

# The Effect of Methyl Donor Nutrition on Global DNA Methylation Levels in Different Photoperiods in Syrian Hamsters

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## Abstract

Throughout their lives, all organisms are affected by environmental factors. The duration of the photoperiod and the type of diet to which the organism is exposed are among the most important parameters of the interaction between genes and the environment. In this study, Syrian hamsters were left in two separate light conditions, 16L (04.00-20.00) and 8L (12.00-20.00). Adult hamsters were randomized into two major groups and fed with *ad-libitum* and methyl-rich diets. Daily feed intake and weekly body weights were monitored. At the end of 8 weeks, the hypothalamus tissues of Syrian hamsters were isolated by decapitating them at four different times of the day. lights on, mid-day, lights off, and midnight. As shown in the results of DNA materials by the Trisole method, global methylation amounts were measured by ELISA. Hamsters fed a diet high in methyl consumed more nutrients than those supplied *ad-libitum*. ( $p < 0,05$ ). There was no effect of diet type on body weight, The amount of methylation increased in both long and short photoperiods during the hours when the lights were off ( $p < 0,05$ ). Although each content of methyl-rich foods affects different enzymes or hormones, it has an important role in metabolic activities. Photoperiodically varying methylation balances were also shown to vary widely in multiple ways depending on the methyl level in the environment.

**Keywords.** Photoperiod, Global methylation, *Mesocricetus auratus*, Syrian hamster.

## 1. Introduction

Epigenetic regulations are biochemical processes that modulate gene expression without changes in the DNA sequence (Jaenisch and Bird, 2003). Among these regulations, DNA methylation plays a critical role in gene silencing and expression (Newell-Price et al., 2000; Retis-Resendiz et al., 2021). DNA methylation occurs through the addition of methyl groups to cytosine bases in DNA, and this process is a significant mechanism in regulating gene expression (Moore et al., 2013; Siegfried and Simon, 2010). Photoperiods influence biological rhythms in many animal species, leading to physiological and behavioral changes (Dunlap et al., 2004; Kriegsfeld and Bittman, 2010). Particularly, seasonal animals like Syrian hamsters (*Mesocricetus auratus*) are sensitive to light durations, and changes in these durations can affect reproduction, metabolism, and other biological functions (Goldman and Nelson, 1993; Bradshaw and Holzapfel, 2007). Studies on the effects of photoperiods on DNA methylation have shown that these durations can lead to changes in epigenetic regulations (Stevenson and Prendergast, 2013; Pegoraro et al., 2016).

Nutrition, especially diets containing methyl donor substances, has a decisive impact on epigenetic mechanisms (Anderson et al., 2012; Waterland, 2006; Zhang, 2015). Methyl donor foods provide the necessary methyl groups for methylation processes and directly affect these processes. Therefore, the impact of nutrition on DNA methylation is a crucial factor in understanding epigenetic regulations (Anderson et al., 2012; Waterland, 2006). Because methylation affects gene expression and is transmitted to the next generation, nutrients may have various effects on gene regulation. For example, folate, vitamin B12, choline, betaine, and methionine may be donors in providing methyl groups. (Wolff et al., 1998). These reactions are based on a single carbon exchange; even the microchanges involved in nutrient uptake affect the amount of methylation. Folate is involved in carbon metabolism pathways through the interaction of cofactors such as choline, betaine, and B12. This in turn affects DNA methylation and therefore all reactions, including the amount of mRNA (Selhub, 1999; Maunakea et al., 2010).

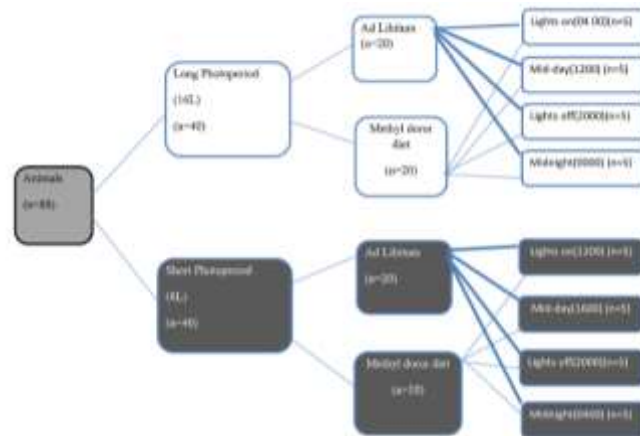
This study aims to investigate the effects of different photoperiods on DNA methylation levels in Syrian hamsters and how this effect can be modulated by methyl donor nutrition. Previous studies have separately examined the impacts of photoperiods and nutrition on epigenetic processes; however, research examining the interaction of these two factors together is limited.

## 2. Material Method

Male adult Syrian hamsters (*Mesocricetus auratus*) used in the experiments were obtained from colonies in the Hamster and Gerbil unit of Çanakkale Onsekiz Mart University Experimental Animals Research and Application Center (ÇOMUDAM). The food and water were *ad-libitum* and the temperature in the rooms was standardized to  $22 \pm 2$  °C. The animals were kept under at least 200 lux of white fluorescent light in the light period in the photoperiod chambers. All methods were carried out with the decision number 2019/06-08 taken from Çanakkale Onsekiz Mart University, Experimental Animals Ethics Committee.

The study was conducted in two separate photoperiods. In the long photoperiod (16 L = 16 hours of light; (from 04.00 to 20.00, the lights are on for 16 hours, 8 hours off) (n = 40)) and in the short photoperiod (8L = 8 hours of light; at 12.00). from 20.00 to 20.00 (lights on for 8 hours, 16 hours off) (n=40) animals were divided into two groups. Methyl Donor nutrient-fed group (n=20) and the Standard nutrient-fed group (n=20). Depending on the time of collection times of tissue and blood samples in each group; Before the lights are turned on (16L;04.00, 8L;12.00) (n=5), after the lights are turned off (16L and 8L;20.00) (n=5), midday (16L;12.00, 8L; 16.00) (n=5) and midnight (16L;00.00, 8L;04.00) (n=5) is divided into four subgroups (Table 1)

*Table 1 Experimental design of male Syrian hamster (Mesocricetus auratus) examining the influence of ad libitum and methyl donor diet supplementation during long(16L) and short (8L) photoperiods. Also decapitations times given as; lights on, middle of the day light, lights off and middle of the night time.*



The feeding process continued for 2 months in total, and at the end of the experiment, hypothalamic tissues were taken from the animals (when the lights turned on in, the middle of the day, the lights turned off, and in the middle of the night) within the periods specified in the subgroups.

### Testes Measurements

Testicular weight was calculated by measuring testicular dimensions with the help of calipers according to the formula in their study by Gündüz and Stetson (2002). Firstly, single testicle volume (STV) was calculated and then paired testicle weights (PTW) were calculated.

### Body Weight Measurement

Hamsters fed with standard nutrients and methyl-rich nutrients were followed by weighing on a weekly basis. Weighing operations were carried out at the hour corresponding to midday (16L; 12.00, 8L; 16.00) for both photoperiods.

### Food Intake

All individuals were taken to single cages in order to make feed follow-ups. A certain amount of feed was placed in each cage and the remaining amount was measured the next day. During the measurement process, the baits in the mouth of the hamsters were emptied and crumbs in the cage were collected. This process was carried out at the time of day corresponding to the middle of the day for both photoperiods. Feed monitoring during the feeding phase, which lasts about 2 months, was done every day.

### Tissue Samples

At the end of the experiment, hamsters were decapitated and the brain was quickly removed. The hypothalamus region of the brain was quickly removed with microdissection scissors under a stereo dissecting microscope in 0.9% NaCl solution, which ensured the survival of the tissue. Tissues taken for each group were taken separately to the eppendorf and immediately frozen in liquid nitrogen and stored in the DNA isolation - up to 84°C.

### DNA Isolation

Total DNA isolation was carried out using the trisole method. Trizol solution was added to the tissue samples, providing 500 µl of Trizol reagent per 50 milligrams of tissue in 2 ml tubes. Chloroform, ethanol, sodium citrate, and the last obtained pellet 0.3-0.6 mL 8 mM NaOH are added and suspended by pipetting up and down. The concentrations and purities of the obtained DNAs were determined by microdrop (Multiskan™ GO Microplate Spectrophotometer, Thermo Scientific™, Finland).

### Measuring whole genome methylation

Global methylation analyses include methods for analyzing the density of all 5m-Cs present in the genome of an organism. Global DNA methylation analysis was performed after DNA denaturation with a commercial kit.

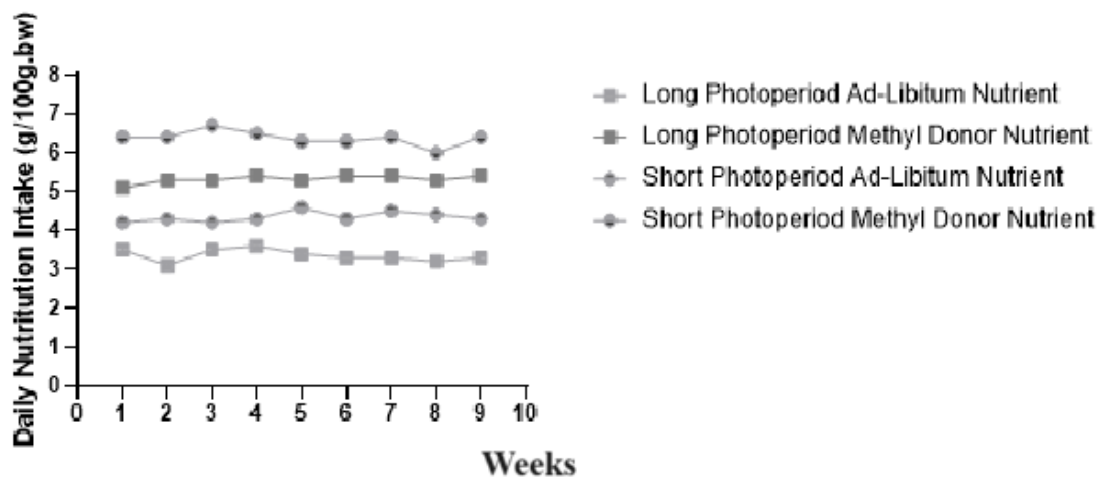
Quantified DNA samples were transferred to the wells as 100ng after calculations were made. Then the final volume was added to the primary antibody to be 100l, and the amount of methylation was determined using the Zymo Research 5mC DNA ELISA kit (California, USA). Samples added to 5-mC containing anti-5-methylcytosine and secondary antibody were incubated for 10-60 minutes of color change. Measurements were made on an ELISA Plate reader ((Multiskan™ GO Microplate Spectrophotometer, Thermo Scientific™, Finland) at 405-450 nm absorbance value. The samples were evaluated by comparison with the negative and positive control DNA samples provided in the kit.

### 3. Results

#### Testicular Weight

Testicular weights were measured on a weekly basis to observe whether the hamsters in the long photoperiod adapted to the short photoperiod.

Testis weight, which was approximately 2 grams in the long photoperiod, shrank over time after being placed in the short photoperiod and decreased to 0.5 grams at the end of the 8th week (Figure 1).

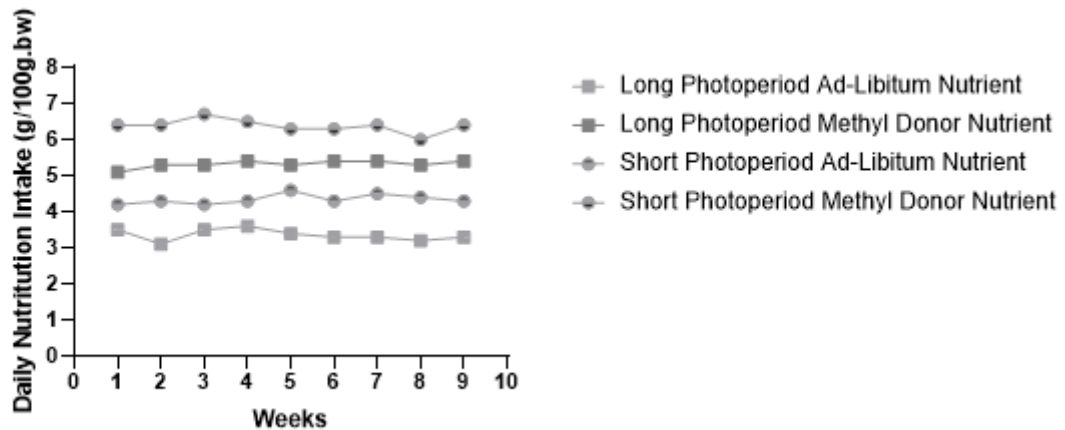


**Figure 1 Paired Testes Weight (PTW) of *Mesocricetus auratus*, which were transferred from 16L photoperiods to 8L photoperiods, were measured weekly to observe seasonal rhythm adaptations. Each point represents mean±SD of n=40 individual for short (8L) photoperiods.**

Syrian hamsters, which were being cared for in a 16L long photoperiod environment, were placed in an 8L (8 hours light-16 hours dark) short photoperiod environment, and their testicular dimensions were followed for 8 weeks. Individuals adapted to the short photoperiod were randomly selected and divided into groups, and experimental groups were formed by feeding both *ad-libitum* and methyl-rich nutrients for 8 weeks.

#### Food Intake

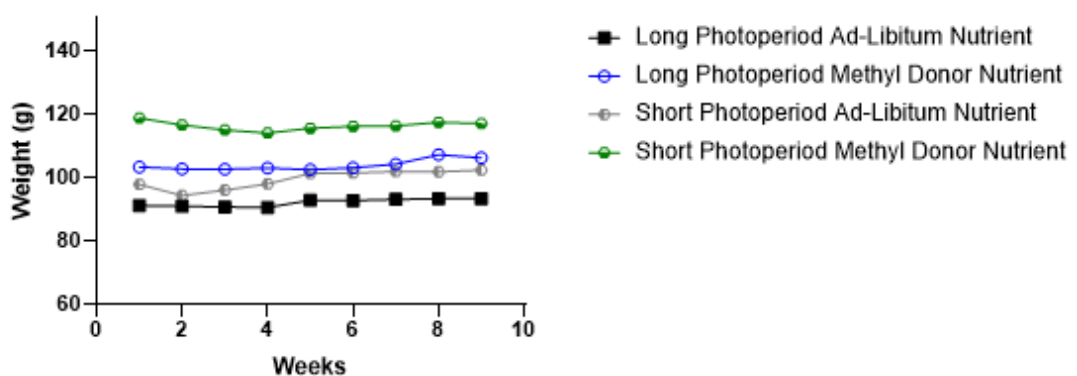
While it was observed that hamsters consumed more food in the short photoperiod ( $p < 0.05$ ), hamsters consuming methyl-rich food consumed more food than those fed *ad-libitum* food ( $p < 0.05$ ) (Figure 2.).



**Figure 2. The ad libitum and Methyl Donor Nutrient Intake of *Mesocricetus auratus* on Long (16L) and short (8L) photoperiods. Food intake was measured daily in single housed animals although data shown here in the figure represented weekly. Values are reported as the mean  $\pm$ SD (n=20)**

### Body Weights

The weight of the individuals in both long and short photoperiods was monitored weekly. In individuals fed with *ad-libitum* food in a long photoperiod (16L), the individuals weighed approximately 90g, and no change was observed in their weight in the following weeks ( $p > 0.05$ ), while in individuals fed with methyl-rich food, an increase was observed after the 6th week ( $p < 0.05$ ). In the short photoperiod (8L), it was observed that the weight of individuals fed with *ad-libitum* food first decreased and then increased slightly after the 4th week, but stabilized again after the 6th week ( $p < 0.05$ ). Additionally, as seen in Figure 3, body weights increased in the short photoperiod compared to the long photoperiod ( $p < 0.05$ ) (Figure 3.).

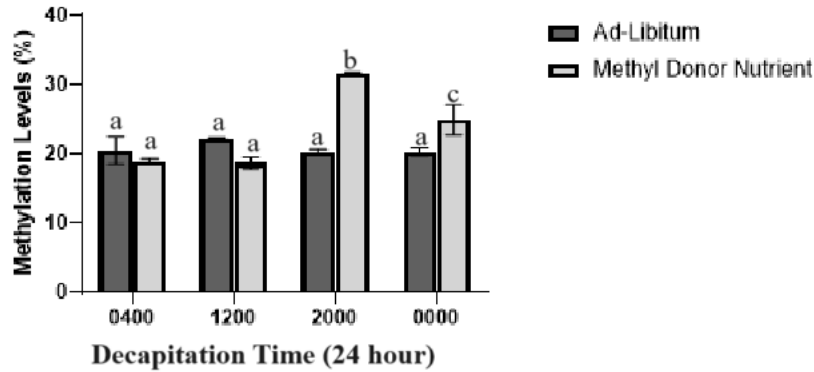


**Figure 3. . The body weight changes of ad libitum and Methyl Donor Nutrient group of *Mesocricetus auratus* on Long (16L) and short (8L) photoperiods. Body weight was measured every week. Values are expressed as the mean  $\pm$ SD (n=20)**

### Methylation percentage

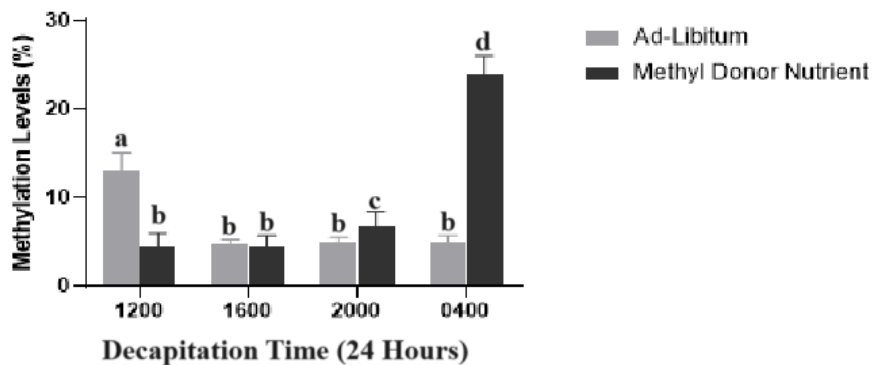
Hamsters are decapitated at 4 different times of the day (when the lights are turned on, mid-day when the lights turn off, and at midnight). After DNA was isolated from hypothalamus tissues, 5mC global

methylation amounts were measured with the help of a kit. The methylation amounts of both short and long photoperiods are given in the table (Figure 4-5).



**Figure 4 Methylation percentage of 16L long photoperiod group fed ad libitum and Methyl Donor Supplemented of *Mesocricetus auratus*. Tissue samples were collected from animals (n=5) at 04.00 (lights on), 12.00 (middle of the day), 20.00 (lights off) and 00.00 (middle of the night) and the experiments tripped each. Values are shown as mean ±SD. Different letters indicate statistical significance (Dunn's post hoc test, p<0.05).**

Methylation percentages in individuals fed with *ad-libitum* and methyl-rich food in a long photoperiod (16L) are given in Figure 4. While the amount of methylation does not change in the control groups ( $p>0.05$ ), the amount of methylation in the 20.00 and 00.00 groups with methyl donor nutrition is higher than in the other groups. The time when the most methylation occurs is when the lights are turned off (20.00;  $p<0.05$ ).



**Figure 5 Methylation percentage of 8L long photoperiod group fed ad libitum and Methyl Donor Supplemented of *Mesocricetus auratus*. Tissue samples were collected from animals (n=5) at 12.00 (lights on), 16.00 (middle of the day), 20.00 (lights off) and 04.00 (middle of the night) and the experiments tripped each. Values are shown as mean ±SD. Different letters indicate statistical significance (Dunn's post hoc test, p<0.05).**

In the short photoperiod, the maximum methylation level is seen to be hypermethylation at the hours corresponding to the dark phase (20.00, and 04.00) ( $p<0,05$ )(Figure 5). In the *ad-libitum* nutrient-fed

group, hypermethylation was observed in 1200, which is the time when the lights were turned on, and there was no statistically significant difference in other periods ( $p > 0.05$ ).

Methylation percentages in hamsters fed with *ad-libitum* and methyl-rich food in a long photoperiod (8L) are given in Figure 5. In *ad-libitum* nutrition groups, the amount of methylation increased only in the 12.00 group. In the groups fed with methyl donors, although the increase was in the 20.00 and 04.00 groups, the highest increase occurred in the 04.00 group (hypermethylation). In the short photoperiod, methylation amounts decreased in both the control and methyl donor-fed groups varying between 37% up to 60% in each group.

#### 4. Discussion

Environmental factors and dietary habits have been shown to significantly influence epigenetic regulations (Faulk and Dolinoy, 2011).

Significant changes in testis size in hamsters exposed to a short photoperiod demonstrate the strong influence of photoperiod on biological rhythms. These findings, consistent with existing literature, support the regulatory role of photoperiod on reproductive functions (Butler et al., 2008; Nelson and Zucker, 1987).

Figure 2. and Figure 3. When evaluated together, it is seen that the weights of individuals increase depending on the season. Feeding capacity increased in direct proportion to the changing body mass. In the study conducted by Karakaş et al. in 2005, it was reported that the eating capacity of the individuals is directly proportional to the size of the individuals. Our study supports this hypothesis. This seasonal pattern of weight gain and loss has been observed in various studies on golden hamsters, and it is believed to be an adaptive response to changing environmental conditions. (Ithinji et al., 2022; Smit and Smit-Vis, 1969; Hoffman et al., n.d; Gordon et al., 1986; Figala et al., 1973; Rosen, 1973) Since these rhythmic changes occur concerning metabolic activities, it comes to mind that they may play a role in monitoring gene expression (Wang and Zhou, 2021; LaSalle et al., 2013).

The content of methyl-rich food is slightly different than *ad-libitum* eating, which may be high due to the proportional work of appetite-stimulating hormones and growth hormones. Studies on both humans and animals have shown that the developmental functions of methyl-rich at a young age are positively affected (Irvine et al., 2022; Ryan et al., 2018; Ishii et al., 2014; Paternain et al., 2016; Saha et al., 2019).

The findings of this study revealed that the hamsters fed the methyl donor-supplemented diet exhibited a significant reduction in *ad-libitum* food intake compared to the control group (Chayama et al., 2016). Additionally, the supplemented group showed a slower rate of body weight gain over time, suggesting that the methyl donors may have had a regulatory effect on energy metabolism and body weight homeostasis (Kohguchi et al., 2004; Steinlechner et al., 1983).

DNA methylation patterns are generally stable, but recent evidence suggests that they can undergo dynamic changes in response to various environmental cues (Szyf and Bick, 2012). Importantly, the dynamic nature of DNA methylation is not limited to the early stages of development. Studies have revealed that methylation patterns exhibit distinct circadian rhythms, fluctuating cyclically over the course of the day (Wang and Zhou, 2021; LaSalle et al., 2013) This intriguing phenomenon suggests that the epigenome is not a static entity, but rather a highly responsive and adaptive system, capable of aligning its activity with the external environment (Wang and Zhou, 2021; LaSalle et al., 2013).

Understanding the epigenetic mechanisms underlying the relationship between diet and gene expression is crucial for developing targeted interventions to promote health. Recent research has highlighted the role

of DNA methylation, a key epigenetic modification, in mediating the effects of dietary factors on gene regulation and phenotypic outcomes (Daskalakis and Yehuda, 2014; Roth, 2013). Specifically, studies in animal models have demonstrated that exposure to different dietary regimes, such as *ad-libitum* feeding versus methyl donor supplementation, can induce both transient and persistent changes in DNA methylation patterns (Eckstein et al., 2017; Moghadam et al., 2015) changes that may have significant implications for the development and progression of stress-related psychiatric disorders (Klengel et al., 2014; Weaver, 2009).

In the golden hamster, a well-established model organism for investigating the influence of environmental factors on epigenetic processes, researchers have begun to elucidate the complex relationship between dietary exposure and epigenetic reprogramming (Hing et al., 2014). Exposure to *ad-libitum* feeding during critical developmental windows has been linked to aberrant DNA methylation profiles in genes associated with the hypothalamic-pituitary-adrenal axis, a key neuroendocrine system involved in the stress response (Bick et al., 2012). Conversely, supplementation with methyl donor compounds, such as folate, vitamin B12, and betaine, has been shown to counteract these diet-induced epigenetic changes, potentially conferring resilience to the detrimental effects of stress exposure (Klengel et al., 2014; Roth, 2013).

The percentage of methylation depends on the presence of methyl donor and methyl transferase in the environment. Diet may modify DNA methyltransferase activity. Within the scope of the study, the amount of methylation increased when the lights were turned off in both photoperiods (Figure 4-5). In the study conducted by Xia et al. on mice in 2015, methylation levels in the liver tissue were shown to peak when the lights turned off and in the dark phase. The data we obtained in our study match the literature information. In cases of hypermethylation, gene expression is also shut down as CpG sites are methylated and turned off (Mathers, 2007). Considering that the Syrian hamster is nocturnal, it is not yet known which gene regions were turned off.

Although each ingredient affects a different enzyme or hormone, studies show that methyl-rich foods are effective in metabolism (Wu et al., 2020; Villatoro-Santos et al., 2020). Taken together, these findings suggest that optimal methyl donor nutrition, in combination with appropriate environmental cues, may be essential for maintaining reproductive health and performance in *Mesocricetus auratus*. In future studies, it is planned to examine epigenetic changes at the gene-specific level.

## 5. Acknowledgments

GÜLSÜM AKKUŞ, who took part in this study, was supported by the Council of Higher Education (YÖK) throughout her PhD Program, within the scope of YÖK 100/2000 Doctoral Scholarship Program in the field of Human Brain and Neuroscience, and within the scope of TÜBİTAK (Turkish Scientific and Technological Research Council) 2211-A Domestic Doctoral Scholarship Program.

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