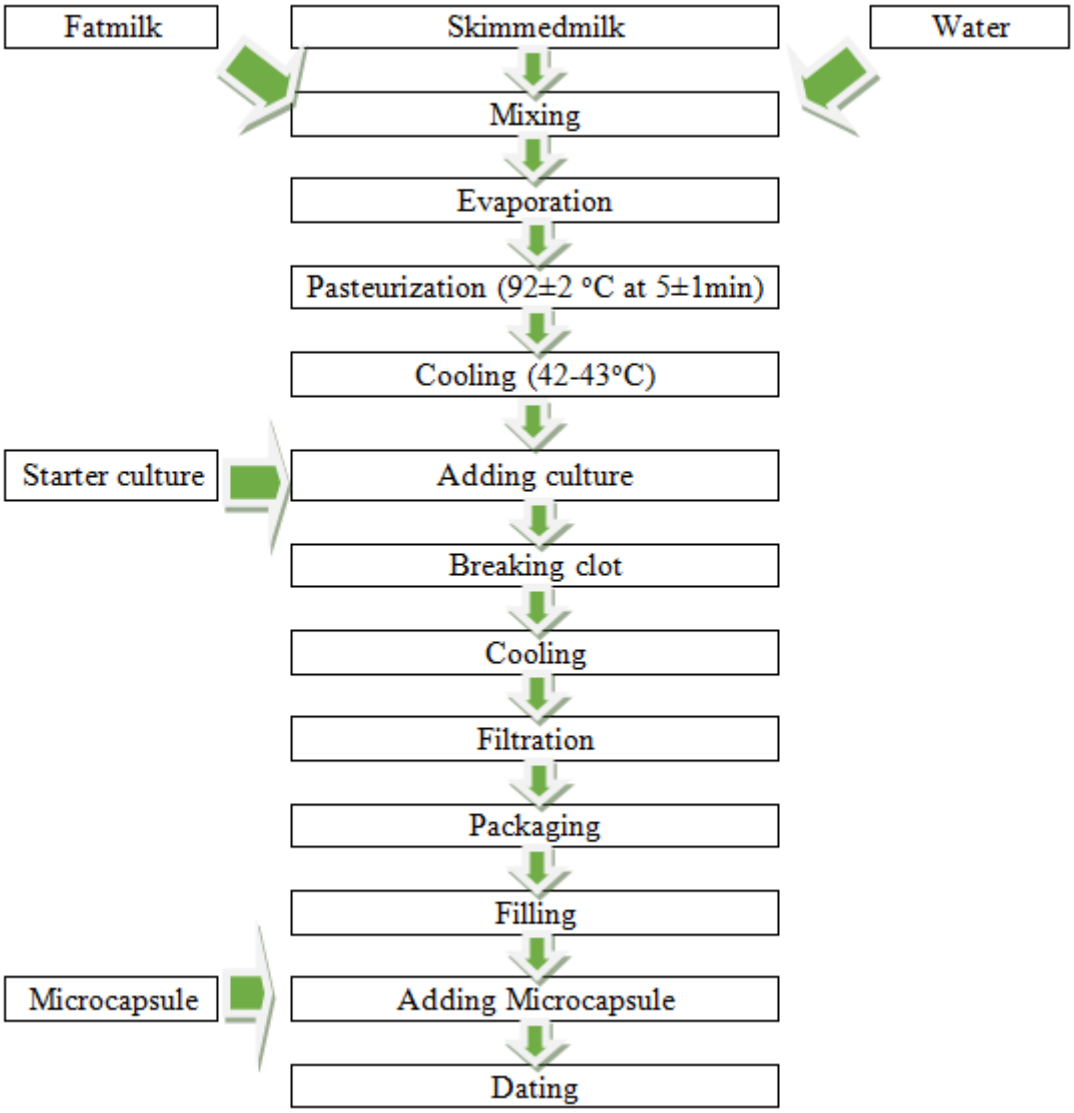

Figure 3. Production of drinking yoghurt

2.5. Total Phenolic Compound Analysis

The total phenolic compound analysis of the samples was carried out according to Singleton and Rossi (1965). Distilled water (2.4 mL) and Folin-Ciocalteau solution (200 µL) were added to 40 µL of HS extract (1 $mgmL^{-1}$). Then, 600 µL of saturated sodium carbonate and 760 µL of distilled water were put into the mixture. The absorbance values were measured at 760 nm (using Standard gallic acid solution) in a spectrophotometer (UV-1601 Shimadzu). The results were expressed as mg GA $equivalentg^{-1}$ of extract.

2.6. DPPH Radical Scavenging Activity

Diluted DPPH solution (3.8 mL) was added to 200 µL of filtrate and then it was vortexed for 15s and left in the dark for 60 min. Phase seperation was observed at the end of the period. Supernatant was carefully taken from the mixture, and the absorbance values were recorded at 515 nm (Aksoylu, 2012). DPPH scavenging activity was calculated as percent inhibition for the extract, micocapsules and ayran samples as well.

2.7. Sensory Analysis

Color–appearance, texture–consistency, taste-smell, and general taste characteristics of the samples were evaluated by panelist group. HS microcapsule supplemented with ayran samples were evaluated by a panelist group consisting of 10 people. The evaluation was made in a range of points from 1 (quite bad) to 5 (quite nice) (Anonym, 1982).

2.8. Statistical Analysis

All the analyses were performed at least two technical experiments and mean standard deviations were calculated. The results were analysed with SPSS 16.0 package program and Tukey Test was used for as post-hoc test. The significance levels of the groups were evaluated at $p < 0.05$ (SPSS, 2011).

3. Results and Discussion

Total phenolic content was calculated as 149.67 mgg^{-1} GAE for HS extract and 435.89 mgg^{-1} GAE for its microcapsule (Table 1), respectively. The complete removal of water by the encapsulation process and the fact that the coating core ratio was 1/1 caused an increase in the amount of extract in the microcapsule and thus an increase in the total amount of phenolic substances viceversa. The experimental results showed that HS ethanol-water extract contains 149.67 mgg^{-1} GAE of phenolics, a lower value compared with those reported for aqueous (186.94 mg $GAEg^{-1}$ extract), methanol (171.00 mg $GAEg^{-1}$ extract), and ethanol (262.00 µg $GAEmg^{-1}$ extract) extracts of HS (Barış et al., 2011; Karatoprak, 2019). The phenolic content of the plant depends on several factors such as the parts used, collection region, collection time, extraction conditions, etc.

Table 1. TPC results and DPPH radical scavenging activity of HS extract and microcapsule

Sample	TPC ($mg_{GAE}/g_{extract}$)	DPPH (% Inhibition)
HS extract	149.67 ± 3.42	79.17± 0.27
HS microcapsule	435.89 ± 3.43	77.07±2.21

Data are expressed as mean ± Standard deviation (n = 3)

Phenolics are important compounds that have the ability of scavenge free radicals. DPPH is an easy and fast method to evaluate the antioxidant activity. The reduction of DPPH free radicals is based on the capacity of some hydrogen-donating compounds including phenolics such as flavonoids, phenolic acids and tannins by donating hydrogen atom (Barış et al., 2011). DPPH scavenging activity was found as 79.17% in HS extract and 77.07% in encapsulated form, respectively. Several studies revealed that DPPH activity is correlated with TPC (Yeo and Shahidi, 2015; Parikh and Patel, 2017; Parikh and Patel, 2018). Although HS microcapsule contain higher amounts of TPC than HS extract, both of them exhibit similar DPPH radical scavenging activities (Table 1). It may be due to the fact that antioxidant compounds are adversely affected by the heat applied during encapsulation. Application of 180^{o}C temperature during the encapsulation process may damage the chemical structure of the compounds that provide DPPH activity and therefore cause a decrease in % inhibition. Especially hyperforin, one of the bioactive compounds, is a very sensitive compound to heat and temperature, and the increasement in temperature to high degrees causes degradation of the active compounds and decrease in inhibition (%).

DPPH activity results of the present study are approximately similar as obtained by Unal et al. (2008). Unal et al., (2008) determined the DPPH activity of the ethanol extract of HS as 78% while we found 79% and 77% for HS extract and microcapsule, respectively. On the other hand, Baris et al. (2011) obtained higher DPPH scavenging activity (90%) for the ethanol extract of HS. HS extract is a powerful source of antioxidant on the account of its composition. Omidi et al. (2020) determined that HS contains flavonoids such as quercitrin and quercetin that can scavenge the free radicals. Previous studies revealed that TPC amount of microcapsule is quite high when compared with its extract form. It is also reported that air inlet temperature of encapsulation process, coating material, the core composition, and ultrasonic power effect the TPC of the samples (Franceschinis et al., 2014; Peanparkdee et al., 2016; Tatar, 2016).

Ayran samples supplemented with different concentrations of HS microcapsule were examined in terms of their TPC and DPPH values. The results were found statistically significant ($p<0.05$) (Table 2). TPC was increased with the increasing concentration of HS microcapsule. The control group has the lowest TPC value (10.58 mg GAE100mL^{-1}), while HS6 contains the highest TPC value (268.86 mg GAE100mL^{-1}). It is also observed that, DPPH activity of the ayran samples were increased with increasing concentration of HS microcapsule. In literature, there is no study related with the addition of HS microcapsule to ayran. However, similar to our results, the studies related with the comparison of TPC values of different plant/food extract with its supplemented form in food revealed that TPC was increased with the increase of supplemented ratio (Aydemir, 2015; Jalal, 2018). Encapsulation of plant extract provides high inhibition values for the final product by protecting the antioxidant compounds (Barretto et al., 2020).

Table 2. TPC and DPPH radical scavenging activity of ayran samples.

Sample	TPC (mg GAE/100mL)	DPPH (%Inhibition)
C	$10.58^{f} \pm 0.33$	$5.75^{f} \pm 0.14$
HS2	$77.58^{e} \pm 0.37$	$35.48^{e} \pm 0.31$
HS3	$130.58^{d} \pm 0.22$	$56.49^{d} \pm 0.30$
HS4	$202.41^{c} \pm 0.49$	$67.48^{c} \pm 0.18$
HS5	$230.53^{b} \pm 0.26$	$75.49^{b} \pm 0.32$
HS6	$268.86^{a} \pm 0.58$	$78.05^{a} \pm 0.66$

*: mean of standard ± deviation.
a-f: The lower case letters in the same column are the comparison of the ayran samples and the same letters show that there is no statistical difference between the samples. (P> 0.05)
C: Control ayran, HS2: ayran produced by 2 % HS supplemented microcapsule, HS3: ayran produced by 3 % HS supplemented microcapsule, HS4: ayran produced by 4 % HS supplemented microcapsule, HS5: ayran produced by 5 % HS supplemented microcapsule, HS6: ayran produced by 6 % HS supplemented microcapsule

TPC amount of HS microcapsule (435.89 ± 3.43) was higher than all HS microcapsule supplemented with ayran samples (Table 1 and Table 2). This result can be explain by the fact that ayran is a fermented product, it is acidic and thus, causes the amount of phenolic compounds to be lower in the final product by causing dissolution in the microcapsule. Tseng and Zhao (2013) stated that oxygen, pH, temperature, light, metal ions, enzymes and moisture content are among the main factors affecting the stability of polyphenols which are more stable at lower pH values. This reduction can be prevented by changing the coating material and coating material ratios used or by using different methods such as encapsulation method.

Table 3. The sensory properties of ayran samples.

Sample	Color and appearance	Texture – consistency	Taste – aroma	General taste
C	$3.60^{a} \pm 0.54$	$3.00^{b} \pm 0.00$	$3.40^{ab} \pm 0.89$	$3.60^{ab} \pm 0.89$
HS2	$3.60^{a} \pm 0.89$	$4.40^{a} \pm 0.89$	$4.00^{a} \pm 0.00$	$4.00^{a} \pm 0.00$
HS3	$3.60^{a} \pm 0.54$	$4.00^{ab} \pm 0.70$	$3.60^{ab} \pm 0.54$	$3.20^{b} \pm 0.44$
HS4	$3.60^{a} \pm 0.54$	$3.80^{ab} \pm 0.83$	$3.20^{b} \pm 0.44$	$3.20^{b} \pm 0.44$
HS5	$3.60^{a} \pm 0.54$	$3.60^{ab} \pm 0.54$	$2.40^{c} \pm 0.54$	$2.20^{c} \pm 0.44$
HS6	$3.60^{a} \pm 1.14$	$3.80^{ab} \pm 1.09$	$2.00^{c} \pm 0.00$	$2.00^{c} \pm 0.00$

*: mean standard ± deviation.
a-f: The lower caseletters in the same column are the comparison of the ayran samples and the same letters show that there is no statistical difference between the samples. ($P > 0.05$)
C: Control ayran, HS2:ayran produced by 2 % HS supplemented microcapsule, HS3: ayran produced by 3 % HS supplemented microcapsule, HS4:ayran produced by 4 % HS supplemented microcapsule, HS5: ayran produced by 5 % HS supplemented microcapsule, HS6: ayran produced by 6 % HS supplemented microcapsule.

The sensory properties of microcapsule supplemented ayran samples are given in Table 3. There is no statistical difference in the color-appearance scores of the ayran samples. It is observed that the increase in the amount of microcapsule did not have a negative effect on the color-appearance. Thus, the encapsulation process prevented the passage of the dark yellow color of the HS extract into the product, ensuring high scores. The highest scores for texture-consistency and taste-aroma were gained with HS2 and HS3, respectively. All HS microcapsule supplemented ayran samples had higher texture-consistency values than control. On the other hand, lower taste-aroma scores were achieved for HS4, HS5 and HS6, respectively. The highest general taste value was obtained with HS2 while HS6 gave the lowest value.

4. Conclusion

In recent years, oxidative stress and its effects on human health are important issues that have been emphasised. The increase in diseases such as obesity, cancer, immune system and the desire of people to have more healthier life canalized researcher stop roduce functional and healthy foods (Essa et al., 2021). Therefore, the interest in natural additives and healthy foods is increasing day by day. In this study, *H. scabrum* plant, which is used in the field of health but has not been studied enough, was investigated in terms of its usage in the production of healthy functional foods. It is suggested that HS microcapsule which was coated with maltodextrin and gum arabic can be used as a new additive food supplement with high TPC. The amount of TPC and antioxidant capacity of the final product can be increased with different coating materials or different encapsulation methods.

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Research Article

Animal product consumption habits of university students: Suluova Vocational School

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Abstract

The aim of this study is to determine the animal product consumption habits of associate degree students at Amasya University Suluova Vocational School. For this purpose, a face-to-face survey was conducted with a total of 285 students, which 159 students from the Veterinary Department and 126 students from the Property Protection and Security Department, between January and March 2020. When the opinions of the students about the food groups were evaluated, it was observed that 71.2% of them thought that the animal-based foods were healthier and 94.7% thought that the products of animal origin had a better protein quality. Furthermore, it was determined that the majority of the students (42.5%) were consuming foods of animal origin because they were healthy. When the animal-based food consumption frequency of the participants was examined, it was seen that those who consumed cheese (37.9%) and eggs (31.2%) every day were in the majority. While most of the participants consumed yoghurt (25.8%), ayran (26.4%), chicken meat (26.3%) and sausage (28.4%) 1-2 times a week, it was found that consumption of beef-veal (30.2%), lamb-mutton (39.6%), fish (48.8%), salami (21.4%) and sausage (29.5%) was rare. Moreover, 25.8% of the participants did not consume butter, 72.3% did not consume turkey meat and 51.9% did not consume pastrami at all. It was found that the majority of university students did not consider any of the criteria of taste, price, brand, quality, hygiene, ease of preparation, smell-color and personal health when buying red and white meat. Finally, when the participants were examined in terms of their milk consumption preferences, it was seen that the majority (47.4%) preferred street milk.

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1. Introduction

The intake of various nutrients into the body in order to maintain life, growth, development and protection of health is defined as nutrition (Baysal, 2015; Tayar et al., 2015). For an adequate and balanced diet, the nutritional elements needed by individuals must be taken at an adequate level and used appropriately in the organism (Tayar et al., 2015). Malnutrition not only affects physical and mental development but also reduces resistance to infections (Ndlovu, 2010). People get the nutrients they need from plant and animal sources. Among these, foods of animal origin are an indispensable element of the human diet with their macro (protein, carbohydrate, fat) and micro (vitamins and minerals) nutrients (Ndlovu, 2010). Moreover, the bioavailability of these foods is high (Flachowsky et al., 2017). It is recommended to meet 1/3 of the daily protein needs of people with animal origin products (Flachowsky et al., 2017).

There are many factors that affect the consumption level of animal products, such as nutritional habits and income level. Considering the 2011 data of FAO, it has been reported that there will be an increase in meat, egg (73%) and milk consumption (58%) until the middle of the 21st century (Makkar, 2016). This study was carried out to determine the animal product consumption level and habits of Amasya University Suluova Vocational School students.

2. Materials and Methods

2.1. Type, Place and Time of the Research

The research is a descriptive study conducted to determine the animal origin food consumption levels of students from Amasya University Suluova Vocational School between January 2020 and March 2020.

2.2. Ethical Aspect of the Research

To conduct the research, written permission from the Rectorate of Amasya University and approval with the letter of Amasya University Non-Interventional Clinical Research Ethics Committee, numbered 15386878-044 and dated 06.01.2020, were taken. The students participating in the research were included after they were informed about the study and their consent was obtained.

2.3. Population and Sample of the Research

The research population consisted of Amasya University Suluova Vocational School Property Protection and Security Department students and Veterinary Department students. In this research, the sampling method was not used, and 285 students studying in the departments of Veterinary Medicine (n=159) and Property Protection and Security (n=126) who agreed/voluntarily participated in the study were included.

2.4. Data Collection Tools

A questionnaire form was used to collect research data. While preparing the questionnaire, the Dietary Guidelines (Baysal et al., 2014) was used. The questionnaire includes questions about students' demographic information (age, gender, department, housing status), students' views on food groups (which food groups are healthy, evaluation of food groups according to protein quality, reasons for consumption of animal origin food), the frequency of animal-based food consumption (cheese, yoghurt, ayran, butter, eggs, beef-veal, lamb-mutton, chicken, fish, turkey, soudjouk, salami, sausage, pastrami), the points that students pay attention to when buying red and white meat (taste, brand, price, quality, hygiene, ease of preparation, smell-color, personal health criteria), and their milk consumption preferences (UHT milk, packaged milk, street milk).

2.5. Evaluation of Data

Research data were analyzed using SPSS 22.0 (Statistical Package for Social Sciences) for Windows 22.0 program (IBM Corp., 2011). While evaluating the data, numbers, percentages and mean values were used.

3. Results and Discussion

In this study, which was conducted to determine the animal origin food consumption levels of Amasya University Suluova Vocational School students, when the demographic characteristics of the students were examined, it was seen that the average age was 20.09. When other demographic characteristics were analyzed, it was observed that 89 (31.2%) of the participants were female, 196 (68.8%) were male, 159 (55.8%) were student Veterinary

Department, 126 (44.2%) of them were a student at Property Protection and Security Department. Moreover, 135 (47.4%) of them were living at home-apartment, 98 (34.4%) were living in dormitories, and 15 (5.3%) of the participants were living with their relatives (Table 1).

Table 1. Demographic characteristics of the participants

Yaş		
Minimum age	18	
Maksimum age	28	
Average age	20.09	
Gender	**N**	**%**
Female	89	31.2
Male	196	68.8
Department	**N**	**%**
Veterrinary	159	55.8
Property protection	126	44.2
Housing Status	**N**	**%**
With family	37	13
With a relative	15	5.3
At home-apartment	135	47.4
In the dormitory	98	34.4

When the opinions of university students about food groups were examined, it was determined that 203 (71.2%) of them thought that animal-based products were healthier, while 270 (94.7%) of them thought that animal-based products contained good quality protein. While 121 (42.5%) of the participants were consuming foods of animal origin because they thought it was healthy, 109 (38.2%) of them were consuming because they were delicious, and 23 (8.1%) of them were consuming foods of animal origin due to familial habits (Table 2).

Table 2. Students' opinions on food groups

Which food group do you think is healthy?		
Food groups	**N**	**%**
Animal origin products	203	71.2
Herbal origin products	82	28.8
Evaluation of food groups according to protein quality?		
Food groups	**N**	**%**
Animal origin products	270	94.7
Herbal origin products	15	5.3
Reason for consumption of animal origin food		
Reason for consumption	**N**	**%**
Because it's healthy	121	42.5
Because it's delicious	109	38.2
Because it's satisfying	32	11.2
Familial habits	23	8.1

The frequency of food consumption of animal origin by the students is given in Table 3.

Table 3. Animal source food consumption frequency of students

	Every day		5-6 times a week		3-4 times a week		1-2 times a week		Once in 15 days		Rarely		Never	
Food groups	**N**	**%**	**N**	**%**	**N**	**%**	**N**	**%**	**N**	**%**	**N**	**%**	**N**	**%**
Cheese	108	37.9	50	17.5	38	13.3	41	14.4	8	2.8	25	8.8	13	4.6
Yoghurt	63	22.3	34	12	49	17.3	73	25.8	12	4.2	45	15.9	7	2.5
Ayran	36	12.7	41	14.4	58	20.4	75	26.4	21	7.4	49	17.3	4	1.4
Butter	46	16.2	24	8.5	33	11.6	39	13.7	29	10.2	68	23.9	45	25.8
Egg	89	31.2	48	16.8	64	22.5	35	12.3	14	4.9	19	6.7	16	5.6
Beef-Veal	15	5.3	17	6	22	7.7	71	24.9	40	14	86	30.2	34	11.9
Lamb-Mutton	5	1.8	7	2.5	11	3.9	26	9.1	35	12.3	113	39.6	88	30.9
Chicken	20	7	49	17.2	63	22.1	75	26.3	33	11.6	39	13.7	6	2.1
Fish	2	0.7	4	1.4	9	3.2	39	13.7	42	14.7	139	48.8	50	17.5
Turkey	2	0.7	5	1.8	2	0.7	3	1.1	3	1.1	64	22.5	206	72.3
Soudjouk	23	8.1	37	13	53	18.6	81	28.4	30	10.5	48	16.8	13	4.6
Salami	23	8.1	22	7.7	41	14.4	55	19.3	38	13.3	61	21.4	45	15.8
Sausage	9	3.2	15	5.3	32	11.2	39	13.7	29	10.2	84	29.5	77	27
Pastrami	7	2.5	5	1.8	3	1.1	9	3.2	9	3.2	104	36.5	148	51.9

When the dairy products consumption frequency of the students was examined, it was determined that 108 (37.9%) of them were consuming cheese every day, 8 (2.8%) of them were consuming cheese once in 15 days, and 13 (4.6%) of them were not consuming cheese at all.

Moreover, 63 (22.3%) of students were consuming yoghurt every day, 73 (25.8%) were consuming yoghurt 1-2 times a week and 7 (2.5%) of them were not consuming yoghurt at all. While 75 (26.4%) of the students were consuming ayran 1-2 times a week, 4 (1.4%) of them were not consuming ayran at all. While 46 of the students (16.2%) were consuming butter every day, 45 (25.8%) of them were not consuming butter at all.

In a study examining the milk and dairy products consumption habits of Kafkas University students, Çetinkaya (2010) reported that the rate of milk consumption of students was 33%, the rate of those who never consumed milk was 67%, and the rate of choosing dairy products instead of milk was 76%. Çetinkaya (2010) stated that the majority of the students did not have the habit of consuming milk and that more cheese and yoghurt were consumed than dairy products.

Selçuk et al. (2003) investigated the dairy products consumption habits of Yüzüncü Yıl University undergraduate students and determined that the students generally found the prices of dairy products expensive. In another study investigating the milk and dairy products consumption habits of university students, Şahinöz and Özdemir (2017) revealed that 41.7% of the students had a habit of drinking milk, while 30% were not consuming milk at all. In a study by Tarakçı et al. (2003), in a study examining the drinking milk consumption habits of Yüzüncü Yıl University students, it was reported that the students had limited knowledge about the milk they drink.

When the egg and meat consumption frequency of the students was examined, it was seen that the majority of them rarely preferred beef-veal (30.2%) and lamb-mutton (39.6%). While 75 (26.3%) of the participants were consuming chicken 1-2 times a week, 39 (13.7%) of them were consuming it infrequently. While there were 2 students (0.7%) who were consuming turkey and fish every day, 50 (17.5%) of them were not consuming fish at all. The number of participants who were not consuming turkey at all was 206 (72.3%).

The consumption levels of soudjouk, salami, sausage and pastrami of the participants are given in Table 3. Among the participants, 23 (8.1%) of them were consuming soudjouk and salami every day. Thirteen (4.6%) of the participants were not consuming soudjouk and 45 (15.8%) of them were not consuming salami at all. Furthermore, while 84 (29.5%) students were consuming sausage infrequently, 77 (27%) of them were not consuming sausage at all.

When the pastrami consumption levels of the participants are examined, it is seen that 148 (51.9%) of them were not consuming this product at all.

In another study investigating the animal product consumption patterns and habits of Erciyes University Faculty of Veterinary students, Sarıözkan et al (2007) revealed that students were consuming more beef-veal and sausage than red meat and its products. In the same study (Sarıözkan et al., 2007), it was reported that 20% of the students did not have the habit of consuming milk.

In a study investigating the chicken preferences of Çoruh University students, İskender et al. (2015) determined that the weekly average chicken consumption was 1.3 kg and the vast majority (71.8%) were not consuming poultry meat other than chicken. In the same study (İskender et al. (2015), it was revealed that 77.1% of the students preferred village chicken.

In a study on university students by Işkın and Sarıışık (2017), it was reported that students were consuming cheese, chicken and eggs very often, and products such as offal, fish and soudjouk -sausage were being consumed at a minimum level.

On the other hand, Taşkın et al. (2020) determined the meat consumption preferences of students studying at different faculties at Ege University. It was revealed that most of the students participating in the survey (92.4%) were consuming red meat, while 7.6% were not (Taşkın et al., 2020). In the same study, they reported that students preferred more beef (95.2%) in the red meat group.

In a study in which the fish consumption levels of the students of Çine Vocational School were determined, Özuğur et al. (2019) stated that students were consuming fish because it was both delicious and healthy. In the same study, they reported that the students were above the average fish consumption level in Turkey (Özuğur et al., 2019).

In a study, İskender and Kanbay (2014) determined that the majority of students (91.9%) consumed eggs and their weekly egg consumption was 3.4 on average. In the same study, it was also revealed that 81.2% of the students had knowledge about organic eggs.

When the points that students pay attention to in red meat consumption are examined, it is seen that the majority of the participants did not pay attention to taste (72.3%), brand (81.8%), price (65.6%), quality (56.8%), hygiene (64.9%), ease of preparation (93.7%), smell-color (75.4%) and personal health criteria (85.3%). Only a low percentage of the participants took these criteria into account when buying red meat. Besides, 79 (27.7%) students paid attention to the taste, 52 (18.2%) of them to the brand, and 98 (34.4%) of them to the price of meat. While 123 students (43.2%) considered quality in their meat selection, 100 (35.1%) of them considered hygiene. Eighteen students (6.3%) prioritized the ease of preparation in their red meat consumption. While buying red meat, the smell-color criterion was considered as important by 70 students (24.6%), 42 students (14.7%) shaped their meat consumption as a result of personal health criteria (Table 4).

Table 4. Considerations for students while buying red meat

Considerations while buying red meat		
Taste	**N**	**%**
Yes	79	27.7
No	206	72.3
Brand	**N**	**%**
Yes	52	18.2
No	233	81.8
Price	**N**	**%**
Yes	98	34.4
No	187	65.6
Quality	**N**	**%**
Yes	123	43.2
No	162	56.8
Hygiene	**N**	**%**
Yes	100	35.1
No	185	64.9
Ease of preparation	**N**	**%**
Yes	18	6.3
No	267	93.7
Smell-Color	**N**	**%**
Yes	70	24.6
No	215	75.4
Personal health criteria	**N**	**%**
Yes	42	14.7
No	243	85.3

The majority did not pay attention to taste (75.1%), brand (72.3%), price (70.5%), quality (55.8%), hygiene (67%), ease of preparation (93.3%), smell-color (78.2%), and personal health criteria (87.4%) on white meat consumption (Table 5). Moreover, a low percentage of students

paid attention to taste (24.9%), brand (27.7%), price (29.5%), quality (44.2%), hygiene (33%), ease of preparation (6.7%), smell-color (21.8%) and personal health criteria (12.6%) while buying white meat.

Table 5. The points students consider while buying white meat

Considerations when buying white meat		
Taste	**N**	**%**
Yes	71	24.9
No	214	75.1
Brand	**N**	**%**
Yes	79	27.7
No	206	72.3
Price	**N**	**%**
Yes	84	29.5
No	201	70.5
Quality	**N**	**%**
Yes	126	44.2
No	159	55,8
Hygiene	**N**	**%**
Yes	94	33
No	191	67
Ease of preparation	**N**	**%**
Yes	19	6.7
No	266	93.3
Smell-Color	**N**	**%**
Yes	62	21.8
No	223	78.2
Personal health criteria	**N**	**%**
Yes	36	12.6
No	249	87.4

In a survey conducted with Siirt University students, Örük (2021) indicated that the factors that students pay the most attention to when purchasing animal products were hygiene, freshness and product smell, respectively. İskender et al. (2015) reported that as a result of a survey they conducted with Coruh University students, they preferred the expiration date of the product when buying chicken (32.8%), while the brand was in the second place (28.8%). In another study, Taşkın et al. (2020) reported that hygiene (82.6%), freshness (82.3%), hygiene (81.8%) and reliability (72.4%) factors came to the fore in students' red meat purchase.

When the milk consumption preferences of the students are examined, 135 students (47.4%) preferred street milk, 120 students (42.5%) preferred pasteurized milk and 30 students (10.5%) preferred UHT milk (Table 6).

Table 6. Students' milk consumption preferences

Preferences	**N**	**%**
UHT milk	30	10.53
Packaged pasteurized milk	120	42.11
Street milk	135	47.37

4. Conclusion

According to the results obtained from the research, the majority of Suluova Vocational School students found foods of animal origin healthier. Furthermore, there were more students who thought that animal proteins have higher quality. It was observed that most of the students were consuming beef-veal, lamb-mutton, fish, salami and sausage infrequently, while the majority of them were not consuming butter, pastrami and turkey at all. It was revealed that the majority of the students did not consider many criteria (taste, price, brand, quality, hygiene, ease of preparation) when buying white and red meat. Besides, it was observed that most of the students preferred street milk compared to UHT and packaged milk.

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Research Article

New additional aphid (Hemiptera: Aphidoidea: Aphididae) records for Samsun (Turkey) province

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Abstract

The current study aimed to contribute to aphid fauna of Turkey by surveying the Samsun region. As a result of the study on aphids conducted in Samsun province, 78 aphid species were determined as new recods for Samsun province. Of these species, 49 species are in Aphidinae subfamily, one species is in Anoeciinae subfamily, eight species are in Calaphidinae subfamily, eight species are in Chaitophorinae subfamily, one species is in Drepanosiphinae subfamily, one species is in Phyllaphidinae subfamily, six species are in Lachninae subfamily, two species are in Eriosomatinae subfamily and two species is in Thelaxinae subfamily.

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Keywords

Aphid,
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1. Introduction

Aphids are one of the most important sap-sucking and invasive insect group in the world. They can cause damage both through their direct phloem feeding as well as through injecting of toxic saliva compounds and by the build-up of excreted honeydew causing sooty mould and plant viruses transmission (Blackman and Eastop, 2007). The known world fauna of aphids reached about 5100 species, placed in 510 currently accepted genera (Blackman and Eastop, 2021; Favret, 2018). Also, many studies have been conducted in Turkey. The studies on aphids in Turkey that started in early 19th century were collected in the checklist prepared by Görür, 2012. After this checklist, Şenol et al. (2014, 2015a, 2015b, 2017, 2021), Görür et al. (2017, 2020), Özdemir and Barjadze (2015), Akyürek et al. (2019), Kök and Kasap (2019), Özdemir (2020), Blackman and Eastop (2021), Kök and Özdemir (2021), Patlar et al. (2021) have added significant contributions to the the aphid fauna of Turkey. With these contributions, Turkey aphid fauna reached to nearly 605 species.

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Considering Turkey's geographical features and climatic differences, it is expected that Turkey aphid diversity has to be richer than this number, so there is need to be more detailed researches from different areas of Turkey. Since the Central Black Sea Region is a climate transition region, these studies have accelerated in recent years and this study has been carried out with the belief that new aphid species can be discovered in this region.

2. Materials and Methods

This study was carried out in Samsun and its districts. The samples belonging to the family Aphididae were colltected periodically from herbaceous and woody natural plants and cultivated plants in localities which have different vegetation, plant cover, habitat, elevation, slope, topographic charactertistics in the research field. The samples were taken into eppendorf tubes containing 80% ethyl alcohol. During the field work, the natural apperances of the specimens on the host plants were photographed. In addition, information that may help to diagnosis such as host plants, the part of the host plant that specimens are on it and density of the colony were noted. The collected aphid samples were prepared permanently in the laboratoy to the Martin (1983) method. All samples were identified according to Blackman and Eastop, 2021. Taxonomic status and host plants were verified by checking, Holman, 2009 and Farvet, 2018. Voucher specimens have been deposited at the Entomology Laboratory of Biology Department of Ondokuz Mayis University.

3. Results and Discussion

As a result of the examination of all the prepared samples under the microscope, a total of 141 species belonging to 10 subfamilies, nine tribes, two subtribes and 47 genera of the familiy Aphididae were identified in Samsun. Of these species, 78 species were determined as new records for Samsun province. These are:

Family: Aphididae

Subfamily: Aphidinae

Tribe: Aphidini

Genus: Aphis

***Aphis affinis* del Guercio, 1911:** Collected from stem of *Mentha* sp. in Ayvacık, Yukarıyenice, 22.07.2010.

***Aphis brotericola* Mier Durante, 1978:** Collected from shoot annd flowers of *Euphorbia*

sp. in Asarcık, Aydın village, 04.07.2009.

***Aphis brunellae* Schouteden, 1903:** Collected from flowers of *Prunella* sp. in Terme, Sakarlı, 10.07.2009.

***Aphis chloris* Koch, 1854:** Collected from flowers of *Hypericum* sp. in Asarcık, Aydın village, 04.07.2009 and Yakakent, 05.07.2009.

***Aphis craccae* Linnaeus, 1758:** Collected from leaves on shoot of *Vicia cracca* in Tekkeköy, 15.05.2010 and from flowers of *Vicia cracca* and *Vicia sativa* in Atakum, Mimar Sinan N., 19.05.2010.

***Aphis cytisorum* Hartig, 1841:** Collected from stem of *Coronilla varia* and flowers of *Sophora* sp. in Ladik Lake, 03.07.2009, and from stem of *Spartium* sp. in Yakakent, 05.07.2009.

***Aphis fabae* Scopoli, 1763:** Collected from stem and leaves of *Papaver* sp. in Kurupelit Campus Area, 18.05.2009; from stem of *Petroselinum crispum* in Atakum, Denizevleri N., 19.05.2009; from stem of *Sium* sp. in Atakum, Mimar Sinan N, 25.05.2009; from stem of *Matricaria* sp. in Vezirköprü, Köprübaşı, from flowers of *Helianthus* sp. in Havza-Vezirköprü Road, from shoot and stem of *Chenopodium* sp. and stem of *Echium* sp. in Vezirköprü Center, from under the leaves and stem of *Phaseolus* sp. in Vezirköprü, Yağanözü, 02.07.2009; from stem of *Centaurea* sp. and *Eryngium creticum* in Ladik- Amasya Kılıçarslan Pass, from flowers and stem of *Pimpinella saxifraga* in Ladik Lake, 03.07.2009; from flowers and stem of *Pimpinella saxifraga* in Asarcık, Aydın village, 04.07.2009; from under the leaves of *Clematis* sp., branch of *Cichorium* sp., stem of *Chenopodium* sp. and corncob of *Zea mays* in Bafra, Kolay and from shoot of *Lysimachia vulgaris* in Bafra, İkizpınar village, 06.07.2009; from flowers and stem of *Pimpinella* sp. in Salıpazarı, Kocalar village, 09.07.2009; from flowers of *Pimpinella* sp. in Terme, Sakarlı, 10.07.2009; from stem of *Cichorium* sp. in Ladik, Aşağıgölyazı, 30.08.2009; from shoot and stem of *Digitalis* sp. in Vezirköprü, Kunduz Forest, 11.10.2009; from under the leaves and shoot of *Hedera helix* in Atakum, 19.12.2009; from under the leaves of *Rumex* sp. in Atakum, 08.02.2010; from shoot of *Hedera helix* in Atakum, Liman N, 20.03.2010; from shoot of *Chenopodium* sp. in Atakum, Mimar Sinan N., 26.03.2010; from stem of *Rumex* sp. in Çarşamba, Ağacabey, from stem of *Ranunculus* sp. in Çarşamba, Acıklı village, from stem of *Silene* sp. in Tekkeköy, Büyüklü village, 15.05.2010; from flowers and leaves of *Trifolium repens* in Atakum, Balaç village, 19.05.2010; from shoot of *Conium maculatum* in Atakum, Balaç village, 22.05.2010; from branch of *Phaseolus* sp. in Terme, Gündoğdu village, 23.05.2010; from stem of *Papaver* sp. in Kavak, Demirci village, 26.05.2010; from shoot and flowers of *Genista* sp. in Yakakent Center, from stem of *Vicia*

faba in Yakakent, Akgüney village, 28.05.2010; from stem of *Zea mays* in Asarcık, Musaağa and Midilli, 12.07.2010.

***Aphis genistae,* Scopoli, 1763:** Collected from stem of *Genista* sp. in Ladik, İbi village, 21.07.2010.

***Aphis hederae* Kaltenbach, 1843:** Collected from shoot and stem of *Hedera helix* in Çarşamba, Çınarlık, 15.05.2010.

***Aphis hillerislambersi* Nieto Nafria & Mier Durante, 1976:** Collected from under and over the leaves of *Euphorbia* sp. in Bafra, Doğanca, 28.08.2009; from under the leaves of *Euphorbia* sp. in Vezirköprü, Kunduz Forest, 11.10.2009; from stem of *Euphorbia* sp. in Çarşamba, Acıklı village, 15.05.2010; from flowers of *Euphorbia* sp. in Atakum, Balaç village, 22.05.2010.

***Aphis intybi* Koch, 1855:** Collected from stem of *Cichorium* sp. in Atakum, Balaç village, 19.05.2010.

***Aphis punicae* Passerini, 1863:** Collected from branch, under and over the leaves of *Punica granatum*, in Atakum, Alparslan Boulevard, 21.05.2010.

***Aphis sambuci* Linnaeus, 1758:** Collected from stem of *Sambucus ebulus* in Çarşamba, Acıklı village, 15.05.2010; from stem of *Sambucus ebulus* in Ayvacık, Yukarıyenice, 22.07.2010.

***Aphis sanguisorbae* Schrank, 1801:** Collected from flowers and stem of *Sanguisorba minor* in Ladik, Çerkezler N., 03.07.2009.

***Aphis solanella* Theobald, 1914:** Collected from stem of *Centaurea iberica* in Ladik-Amasya, Kılıçarslan Pass, 03.07.2009; from stem of *Centaurea* sp. in Asarcık, Aydın village, 04.07.2009; from under the leaves of *Solanum nigrum* in Terme, Sakarlı and from shoot of *Solanum* sp. in Terme, Hüseyinmescit, 10.07.2009; from stem and shoot of *Solanum* sp. in Ladik, Aşağıgölyazı, 30.08.2009; from stem of *Papaver* sp. in Atakum, Mimar Sinan N., 19.05.2010; from stem of *Urtica* sp. in Atakum, Türkiş, 21.05.2010.

***Aphis thomasi* (Börner, 1950):** Collected from flowers of *Scabiosa* sp. in Ayvacık, Döngel village, 24.05.2010.

***Aphis urticata* J. F. Gmelin, 1790:** Collected from shoot of *Urtica* sp. in Çarşamba, Acıklı village, 15.05.2010; from stem of *Urtica* sp. in Atakum, Balaç village, 22.05.2010.

***Aphis verbasci* Schrank, 1801:** Collected from flowers of *Verbascum* sp. in Terme, Gölardı, 23.05.2010.

Subtribe: Rhopalosiphina

Genus: *Hyalopterus* Koch, 1854

***Hyalopterus pruni* (Geoffroy, 1762):** Collected from curled leaves on shoot of *Prunus* sp. in Alaçam, Etyemez, 05.07.2009, in Atakum, Balaç village, 19.05.2010; from under the leaves of *Prunus* sp. in Atakum, Alparslan Boulevard, 21.05.2010; from shoot of *Prunus* sp. in Ayvacık, Center, 24.05.2010; from under the leaves of *Prunus persica* in Yakakent, Center, 28.05.2010.

Genus: *Rhopalosiphum* Koch, 1854

***Rhopalosiphum maidis* (Fitch, 1856):** Collected from under the leaves of a member of Greminea in Alaçam, Karlı village, 28.08.2010.

Genus: *Schizaphis* Börner, 1931

***Schizaphis* (*Schizaphis*) *pyri* Shaposhnikov, 1952:** Collected from under the leaves of *Pyrus* sp. in Alaçam, Etyemez, 05.07.2009.

***Schizaphis rotundiventris* (Signoret, 1860):** Collected from flowers of *Palmae* sp. in Ayvacık, Center, 24.05.2010.

Genus: *Melanaphis* van der Goot, 1917

***Melanaphis pyraria* ((Passerini, 1861):** Collected from shoot of *Pyrus* sp. in Yakakent, Center, 28.05.2010.

Tribe: Macrosiphini

Genus: *Acytrhosiphon* Mordvilko, 1914

***Acyrthosiphon* (*Acyrthosiphon*) *malvae* (Mosley, 1841):** Collected from shoot of *Solanum* sp. in Bafra, 06.07.2009; from shoot of *Solanum* sp. in Çarşamba, Kayadibi village, 09.07.2009.

***Acyrthosiphon* (*Acyrthosiphon*) *pisum* (Haris, 1776):** Collected from shoot of *Vicia* sp. in Bafra, Altınkaya Barrage, 12.05.2009; from leaves of *Pisum sativum* in Atakum, Mimar Sinan N., 21.05.2010; from stem of *Trifolium resupinatum* and stem of *Melilotus* sp. in Atakum, Balaç village, 22.05.2010; from flowers and stem of *Galega* sp. in Salıpazarı, Alanköy, 23.05.2010.

Genus: *Aphidura* Hille Ris Lambers, 1956

***Aphidura picta* Hille Ris Lambers, 1956:** Collected from flowers of *Silene dichotoma* in Ladik, Merkez, 26.05.2010.

Genus: *Brachycaudus* van der Goot, 1913

***Brachycaudus* (*Brachycaudus*) *helichyrsi* (Kaltenbach, 1843):** Collected from flowers of *Anthemis* sp. in Bafra, Altınkaya Barrage, 12.05.2009; from stem of *Matricaria chamomila* in Atakum, Mimar Sinan N., 24.05.2009; from shoot of *Prunus* sp. in Ladik, Çerkezler N., 03.07.2009; from flowers and shoot of *Cynoglossum* sp. and under of leaves and inflorescenses of *Calendula arvensis* in Atakum, Mimar Sinan N., and from curled leaves on shoot of *Prunus* sp. in Atakum, Balaç village, 19.05.2010; from flowers of *Cynoglossum* sp. in Terme, Altunlu village, 23.05.2010; from leaves of *Pallenis spinosa* in Yakakent, 28.05.2010.

***Brachycaudus lateralis* (Walker, 1848):** Collected from stem of *Cirsium* sp. in Asarcık, Aydın village, 04.07.2009.

***Brachycaudus* (*Acaudus*) *lychnidis* (Linnaeus, 1758):** Collected from stem of *Silene* sp. in Kurupelit Campus Area, 25.05.2009; from stem of *Silene* sp. in Bafra, Darboğaz village, 06.07.2009; from stem of *Silene* sp. in Atakum, Mevlana N., 19.05.2010.

Genus: *Capitophorus* van der Goot, 1913

***Capitophorus elaeagni* (del Guercio, 1894):** Collected from shoot of *Elaeagnus* sp. in Atakum, Balaç village, 19.05.2010.

***Capitophorus hippophaes* (Walker, 1852):** Collected from under of leaves of *Polygonium* sp. in Bafra, Altınova village, 06.07.2009; from leaves of *Elaeagnus* sp. in Canik, Doğupark, 24.05.2010.

Genus: *Dysaphis* Börner, 1931

***Dysaphis* (*Dysaphis*) *foeniculus* (Theobald, 1923):** Collected from stem of *Eryngium creticum* in Alaçam, Esentepe N., and under the leaves of *Malus* sp. in Yakakent Center, 05.07.2009.

***Dysaphis* (*Pomaphis*) *plantaginea* (Passerini, 1860):** Collected from shoot of *Malus* sp. in Terme, Sakarlı, 10.07.2009; from under the leaves of *Sorbus torminalis* in Vezirköprü, Kunduz Forest, 11.10.2009; from curled leaves of *Malus* sp. in Ladik, 26.05.2010.

Genus: *Hyperomyzus* Börner, 1933

***Hyperomyzus lactucae* (Linnaeus, 1758):** Collected from stem of *Picris* sp. in Samsun Center, Başalan village, 04.07.2009; from shoot and branch of *Sonchus* sp. in Atakum, Mimar Sinan N., and stem and shoot of *Cirsium* sp. in Atakum, Balaç village, 19.05.2010.

Genus: *Macrosiphoniella* Del Guercino, 1911

***Macrosiphoniella sanborni* (Gillette, 1908):** Collected from shoot of *Artemisia vulgaris* in Çarşamba and Terme, 15.05.2010; from stem of *Chrysanthemum* sp. in Terme, Karamahmut village, 23.05.2010.

Genus: *Megoura* Buckton, 1876

***Megoura viciae* Buckton, 1876:** Collected from fruit of *Lathyrus* sp. in Atakum, Mimar Sinan N., 19.052.2010.

Genus: *Myzus* Passerini, 1860

***Myzus* (*Nectarosiphon*) *ascalonicus* Doncaster, 1846:** Collected from under the leaves of *Salvia* sp. in Samsun Center, 02.10.2009.

***Myzus* (*Myzus*) *lythri* (Schrank, 1801):** Collected from stem and flowers of *Lythrum salicaria* in Bafra, Kolay, 06.07.2009, in Ladik Lake, 30.08.2009, in Asarcık, Şeyhli, 12.07.2010 and in Salıpazarı, Bereket N., 24.07.2010.

***Myzus ornatus* Laing, 1932:** Collected from branch of *Punica* sp. in Ayvacık Center, 24.05.2010; from over the leaves of *Rhododendron luteum* in Asarcık, 25.05.2010.

***Myzus* (*Myzus*) *varians* Davidson, 1912:** Collected from curled leaves of *Prunus persica* in Atakum, Mimar Sinan N., 04.07.2009; from leaves of *Clematis* sp. in Kavak, Kaya village, 02.10.2009.

Genus: *Nasonovia* Mordvilko, 1914

***Nasonovia ribisnigri* (Mosley, 1841):** Collected from leaves of *Lactuca* sp. in Bafra, 06.04.2010; from under and over the leaves of *Lactuca* sp. in Yakakent, Güvenli village, 28.05.2010.

Genus: *Ovatus* van der Goot, 1913

***Ovatus crateagarius* (Walker, 1850):** Collected from shoot and under the leaves of *Cydonia* sp. in Terme, Karamahmut village, 23.05.2010.

Genus: *Phorodon* Passerini, 1860

***Phorodon humuli* (Schrank, 1801):** Collected from shoot and under the leaves of *Prunus* sp. in Çarşamba, Acıklı village and Manamut village, 15.05.2010, in Atakum, Mevlana N., 21.05.2010, in Atakum, Balaç village, 22.05.2010, in Salıpazarı, Yenidoğan village and Yavaşbey village, 23.05.2010, in Canik, Doğupark, 24.05.2010, in Asarcık, Gökköy, 25.05.2010, in Havza Center-Karageçmiş village, Vezirköprü-Yukarınarlı village and Kavak-Çayırlı, 26.05.2010, in Yakakent, Asmapınar village and Akgüney village, 28.05.2010, in Ladik, Hamamayağı, 12.07.2010.

Genus: *Sitobion* Mordvilko, 1914

***Sitobion fragariae* (Walker, 1848):** Collected from ear of *Avena* sp. and *Cynosourus* sp. in Tekkeköy, Ant yeri, from ear of *Bromus* sp. and *Hordeum* sp. in Tekkeköy, Büyüklü village, 15.05.2010; from ear of *Cynosourus* sp., *Bromus* sp., *Hordeum* sp. and *Phleum* sp. in Atakum, Mimar Sinan N. and ear of *Avena* sp. in Atakum, Mevlana N., 19.05.2010.

Genus: *Uroleucon* Mordvilko, 1914

***Uroleucon* (*Uromelan*) *aeneum* (Hille Ris Lambers, 1939):** Colected from stem and under the leaves of *Carduus* sp. in Atakum, Mimar Sinan N., 04.05.2009, in Bafra, Derbent Barrage, 12.05.2009; from stem of *Cirsium* sp. in Tekkeköy, Ant yeri, 15.05.2010, in Ayvacık, Hasan Uğurlu Barrage, 24.05.2010.

***Uroleucon ambrosiae* (Thomas, 1878):** Collected from under the leaves of *Eriobotrya* sp. in Atakum, Alparslan Boulevard, 21.05.2010; from flowers of *Butomus umbellatus* in Ladik Lake, 21.07.2010.

***Uroleucon* (*Uromelan*) *jaceae* (Linnaeus, 1758):** Collected from stem of *Centaurea solstitialis* in Havza, Güvenbeli Pass, 02.07.2009.

***Uroleucon* (*Uroleucon*) *picridis* (Fabricius, 1775):** Collected from stem and shoot of *Crepis foetial foetidia* in Alaçam, Karlı village, 28.08,2009; from stem of *Picris* sp. in Ladik Lake, 21.07.2010.

***Uroleucon scorzanerae* Danielsson, 1974:** Collected from stem of *Scorzanerae* sp. in Asarcık, Kadirli, 12.07.2010.

Subfamily: Anoeciinae

Genus: Anoecia Koch, 1857

***Anoecia* (*Anoecia*) *corni* (Fabricious, 1775):** Collected from under the leaves of *Cornus mas* in Kavak, Kaya village, 02.10.2009.

Subfamily: Calaphidinae

Tribe: Calaphidini

Genus: *Neobetulaphis* A. N. Basu, 1964

***Neobetulaphis pusilla* A. N. Basu, 1964:** Collected from under teh leaves of *Alnus* sp. in Samsun Center, Gökçepınar village, 02.10.2009, in Terme, Gölardı, 23.05.2010.

Tribe: Panaphidini

Genus: *Pterocallis* Passerini, 1860

***Pterocallis alni* (De Geer, 1773):** Collected from under the leaves of *Alnus* sp. in Terme - Şüayip village, in Salıpazarı - Yenidoğan village and Terme - Gölardı, 23.05.2010.

Genus: *Chromaphis* Walker, 1870

***Chromaphis juglandicola* (Kaltenbach, 1843):** Collected from under the leaves of *Juglans* sp. in 19 Mayıs, Yeşilköy, 05.09.2009, in Terme, Karamahmut village and Salıpazarı,

Yenidoğan village, 23.05.2010, in Çarşamba, Sefalı, 24.05.2010, in Asarcık, Gökköy, 25.05.2010 and in Havza, Dereköy, 26.05.2010.

Genus: *Eucallipterus* Schouteden, 1906

***Eucallipterus tiliae* (Linnaeus,1758):** Collected from leaves of *Tilia* sp. in Samsun Center, Çiftlik, 06.09.2009, in Asarcık, Şeyhli, 02.10.2009, in Vezirköprü, Güvenbeli Pass, 11.10.2009, in Atakum, Mevlana N., 19.05.2010, in Asarcık, Aydın village, 25.05.2010 and in Havza, Sofular village, 26.05.2010.

Genus: *Myzocallis* Passerini, 1860

***Myzocallis carpini* (Koch, 1855):** Collected from leaves of *Carpinus* sp. in Tekkeköy, Çimenli village, 15.05.2010, in Atakum, Balaç village, 22.05.2010, in Salıpazarı, Alanköy, 23.05.2010, in Asarcık, 25.05.2010, in Vezirköprü, Kunduz Forest, 26.05.2010 and Yakakent, Güvenli village, 28.05.2010.

***Myzocallis* (*Myzocallis*) *coryli* (Goeze, 1778):** Collected from leaves of *Corylus* sp. in Taflan, 21.05.2009, in Çarşamba, Ağacabey, 15.05.2010, in Atakum, Alparslan Boulevard, 21.05.2010, in Salıpazarı, Tikencik village, 23.05.2010, in Çarşamba, Sefalı, 24.05.2010 and Havza, Yenice village, 26.05.2010.

***Myzocallis* (*Myzocallis*) *glandulosa* Hille Ris Lambers, 1948:** Collected from leaves of *Quercus* sp. in Kurupelit Campus Area, 25.11.2009.

Genus: *Tinocallis* Matsumura, 1919

***Tinocallis takachihonensis* Haguchi, 1972:** Collected from under the leaves of *Ulmus* sp. in Terme, Beşikli village, 23.05.2010.

Subfamily: Chaitophorinae

Tribe: Chaitophorini

Genus: *Chaitophorus* Koch, 1854

***Chaitophorus euphraticus* Hodjat, 1981:** Collected from over the leaves and fruit of *Populus nigra* in Atakum, Türkiş, 21.05.2010.

***Chaitophorus longisetosus* Szelegiewicz, 1959:** Collected from over and under the leaves of *Populus* sp. in Vezirköprü, Kunduz Forest, 11.10.2009, in Ayvacık Center, 24.05.2010, in Kavak, Ayvalı village, 26.05.2010, in Alaçam Center, 28.05.2010; from shoot if *Populus tremula* in Ladik, İbi village, 21.07.2010.

***Chaitophorus melanosiphon* Pintera, 1789:** Collected from over and under theleaves of *Populus* sp. in Alaçam, Etyemez village, 05.07.2009, in Alaçam, Karlı village, 28.08.2009, in Kavak, Kaya village, 02.10.2009, in Atakum, Balaç village, 22.05.2010.

***Chaitophorus populeti* (Panzer, 1804):** Collected from shoot of *Populus* sp. in Ladik, 03.07.2009.

***Chaitophorus populialbae* (Boyer de Fonscolombe, 1841):** Collected from under the leaves of *Populus* sp. in Çarşamba and Terme, 15.05.2010; from under and over the leaves and petiole of *Populus* sp. in Atakum, Balaç village, 22.05.2010.

***Chaitophorus saliciniger* (Knowlton, 1927):** Collected from shoot and branch of *Salix* sp. in Ladik, Aşağıgölyazı, 30.08.2009.

Genus: *Periphyllus* van der Hoeven, 1863

***Periphyllus aceris* (Linnaeus, 1758):** Collected from under the leaves of *Acer* sp. in Salıpazarı, Kocalar village, 09.07.2009; from under and over the leaves and fruit of *Acer campestre* in Asarcık, Armutlu, 25.05.2010, in Vezirköprü, Kunduz Forest and Ladik, Akdağ 26.05.2010, in Yakakent, Karlı village, 28.05.2010.

***Periphyllus testudinaceus* (Fernie, 1852):** Collected from under the leaves and fruit of *Acer campestre* in Ladik, Akdağ, 26.05.2010 and in Ladik, Hamamayağı, 12.07.2010.

Subfamily: Drepanosiphinae

Genus: *Drepanosiphum* Koch, 1855

***Drepanosiphum oregonensis* Granovsky, 1939:** Collected from under the leaves of *Acer* sp. in Ladik, Hamamayağı, 12.07.2010.

Subfamily: Phyllaphidinae

Genus: *Phyllaphis* Koch, 1856

***Phyllaphis fagi* (Linnaceus, 1758):** Collected from under the leaves and petiole of *Fagus* sp. in Asarcık, Aydın village and Gökköy, 25.05.2010, in Salıpazarı, Yavaşbey village, 23.05.2010, in Vezirköprü, Kunduz Forest and Ladik, Akdağ, 26.05.2010, in Terme, Mescitli

village, 24.07.2010.

Subfamily: Lachninae

Tribe: Eulachnini

Genus: *Eulachnus* del Guercio, 1909

***Eulachnus agilis* (Kaltenbach, 1843):** Collected from leaves in neddle from of *Pinus sylvestris* in Vezirköprü, Kunduz Forest, 26.05.2010.

Genus: *Cinara* Curtis, 1835

***Cinara* (*Cinara*) *brauni* Börner, 1940:** Collected from branch of *Pinus sylvestris* in Kavak, Mahmutlu village, 11.10.2009, in Asarcık, Aydın village, 02.10.2010.

***Cinara* (*Cupressobium*) *cupressi* (Buckton, 1881):** Collected from branch of *Cupressus* sp. in 19 Mayıs, Yeşilköy, 05.09.2009; from branch of *Thuja* sp. in Atakum, Balaç village, 19.05.2010 and in Atakum, Mevlana N., 21.05.2010.

***Cinara* (*Cupressobium*) *juniperi* (de Geer, 1773):** Collected from branch of *Juniperus communis* in Vezirköprü, Kületek village, 02.07.2009; from branch of *Juniperus oxycedrus* in Vezirköprü, Kunduz Forest, 26.05.2010.

***Cinara pilicornis* (Hartig, 1841):** Collected from branch of *Picea* sp. in Atakum, Mevlana N., 19.05.2010 and in Canik, Doğupark, 24.05.2010.

Genus: *Schizolachnus* Mordvilko, 1909

***Schizolachnus pineti* (Fabricius, 1781):** Collected from leaves in needle form of *Pinus* sp. in Ladik, Aşağıgölyazı, 30.08.2009, in Kavak, Mahmutlu village and Vezirköprü, Kunduz Forest, 11.10.2009.

Subfamily: Eriosomatinae

Tribe: Eriosomatini

Genus: *Eriosoma* Leach, 1818

***Eriosoma lanigerum* (Hausmann, 1802):** Collected from stem and braches of *Malus* sp. in Asarcık, Aydın village, 02.10.2009, in Atakum, Mevlana N., 19.05.2010, Atakum, Türkiş, 21.05.2010, in Salıpazarı, Yavaşbey village, 23.05.2010, in Kavak, Ayvalı village, 26.05.2010

and in Yakakent Center and Gülkaya village, 28.05.2010.

Genus: *Tetraneura* Hartig, 1841

***Tetraneura ulmi* (Linnaeus, 1758):** Collected from galls on the leaves of *Ulmus* sp. in Salıpazarı, Alanköy, 23.05.2010.

Subfamily: Thelaxinae

Genus: *Thelaxes* Westwood, 1840

***Thelaxes californica* (Davidson, 1919):** Collected from over the nutgall of *Quercus* sp. in Kavak, Mahmutbeyli, 02.10.2009.

***Thelaxes suberi* (del Guercio, 1911):** Collected from over the gall and shoot of *Quercus petrea* and *Quercus cerris* in Asarcık, Gökköy, 04.07.2009, in Ladik, Ahmetsaray village, 21.07.2010.

Until this study was carried out, it was known that 80 species belonging to the Aphididae family were distributed in Samsun according to the literature (Çanakçıoğlu, 1966, 1972, 1975; Çanakçıoğlu and Eastop, 1972; Tuatay, 1988, 1990, 1991, 1993, 1999; Tuatay and Remaudiere, 1964; Tuatay et al. 1967; Akyürek et al., 2011, 2019). When the results of these studies and literature information are evaluated together, it is revealed that the number of aphid species in Samsun is 158.

Because the species belonging to the family Aphididae cause serious damage to many cultivated plants and cause great economic losses in agricultural products, studies on the Aphididae family have increased rapidly in the world in recent years. In most of the studies on aphid species in Turkey, new species have been described, and many new records have been determined to the literature on the basis of provinces and regions. The introduction of many new species records into the literature, even in recent studies in Samsun, has shown that aphid fauna has not yet been fully determined for Turkey. For this reason, in order to determine the aphid fauna of Turkey exactly, it has become necessary to conduct recearches in areas where no studies have been conducted yet. Also, It should not be overlooked that every new species that will enter the literature will play a key role in the fight against these species. The results of studies on aphids will contribute to future studies on Turkey's biodiversity and Aphididae

family and will guide researchers.

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Review Article

Effects of antioxidant use on semen storage in honey bees

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Abstract

Although there are many studies for the storage of semen in honey bees, the desired success has not been achieved, and more study is needed in this area. It has been reported that the percentage of bee egg-laying in queen bees fertilized with stored semen, especially in long-term storage conditions is below the expected rate despite the viability rate. The reason for this situation is that honey bee spermatozoa are negatively affected by freezing processes due to their very fragile and sensitive structure. However, due to natural mating, honey bee semen can remain healthy for years in the queen bee's spermatheca. It is known that there are many enzymatic antioxidants and special proteins in the spermatheca, as well as a suitable environment for spermatozoa. Manipulating made during the stored of honey bee semen has adverse effects on spermatozoon function and fertility. In particular, current antioxidant substances against cold shock, which are among the damages that occur during freezing of semen, are being investigated, and their protective effects on semen are determined. For this purpose, it is aimed to improve the storage conditions of honey bee semen by using substances with antioxidant properties. The purpose of this review is to give information about the use of antioxidant substances in the storage of honey bee semen.

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Keywords

Antioxidant,
Cryopreservation,
Honey bee,
Semen

1. Introduction

Free radicals include reactive oxygen species and reactive nitrogen compounds. They are essential for important physiological activities in the body. To achieve this, the harmony between free radicals and antioxidants must not be disturbed. In case this situation deteriorates in favor of free radicals, negative effects due to oxidative stress may develop (Lobo et al., 2010).

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Free radicals arise as a result of metabolic byproducts and activities such as phagocytosis. They contain unpaired electrons and are unstable. In aerobic respiration cells, they occur as hydroxyl radicals, superoxide radicals, hydrogen peroxide, and transition metals. It is known that when reactive oxygen species (ROS) originating from free radicals are within the required limits, they involve in a series of metabolic events required for spermatozoa to gain fertilization ability such as activation, capacitation, and oocyte fusion, which occurs when encountering the ovum. However, harmful effects are seen when ROS is produced more than necessary. Besides causing some differences in the membrane structure of the spermatozoa, ROS also cause DNA damage in the spermatozoon. Moreover, low motility may cause impaired oocyte penetration ability and decrease the fertilization ability of spermatozoa. (Duru et al., 2000; Tremellen, 2008). The decrease in the antioxidant capacity of the cell, the disruption of the enzyme activities necessary for the cells to receive oxygen and the ion transition in the cell wall, the increase in metabolic activities in the cell, the occurrence of inflammatory conditions in the cell, and the presence of negativities such as radiation cause cell damage by ROS compounds. The cold shock that is seen during the cryopreservation of semen also increases the formation of ROS. Hence, it has been stated that antioxidant substances added to semen during semen storage have protective effects against ROS (Petruska et al., 2014).

2. Storage of Honey Bee Semen and Oxidative Stress

Queen honey bees can retain spermatozoa in their spermatheca for years after mating with drones. In this process, it was stated that antioxidants in the body of the queen honey bee have protective effects against the harmful effects caused by the interaction of ROS (Collins et al,, 2004). In particular, it was emphasized that antioxidant functions developed in the spermatheca of the mating queen bee in the order Hymenoptera. Furthermore, after becoming an adult, fertilized queen bees have the ability to store spermatozoa in their spermatheca throughout their lives (Gotoh et al., 2017). Honeybee semen contains antioxidative enzymes and proteins that regulate the functions of enzymes and balance various metabolic activities (Zareie et al., 2013). ROS production occurs due to the negative effects (such as pollen, nectar and oxidation of various chemical agents) that honey bees are exposed to outside the colony. The antioxidant level in the cell also changes according to the level and content of the harmful effect that causes the formation of ROS (Korayem et al., 2012). Besides, it was stated that there are antioxidative defense systems against free radicals in semen (Storey, 1997). However, all this internal balance

can be disrupted by various external negative and pathological effects. For this reason, the importance of using synthetic antioxidants from outside was emphasized (Lobo et al., 2010).

As in mammals, various freezing methods are used for the long-term storage of semen in honey bees. For this purpose, the positive effects of the use of antioxidants added to different diluents were reported (Taylor et al., 2009). When the honey bee spermatozoa are examined morphologically, it is seen that the proportional length between the head and the flagellum is quite high compared to the spermatozoa structure of mammals (Gontarz et al., 2016). In addition, it was reported that honey bee semen is negatively affected by environmental factors due to its high density and tendency to aggregate (Cobey et al., 2013). It has been reported that applications such as cold shock, which occur during the cryopreservation of semen, different diluent contents, cryoprotectant substances, and centrifugation adversely affect honey bee spermatozoa (Taylor et al., 2009; Paillard et al., 2017). It was reported that the levels of catalase, glutathione peroxidase, superoxide dismutase and malondialdehyde (MDA), which is the end product of lipid peroxidation increase in spermatozoa when exposed to chemical agents that cause oxidative stress in honey bees (Abdelkader et al., 2019). Furthermore, it was observed that there was a decrease in semen density and protein amount in drones due to stress-related reasons. On the other hand, it was stated that there was an increase in the level of superoxide dismutase (Abdelkader et al., 2014).

Queen honey bees are exposed to the effects of ROS due to the fact that the spermatozoa in the spermatheca need the oxygen necessary for respiration. The negative effects of this situation can be minimized by various antioxidative enzymes. It was observed that the levels of catalase and glutathione transferase in mated queen bees were 10 times higher than in unmated queen bees (Collins et al., 2004). It was determined that the addition of some antioxidant and protective substances against the harmful effects of oxidative stress in honey bees is effective in the protection of honeybee semen (Taylor et al., 2009). Balieira et al. (2018) reported that caffeine in honey bees caused a partial decrease in MDA level due to its antioxidant property against oxidative stress caused by the effect of imidacloprid. In a study conducted to determine the effects of soy lecithin and egg yolk added to honeybee semen diluent on semen storage, Dadkhah et al. (2016) reported that there was a positive effect on semen motility and viability in the study groups where different doses of egg yolk and soy lecithin were added compared to the control group. Besides, Almeda and Espencer (2002) stated that coconut water added to the semen diluent under short-term storage conditions is effective on the storage of semen. In the

study investigating the efficacy of trehalose added to the Kiev solution, which is a semen diluent, it was reported that there was an increase in the short-term retention time and semen motility values in honeybee semen (Yániz, et al., 2019). It was also reported that trehalose had a protective effect against damage to the spermatozoon plasma membrane (Ahmad and Aksoy, 2012). In a study investigating the effects of TL HEPES-based diluent with BSA added to the semen diluent at different rates in honey bees, it was reported that the diluent had a positive effect on semen motility, spermatozoa plasma membrane integrity and acrosome integrity. In addition, it was demonstrated that with the increase in the ratio of the substance used in the study, there was a positive increase in spermatological parameters (Alçay et al., 2019a).

3. Antioxidant Substances Used in Studies on Honey Bees

Over the past 40 years, various extenders and antioxidants have been used to improve honey bee spermatologic parameters in the cooled or cryopreserved. Because of temperature changes, cold shock, and ice formation, cryopreservation techniques damage spermatozoa. The sperm quality characteristics of spermatozoa are reduced as a result of these consequences. Furthermore, during the cryopreservation process, ROS are produced as a result of lipid peroxidation in the cytoplasmic membrane. The sperm membrane is stressed in various ways by the free radicals that arise. Antioxidants have been added to honey bee semen extenders and nutrients in recent years to prevent lipid peroxidation and induce spermatologic quality parameters (Wegener and Bienefeld, 2012). Antioxidant substances used in studies on honey bees are shown in Table 1.

Table 1. Antioxidant substances used in studies on honey bees

Spermatological Parameters	Antioxidant Substances	Effects	References
Semen Paremeters (motility, viability)	Catalase	Insignificant	(Taylor et al., 2009)
Sperm Concentration	Pollen	Insignificant	(Rousseau and Giovenezzo, 2016)
Sperm Motility	Soybean Lecithin	Beneficial effects	(Dadkhah et al., 2016)
Semen Parameters (motility, plasma membrane and, acrosomal integrity)	Bovive Serum Albümine (BSA)	Beneficial effects	(Alcay et al., 2019a)
Semen Parameters (motility, plasma membrane and, acrosomal integrity)	Royal Jelly	Beneficial effects	(Alcay et al., 2019b)
Semen Parameters (motility, plasma membrane, integrity, mitocondrial function)	L-Carnitine	Beneficial effects	Alcay et al., 2021)

5. Conclusion

As in mammals, the use of antioxidant substances in studies related to the storage of honey bee semen is an important factor that increases the success of semen freezing. When the structure of honey bee spermatozoa is evaluated morphologically, it is quite fragile and sensitive to environmental factors compared to mammalian spermatozoa. Many factors cause oxidative stress, especially the cold shock that occurs during the freezing and storage of semen, the centrifugation process applied to the semen, and the negative effects of different diluents on spermatozoa. Moreover, the stress environment that drones are exposed to outside the colony, contamination of nectar and pollen sources in the region with harmful external factors such as pesticides or carbon monoxide, spraying of colonies against parasites such as varroa destructor and similar conditions negatively affect semen quality. Although the enzymatic antioxidants and some proteins in honey bee semen help reduce the oxidative effects, they cannot eliminate all the damage that occurs depending on the content and duration of the damage. In this case, additional antioxidant substances are needed from the outside, especially for the storage of honey bee semen. For this purpose, various antioxidants or substances that strengthen the antioxidant effect are added to the nutrients or semen diluent in honey bees. As a result, it is

seen that antioxidant substances have important protective effects on the storage conditions of honeybee semen and spermatological parameters after solution. Studies on the storage of semen in honey bees should be increased. In studies to be conducted in this area, the activities of antioxidant substances are key in improving the storage conditions of honeybee semen.

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Review Article

miRNAs as biomarkers in human diseases

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Abstract

RNA interference (RNAi) is one of the primary machineries involved in the regulation of gene expression using small double-stranded RNA (dsRNA) in eukaryotic cells. MicroRNA (miRNA) is a class of small non-coding RNAs, regulating gene expression through canonical and non-canonical ways. Previous studies have shown that miRNA coding sequences make up 1% of the human genome and currently 1917 human miRNAs are displayed in the miRBase database. Expression levels of circulating miRNAs are related to various pathophysiological conditions such as cancer, infectious conditions, cardiovascular diseases, neurodegenerative diseases, and many more. Therefore, it is important to identify, detect and analyse miRNAs by using *in silico* and experimental analyses. In this review, after a brief description, we discuss the use of miRNAs for diagnosis and prognosis as biomarkers and biosensors in addition to miRNA-based therapies.

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Biomarker,
Biosensor,
Gene Expression, Regulation,
Small Non-Coding RNAs

1. Introduction

RNA interference (RNAi) is one of the primary machineries related to gene expression regulation in eukaryotic cells. RNAi delivered into cells initiate the degradation of messenger RNA (mRNA) via the cells' inner mechanism (Figure 1). This mechanism limits the gene expression by either suppressing transcription or activating a sequence-specific RNA degradation process (Almeida and Allshire, 2005; Deng et al., 2014; Xin et al., 2017).

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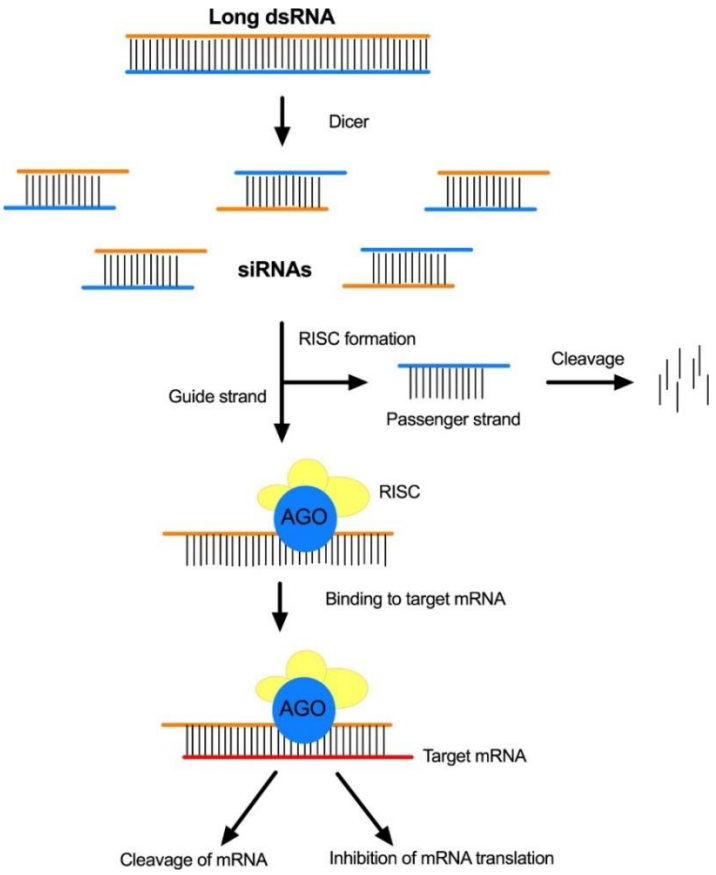

Figure 1. RNAi mechanism (Brüggenwirth and Martins, 2020)

In the process of gene expression regulation, Dicer protein binds to dsRNA, cleaving it into small pieces named as siRNA. These siRNAs bind to an Argonaute (Ago) protein which is part of the RNA-induced silencing complex (RISC). RISC divides siRNAs into two parts including passenger strand and guide strand. The passenger strand is degraded while the guide strand serves as a search probe that connects RISC for complementary RNA targets. After this identification, targeted gene expression could be regulated by various mechanisms (Brüggenwirth and Martins, 2020).

2. Small non-coding RNAs (sncRNAs)

sncRNAs with 20-25 nt in length including microRNA (miRNA), small interfering RNA (siRNA), and short hairpin RNA (shRNA) etc. have gained considerable attention in a wide range of applications in plant, animal and human (Liu and Paroo, 2010; Castel and Martienssen, 2013; Inal et al., 2014; Movahedi et al., 2018). These sncRNAs perform their mechanisms via both transcriptional gene silencing (TGS) and post-transcriptional gene silencing (PTGS) (Axtell and Bowman, 2008; Covarrubias and Reyes, 2010). Both *in silico*

and experimental analyses have been performed to identify novel sncRNAs and their targets playing important roles in different metabolic pathways (Chetta et al., 2020; Zhang et al., 2020; El-Kafrawy, et al., 2021; Garcia et al., 2021; Rana et al., 2021). Obtaining data from these investigations provide new insight to figure out the complexity and functions of sncRNAs (Wittmann and Jäck, 2010).

2. miRNA

miRNAs are 17-25 nt in length, constituting up to 1% of the human genome (Friedman et al., 2009) and currently 1917 human miRNAs are displayed in the miRBase database (Kozomara et al., 2019). They regulate gene expression by binding to seed sequences of target mRNAs (Figure 2) (Bartel, 2004; Bartel and Chen, 2004). In eukaryotic organisms, miRNAs target mRNA involved in various metabolic pathways such as growth, development, abiotic and biotic stress (Eren et al., 2016; İlhan et al., 2016; Jian et al., 2017; Stepien et al., 2017).

Because of technological limitations to investigate miRNAs, the significance of miRNAs was understood after the discovery of lin-4 and let-7 which control nematode (*Caenorhabditis elegans*) development via incomplete base pairing to the 3′ UTRs of target mRNAs to prevent translation (Lee et al., 1993; Reinhart et al., 2000). For plants, Reinhart et al. (2002) detected miRNAs in a model organism, *Arabidopsis*. They revealed that ncRNAs could be arisen early in eukaryotic evolution (Reinhart et al., 2002). A single miRNA might potentially target several mRNAs whereas one mRNA might contain multiple binding sites for miRNAs (Chevillet et al., 2014).

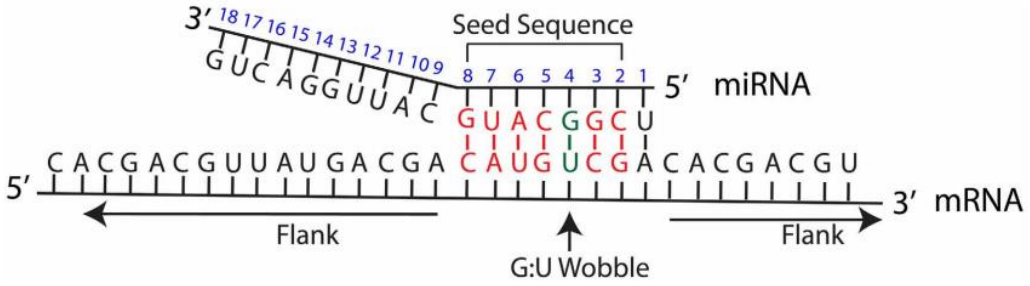

Figure 2. miRNA:target mRNA relationships. Blue numbers indicate miRNA position. The seed sequence is between 2-8 nt in miRNA position. Flank regions correspond to mRNA regions are found on either side of the seed region. Watson-Crick (WC) matches in the seed region are presented as red colour, and green colour for G:U wooble (Peterson et al., 2014)

3.1. miRNA Biogenesis

Biogenesis of miRNA starts with the processing of RNA pol II and III affecting primary miRNA (pri-miRNA) in the nucleus (Lee et al., 2004; Krol et al., 2010). Nearly half of the identified miRNAs are found in intragenic regions. They could be processed from introns and a few exons (de Rie et al., 2017). On the other hand, the remaining miRNAs are in intergenic regions regulated by their promotors (Kim and Kim, 2007). There are two different miRNA biogenesis classified as canonical and non-canonical pathways (Figure 3). miRNA-mediated silencing complex (miRISC) complex containing the sense miRNA strand which binds to the target mRNA through its 3′-untranslated regions (3 ′-UTR) in canonical pathways (Chevillet et al., 2014). On the other hand, about 60% of the relations between the miRISC complex and mRNA are non-canonical in human (Helwak et al., 2013) which means their chains aren't always entirely complementary to each other (Jonas and Izaurralde, 2015).

The production of pri-miRNA transcript is produced at the beginning of canonical RNA biogenesis and then microprocessor complex consisting of Drosha and DiGeorge Syndrome Critical Region 8 (DGCR8) cuts the pri-miRNA. As a result of cutting, precursor-miRNA (pre-miRNA) is produced and exported to the cytoplasm via Exportin5/RanGTP-dependent manner. In the cytoplasm, pre-miRNA duplex is bound to Ago family of proteins to form a miRISC. Another pathway, non-conical pathway is classified in Drosha/DGCR8-independent and Dicer-independent pathways. shRNAs are cut by microprocessor complex and exported to the cytoplasm via Exportin5/RanGTP similar to canonical pathway. Moreover, shRNAs were further processed via AGO2-dependent but Dicer-independent manner. Mirtrons and 7-methylguanine capped (m^7G)-pre-miRNA are processed to obtain mature miRNAs and this process is carried out by Dicer. Exportin5/RanGTP carries Mirtrons while Exportin1 is important for m^7G -pre-miRNA (Hayder et al., 2018).

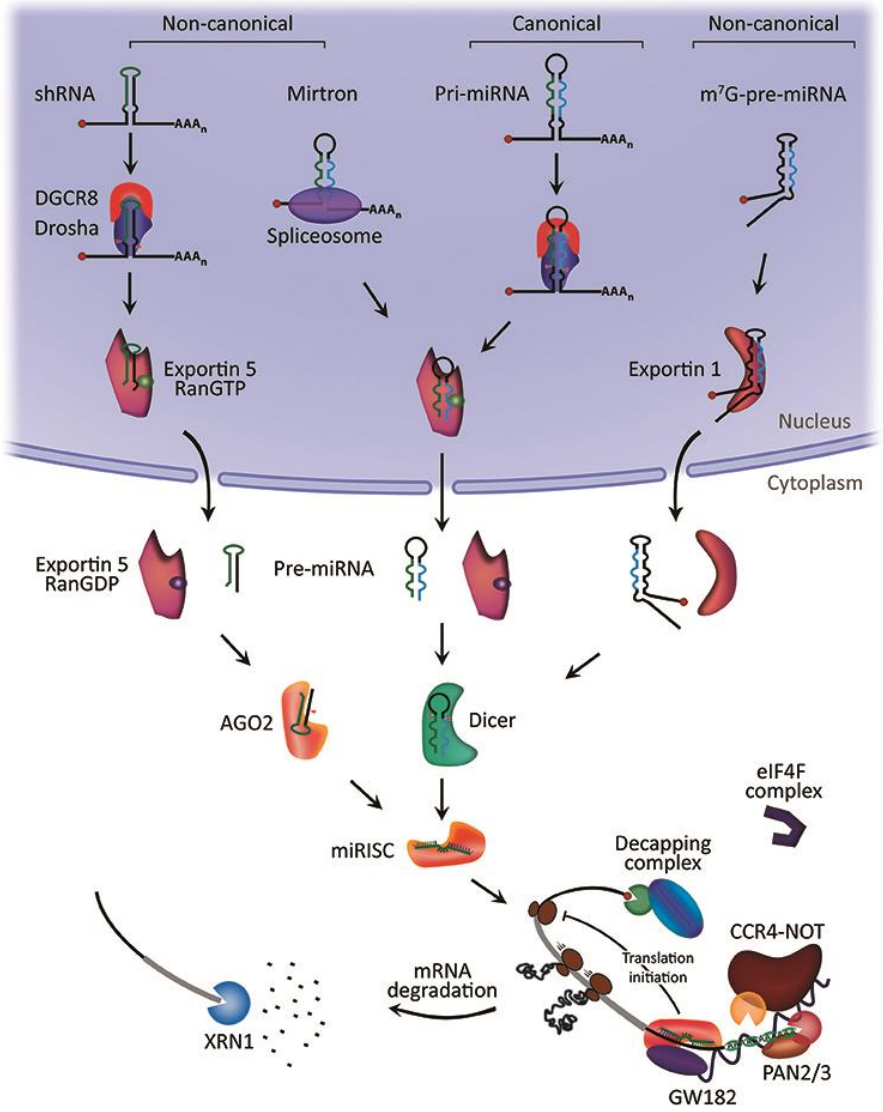

Figure 3. miRNA biogenesis (O'Brien et al., 2018)

3.2. miRNA Detection Methods

Novel miRNAs can be identified and analysed by using different methods. These methods are generally divided into groups *in silico* analyses and experimental analyses. Different miRNAs databases such as miRBase (Kozomara et al., 2019) miRandola (http://mirandola.iit.cnr.it/) and human infections (http://mir2disease.org/) etc. have been commonly used for detection of novel miRNAs related to disease and different metabolic pathways. By using reference sequences obtained from these databases, NCBI-BLAST, RNAfold, RNAHybrid, BLAST2GO and other related programs have been widely used for identification of miRNA and their targets via *in silico* analyses (Altschul et al., 1990; Kruger and Rehmsmeier, 2006; Conesa and Götz, 2008; Lorenz et al., 2011).

Northern blot analysis (Sempere et al., 2004; Válóczi et al., 2004), *in situ* hybridization (Kloosterman et al., 2006), real-time PCR (Chen et al., 2005; Wang et al., 2009), miRNA microarray (Thomson et al., 2004; Wang et al., 2014), and next-generation sequencing (NGS) (Wang et al., 2015) utilising massive parallel sequencing on platforms such as Illumina Genome Analyzer, ABI SOLiD, or Roche/454 Genome Sequencer FLX (Mardis, 2008) are employed in experimental analyses. Since each technology has strengths and weaknesses, Hong et al. (2021) proposed that miRNA-Seq for miRNA biomarker discovery and even identification of novel miRNAs. It is worth noting that miRNAs' expression profiles in plasma and serum have important for the potential usage of them as biomarkers for early disease diagnostics and the treatment of diseases (Nik and Shahidan, 2019).

3.3. Application of miRNAs as a Biomarker for Human Diseases

miRNAs are stable and tissue-specific molecules in extracellular compartment. These properties make circulating cell-free miRNAs as a promising class of non-invasive biomarkers for human (Hong et al., 2021). Circulating miRNAs are covered by membrane-bound vesicles such as exosomes. Numerous investigations showed that different pathophysiological conditions including cancer, liver damage, contagious conditions, cardiovascular diseases, neurodegenerative disease are related to expression of circulating miRNAs (Bhardwaj et al., 2013; Zeng et al., 2017; Aghili et al., 2018; Biswas et al., 2019; Coban et al., 2020; Teksoy et al., 2020).

3.3.1. miRNA-based biomarkers for diagnosis and prognosis

Circulating miRNAs might be used for diagnosis of infectious diseases such as Dengue, Ebola and others (Duy et al., 2016; Ouyang et al., 2016; Trilobet et al., 2020). Biswas et al. (2019) investigated 372 microRNAs in plasma samples from HIV-1 infected individuals to detect early/acute HIV-1 infection. They reported a miRNA panel (PeHIV-1) containing four differentially expressed miRNAs (miR-16-5p, miR-20b-5p, miR-195-5p, and miR-223-3p) in infected individuals when compared to healthy controls. Another frequent form of malignant disease, ovarian cancer, cause more than 150,000 women to die every year. On the other hand, this disease is diagnosed with late-stage disease. Therefore, it is important to improve early diagnosis biomarker systems. Berner et al. (2021) aimed to identify microRNAs to

detect ovarian cancer reporting that miR-15a and let-7a are highly suitable for ovarian cancer patients.

In addition to diagnosis, miRNAs have also been utilised as novel biomarkers in the prediction of prognosis (Zhang et al., 2019; Sun et al., 2019) and integrating multiple miRNAs might be more effective than single ones to predict prognosis (He et al., 2019). It is suggested that miR-103a-3p might be a potential non-invasive diagnostic and prognostic biomarker (Liu et al., 2022). Moreover, hsa-miR-584-5p detected as tumor suppressor miRNA is a potential candidate biomarker for coronary artery diseases (Coban et al. 2020).

3.3.2. miRNA-based biosensors

In recent years, biosensors with increased sensitivity via nanomaterials have gained more attention especially for early detection of diseases to prevent the progression. One of these investigations was performed by Aghili et al. (2018). They improved an electrochemical nanobiosensor based on the quantification of the circulating biomarker miR-195 for the early detection of Parkinson's disease. Specific and sensitive biomarkers are also very important for early cancer diagnosis. Zeng et al. (2017) demonstrated an ultrasensitive electrochemical biosensor, detecting microRNA biomarkers related to multiple pancreatic carcinomas. Four miRNAs, miRNA21, miRNA155, miRNA196a, and miRNA210, distinguished from healthy controls with very high sensitivity.

Exosomes found in human biological fluids have clinical importance in the diagnosis of various diseases. Song et al. (2021) examined the effect of the combination of exosomal miRNA-125b and miRNA-361 for the progression of Alzheimer's disease. They reported that sensor depending on these sequences can be applied clinically for AD diagnosis and has the potential to outstanding the field of dementia research and treatment in the future.

3.3.3. miRNA-based therapies

miRNA therapeutics in combination with chemotherapy could be used in the effective treatment of certain diseases. Apurinic/apyrimidinic endodeoxyribonuclease 1 (APEX1) is overexpressed in gastric cancer, performing several functions. Since miR-27a-5p inhibits this enzyme’s activity, He et al. (2021) studied with APEX1/miR-27a-5p axis, suggesting this axis

as a new therapeutic agent. Another investigation was performed by Zhang et al. (2020). They showed that the inhibitory role of miRNA-5119 mimic-engineered dendritic cell vaccines and also increasing role in anti-tumor immune response for mouse breast cancer model. In addition to miRNA/DC-based immunotherapy, platinum-based chemotherapy response depending on miRNA variants to detect the lung cancer susceptibility was also examined. Obtaining findings indicated that SNPs rs71428439 (miR-149), rs2910164 (R-146a), rs928508 (mir-30c-1) and rs629367 (let-7a-2) related with polymorphisms of rs11614913 (miR-196a-2) and rs9280508 (miR-30c-1) notably affected the patients' response, serving as potential clinical biomarkers to predict lung cancer risk (Fang et al., 2018).

5. Conclusion

In this review, we summarised the utilisation of miRNAs as biomarkers and biosensors, and even miRNA-based therapies for diagnosis and prognosis of diseases. Detection of miRNAs for a specific disease and also identification of differently expressed miRNAs between control and experimental groups provide new insight, especially for early detection. The results might be integrated into personalized medicine applications.

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Review Article

Circadian rhythm and obesity

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Abstract

The earth rotates around its axis for 24 hours, this process creates physiological, biochemical, and behavioral rhythms in living things. These one-day periods are called the circadian rhythm. The circadian rhythm regulates human physiology and behavior by responding to environmental stimuli around the clock. The circadian system and sleep/wake phases are intertwined, and conditions such as sleep disorders, exposure to artificial light, jet lag, and shift work may cause disruptions in the circadian rhythm. Circadian rhythm; It is effective in gastrointestinal system physiology such as cell proliferation, electrolyte balance, digestion, absorption, motility. Disturbances in the circadian rhythm can cause imbalances in the intestinal flora, which can lead to disruptions in both the immune system and the absorption and digestion of macronutrients. In the human body, many endocrine factors are secreted in 24-hour periods and the amount of secretion reaches its peak at certain hours during the day. Disturbances in the circadian rhythm can cause many pathological conditions such as obesity and diabetes by causing disorders in the gastrointestinal system physiology, the secretion function of appetite hormones, and other endocrine factors secreted in 24 hours. Our aim in this review is to discuss the effects of circadian rhythm on gastrointestinal health and the relationship of hormones fluctuating with 24-hour circadian rhythm and obesity. The search was conducted in peer-reviewed journals PubMed, Web of Science, and Google Scholar. For this purpose, the keywords circadian rhythm and obesity were used together and research articles were included in this study.

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1. Introduction

Biological rhythms are a fundamental feature of life and shape many functions of living systems, from the molecular level to the organismal level (Mat et al., 2020). There are multiple time scales studied in nature that show rhythmic oscillations. Generally, they are divided into ultradian rhythm, infradian rhythm, circannual rhythm (Amanpour et al., 2021), and circadian rhythm. Short-period rhythms are expressed as ultradian rhythm, rhythms lasting weeks or more than 24 hours infradian rhythm, that repeat annually are expressed as circannual rhythm. (Stevenson, 2018; Amanpour et al., 2021).

In this review, the effects of circadian rhythm on gastrointestinal health and its relationship with obesity, hormones fluctuating with 24-hour circadian rhythm, and the effects of these hormones on obesity were discussed.

1.1. Circadian Rhythm

The term circadian rhythm comes from the circa (about) and dies (day). It is used to express the approximately 24-hour solstice. The circadian rhythm coordinates the internal timing of the organism with the outside world and is the main regulator of many physiological processes (Jaganath et al., 2017). The timing of the circadian rhythm is regulated by conditions such as light/dark, social/environmental, food/nutrition, temperature, chemical factors, mechanical stimuli. Light and the light-related sleep/wake cycle are the most effective circadian rhythm regulators (Akıncı and Orhan, 2016; Reid, 2019; Xie et al., 2019).

Circadian rhythm can be examined under two headings as central and peripheral clock. The central clock is located in the hypothalamus's suprachiasmatic nucleus (SCN). Many cells with molecular clocks in the body are regulated and protected by the main pacemaker located in the SCN (Serin and Acar Tek, 2019). The primary regulator of the SCN is the light stimulus. In mammals, light entering the eye first reaches the retina. (Wirz-Justice et al., 2021). The light is then received by special melanopsin-producing intrinsically photoreceptive retinal ganglion cells (ipRGC) in the eye. ipRGC transmits this information via the retinohypothalamic pathway to the SCN and other parts of the brain. The SCN transmits it to other organs of the body (Figure 1) (Logan and McClung, 2019). In this way, the organism distinguishes between day and night. Situations such as exposure to abnormal light, exposure to artificial light, and disruption of

sleep patterns may disrupt the relationship of the SCN with peripheral clocks and cause adverse health conditions such as psychological disorders, cancer, and metabolic diseases (Bedrosian and Nelson, 2017). Exposure to artificial light at night can also disrupt the secretion pattern of melatonin secreted from the pineal gland. Melatonin is responsible for circadian rhythm synchronization and has functions such as regulation of the sleep/wake cycle, modulation of pituitary and adrenal hormones, and regulation of the immune system. Therefore, the disorder in its release can cause many health problems (Vasey et al., 2021).

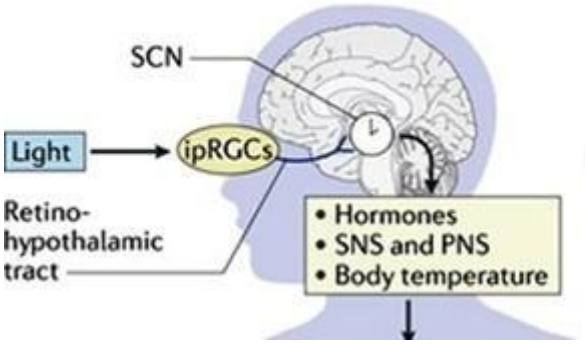

Figure 1. Light editing of the SCN (Logan & McClung, 2019)

Peripheral clocks are; direct the circadian expression of specific genes involved in many physiological functions (Serin and Acar Tek, 2019). Mammalian molecular clock; circadian locomotor output cycle includes a series of transcriptional-translational feedback loops including clock components such as hood (CLOCK), brain and muscle ARNT-like protein 1 (BMAL 1), Period (PER1-3), and cryptochrome (CRY1-2) (Stokes et al., 2017). In the primary feedback loop, CLOCK and BMAL1 form heterodimer complexes in the cytosol of SCN cells. This complex enters the nucleus and binds to regulatory elements of DNA containing E-Box. This binding activates the expression of the PER and CRY genes. PER and CRY then form a trimeric complex with casein kinaseIε/δ (CKI) and enter the nucleus. The PER-CRY complex acts on the CLOCK-BMAL1 complex and represses its transcriptions (Gul et al., 2021). In the second main transcriptional cycle, CLOCK and BMAL1, REV-ERBα and REV-ERBβ activate the transcription of nuclear receptors. The protein products compete with retinoic acid-associated, ligand unknown (orphan) receptors (RORα, RORβ, and RORγ) for binding sites (ROR-binding elements) in the BMAL1 gene. REV-ERB represses BMAL1 transcription; ROR increases BMAL1 activation. The third feedback loop includes D-box binding protein (DBP) binding to D-box elements and nuclear factor, interleukin-3-regulated protein (NFIL3/E4BP4). This cycle is regulated by CLOCK/BMAL1 and CRY1 (Figure 2) (Cox and Takahaski, 2019).

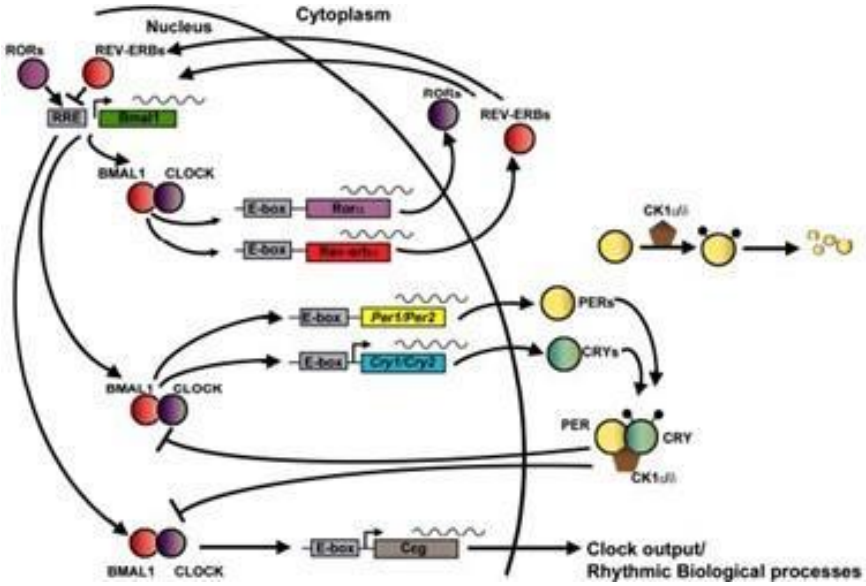

Figure 2. Mammalian circadian clock transcriptional and translational feedback network (Bhadra et al., 2017)

When the balance in the organism's body is observed, the biological/circadian clock from the molecular to the behavioral level must adapt to environmental changes. Conditions such as jet lag, shift work, and exposure to artificial light at night can cause misregulations in the circadian rhythm, disrupting the body balance and causing many adverse health problems. In addition, eating a high-fat diet and food intake at the wrong time can disrupt temporal physiological regulation (Figure 3) (Maury, 2019). The most studied and most common condition in the irregular circadian rhythm is the disruption of the sleep/wake cycle (Baron and Reid, 2014).

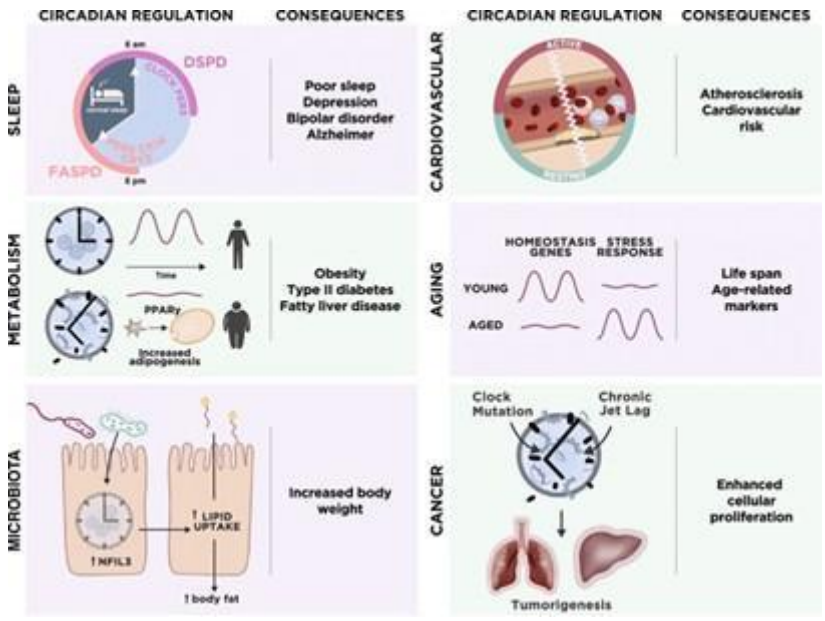

Figure 3. Results of circadian regulation and dysfunction in different physiological systems (FASPD: Familial advanced sleep phase disorder, DSPD: Advanced sleep phase disorder, NFIL3: Regulates lipid intake and body fat) (Rijo-Ferreira and Takahashi, 2019)

Another cause of disruptions in the circadian system is mutations or disorders in the circadian clock genes. This may result in a disturbance in human metabolism, as seen in Table 1.

1.2. Circadian Rhythm and Gastrointestinal System

Many mechanisms in the digestive system show circadian variations. For example, the circadian rhythm can maintain the intestinal barrier and regulate digestive physiology. In addition, depending on the circadian rhythm, the volume of saliva produced in the morning is more important than in the evening; gastric emptying in the stomach takes longer in the evening than in the morning (Codoñer-Franch and Gombert, 2018). In a study examining the effects of circadian rhythm on food intake; attenuation in feeding rhythm and hyperphagia were observed in BMAL1, Cry1–2, and Per2 mutant mice. Similar results were also obtained in mutant CLOCK mice (Page, 2021). According to experiments in mice in the regulation of gastrointestinal motility, it has been observed that the motility of organs such as the stomach, small intestine, the large intestine is closely related to the circadian rhythm. Mutations were made in the Per1/2h genes of the mice, resulting in changes in colonic muscle contraction. In addition, polymorphisms in components of circadian clock genes such as CLOCK and Per3 are associated with poor gastric motility in humans (Voigt et al., 2019). The gut is our

gastrointestinal tract organ, colonized by approximately 100 trillion microbes and mainly dominated by bacteria. The circadian rhythm is effective in regulating the intestinal microbiota. Jet lag etc. it has been shown by experiments on mice that circadian disruptions can cause dysbiosis in the intestinal microbiota due to various reasons. Circadian arrhythmic Per1 −/−; While it was observed that there were changes in the gut microbiota of mice with Per2 −/− pair disabled, it was observed that the gut microbiota also affected the circadian rhythm, and it was concluded that there was a bidirectional relationship between them (Rosselot et al., 2016).

Table 1. Metabolic consequences of circadian rhythm genes dysfunction, disruption, or gene mutations (Lee et al., 2015; Fatima and Rana, 2020)

Circadian Clock Genes	Conclusion
Stopping the BMAL1 function	Specific to liver tissue; elevated serum triglycerides, increased hepatic lipid, impaired circulating glucose clearance. Specific to smooth muscle tissue; impaired blood pressure rhythms. Specific to adipose tissue; changing feeding behavior and obese phenotype. Specific to the pancreas; altered insulin secretion. Specific to skeletal muscle; impaired glucose metabolism in muscles. In the whole body; increased free fatty acids and cholesterol, insulin resistance, glucose intolerance, weight gain, endothelial dysfunction. Impaired gluconeogenesis, adipocyte differentiation.
Stopping the CLOCK function	Slight weight gain
CLOCK mutation	Glucose intolerance. Disturbance in the size of pancreatic islets and defective proliferation. Vascular injury, endothelial dysfunction. Impaired circadian blood pressure.
CRY1 mutation	Diabetes
PER2 stop working	Deterioration in eating behavior, predisposition to obesity. Altered lipid profile, increased adipocyte differentiation. Decreased insulin clearance.
PER1/2/3 stop working	Predisposition to obesity.
Disruption of CRY1/2 functioning	Glucose intolerance
Rev-erbα/β dysfunction	Hepatic steatosis and dyslipidemia

It has also been observed that circadian rhythm dysfunction stimulates intestinal flora imbalance, independent of the nutrient source, and accordingly, it causes deterioration in the

immune system, and causes various pathological conditions (Cui et al., 2016). Circadian rhythms in innate adaptive immunity; it is effective by regulating events such as phagocytic activity, production of cytokines, number of circulating immune cells, and development of specific immune cell lines. For example, the circulating amounts of molecules that can respond to acute inflammatory action, such as lipopolysaccharides (LPS) in the outer membrane of gram-negative bacteria, or molecules such as Toll-like receptors TLR, increase at certain times of the day (Voigt et al., 2019).

The absorption and digestion of lipid, protein, carbohydrate macronutrients are under the control of the circadian clock to ensure simultaneous availability of nutrients in the gastrointestinal tract. Thus, enzymes involved in the digestion of these three crucial macromolecules are secreted in high amounts during food intake (Martchenko et al., 2020). Absorption of peptides transported across the intestinal barrier via the low-affinity lipid transporter 1 (PEPT1) is dependent on the sodium-proton exchanger (NHE3). The NHE3 gene, which is affected by the presence of food, contains E-Box and is under circadian control. Thus, it can be mentioned that there is a circadian rhythm in protein absorption. There is a lot of evidence that the circadian rhythm is also effective in regulating carbohydrate and lipid absorption. For example, absorption of monosaccharides from the intestinal lumen occurs with transporters such as sodium-dependent glucose transporters (SGLT1/2), enterocytes, glucose transporters 5 and 3 (GLUT5 and GLUT3), and the circadian clock is effective in these transporters (Voigt et al., 2019). In the absorption of lipids, three molecules named MTP, apo A-IV, and nocturnal were identified and it was observed that oscillators stimulated by both light and food affect the expression of these clock genes (Figure 4) (Hussain and Pan, 2015).

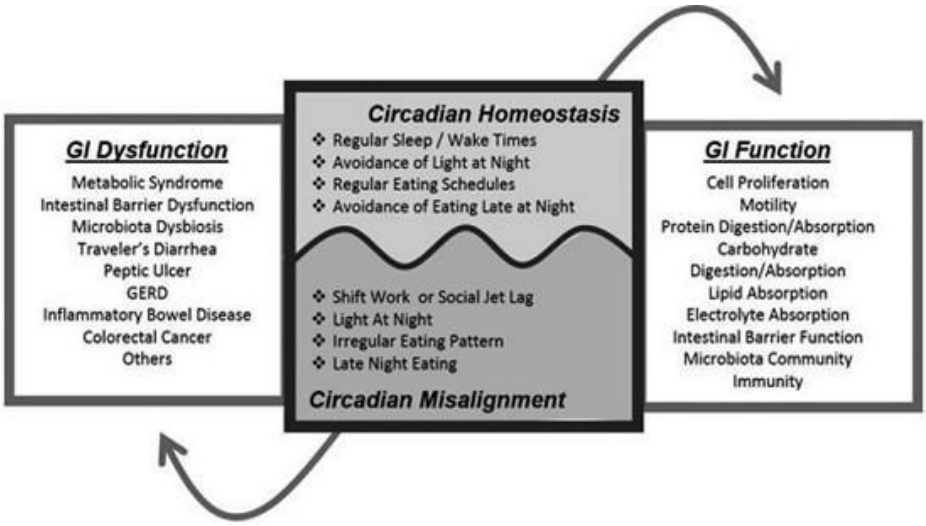

Figure 3. Circadian regulation of gastrointestinal function and dysfunction (Voigt et al., 2019) Various dysfunctions in the gastrointestinal tract may occur in the disruption of the circadian rhythm, which enables its biological functions to adapt to environmental conditions and predictable changes.

1.3. Circadian Rhythm and Obesity

According to the World Health Organization (WHO), obesity is defined as "abnormal or excessive fat accumulation that poses a health risk"; The World Obesity Federation, on the other hand, stated that obesity is "a chronic, recurrent progressive disease". In addition, epidemiological studies have shown that obesity can cause pathological conditions such as insulin resistance, cardiovascular disease, metabolic syndrome, and diabetes (Blüher, 2020). Disruptions in energy metabolism and circadian rhythm integrated with the environment can cause various pathological conditions, one of which is obesity (Sridhar and Sanjana, 2016). People with appropriate and inadequate sleep/wake cycles, such as shift workers, are more prone to conditions such as obesity. The most important accelerator of the disrupted circadian rhythm is exposure to light (LAN) at night, and light-sensing retinal ganglion cells reflect on SCN neurons, thus evoking a signal that leads to changes in Per1 and Per2 CLOCK gene expression (Noh, 2018). Imbalances in meal timing, such as shift work, or situations such as skipping meals can also lead to disruptions in biological rhythms. It has been reported that skipping breakfast, which is the first meal of the day, increases the risk of obesity more than skipping lunch or dinner (Orihara et al., 2020). In fact, in a study, it was observed that weight loss was higher when participants who consumed a single 2000 kcal meal in the morning were compared with those who consumed it in the evening (Halberg et al., 1995). In addition, studies

have shown that people exposed to artificial light at night have impaired secretion of plasma melatonin and leptin. In addition, it was observed that while plasma glucose levels were at high levels during the night, decreases in insulin secretion occurred. As a result, these studies have shown that exposure to light at night or working in shifts impairs insulin response (Pagano et al., 2017). Studies show that CLOCK gene expression in mouse adipocytes is decreased in the presence of diabetes and obesity. In addition, a decrease in adiponectin levels and a loss in the circadian rhythm of expression of adipokines were observed (Scott, 2015). Genes exhibiting circadian expression in visceral adipose tissue; CLOCK genes such as adiponectin, leptin, resistin, and visfatin. The functions of adiponectin and resistin in obese mice were greatly atrophied. In rats fed a high-fat diet, melatonin reduced plasma leptin levels in response to diet for three weeks. It has been observed that the circadian rhythm associated with this information regulates body weight by influencing hormone expression (Froy, 2010).

When the relationships between sleep-wake times and obesity depending on the circadian rhythm are investigated, it is generally thought that long sleep causes obesity, but according to these studies, it has been observed that subjects prone to obesity who claim to sleep longer spend more time in bed and sleep duration is short (Ogilvie and Patel, 2017). The relationship between short sleep duration and obesity in both adolescents and children is supported by the available literature. In addition, it has been observed that restriction during the sleep process changes orexigenic hormone functions, disrupts energy-glucose metabolism, and causes them to develop more unhealthy dietary behaviors (Hayes et al., 2018).

1.4. Hormones with Circadian Rhythm and Their Effects on Obesity

1.4.1. Melatonin

Melatonin, is a sleep regulator in humans and many living species, is secreted in a circadian rhythm and its secretion is regulated by the pineal gland (Morris et al., 2012). Melatonin levels rise at night and decrease during the day. About 2 hours after the production of melatonin, the deep sleep process begins. The circadian rhythm of melatonin is effective in individuals with and without visual impairment (Zisapel, 2018). Exposure to light at night can reduce melatonin levels and disrupt many physiological processes. Melatonin, which has effects on suppressing ultraviolet (UV) damage in skin cells, wound healing, hair growth, and anticancer, can also be taken orally to induce sleep (Lyons et al., 2019). In addition, the circadian amplitude of

melatonin may decrease with age (Goswami et al., 2020). Circadian timing, which has positive effects on physical and mental health; melatonin is directly affected by melatonin receptor agonists and light (Emens and Burgess, 2015). It has been shown to enhance the circadian rhythm in newborns through the transfer of melatonin with colostrum. Therefore, the inability of the newborn to be fed with breast milk causes disruptions in the circadian rhythm. In this case, disruptions occur in the physiological processes of the baby, who cannot reach melatonin sufficiently. e.g., excessive weight gain is observed independent of caloric intake. In addition, it has been shown that the secretion rhythm of melatonin is impaired in obese mothers, and similar situations have been found in the children of these mothers (Ivanov et al., 2020). In addition, melatonin receptor dysfunctions (MT1 and MT2 receptors) have been associated with diabetes (Gamble et al., 2014).

1.4.2. Leptin

Leptin is a satiety hormone produced by adipocytes and circulated in proportion to body fat (Patton and Mistlberger, 2013). Leptin has endocrine and paracrine effects (Berger and Polotsky, 2018). After leptin is secreted from adipocytes, it crosses the blood-brain barrier to the brain and binds to its receptor LepR. In addition, leptin is encoded by the obese (ob) gene (Audira et al., 2018).

The circadian rhythm has an important place in the leptin cycle. BMAL1 and CLOCK heterodimers regulate CCAAT-enhancing protein alpha (C/EBPα) activity in adipose tissue. C/EBPα is the most potent transcriptional activator for leptin, and this regulation indicates that the heterodimers of BMAL1 and CLOCK are directly involved in leptin modulation. In addition, this regulation rhythmically enhances the leptin response of the SCN clock Arcuate Core (ARC) neurons in the Central Nervous System (Kettner et al., 2015). While it is low in the early morning, it increases during the day and is at its highest level late at night. Those that affect leptin levels for 24 hours are; sleep loss or prolonged sleep, circadian phase, excessive food intake, or calorie restriction (Nguyen and Wright Jr, 2010). In animal studies, sleep deprivation caused disturbances in leptin secretion, and sleep-deprived rats showed lower leptin levels and increased energy expenditure (Olson et al., 2016). In addition, slow-wave sleep (SWS) increased while REM sleep decreased in leptin-fused rodents. In another study conducted on humans, 4 hours of sleep restriction showed a 23-24% increase in appetite and hunger, and an 18% decrease in leptin hormone (Kim et al., 2015). As a result, chronic or acute

sleep deprivation can cause an increase in food intake and weight, which can lead to obesity, diabetes, etc. can cause pathological conditions.

1.4.3. Ghrelin

Ghrelin, an orexigenic hormone, stimulates the secretion of adrenocorticotropic hormone (ACTH) and growth hormone, thus increasing food intake and appetite. It also increases gastric acid secretion and intestinal motility, affects energy expenditure, contributes to the hedonic aspects of food, and lowers arterial blood pressure (Gray et al., 2019). Ghrelin is secreted by endocrine cells of oxyntic glands in the fundus of the stomach; it is secreted in small amounts by the duodenum, jejunum, stomach body, as well as by the pituitary gland, lungs, and urogenital organs. To produce active ghrelin, the enzyme Grelin-O Acyltransferase octanylates the inactive form then attaches an acyl side chain to the serine residue at position 3. This chain is critical in the gastric emptying and appetite-enhancing functions of ghrelin (Makris et al., 2017).

While plasma ghrelin levels vary according to feeding times, they are highest during the day and lowest at night. Acting on the circadian system, ghrelin is a potential feedback signal for the SCN. It has also been discovered that ghrelin secretion is regulated in Per1 and Per2 in the stomach. Thus, it has been reported that ghrelin can regulate the peripheral circadian rhythm (Wang et al., 2018). Ghrelin-secreting cells are Food Driven Oscillators (FEO) because in mice oxyntic cells express circadian cycles of CLOCK gene expression associated with mealtime (Mistlberger, 2020). In nocturnal animals fed only during the day, there is a large increase in premeal circulating ghrelin (Challet, 2015). In a study conducted to reveal the relationship between sleep and appetite, the difference between 4 hours of sleep in 2 nights and then 10 hours of sleep in 2 nights was examined. While leptin decreased by 18% compared to long sleep during short sleep, it was observed that ghrelin increased by 28% in short sleep. Appetite for high-carbohydrate foods increased after a short nap, with a 32% increase (Leproult and Van Cauter, 2010).

1.4.4. Insulin

Insulin is secreted by β cells in pancreatic islets. It responds to levels of circulating nutrients such as free fatty acids, amino acids, and glucose. If the circulating level of insulin is insufficient

to stimulate glucose uptake from the blood, protein and fat stores are used for energy production (Kolb et al., 2020). Insulin signaling begins with its binding to the cell surface insulin receptor (IR), a tyrosine kinase, and with MEK/ERK, AKT/PI3K/mTOR, NOX4 signaling pathways, lipolysis, gluconeogenesis, lipid/protein/glucose synthesis, and glucose uptake are effective in cell proliferation (Posner, 2017; Kolb et al., 2020). While it is thought that there is a connection between the increasing obesity problem in the world and insulin resistance, the underlying causes of this connection have been investigated and it has been observed that melatonin is secreted less in insulin-resistant individuals (Otamas et al., 2020). In studies with rodents, melatonin receptors (MT1 and MT2) and melatonin interactions on the beta-cell surface have been shown to inhibit insulin secretion from beta cells (Onaolapo and Onaolapo, 2018).

According to the experiments, reasons such as sleep restriction, circadian disruption, behavioral and environmental evening causes problems in insulin sensitivity (Mason et al., 2020). According to an experiment conducted with volunteers over 28 weeks, insulin and glucose levels increased independently of the time after a meal, while their levels decreased during sleep. They also found that circadian disruption may be effective in insulin resistance, which may cause weight gain and obesity (Mesarwi et al., 2013).

1.4.5. Adiponectin

Adiponectin found in adipose tissue and encoded by the Adipo Q gene has 224 amino acids and weights 28 kDa. Adiponectin, which is effective in the metabolism of carbohydrates and lipids, especially in muscle and liver, as well as energy homeostasis; has also been found to have anti-atherogenic and anti-inflammatory effects. Adiponectin also; It has also been detected in lymphocytes, adrenal glands, osteoblasts, skeletal muscle, cardiomyocytes, testis, ovary, and placenta (Nguyen, 2020). Adiponectin, which has two receptors as AdipoR1 and AdipoR2, plays a crucial role in reducing pathological conditions such as obesity-related type 2 diabetes/insulin resistance and cardiovascular diseases. While adiponectin is positively associated with insulin sensitivity, a decrease in serum levels of adiponectin is observed in obesity (Achari and Jain, 2017; Straub and Scherer, 2019).

Adiponectin is one of the fat-derived endocrine factors (i.e. adipokine) and has a time-of-day rhythm. This rhythm is also associated with increased insulin secretion later in the day (Gamble et al., 2014). In a study with experimental animals, although adiponectin showed

minimum expression around 10:00 and 11:00 in the morning, high adiponectin expression was observed in humans at the same hours. This showed that circadian secretion of adiponectin occurs in opposite phases between night-active mice and day-active humans (Gómez-Abellán et al., 2010). Adiponectin expression is regulated by the circadian clock and its co-activator PGC1α and circadian expression of the transcription factor PPARγ (Barnea et al., 2015). In a study, adipose tissue was taken from obese women, and AdipoQ, AdipoR1, and AdipoR2 genes were examined. According to the results of this experiment, it was concluded that these three genes exhibited 24-hour rhythmicity and adiponectin expression was regulated at the mRNA level and time-dependently (Gómez-Abellán et al., 2010; Barnea et al., 2015). Apart from nutrient-sensitive hormones that have a direct effect on obesity and appetite, many endocrine factors respond to environmental conditions and are secreted over 24 hours. At certain times of the day, the secretion of these endocrine factors reaches its peak and becomes effective on the organism and metabolism (Table 2).

2. Conclusion

The circadian rhythm, which regulates many physiological functions of the organism, is particularly affected by the light/dark cycle, and therefore by the sleep/wake phases. According to the results of the literature review; conditions such as exposure to artificial light at night, social jet lag, shift work, and eating late can lead to circadian disruptions, thus deterioration in gastrointestinal functions such as absorption, digestion, motility, and this may result in various pathological conditions. Although obesity is known as an excessive amount of fat accumulation in the body, it can bring conditions such as metabolic syndrome, insulin resistance, diabetes, and cardiovascular diseases. Recently, it has been thought that circadian rhythm and sleep/wake processes may be associated with obesity, and many studies have been conducted. Although it is generally thought that long sleep periods play a role in the process leading to obesity, studies have shown that short sleep (less than 6 hours of sleep) rather than long sleep may be more effective on obesity. The shortening of the sleep period resulted in spending more time on eating, more tendency to carbohydrate foods, and the appetite hormones secreted with the circadian rhythm and the secretion of the melatonin hormone, which plays a very important role in the circadian rhythm, causing disorders in the secretion, increasing the appetite and inviting obesity. The circadian rhythm is highly effective on obesity. Although it is not known for certain whether circadian disruption causes functional disorders in the gastrointestinal system of the individual or causes disorders in the secretion of endocrine factors secreted by the

circadian rhythm, it should be accepted that it is one of the important underlying causes of obesity and further research is required considering the contributions it will provide to the literature.

Table 2. Other endocrine factors known to be released over 24 hours in humans (Gamble et al., 2014)

Hormones	Rhythm Based on Time of Day	Peak Point	Function in the Body
Cortisol	Yes	07.00-08.00 a.m.	Stress response İmmune response Glucose and protein homeostasis (Thau et al., 2021)
GH	Yes	increased secretion at night	Growth, Cell division and regeneration Regulation of metabolism Effective in Immunity, Reproduction, Cardiovascular System (Lin et al., 2018)
Testosterone	Yes	07.00 a.m.	Male reproductive organs development and hair growth Increasing bone, muscle mass (Banihani, 2018)
TSH	Yes	01.00-02.00 a.m.	Regulates the work of the thyroid gland, provides the secretion of Thyroxine and Triiodothyronine
PRL	Yes	02.00 a.m. (amplitude is greater in females)	Milk production and development of the mammary glands Maintaining homeostasis (Al-Chalabi et al., 2020)
T_3	Yes	02.30-03.30 a.m.	Increases basal metabolic rate Regulates growth (Mullur et al., 2014)
Vasopressin	Yes	Midnight	Body water balance Blood pressure regulation (Bankir et al., 2017)
FGF21	Yes	05.00-08.00 a.m.	Regulates simple sugar intake and tendency to sweet foods (Tezze et al., 2019)
RAAS	Yes	Early morning	Regulates blood volume and blood pressure (Ames et al., 2019)

*GH, growth hormone; TSH, thyroid-stimulating hormone; PRL, prolactin; T3, triiodothyronine; RAAS, the renin-angiotensin-aldosterone system; FGF21, fibroblast growth factor 21

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Review Article

Semen collection from small breed birds and some parameters related to passerine bird semen

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Abstract

It is much more difficult to collect semen from small breed birds than large breed birds. The biggest reason for this situation is that small breed birds are very active and their cloaca is smaller. To receive semen from passerine birds, the birds must be in the breeding season. Having active females around during this period will increase libido due to male competition. Trimming the hair around the cloaca to collect semen both prevents the risk of contamination and provides adequate viewing angles. The most commonly used method of obtaining semen is cloacal massage. Massage should be done cranially from the abdomen towards the cloaca. When the bird ejaculates, it is seen that the semen comes out of the cloaca from the seminal glomera with the pressure applied laterally on the cloaca. It collects the exiting semen with the help of a sterile hematocrit capillary tube. Sperm contaminated with feces should not be taken as it will be contaminated. In bird semen, motility examination is important in the direction of movement, speed of movement, and rate of movement of the spermatozoa. Due to this situation, it is seen that there is a relationship between motility and the morphological structure of spermatozoa in passerine birds. This study aims to give information about semen collection by a cloacal method in small breed canaries and some motility and morphological examination methods in passerine bird semen.

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1. Introduction

It is thought that anatomical differences in the reproductive tract in birds may be related to semen values. The importance of knowing the male reproductive system has been emphasized (Briske, 1993). Different semen collection methods have been developed, such as semen expelled from passerine birds, using a female mannequin, or a cloacal massage technique. It has been reported that semen collection by cloacal massage method is more advantageous in

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small breed birds (Girndt et al., 2017). The rate of motile cells and the swimming speed of spermatozoa seem to be important factors in fertilization success (Cramer et al., 2019). It is also thought that there may be a relationship between the morphological structure of the sperm and its motility (Cramer et al., 2021).

2. Male Reproductive System in Passerine Birds

It has been stated that the semen produced in the testicles of birds are transmitted from the epididymis to the vas deferens. It has been emphasized that there is no seminal gland on the vas deferens in birds (Blesbois and Brillard, 2007). In birds, the semen produced in the testicles is stored in a special structure called the glomera, which is located around the cloaca, until the time of mating. It has been observed that the cloacas of polygamous birds are larger than those of monogamous ones. However, it has been reported that the number of spermatozoa stored compared to semen varies according to bird species (Briskie, 1993). In a study conducted in passerine birds, it was observed that there was not much difference in total sperm count between the 2 seminal glomera (Birkhead et al., 2006). It has been emphasized that the seminal glomera in passerine birds can be of different sizes during the breeding period and out of season. It is known that the seminal glomera, which is active during the mating season, can be larger than its size outside the breeding season. In direct proportion to this situation, the amount of sperm in the ductus ejaculate may change (Chiba et al., 2011). The male reproductive system in the passerine birds is shown in Figure 1.

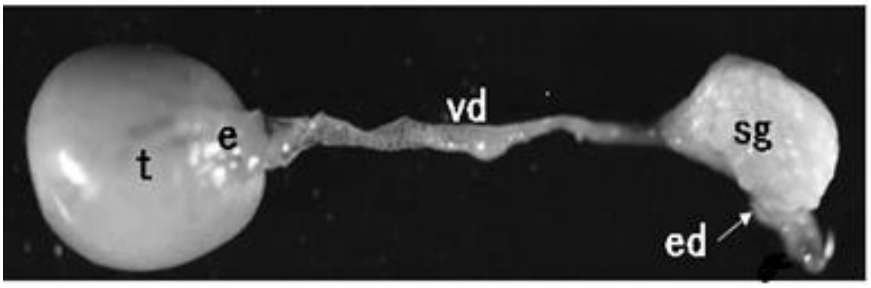

Figure 1. Male reproductive system in passerine bird (Chiba et al., 2011). T, testis; e, epididymis; vd, vas deferens; sg, seminal glomera, ed, ductus ejaculate

3. Semen Collection Method

It has been emphasized that male competition in birds will increase success (Grndt et al., 2017). Semen retrieval in passerine birds is usually obtained from dissected seminal glomera of birds that have undergone colossal massage or euthanasia (Birkhead et al., 2006; Yang et al., 2019). In the cloacal massage method in passerine birds, semen can be collected with the help of pressure applied to the right and left sides of the cloaca. It has been emphasized that the seminal glomera, which stores sperm in birds, is associated with the left and right ductus ejaculate (Samour, 2004). The seminal glomera and cloaca view are shown Figure 2. Before semen is collected by cloacal massage, it is important to clean the ventral periphery of the cloaca with 0.9% isotonic NaCl solution for the study to be carried out properly. Capillary tubes are used to collect sperm from the cloaca (O'Brien et al., 1999). In the modified cloacal massage method, the bird is stimulated by stroking the ventral root of the tail. After the accumulation of semen in the ductus ejaculate, semen can be taken with the help of gentle pressure applied to the cloaca by massaging the cloaca from the base of the abdomen with the thumb and index fingers (Brock, 1991). In addition, it has been reported that semen can also be obtained from sparrows by using a female bird dummy in addition to the cloacal massage method (Girndt et al., 2017). To determine the sperm parameters, the semen taken is diluted with the appropriate extender. Although PBS (phosphate buffer saline) is preferred as the diluent, it has been stated that Dulbecco's modified Eagle Medium is widely used (Cramer et al., 2019).

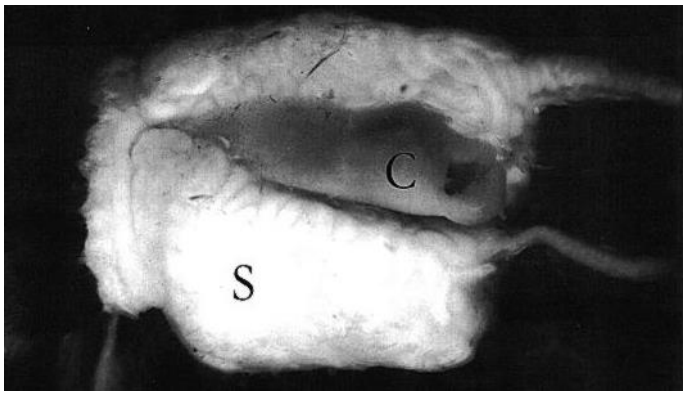

Figure 2. Seminal glomera (S) and cloaca (C) view (Briske, 1993)

4. Sperm Morphological Evaluation

In the morphological examination, the length of the spermatozoon can be evaluated as well as the length of the head and middle part (Lüpold et al., 2009). Morphological examination in semen collected from passerine birds is necessary to determine the abnormal spermatozoa rate (Hermosell et al., 2013). After the semen sample taken for morphometric examination is fixed in 5% formalin solution, it is observed under the light microscope at 160X-320X magnification and evaluated as head length, midpiece length, flagellum length, and spermatozoa length (Rowe et al., 2012). It has been emphasized that there is a supportive relationship between the competitiveness of spermatozoa and their morphological structure. When the semen samples were taken by using cloacal massage, female bird dummy and spermatozoa in feces were compared morphologically, it was stated that the head and middle part length of the spermatozoa decreased in the semen sample taken from feces, and it could be concluded that the spermatozoa developed less morphologically from this situation (Girndt et al., 2017). The speed of spermatozoa is thought to affect their competitive ability. Therefore, it was emphasized that morphological structures such as the enlarged middle part (energy component) or the length of the flagellum, or the ratio between the head size and the flagellum may depend on the interaction (Lüpond et al., 2009). It has been reported that the energy amount is proportional to the length of the spermatozoa. However, it has been stated that the increase in energy level is not related to swimming speed (Rowe et al., 2012). In a study conducted on passerine birds, it was mentioned that there may be an inverse correlation between the total length of spermatozoa and sperm competition (Kleven et al., 2008). Figure 3 show the morphological appearance of sparrow spermatozoa.

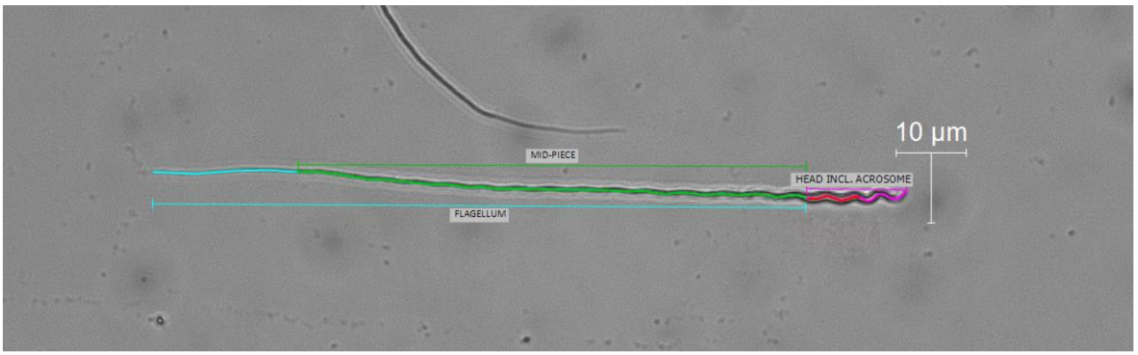

Figure 3. Morphological appearance of sparrow spermatozoa: head, acrosome, mid piece, flagellum (Grndt et al., 2017)

5. Sperm Motility Assessment

It has been reported that sperm motility evaluation in passerine birds is based on sperm motility, sperm motility rate and direction of spermatozoa. The importance of temperature was also revealed in the study. 38°C-40°C temperature was found to be ideal in terms of motility parameters. A decrease in the motility of spermatozoa was noted at 42°C. However, the motility values at 38°C were found to be compatible with 40°C (Yang et al., 2019). It has been reported that the body temperature of passerine birds is around 40°C (Birkhead et al., 2006). It has been stated that pH values between 7 and 8 are positive for passerine birds (Yang et al., 2019). In the motility examination, 5 µl of the semen diluted at a suitable rate is taken on a slide and evaluated under a heated light microscope at 400X magnification, and then expressed as a percentage value (Fischer et al., 2014). In a study conducted on sparrows, the temperature of the waterer used was predicted to be around 40°C (Helfenstein et al., 2010). Motility movement is evaluated by monitoring 30 s (Cramer et al., 2021).

5. Conclusion

The success of studies on semen in birds depends on the provision of conditions for obtaining healthy semen. It is difficult and laborious to obtain semen from small breed birds. Because small birds are very mobile and have a small cloaca, the choice of manipulation is very limited. During semen collection, the bird may be injured or even die. Or the required number of spermatozoa may not be obtained. Therefore, it is necessary to adopt the appropriate semen collection technique and gain sufficient experience. Morphological differences are key in the formation of important parameters affecting success in storage conditions such as viability rate and motility in the external environment. To improve the storage conditions of semen in passerine birds, it may be of great advantage to determine the motility values and morphological structure of spermatozoa according to species, and to reveal the relationship between these parameters. In this review study, information about semen collection techniques and some semen examination methods in small breed birds is given. It seems that more work to be done in this area is needed.

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