VILNIUS UNIVERSITY
LIFE SCIENCES CENTER

NOJUS KATKEVIČIUS

THE EFFECTS OF ARTHROSPIRA PLATENSIS ON WISTAR RATS' PHYSICAL ACTIVITY AND LONGEVITY

Master's thesis

Neurobiology study program

Thesis supervisor
Prof. Osvaldas Rukšėnas

Consultant
Prof. Valentina Vengelienė

Reviewer
Assoc. Prof. Rokas Buišas

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Biophysics Institute of Biosciences
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INTRODUCTION

Arthrospira platensis, more commonly known as *Spirulina,* is a fresh and marinewater cyanobacterium that exhibits nutritional, pharmaceutical, cosmetological and various other properties, its nutritional properties and plant-protein count are unparalleled (F. Jung and A. Kruger-Genge, 2019). Known for its easy protein uptake due to its ease of digestibility (S. Devi, 2018) and its supplemental value when taken along with other proteins it helps increase nutrient uptake when paired with the right diet (A.P. Batista, et al., 2019).

Use of *Spirulina* not only as a dietary supplement, but also as a means to improve health and longevity, its functions in improving immune response (N.A. El-Shall, 2023) and its ability to reduce inflammatory reaction and provision of antiviral properties (R. Tripathi, 2021) has been a subject of investigation for a long time.

The applications of *Spirulina* have been studied on animal subjects, particularly chicken broilers, upon which it has seemingly bestowed increased longevity (El-Hady, 2022). Still, further investigations are required in order to test the full capabilities of this superfood.

In this thesis we will overview the health significance, dietary and pharmacological properties and various other possible applications of *Arthrospira Platensis* using an animal, female Wistar rat, model. The hypothesis is that rats consuming differing amounts of *Spirulina* should show lowered expressions of aging as shown by their locomotor and circadian activity and blood results.

The aim of the study is to measure the expressions of aging in Wistar rats. Therefore, the tasks for this study are:

1. To evaluate the changes on locomotor activity following treatment with *Arthrospira platensis*
2. To determine the effect of *Arthrospira platensis* consumption on the subjects'circadian activity
3. To compare the changes in hematological test parameters following two trials in which venous blood is gathered from puncturing the rats' tails.

1.1. CLASSIFICATION AND MORPHOLOGY OF *ARTHROSPIRA PLATENSIS*

Arthrospira platensis, or more commonly *Spirulina* is a blue-green microalgae that has been used as a food source throughout the ages by many civilizations, the first recorded use of which being in the Mesoamerican Aztec civilization, where Spanish colonisers in the sixteenth century described local fishermen gathering seaweed with nets, drying and shaping it in a sort of cake or bread (Ali & Saleh, 2012). Research into *Spirulina* picked up along with a boom in chemical analyses, at a time when single-cell organisms were thought to be the most efficient form of protein intake; following the discovery that *Spirulina* had a protein content of sixty to seventy per cent of its dry mass, the industry saw a shift in *Spirulina*-based marketing (Matufi & Choopani, 2020).

Arthrospira Platensis is a species of the *Spirulina* genus categorized as follows in Table 1.

Table 1. Systemic classification of *Arthrospira Platensis*

Domain	Bacteria
Phylum	Cyanobacteria
Class	Cyanophyceae
Order	Spirulinales
Family	Spirulinaceae
Genus	*Spirulina*

What sets *Spirulina* apart from other microalgae is that it does not have cellulose cell walls, but rather a thin sheath of murein. This provides benefits and limitations to the application of *Spirulina:* the lack of a cell wall makes it easy to digest and intake its nutrients, but the murein sheath poses a difficulty when attempting to extract any of its components, such as phycocyanin, for pharmacological use (Larrosa et al., 2018).

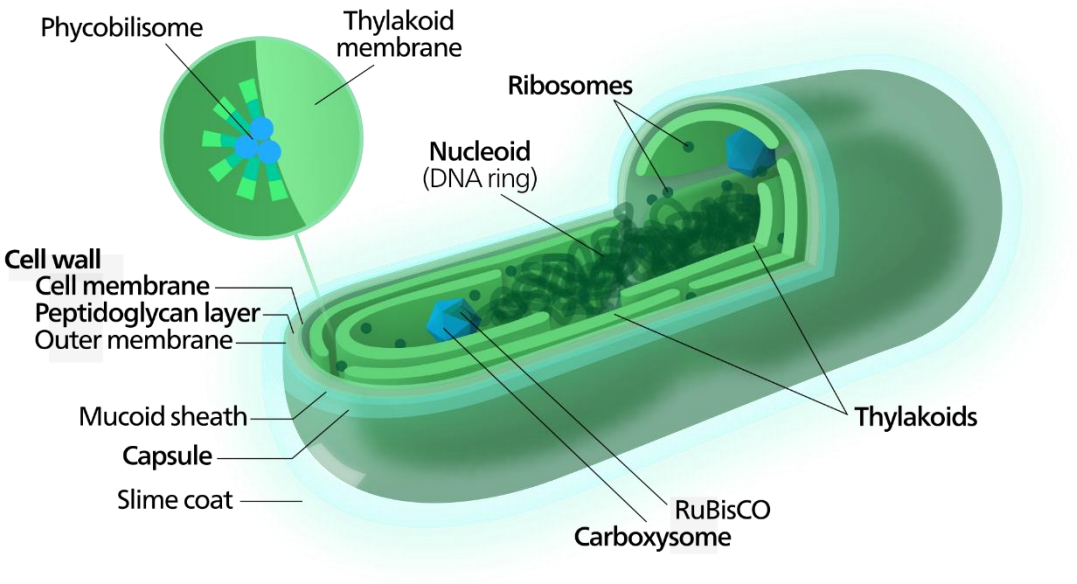

Fig. 1.1. Composition of a *Spirulina* cell 0.3-0.5 µm, illustration adapted from K. Song (2023).

The main morphological and most distinctive feature of the *Spirulina* genus is its arrangement of multicellular trichomes in a helix shape across its whole length; the blue-green filaments of the trichomes are made of cells dividing in one plane and show clear cross-walls, these filaments float freely, move by gliding, and are surrounded by a thin sheath. The ends of the filaments may be rounded or pointed. Their width ranges from about 6 to 12 µm (sometimes up to 16 µm), and they are made of short, cylindrical cells (Ali & Saleh, 2012). *Spirulina's* cell wall is composed of four layers, the first of which is made indigestible to humans as it contains β1 and β2-glucan, which are polysaccharides, also referred to as dietary fibers; these fibers pass the digestive tract without being processed but are rather fermented, thus allowing for a healthier microbiome in the gut through the absorption of its byproducts (Wells et al., 2016).

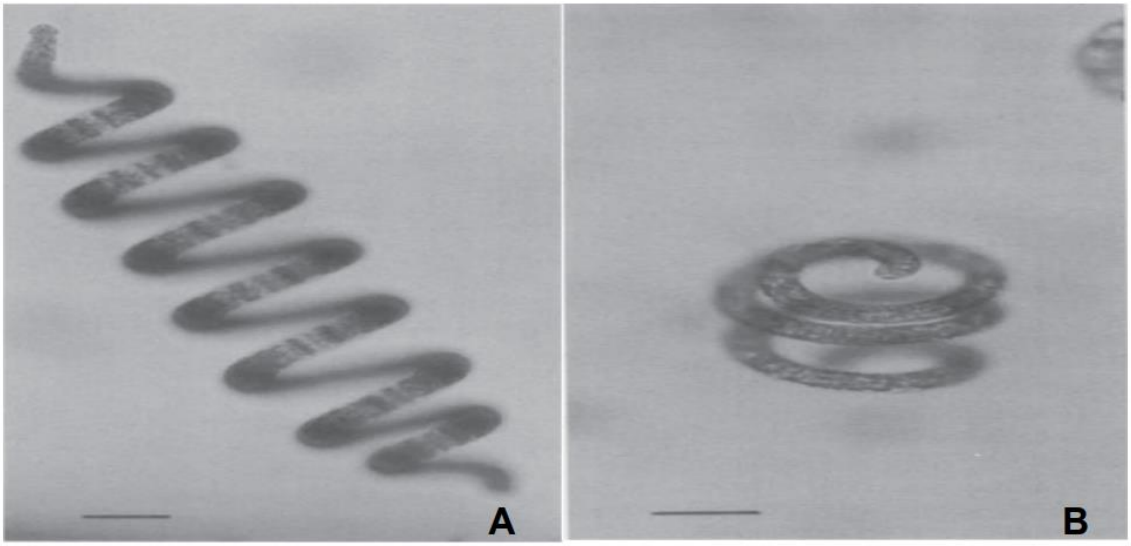

Fig. 1.2. Light micrograph showing *Arthrospira maxima* (A) and *Arthrospira platensis* (B). The bar at the bottom of the image represents 20µm. Light microscopy image adapted from Ali & Saleh (2012).

Murein, or peptidoglycan, is a protective component of the bacterial cell wall present in almost all bacteria. Its main purpose is to maintain cell shape, guard the cell from osmotic pressure and to protect it from any external threats (A. Galinier, et al., 2023). In a normal bacteria cell, peptidoglycan forms a web-like coating around the cytoplasmic membrane and is important for elongation of the cell by expanding the coating and for cell division by forming the septum (S. Garde, et al., 2021). The polymer murein is composed of strands of glycan composed of alternating N-acetylmuramic acid (MurNAc) and N-acetylglucosamine (GlcNAc) units (P.D.A. Rohs, T.G. Bernhardt, 2021).

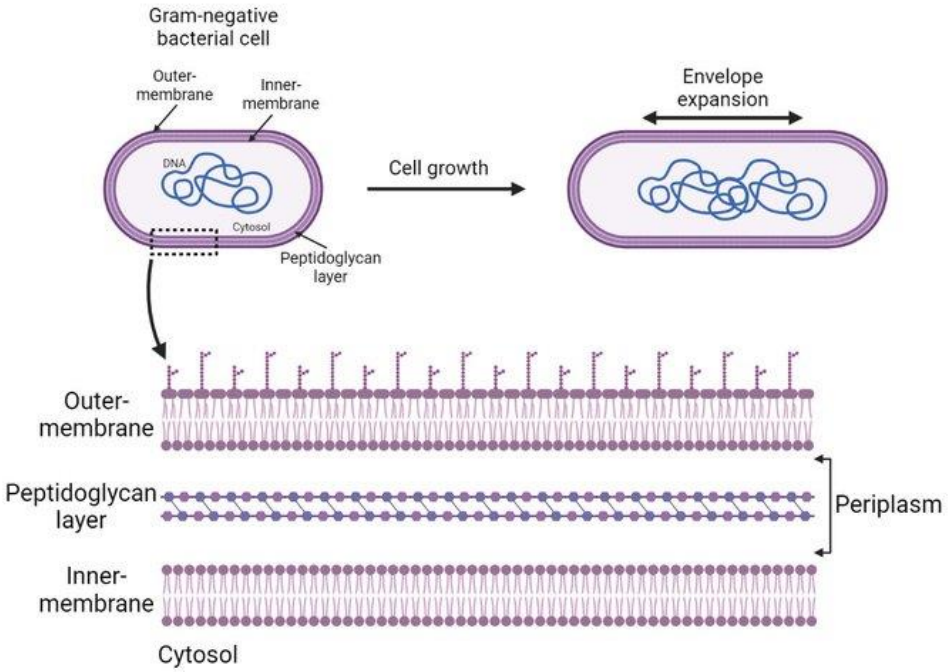

Fig. 1.3. A schematic on bacterial cell envelope in a Gram-negative bacteria, image taken from IndiaBioscience, *Understanding bacterial cell wall expansion offers new antibiotic targets*

1.2. NUTRITIONAL PROPERTIES OF SPIRULINA

Spirulina contains various macro and micronutrients, such as proteins, omega-3 and omega-6 fatty acids, vitamin A, various subgroups of vitamin B and many kinds of minerals, of which iron in particular has a great quantity. It has therefore been labelled a superfood for its near-complete composition of every vital nutrient for the human body (Jung et al, 2019).

The protein variety in *Spirulina*'s composition is complete, meaning it contains every essential amino acid, although relatively lacking in cystine, lysine and methionine when compared to animal-based products. Still, *Spirulina* is vastly superior in protein count when compared to any other plant-based food (F. Jung and A. Kruger-Genge, 2019). Z. Tavakoli et al. (2025) states that *Spirulina's* protein content is as high as approximately 45 grams per 100 grams, with the remainder of the biomass being 21 grams of carbohydrates, 17 grams of lipids and the remainder of ash (~9 grams) and moisture (~7 grams). However, measuring the full macronutrient composition of *Spirulina* may be difficult to perform accurately, as the chemical composition of *Spirulina* is dependent on the microalgae's species, the geographical region from which it originates, its environmental conditions, the cultivation

methods used and the medium in which it's grown, as well as the techniques used for the metabolite extraction (A. Cosenza et al., 2024).

An important part of a food's true nutritional value is its digestibility, which plays a great role in *Spirulina*'s nutritional value, since its cells do not have cellulose walls, but rather a thin murein layer, this allows for a very efficient protein uptake when digesting, up to 85% (S. Devi, 2018). Not only is it easily digestible, but it has also been proven that *Arthrospira Platensis* increases the protein uptake in the organism when taken along with other proteins (A.P. Batista, et al., 2019).

Spirulina has also exhibited potent antioxidant properties, in a study conducted by P. Bermejo et al. (2008), *Spirulina* was found to contain a protean specific to *Spriluna* (*Spirulinaplatensis* protean) and a biliprotein phycocyanin, which inhibited the production of peroxyl radicals, hydroxyl radicals and the lipid peroxidation process. Lipid peroxidation is a process during which lipids are attacked by free radicals which results in the creation of an organic, oxygen-reactive radical called a peroxyl radical (M. Zana et al., 2007). The production of peroxyl radicals leads to irreversible damaging processes upon the cell membrane and eventually cell death (J. Li et al., 2021).

1.3. PHARMACOLOGICAL PROPERTIES OF SPIRULINA

Spirulina has been noted to have various applications in clinical trials in lab animals and human subjects alike. Properties such as inhibition of viral replication, anti-inflammatory effects and strengthening of immune responses have been observed in various *Spirulina*-based experiments.

The anti-inflammatory properties of *Spirulina* are due to its composition, notably phycocyanin, a pigment-protein complex that inhibits inflammatory reaction by preventing cytokine formation, which is responsible for the upregulation of inflammatory immune response, stops cyclooxygeanase-2 expression, thus not allowing prostacyclin precursors to form, causing the inflammatory reaction to halt (R. Tripathi, 2021). *Spirulina* does not only stop inflammation reactions, but also helps immune responses by increasing monocyte and natural killer cell activity, which are vital in maintaining immune response (N.A. El-Shall, 2023).

Small doses of *Arthrospira Platensis* were noted to inhibit the replication of viruses, with higher doses stopping replication altogether. The antiviral properties of this cyanobacterium lie within calcium-spirulan, which has been proven to reduce the virus's ability to penetrate into the cell without causing any toxic effects to the host (S. Singh et al., 2020). In an *in vitro* study conducted by Chen et al. (2016), multiple strains of influenza, including oseltamivir, an antiviral drug, resistant variants were treated with cold water extract of *Spirulina*. Treating the cells with 0.375, 0.75, 1.5 and 3.0

mg/ml of *Spirulina* yielded 12.12%, 22.90%, 58.73%, and 89.00% of viral plaque inhibition respectively for the main strain (A/H1N1) when compared to untreated controls. It has been observed that 3 milligrams of *Spirulina* completely halted the plaque formation in certain strains, including the oseltamivir resistant strain. This bodes well for treatment possibilities of patients who have contracted drug-resistant variants of influenza, possibly halting the spread of the disease completely before it has a chance to elicit symptoms in the organism.

The low toxicity of *Arthrospira Platensis* brings good implications for use of treatment in many dietic and clinical cases. Its low toxicity was observed when Sprague-Dawley rats were given an acute dose of 5000 mg/kg for 14 days. No adverse effects were observed, so the study was repeated again with 3000 mg/kg of *Spirulina* being subacutely administered *per os* for another 14 days. No deaths were observed during the study, leading the researchers to conclude that the lethal dose is above 5000mg/kg. Continuing, no significant changes in body weight were observed in treatment and control groups for the study. Further analyses, including blood and urine sampling did not provide any perceivable disparities apart from alanine transaminase and aspartate transaminase, which are both liver enzymes, whose blood levels were lower than usual (Y.-H., Chen et al., 2016).

Spirulina has been found to possess a property known as chelation, which is the metal-ligand binding process where a metal ion is collected by two or more coordination bonds, with such a binding being able to remove heavy metal toxins from the blood of a person suffering from metal poisoning from metals such as lead, mercury, cadmium and arsenic (J. W. Harrington & S. Bora, 2018). In a number of human artificially-induced heavy metal poisoning trials, where conventional chelating agents were not sufficient, *Spirulina* seemed to alleviate the symptoms of heavy metal intoxication (S. Bhattacharya, 2020).

Spirulina is also known for its hematopoetic properties, where in a study performed by S. Moradi et al. (2023) human adults with ulcerative colitis, a condition often leading to chronic anemia, consumed 1 g of *Spirulina* a day along with a control group consuming a placebo had their blood taken and compared, the results were that the treatment group had significantly ($p=0,04$) higher serum iron than the control group, as well as a greatly ($p=0,004$) higher mean corpuscular volume; meanwhile the control group showed greatly lower red blood cell count ($p=0,01$) and hematocrit ($p=0,03$) following 8 weeks of *Spirulina* consumption. Coagulative properties in an automated blood test were also investigated in a study by P. Koukouraki et al. (2020), where *Spirulina's* effects on thrombin and platelet-activating factor, both factors involved in platelet aggregation by inflammation or injury, were measured to see how they react to the compounds extracted from Spirulina, mainly phycocyanin and phycocyanobilin; the study showed that concentrations of phycocyanobilin ranging from 1 to 50 μg/mL dose-dependently inhibited platelet aggregation induced by collagen (dose of 10

μg/mL) or arachidonic acid (100 μM dose), with an IC_{50} of approximately 10 μg/ml. Comparing to this, however, another study showed that mice being fed either low (0,82 g/kg) or high (4,10 g/kg) doses or Spirulina coupled with Chlorella biomass had relatively no influence on the automated blood test results of mice receiving treatment compared to the control group, as seen in Table 2. (P. Wang, et al., 2025).

Table 1.1. Blood routine assay in mice, P. Wang, et al., 2025. Data is presented as mean±SD, the statistically significant ($p<0,05$) indices are denoted by superscript letter a and b, which indicate which groups are statistically similar or different (a and b are significantly different). Chlorella biomass and Spirulina biomass are marked ChB and SpB with dose indicated by letters –L for low and –H for high (P. Wang, et al., 2025).

	Control	**ChB-L**	**ChB-H**	**SpB-L**	**SpB-H**
HGB (g L^{-1})	156.3 ± 8.1^{a}	153.5 ± 9.8^{a}	149.4 ± 8.8^{a}	152.4 ± 9.8^{a}	154.2 ± 7.9^{a}
WBC (10^9/L)	4.61 ± 0.72^{ab}	3.90 ± 0.60^{b}	3.98 ± 0.84^{ab}	4.38 ± 0.77^{ab}	5.34 ± 1.14^{a}
RBC (10^{12}/L)	9.92 ± 0.37^{a}	10.11 ± 0.53^{a}	9.70 ± 0.77^{a}	9.30 ± 0.99^{a}	10.15 ± 0.46^{a}
HCT (%)	51.21 ± 2.32^{ab}	53.92 ± 1.50^{a}	50.68 ± 2.55^{b}	53.84 ± 1.79^{ab}	50.96 ± 4.64^{ab}
LY (%)	63.07 ± 4.18^{ab}	60.87 ± 2.81^{b}	63.74 ± 5.59^{ab}	58.89 ± 4.68^{b}	66.94 ± 4.88^{a}
BA (%)	0.13 ± 0.05^{a}	0.13 ± 0.05^{a}	0.13 ± 0.05^{a}	0.12 ± 0.04^{a}	0.13 ± 0.05^{a}
EO (%)	3.20 ± 1.00^{a}	3.74 ± 1.50^{a}	4.07 ± 0.99^{a}	4.07 ± 1.35^{a}	3.15 ± 1.08^{a}
NEU (%)	29.60 ± 3.02^{ab}	28.81 ± 2.47^{ab}	32.16 ± 6.48^{a}	28.44 ± 5.04^{ab}	25.26 ± 4.1^{b}
PLT (10^9/L)	1095.2 ± 148.4^{a}	1113.2 ± 127.9^{a}	1083.8 ± 103.5^{a}	1073.4 ± 90.3^{a}	1014.9 ± 111.8^{a}
PCT (%)	0.82 ± 0.15^{a}	0.87 ± 0.16^{a}	0.76 ± 0.11^{a}	0.83 ± 0.12^{a}	0.77 ± 0.17^{a}

Spirulina's effects on aging, cell aging in particular, were investigated in a study conducted by K. Machihara et al. (2023) where it was stated that reactive oxygen species, which are produced by mitochondria, but also impair their function via cellular processes related to cell aging, are scavenged

by *Spirulina's* polysacharide complex, therefore leading to the recuperation of mitochondria and collagen synthesis without the activation of cytokine expression, meaning this method of reactive oxygen species scavenging does not lead to inflammatory processes.

1.4. INVESTIGATION OF SPIRULINA EFFECTS USING ANIMAL MODELS

Different kinds of animal-based experiments have been performed to study the effects and health benefits of *Arthrospira Platensis,* with most findings coming from research focused on chickens. For many years, putting antibiotics in poultry feed was a common practice in order to increase meat animals' resilience to pathogens, disease, to reduce mortality in grown animals, thus increasing efficiency and production rates for meat and animal-based products. There were great problems with this practice, however: bacteria under constant exposure to antibiotics would become resistant to them, thus making treatment in case of an outbreak very difficult, the tissues and cells in the animals' meat would accumulate these agents and continue to pass them down to the consumer, spreading the needless use of treatment substances and potentially making simple pathogens very dangerous. One more aspect to this would be that constant exposure to antibiotics disrupts the natural conditions of the gut bacteria, making people and animals more vulnerable to gastrointestinal tract diseases and other such disorders related to the changes in the natural microflora of the stomach (Haque, 2020). As such, a new method for increasing the lifespan and survivability of domestic fowl (and other farm or otherwise domestic animals), was needed, one that would also provide a healthy and nutritious diet. Recently, an emerging trend focused on simple algae as a substitute for pharmaceutical substances and was found with great results: broiler chickens who were fed small-percentage mixtures of *Spirulina* in their feed were noted to have increased growth rate and immune responses, further growing with higher concentrations of *Spirulina* (El-Hady, 2022).

Continuing the chicken-*Spirulina* research, the antiviral and antipyretic properties of the algae are also quite potent: as demonstrated by S. Singh et al. (2020), low concentrations of *Spirulina* inhibit the multiplication of viruses, with greater doses stopping replication completely. Immunological research performed on broiler chickens (Awad, A. M. et al., 2023) where vaccines for diseases related to the highest poultry mortality were co-administered with small (0,3 % of total feed) doses of the *Arthrospira Platensis* algae showed an immune-stimulating effect by decreasing viral-related mortality.

Another study conducted included broilers being exposed to the mycotoxin Aflatoxin B1 (AFB1) with the additional supplementation of *Spirulina,* using one day-old chicks throughout the experiment for six weeks. It was discovered that while the mycotoxin reduced the animal weight and

increased hepatotoxicity indicating factors such as alanine transaminase (ALT), aspartate transaminase (AST) and alkaline phosphatase (ALP) by as much as 10-40% and kidney performance factors such as urea and creatinine by as much as 50-100% when compared to the control groups. However, these factors were reduced by at least 50% or remained close to control values when AFB1 was administered together with *Spirulina supplementation* (Salah, A. S., et al., 2025).

2. MATERIALS AND METHODS

The study was conducted in Vilnius University Life Sciences Center, where the rats were present for monitoring and study in the faculty's vivarium. Bioethics permission number: G2-189.

2.1. PREPARATION OF SAMPLES

The *Arthrospira Platensis* samples used for this study were provided by JSC Bioneurema who uses their own patented method to prepare and extract *Spirulina* in a way that would allow for its use in the experiment while still maintaining its properties of interest. The spirulina was then put into bottles of varying concentration of 10% or 20% water solutions made using 125µg of *Spirulina.* The substance was made in 400ml bottles and placed into the cages of lab rats. The bottles with *Spirulina* had to be stirred everyday and changed every two days following the instructions of the provider.

2.2. ANIMALS

Twenty-four female Wistar rats, aged 7 months and weighing 270, 281 and 286 grams (group means) were observed in this study following the consumption of either control substance (water) or the differing concentrations of *Spirulina* (10% and 20%). Three study groups divided into six cages of four rats each were therefore gathered for this study.

2.3. BEHAVIORAL EXPERIMENTS

The open field test is designed to monitor rats' physical activity and expressions of anxiety and curiosity-like behavior. How much a rat moves, how much time it spends in the open field or dark corners of the maze are used to evaluate the rats' behavior.

To conduct the behavioral part of the experiment, rats from each cage were placed, four at a time, one rat per cage, into the four sections of the open field maze. Observing four rats at the same time in the open field test allowed for increased efficiency of data analysis for this study. Before beginning the test, the rats were brought into the test chamber to accommodate to the environment for fifteen minutes, then they were placed into the open field maze one cage at a time, then they were recorded for thirty minutes, then removed from the maze, the maze then being cleaned with an alcohol solution and then the rats were continued to be placed in the open field test until all animals were recorded. The illumination at the open field of the maze did not exceed 80 luxes, while the dark corners of the area were no higher than 50 luxes in brightness. The chamber in which the rats were recorded was closed off and isolated as to reduce any stress caused to the rats by outside noise.

2.4. PHYSICAL ACTIVITY MONITORING

Measurement of physical activity was conducted using Biobserve Viewer III software to monitor general movement patterns of the rats, also other indicators of physical activity – grooming and rearing were monitored by observing and counting these occurrences manually while viewing the video material. The recordings lasted 30 minutes each, with four rats participating in a single recording session.

2.5. BLOOD SAMPLES

Blood samples were taken from the rats three months after the start of the experiment. The samples were collected according to all ethical and aseptic guidelines. To gather the sample, a sterile 22G needle was used to puncture the tip of the rat's tail, the blood was then gathered into a glass capillary tube (20 μl) and processed via hematology Boule Exigo Eos Vet 400 veterinary hematoanalyzer.

2.6. STATISTICAL ANALYSIS

Statistical analysis was performed using JASP software provided as freeware by the University of Amsterdam. The selected statistical model for data analysis was the repeated measures ANOVA test. Following the test descriptive statistics were performed. The data underwent assumption checks using the Greenhouse-Geisser sphericity test. Statistically significant data ($p \leq 0,05$) underwent additional post hoc testing using the Bonferroni correction with a confidence interval of 95 percent. Additional significance of correlation between groups was measured using simple main effects.

3. RESULTS

For this experiment measuring the locomotor activity was performed by measuring travel distance in the open field test, as well as tracking the visits to the center and time spent there. Other behavioral parameters were also gathered, such as groomings and rearings, the latter were divided into center rearings, where the rats stood on their rear legs without any support, and wall rearings, where the rats leaned against a wall for support.

For the circadian activity sensor test the amount of times a rat moved as indicated by the sensors as well as the time spent moving were kept track of. These parameters were tracked separately for the light period of the experiment, the dark period as well as the total amount of movement events and time spent moving.

For the hematoanalysis, a general blood count test was performed, but only the hematopoetic factors were analysed for reasons relating to the main goal of the study. These indices include: red blood cell count, hemoglobin and hematocrit.

It is important to mention that while the experiment measured the longevity of the rats, one rat from the 10% treatment group died on the third month of treatment - subject 14.

3.1. LOCOMOTOR ACTIVITY (OPEN FIELD)

The distance traveled in the open field maze throughout the five months of the experiment changed as shown in Figure 3.1. no statistically significant data was found ($p = 0.773$), however, upon closer look it is evident that the parameters changed non-linearly, particularly during the second and third months of treatment, but neither results were significant, with repeated measures ANOVA having returned $p = 0.991$ for Treat2 and $p = 0.745$ for Treat3.

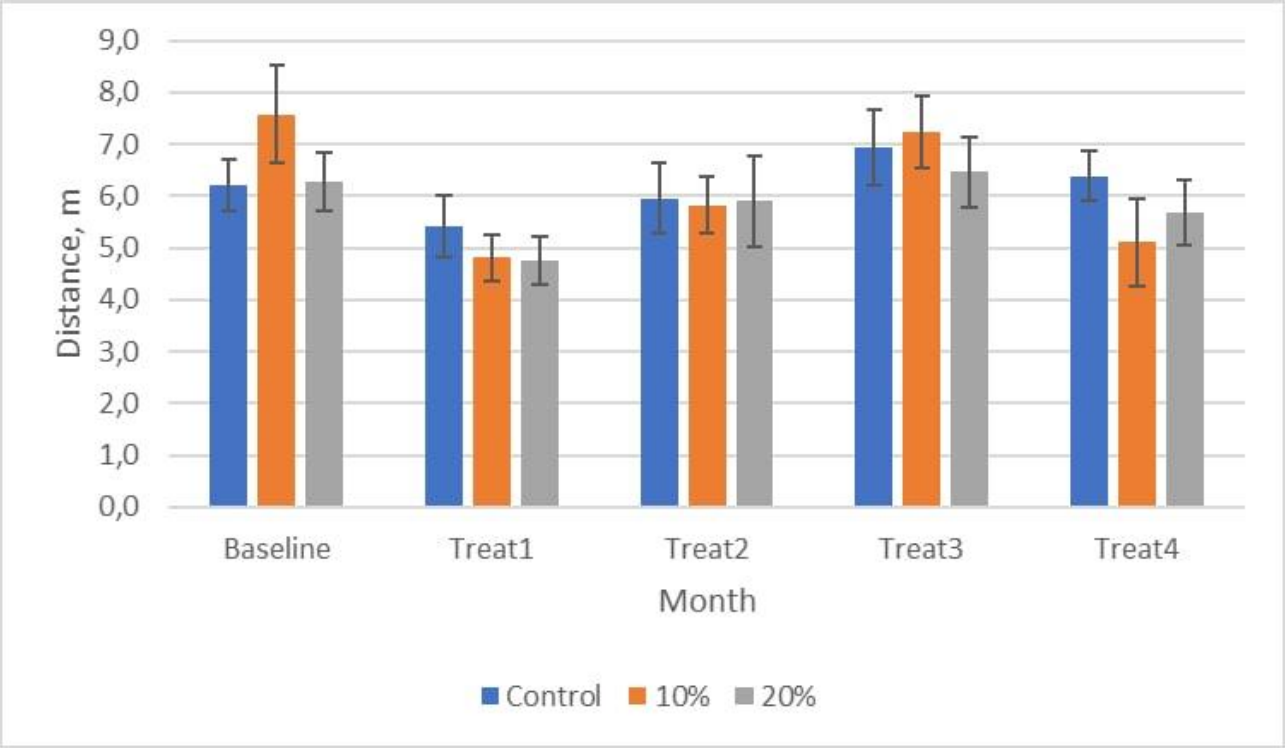

Fig. 3.1. Distance traveled, m. N=23, data presented as mean ± SEM.

Time spent in the center, measured in seconds, had similar baseline results, with the 20% group seemingly lagging behind in the first (3 ± 0,8 s) and second (4 ± 1,9 s) months of treatment, this was lower in the first month of treatment when compared to the control (13 ± 4,1 s) and 10% treatment (9 ± 2.3 s) groups, it was also lower in the second month of treatment when compared to the control (12 ± 4 s) and 10% treatment (9 ± 2,9 s), however statistical analysis showed that the results for the first month of treatment were borderline insignificant with $p = 0.063$ and for the second month the results were insignificant with $p = 0.133$. Overall the results were insignificant ($p = 0.417$) for the whole experiment and consulting Fig. 3.2. it is evident that the results changed non-linearly.

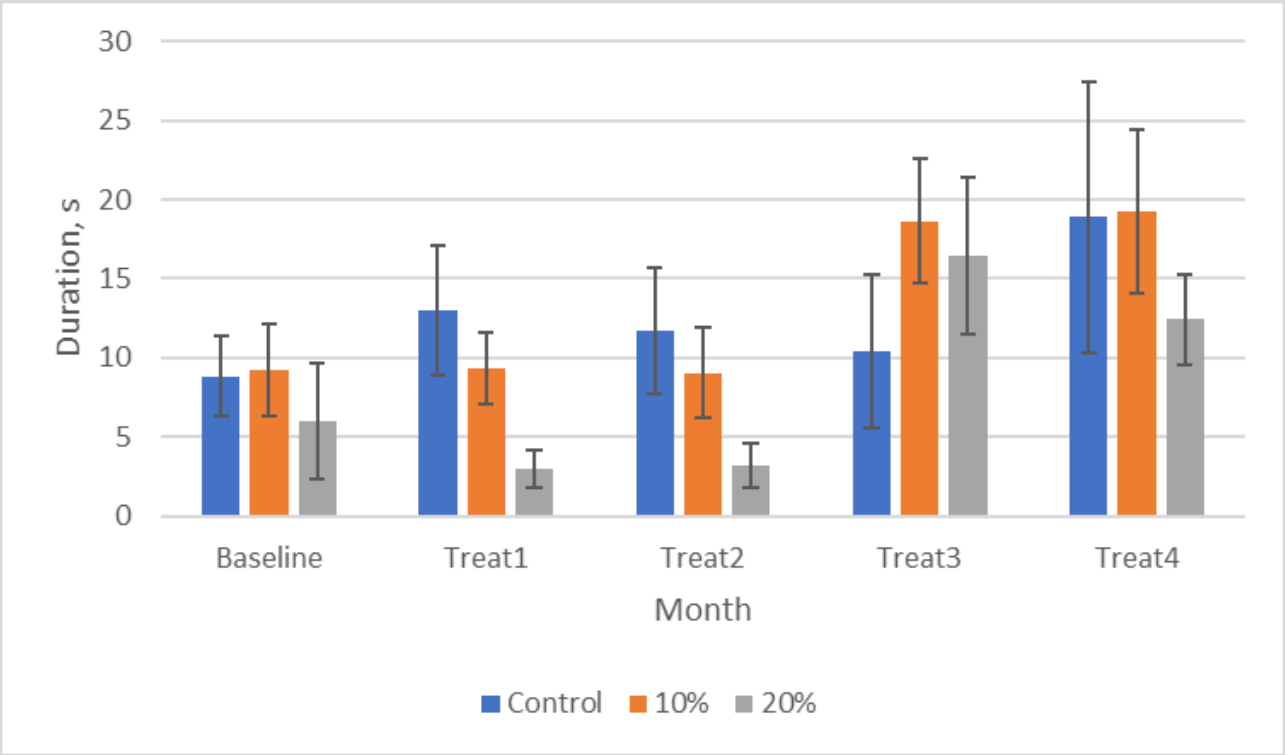

Fig. 3.2. Time spent in center measured in seconds. N=23, data presented as mean ± SEM.

Visits to the center of the maze were counted and the results show that for the baseline and the first two months following treatment the 20% group lagged behind the control and treatment groups. For the baseline the 20% treatment group had an average of 5 ± 1.7 visits, while the control group had 10 ± 3.1 and the 10% treatment group had 11 ± 3.1 visits to the center, this data, however, was confirmed to be insignificant, the ANOVA test having returned a p value of 0.242. Only the second month following treatment showed a clear difference ($p = 0.012$), however, following Bonferroni correction the results came back as $p = 0.133$, leading to believe that the results were not as great as may have been initially thought.

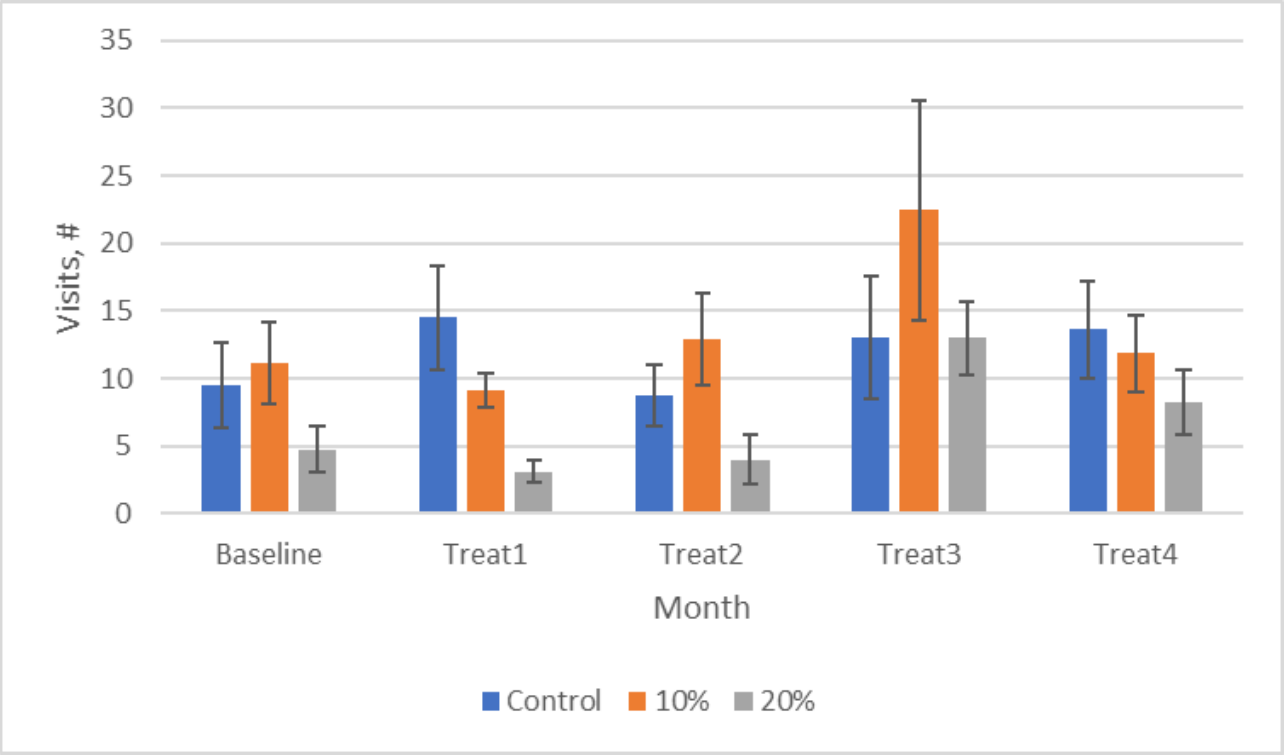

Fig. 3.3. Number of visits to the center of the open field maze. N=23, data presented as mean ± SEM.

Groomings were counted and the results showed that only the baseline was significantly ($p = 0.009$) different between the test subjects, with the control group averaging 5 ± 0.6 groomings, the 10% treatment group 3 ± 0.3 and the 20% treatment group showing 3 ± 0.3 groomings per session, the Bonferroni correction showed that the difference between the control group and the 10% treatment group were the most significant ($p = 0.03$), meanwhile the correction for the 10 and 20% returned a p value of 0.193 and the 20% treatment and control group showing a Bonferroni p value of 1.0.

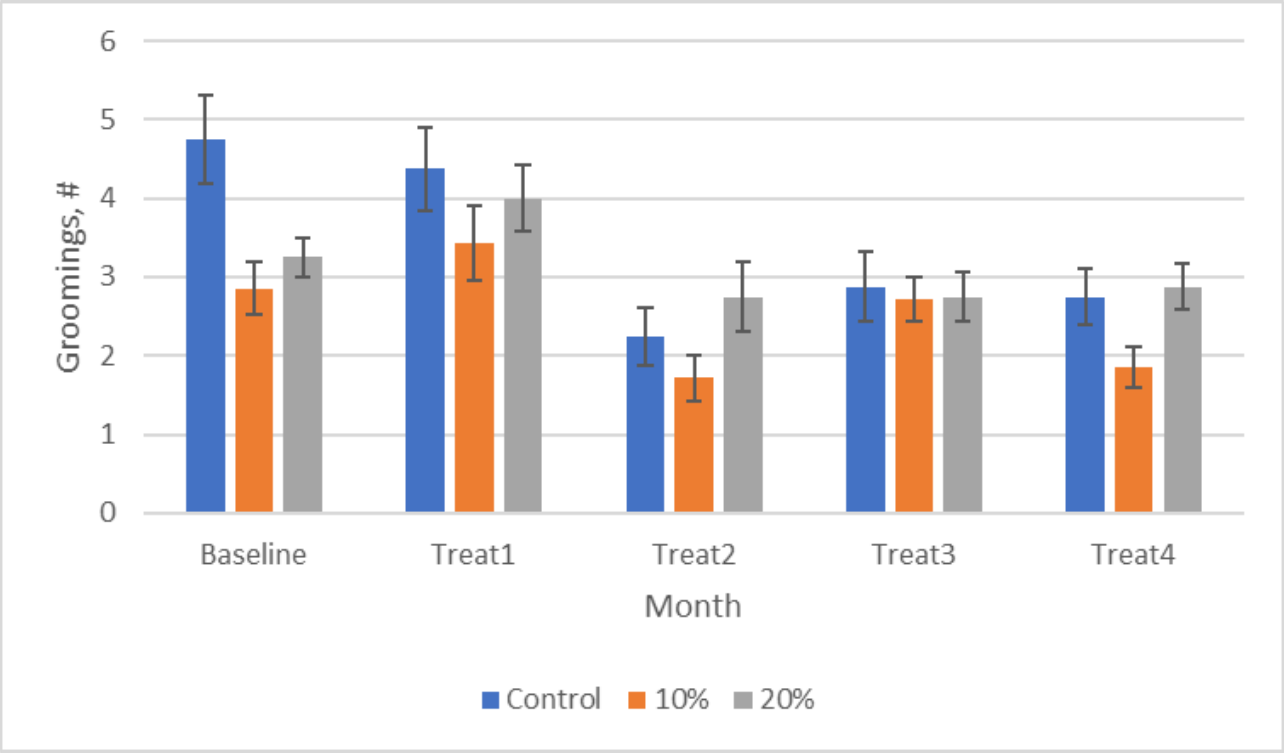

Fig. 3.4. Number of groomings performed during the open field maze test. N=23, data presented as mean ± SEM.

The amount of rearings when leaning against a wall were similar in the control and 10% treatment groups with 60 ± 3.7 and 59 ± 5.3 respectively, while the 20% treatment group fell behind with 52 ± 6.6 wall rearings in the baseline. The average amount of wall rearings seemed to increase in the control group in the months following treatment with 62 ± 9.3 wall rearings, while the 10% treatment group suffered a drop down to 46 ± 6.0 wall rearings per average; the 20% treatment group remained unchanged that month. Following the remaining months of treatment the control group showed more wall rearings and therefore higher physical activity than the 10% and 20% treatment groups. These results, however, were deemed statistically insignificant ($p = 0.224$).

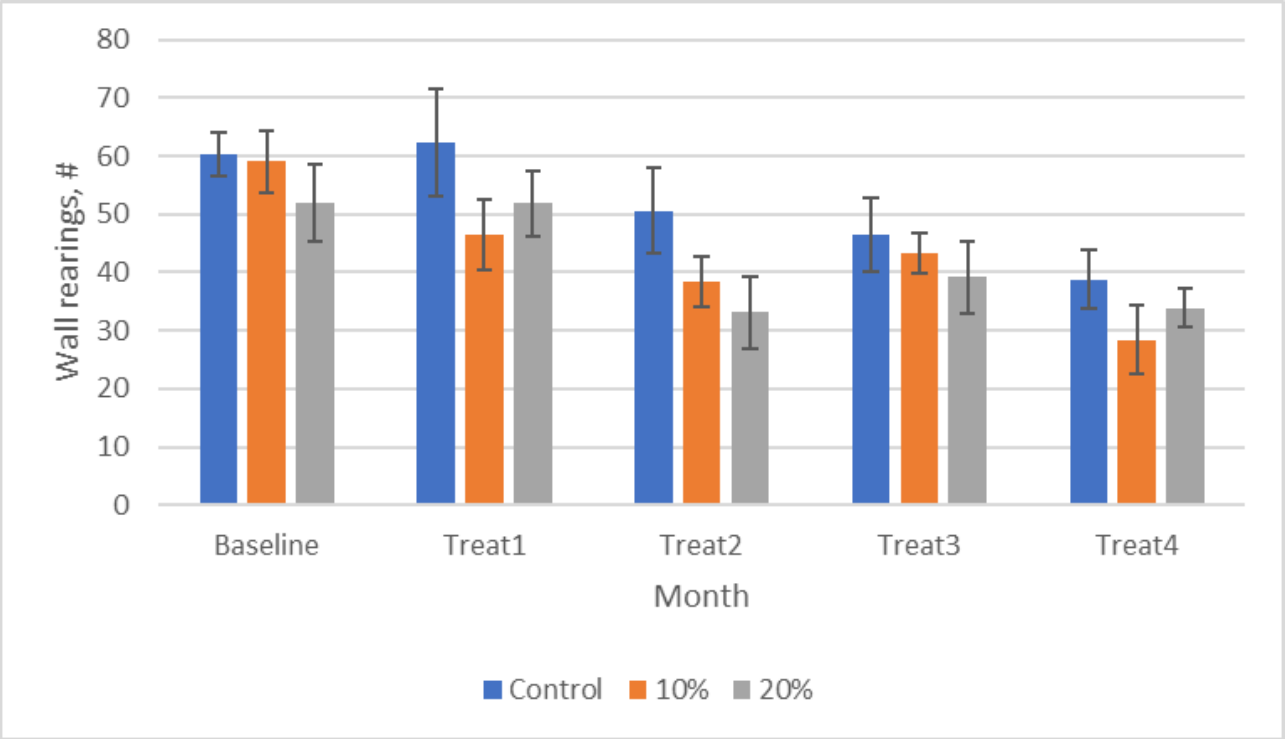

Fig. 3.5. Number of rearings leaning against a wall performed during the open field maze test. N=23, data presented as mean ± SEM.

The amount of center rearings was initially higher among the 10% treatment group's rats with 15 ± 3 center rearings as compared to 10 ± 3.8 and 7 ± 2.4 in the control and 20% groups respectively, however this discrepancy was later evened out following treatment as the control group took the lead with 15 ± 2.2 center rearings per average as compared to the 13 ± 4.2 and 7 ± 2 in the treatment groups. The amount of center rearings remained more or less the same for control and 10% treatment groups during the remainder of the experiment, with the 20% treatment group showing the lowest scores throughout up until the very last month of the experiment, when all values were more or less the same with 7 center rearings in the control group and 5 in both the 10% and 20% treatment groups. These results, however, were statistically insignificant, as the ANOVA test returned a p value of 0.232.

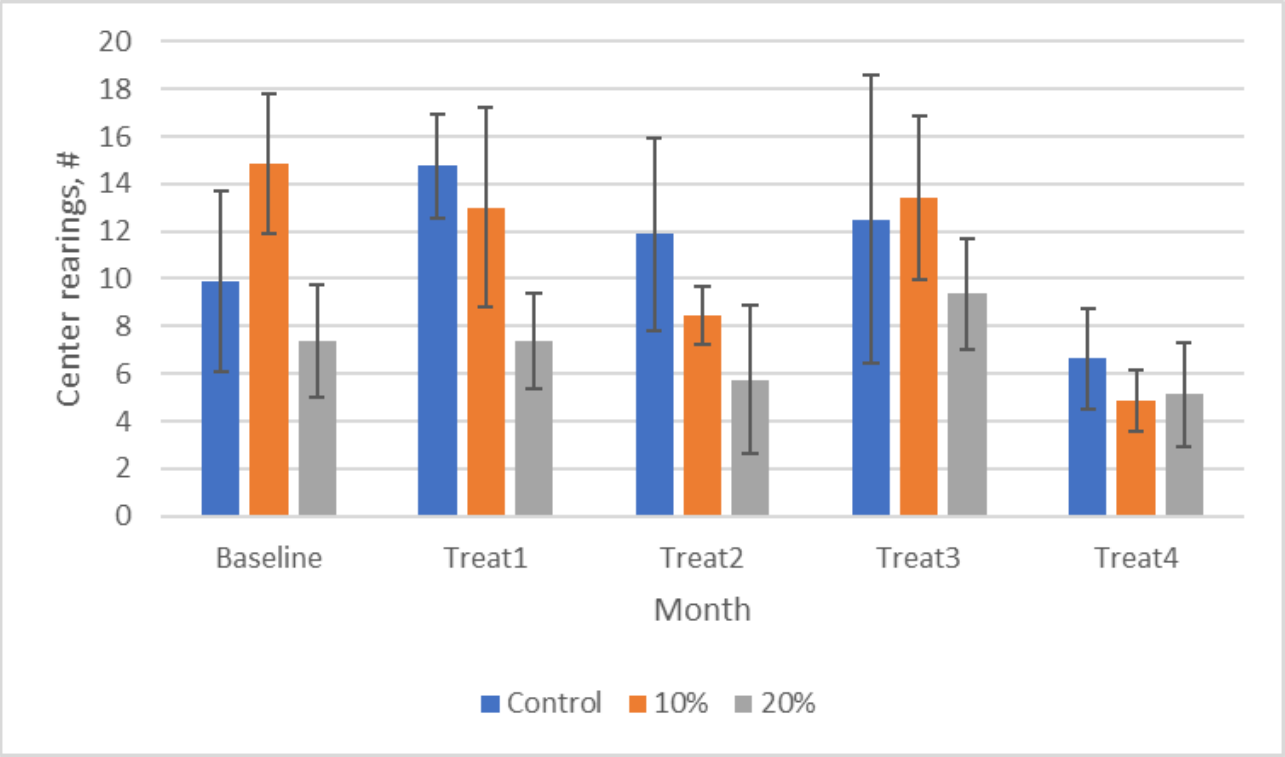

Fig. 3.6. Number of center rearings performed during the open field maze test. N=23, data presented as mean ± SEM.

3.2 CIRCADIAN ACTIVITY

The circadian activity was measured by counting the movement events during the light period, the dark period and by the sum of total activity. During the light period of the recording following the first month of treatment the control group initiated movement an average of 329 ± 37 times, the 10% treatment group – an average of 418 ± 24 times and the 20% treatment group – an average of 521 ± 44 times. The repeated measures ANOVA test showed that these results were not significant ($p = 0.151$) as a between subject effect. No significant data was found for the dark period ($p = 0.525$) or total movement events registered ($p = 0.653$).

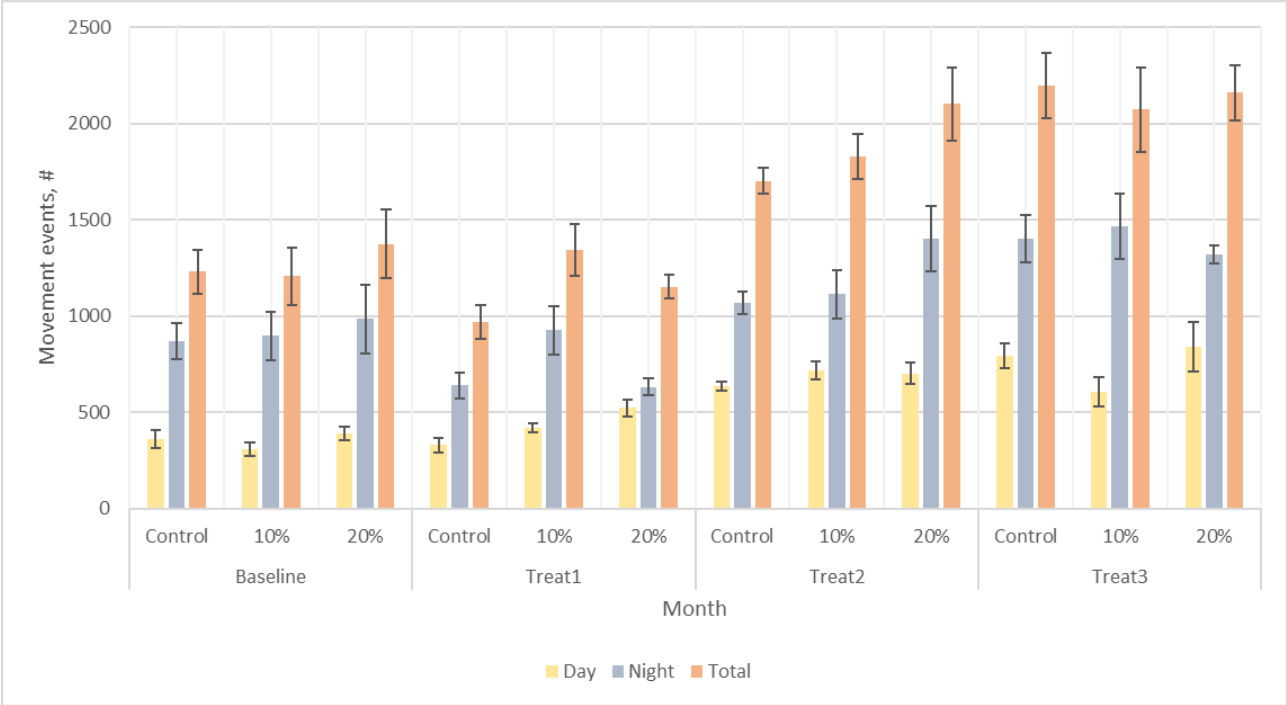

Fig. 3.7. Number of movement events during the circadian activity sensor test. N=23, data presented as mean ± SEM.

The amount of time in the circadian activity sensors was measured in minutes as shown in Figure 8. No significant differences were found neither for the light period movement time ($p = 0.425$), the dark movement period ($p = 0.525$), nor for the total movement time ($p = 0.653$). Upon closer look it seems that following baseline the scores increased linearly.

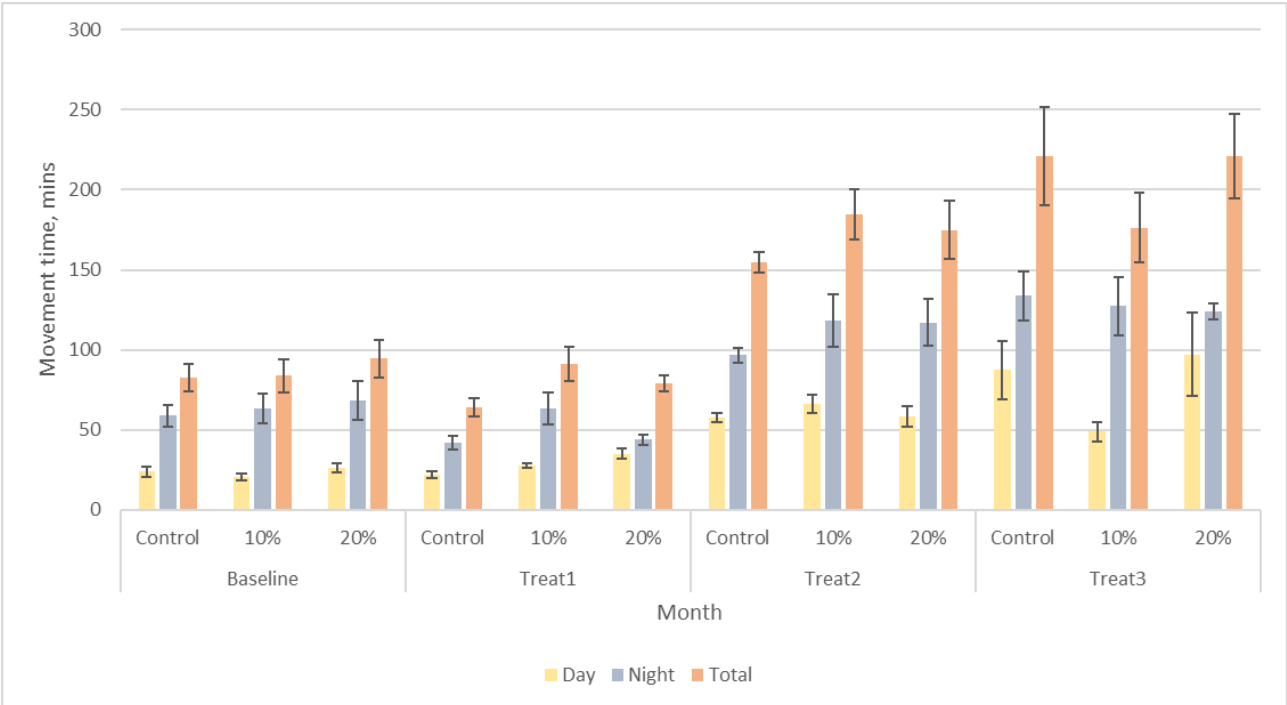

Fig. 3.8. Movement time in the circadian activity sensor test, measured in minutes. N=23, data presented as mean ± SEM.

The running wheel test spins were counted as shown in Figure 3.9.; while the results show no significant changes across the months in light ($p = 0.163$) and total ($p = 0.067$) spins, the dark period activity was seen as significantly (0.046), but after correction via the Bonferroni method it was shown that the differences were not significant (0.067).

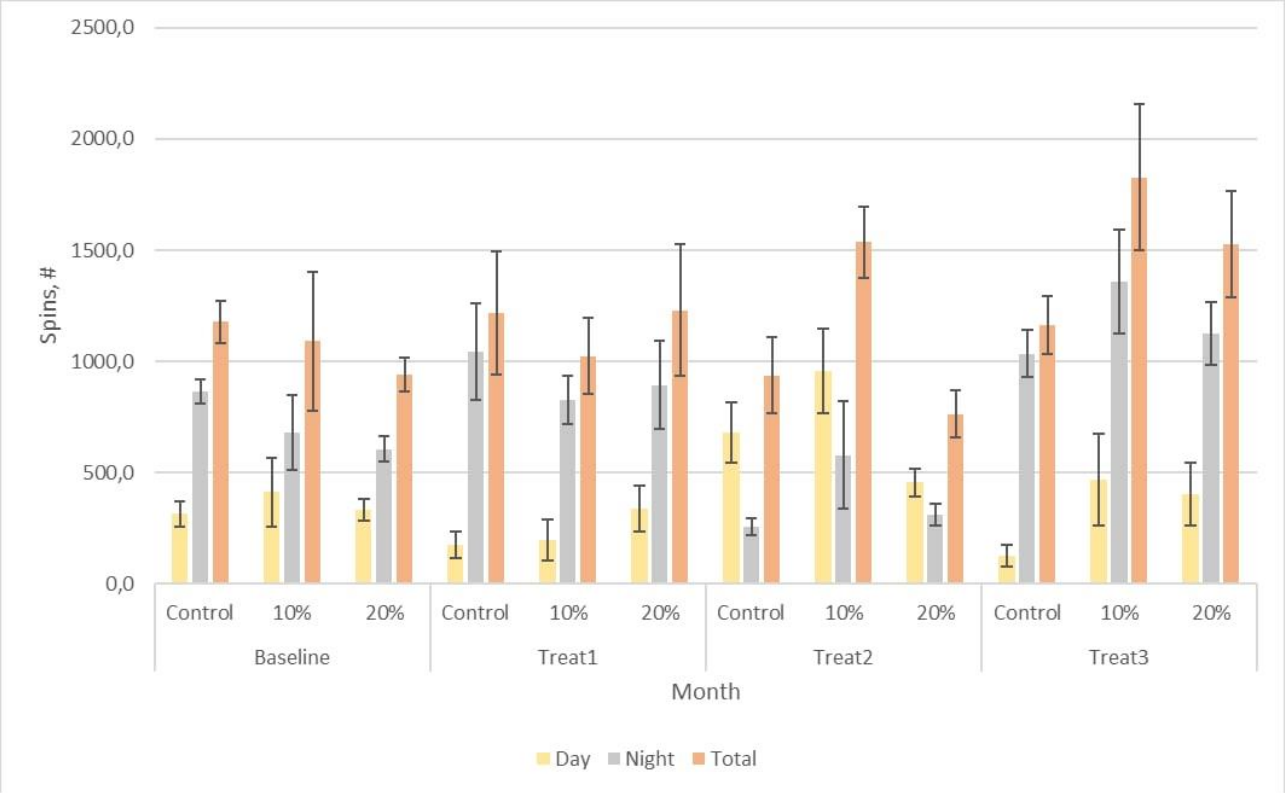

Fig. 3.9. Running wheel test spins. N=23, data presented as mean ± SEM.

3.3. BLOOD RESULTS

In order to interpret the full blood count test results, a standard of ranges for the values must be used. Such a universal standard, however, is not settled. Hence, for the purpose of this research we will draw upon multiple sources to obtain values as objective as possible.

Table 3.1. Compiled blood value norm ranges from *1* Hemoanalyzer Boule Exigo Eos Vet 400 software manual (2018), *2* Patel. S. et al. 2024, *3* Charles River Laboratories 1998, *4* Charles River Laboratories 2008

Source #	1		2		3		4	
	min	max	min	max	min	max	min	max
WBC	5.5	19.5	1.8	6.03	4.6	13	0.96	7.88
LYM	1	7	-	-	-	-	0.68	6.8
MON	0.2	1	-	-	-	-	0.01	0.13
NEU	2.8	13	-	-	-	-	0.15	1.11
EOS	-	-	-	-	-	-	0.01	0.14
LYM %	-	-	40.2	83.27	58	90	48.9	88.1
MON %	-	-	0.38	9.96	0	1	1	3.6
NEU %	-	-	9.8	39.21	10	40	8.8	43.8
EOS %	-	-	0.62	6.27	0	6	0.3	4.7
HGB	8	15	12.64	16.06	15.2	19.3	13.7	17.2
HCT	25	45	39.34	50.83	35	44	38.5	49.2
RBC	5	11	6.71	8.62	6.92	8.78	7.16	9.24
MCV	39	50	53.87	63.43	50	57	50.3	57
MCH	12.5	17.5	17.6	20.23	36	45	17.6	20.3
MCHC	31	38.5	29.5	34.5	18	23	33.2	37.8
RDW	14	18.5	-	-	-	-	10.6	14.6
PLT	200	500	377	963	-	-	599	1144
MPV	8	12	-	-	-	-	6.4	9.5

Evidently, the values for what's considered a normal range differ from one source to another, although some ranges remain fairly close within range of each other. In order to ensure the validity of the data at least three of the four sources' ranges needed to be met before considering a result abnormal.

Cross-comparison between sources showed that the lymphocyte percentage in all groups was lower than the normal minimal range, with 27.89%, 24.74% and 26.81% in the control, 10% Spirulina and 20% Spirulina groups, respectively.

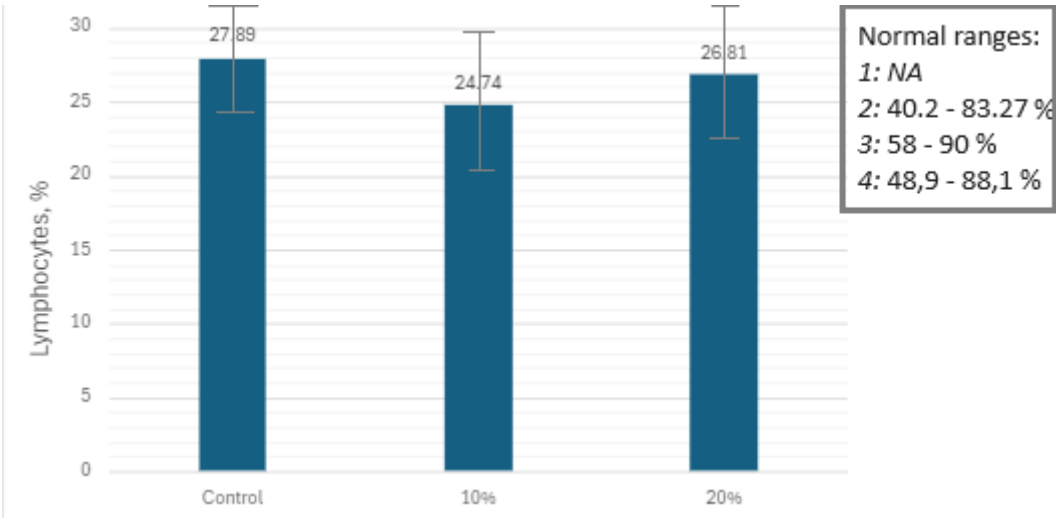

Fig. 3.10. Lymphocyte percentages in all study groups.

Further investigation showed that the neutrophil percentage in all groups was higher than usual, with 54.14%, 58.66% and 57.08% in the control, 10% Spirulina and 20% Spirulina groups, respectively.

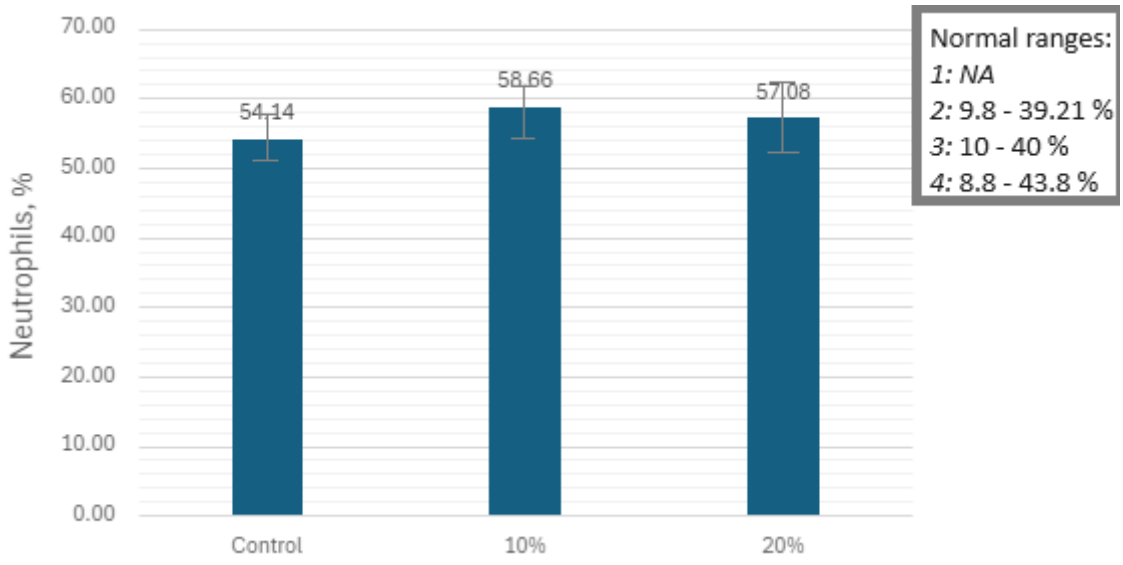

Fig. 3.11. Neutrophil percentages in all study groups.

Mean corpuscular volume (MCV) was noted to be lower across all study groups, with 42.64 fl, 42.00 fl and 43.75 fl in the control, 10% Spirulina and 20% Spirulina groups, respectively.

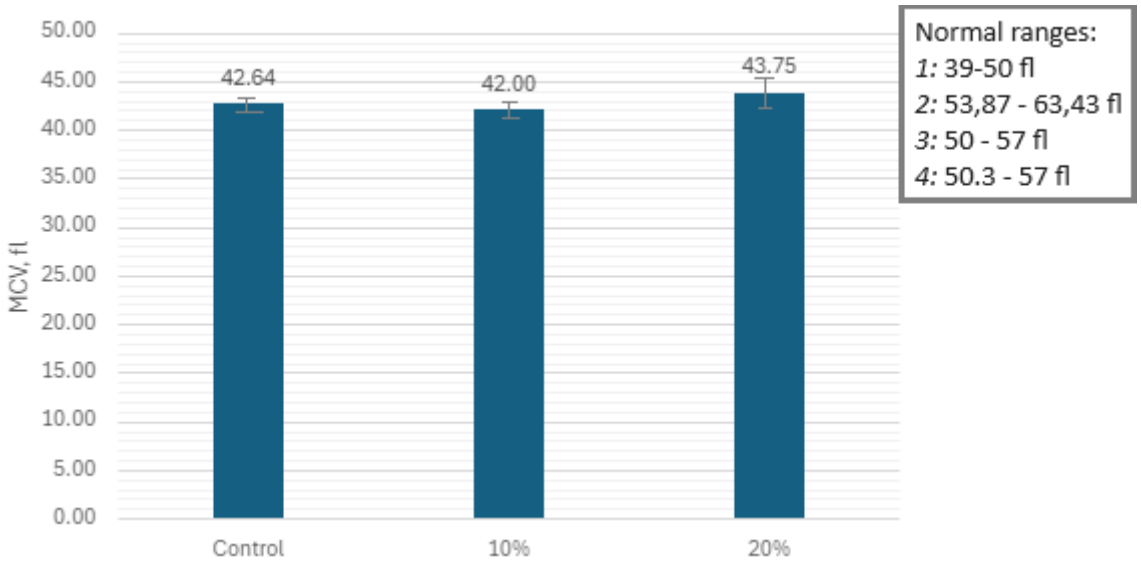

Fig. 3.12. Mean corpuscular volume in all study groups, in femtoliters (fl).

Mean corpuscular hemoglobin concentration (MCHC) was higher in all study groups, with 45,14 g/dl, 45,59 g/dl and 45,08 g/dl in the control, 10% Spirulina and 20% Spirulina study groups, respectively.

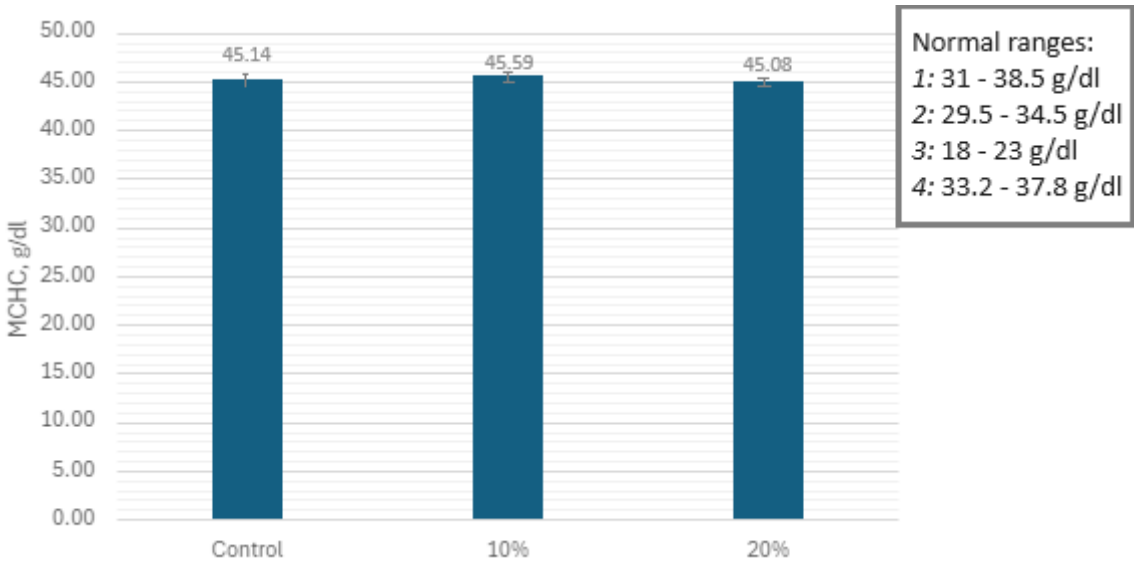

Fig. 3.13. Mean corpuscular hemoglobin concentration in all study groups in grams per deciliter (g/dl).

The platelet count in all study groups was below the norm, with 335.13 bil/l, 298.71 bil/l and 394.25 bil/l in the control, 10% Spirulina and 20% Spirulina groups, respectively.

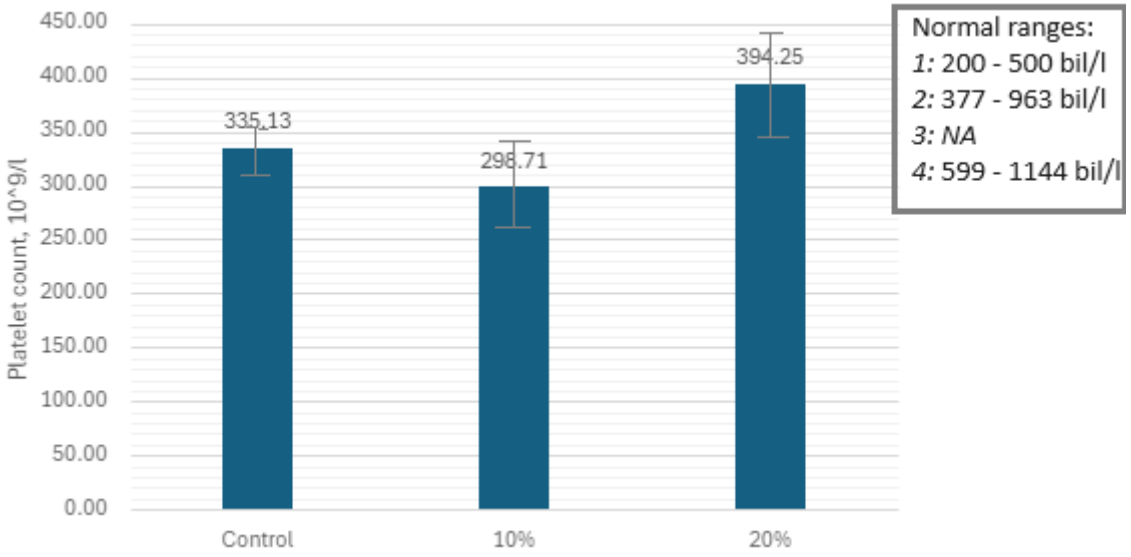

Fig. 3.14. Platelet count in all study groups, measured in billions per liter.

The mean platelet volume was lower than the norm in all study groups, with 5.75 fl, 5.90 fl and 5.86 fl in the control, 10% Spirulina and 20% Spirulina groups, respectively.

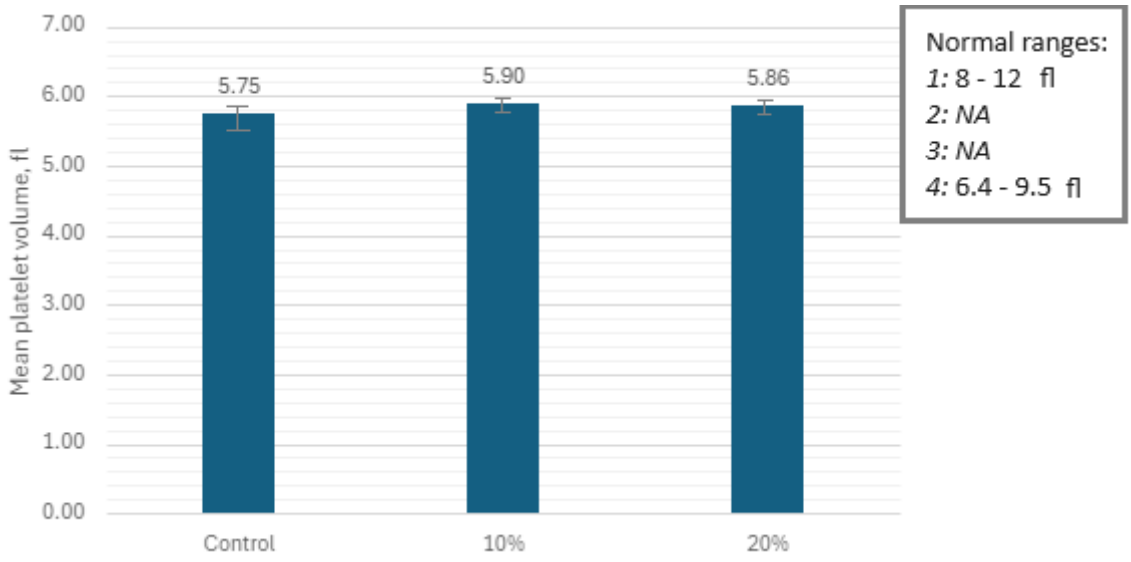

Fig. 3.15. Mean platelet volume in all study groups, measured in femtoliters.

3.4. BETWEEN-SUBJECT COMPARISON OF BLOOD RESULTS

The blood results compared between both trials seeking to find a difference between the groups in the main hematopoetic factors: red blood cell count, hemoglobin and hematocrit. The differences between the groups were statistically insignificant with $p = 0.935$ for red blood cell count, $p = 0.709$ for hemoglobin and $p = 0.650$ for hematocrit. Consulting Figure 15 it is evident that the hematopoetic factors were visually identical between the groups in both trials.

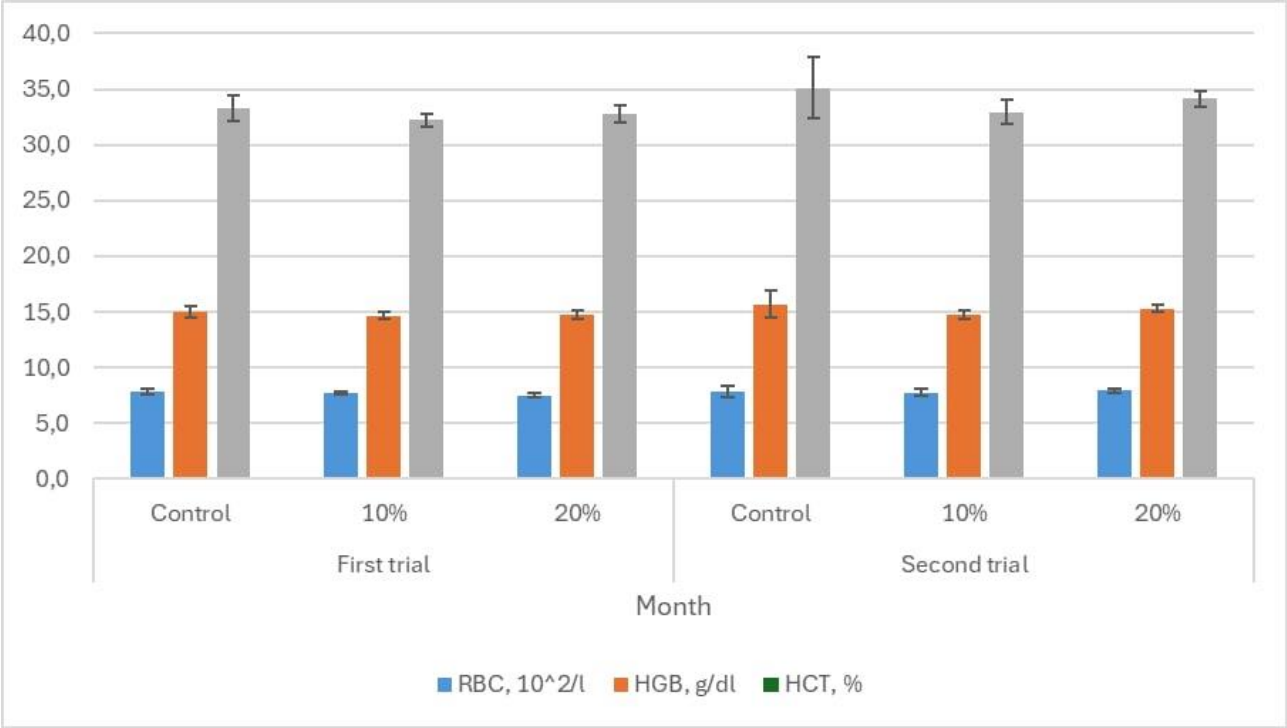

Fig. 3.16. Hematopoetic factors. Here RBC – red blood cell count, HGB – hemoglobin, HCT – hematocrit. N=23, data presented as mean ± SEM.

3.5. WEIGHT RESULTS

The rats were weighed each week since the beginning of the experiment. The baseline values for each group were 270 ± 3.9 g for control, 281 ± 9.4 for 10% treatment and 286 ± 7 for the 20% treatment group. By the end of the experiment the rats weighed an average of 280 ± 4.8 for control, 295 ± 11.3 for 10% treatment and 292 ± 10.5 for the 20% treatment groups. These changes between groups over the entire course of the experiment were not significant ($p = 0.462$) and consulting Figure 16 it is clear that the weight gain was linear across all groups.

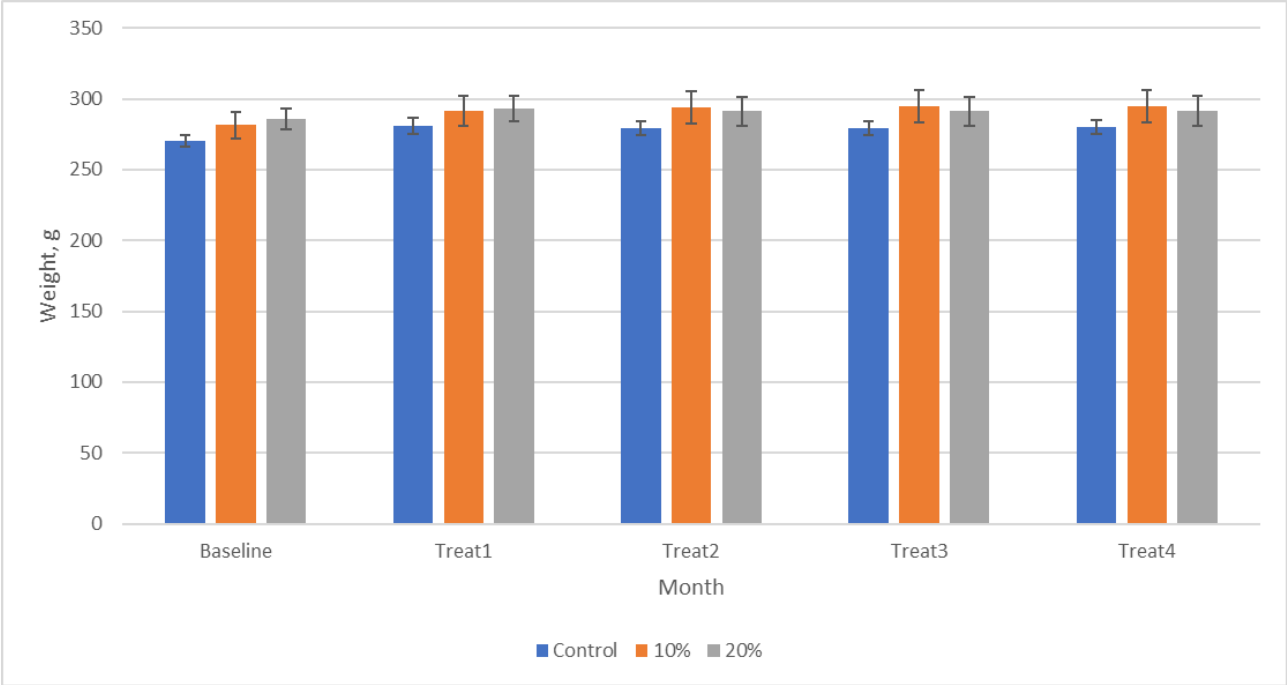

Fig. 3.17. Weight measure in grams. N=23, data presented as mean ± SEM.

4. DISCUSSION

Having completed the analysis of the data that was gathered, it is apparent that significant changes failed to be found. In this section an overlook of all the gathered results will be summarised and discussed.

4.1. NO CHANGE IN LOCOMOTOR ACTIVITY

The results showed that there were no significant changes in the distance traveled in the open field test, nor were there significant changes in behavioral expressions such rearing or visits to or time spent in the center; grooming showed significant ($p = 0.03$) differences, but only in the baseline, meanwhile for the rest of the experiment the values remained more or less the same.

4.2. NO CHANGE IN CIRCADIAN ACTIVITY

The circadian activity sensor test results failed to show any significant changes between the groups for either the light period, dark period or total activity during the test, with the general trend being that activity linearly increased over the course of the experiment across all groups.

4.3. FULL BLOOD COUNT VALUES REMAINED POOR INDIVIDUALLY

Provided in the previous results section were the main findings of the blood analysis study, which included reduced lymphocyte, mean corpuscular volume, platelet and mean platelet volume counts and increased neutrophil and mean corpuscular hemoglobin concentration counts, all the mentioned values were cross-referenced using multiple guidelines for normal general rat blood values, for the values refer to table 1. While according to Patel, S. (2024), all these changes in rat blood values are related to natural aging, further discussion will delve deeper into any other possible reasons for these abnormalities.

4.4. REDUCED LYMPHOCYTE COUNT IN ALL STUDY GROUPS

Reduced lymphocyte count in the bloodstream is often a sign of weakened immune response, although Sharp and Villano (2012) state that lymphocyte counts are usually higher among male Wistar rats than they are among female specimens. Another cause for reduced lymphocyte count may be chronic stress, as corticosterone is released under long-term stress and reduces lymphocyte counts, however this would likely also end up in decreased eosinophil counts - 0, to be exact (Sharp, P. & Villano, J., 2012), and our data showed no such extremes.

Other possible reasons for reduced lymphocyte count are infectious diseases that specifically target lymphocyte cells in the organism, such as the influenza virus or SARS-CoV-2 (Guo, Z. et al.,

2021), though to confirm any such sickness would require additional infection-specific testing and biochemical blood sampling.

4.5. INCREASED NEUTROPHIL PERCENTAGE AMONG ALL STUDY GROUPS

An increase in neutrophils may be an indicator of bacterial or fungal infection (Witter et a.l, 2016), as neutrophils are released from the bone marrow to help combat such diseases. Another suggested cause for a sudden increase in neutrophils might be stress, as it takes only up to six minutes for active immune cells in the bloodstream to reorganise and provide skewed values during a blood test (Dhabhar F.S. et al., 2012), which may indicate that the stress that rats experience before blood sampling or during handling may affect the related blood values.

4.6. CHANGES IN RED BLOOD CELL INDICES IN ALL STUDY GROUPS

Reduced mean corpuscular volume and mean corpuscular hemoglobin concentration are two important indicators of anemia (Burton, A. G., 2024), but the causes underneath may be many.

The first thing to be suspected upon seeing signs of anemia is malnutrition – lack of iron or vitamin B12 intake are the leading causes in the epidemiology of anemia (Turner, J. et al., 2023), however this is unlikely to be the cause since the food rations dispensed to the rats in the current study were nutritionally wholesome and made accordingly to the rats' dietary needs.

Another cause for anemic indices to spike up in the subject rats could possibly be related to immunological responses, as chronic inflammations are a likely cause for anemia (Rivera, S. 2009). Inflammation is responsible for the destruction of red blood cells, the crippling of the ability to form new ones and the inhibition of nutrient uptake (JSI research & training institute, 2022). This would go accordingly with the previous discussion, which suspects a possible infection among the subject rat population.

When discussing anemia, age also plays an important factor. The aging of blood marrow usually results in poorer red blood cell production, aged red blood cells are usually worn down and of lower volume (Patel, S. et al. 2024), this is confirmed by the lower mean corpuscular volume in all the groups.

Another important factor for anemia is lowered blood coagulation, which will be discussed in the next section.

4.7. DECREASED BLOOD COAGULATION INDICES IN ALL GROUPS

Blood coagulation disorders are indicated by reduced platelets and the mean platelet volume in the bloodstream, to which the cause, as in many aforementioned disorders, aging may be the cause, as older bone marrow is incapable of producing blood components and providing them to the organism as efficiently and quickly as would be needed to uphold homeostatic function (Patel, S. 2024).

As in anemia, blood coagulation disorders borrow from many of the same causes and blood coagulation may be a cause for anemia, as anemia may be a cause for blood clotting disorders, since, naturally, the inability to stop bleeding after injury leads to blood loss (Booth, C.J., 2010).

4.8. NO INCREASE IN RAT WEIGHT

Over the course of the experiment no changes in the rats' weight was observed between the groups, with the only changes being that all groups gained weight linearly and similarly over the course of the experiment. As other studies show that *Spirulina* helps increase bodyweight, this experiment's results found no such correlation.

5. CONCLUSIONS

At the end of the experiment after reviewing the results we can conclude that:

- The locomotor activity of rats consuming *Spirulina* did not change compared to the control group.
- The circadian acitivity of rats consuming *Spirulina* did not change when compared to the control group.
- The hematopoetic factors from the complete blood count test did not show any differences between rats that consumed *Spirulina* and the control group.

It is thus that can be concluded that the hypothesis of rats consuming *Spirulina* should show reduced signs of aging was not confirmed.

6. LIMITATIONS

The limitations of the study included:

- Expensive treatment: had the *Spirulina* essence were extracted from a cheaper method or was just not so cost-straining overall, the experiment could have started sooner and gone on longer, perhaps showing clear results in the long run.
- The essence of *Spirulina* quickly losing effectiveness upon making a water-based solution, thus placing the need to make new solutions everyday, which was complicated time and cost-wise.

7. ACKNOWLEDGEMENTS

The author of this work would like to thank prof. Osvaldas Rukšėnas for the planning of this experiment and for the opportunity to participate in this project; prof. Valentina Vengelienė for her continued support, whether in the lab or when performing data analysis; fellow student Elena Kavaliauskaitė for her help in adapting to the experiment and advice given throughout.

VILNIAUS UNIVERSITETAS

GYVYBĖS MOKSLŲ CENTRAS

Nojus Katkevičius

Magistro baigiamasis darbas

***Arthrospira platensis* poveikis Wistar žiurkių fiziniam aktyvumui ir ilgaamžiškumui**

SANTRAUKA

Arthrospira platensis arba tiesiog *Spirulina* yra melsvadumblis jau nuo senovės civilizacijų naudojamas kaip maisto šaltinis, kurio pirmasis rašytinis paminėjimas yra dar šešioliktajame amžiuje, kai ispanų atvykėliai aprašė actekus renkančius jūros dumblius tinklais iš tvenkinių. *Spirulinos* naudojimas išaugo 1970-aisiais, kuomet cheminės analizės parodė, jog *Spirulinos* sausos masės baltymų kiekis siekia net iki 70-ies procentų. Naujausi tyrimai rodo, jog *Spirulina* yra gausi naudingų medžiagų, tokių kaip fikocianinas, kuris stiprina imunines reakcijas ir mažina oksidacinį stresą, kas gali sulėtinti ląstelių senėjimą, bei geležies ir vitamino B12, kurie gali padėti gydyti mažakraujystę, kuri taip pat gali būti ankstyvos mirties priežastimi.

Pagrindinis šio tyrimo tikslas yra įvertinti *Spirulinos* poveikį Wistar modelio žiurkių senėjimui ir ilgaamžiškumui. Darbo uždaviniai: įvertinti žiurkių lokomotorinį aktyvumą gaunant koncentruotos *Spirulinos* papildus; įvertinti cirkadinio aktyvumo pokyčius žiurkėse; įvertinti žiurkių kraujo tyrimų pokyčiuos bendrame kraujo tyrime.

Metodai naudoti tyrime buvo Open Field testas, cirkadinio aktyvumo sensoriai, bendras kraujo tyrimas. Statistinė analizė atlikta naudojant repeated measures ANOVA testą. 125µg *Spirulinos* naudota pagaminti 10% ir 20% tirpalus.

Tyrimas parodė, jog *Spirulinos* papildų skyrimas laikui bėgant nepadidino žiurkių fizinio aktyvumo palyginus su kontroline grupe; žiurkių cirkadinis aktyvumas išliko nepakitęs; atlikti kraujo tyrimai buvo beveik identiški tarp visų tyrimų grupių abiejų bandymų metu.

VILNIUS UNIVERSITY

LIFE SCIENCES CENTER

Nojus Katkevičius

Master’s thesis

The effects of *Arthrospira platensis* on Wistar rats' physical activity and longevity

ABSTRACT

Arthrospira platensis, more commonly *Spirulina* is a blue-green microalgae that has been used by many civilizations throughout the ages, the first recorded use of which was by spanish colonisers in Mesoamerica who described the local aztec fishermen gathering seaweed with fine nets from the lagoon. Research into *Spirulina* picked up in the 1970s, when chemical analyses showed that *Spirulina* has a protein content reaching up to 70 per cent of its dry mass. Latest research suggests that *Spirulina* has a great amount of nutrients, such as phycocyanin that helps immune reaction and reduces oxydative stress which may reduce cell aging, as well as iron and vitamin B12, which reduce anemia, a possible cause in premature death.

The aim of our study was to determine whether *Spirulina* supplementation reduces expressions of aging as shown by the rats' locomotor activity and other indices. The study tasks: to measure the rats' locomotor activity throughout treatment; to measure the effects of *Spirulina* on the rats' circadian activity; to investigate whether the full blood count of rats receiving *Spirulina* shows any changes.

The methods for the study were an open field test, circadian activity sensors, and a full blood count test. 125µg of *Spirulina* were used to make 10% and 20% solutions.

The study showed that *Spirulina* supplementation did not help maintain physical activity over the course of the rats aging throughout the experiment as compared to the control group; the circadian activity remained unchanged between the groups; the full blood count results remained nearly identical between all groups across both blood collection trials.

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