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Physiological and Immunological Changes in Experimentally-Induced Starvation in Rats

Eman H. Al-Fadhili [1], Noor Majeed Abdulhasan [2], Duaa R.M. Al-Safi [3]

1 Pathological Analysis Department, College of Science, University of Wasit, Wasit, Iraq
2 Forensic Evidence Department, College of Science, University of Wasit, Wasit, Iraq
3 Department of Physiology and Pharmacology, College of Veterinary Medicine, University of Wasit, Wasit, Iraq

Corresponding Author: Eman H. Al-Fadhili; E-mail: *eman@uowasit.edu.iq*

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ABSTRACT

This study aims to experimental induction of starvation in rats and investigation of its effect on concentrations of blood antioxidants and immune markers using the quantitative enzyme-linked immunosorbent assay (ELISA). In total, 32 female rats were purchased, acclimated for one week, and divided equally into NC (negative control) and EG (experimental group). In NC, rats were fed and drank normally, while in EG, rats received half the quantity of feed. After 28 days, direct blood was collected, and the obtained sera were tested by ELISA to measurement the concentrations of antioxidants (catalase, glutathione peroxidase, and superoxide dismutase), lipid peroxidation (malondialdehyde), and immune markers (interleukin-4 and interferon-γ). In comparison to values of antioxidants and immune markers of NC, the results of EG were shown a significant reduction in catalase (126.88 ± 8.11 pg/ml), glutathione peroxidase (0.776 ± 0.03 IU/ml), superoxide dismutase (1.195 ± 0.07 U/ml), and interferon-γ (20.11 ± 0.84 pg/ml); while, there was significant elevation in values malondialdehyde (197.87 ± 7.71 ng/ml) and interleukin-4 (57.11 ± 2.44 pg/ml). In conclusion, the results of our experiment showed that starvation caused oxidative stress in rats, which resulted in a lower rate of antioxidants (catalase, glutathione peroxidase, and superoxide dismutase) and a higher rate of malondialdehyde. The feed restriction also influenced the immune system through the lower level of interferon-γ and higher level of interleukin-4. Further studies are of great importance to indicate the effect of starvation on other physiological and immunological markers as well as on the health status of body tissues/organs.

1. Introduction

In the past decades, researchers have employed different terms to describe the cases when animals do not consume food (inanition, fasting, and starvation). These terms can be used to denote certain physiological conditions but more often are employed interchangeably (Pirkmajer & Chibalin, 2011; Galef, 2013). In view of all that is currently known about the behavioral, morphological, and physiological variability in how animals respond to unpredictable availability of food, it is timely to revise the descriptive labels we assign to cases where animals rely solely on their internal physiological reserves to meet their energy needs (Sergio et al., 2018). Starvation can be defined as the state where an animal that is post-absorptive and usually capable of feeding itself is denied its food due to some factors beyond its control. The frequency and the period of natural starvation can be different – there can be short-term and long-term natural starvation. For instance, the starvation cycles may be short and frequent, short and intermittent, long and sporadic, and long and continuous. Starvation has been conventionally divided into three distinct phases based on the rate of mass loss, nitrogen excretion, or the type of primary physiological fuel used in starvation (Bar, 2014; McCue et al., 2017; Dogan et al., 2024). Qualitative terms have also been used to describe the growing intensity of fasting or starvation. While such qualitative definitions may adequately describe the changes in physiological processes in a given species as a function of development, they are not very useful when viewed from a comparative perspective (McCue et al., 2012, 2017).

The immune system is a highly organized structure of cells, tissues, and organs that perform a coordinated function of protecting the body against various pathogens, including bacteria, viruses, fungi, and others (Subramanian et al., 2015; Gombart et al., 2020). “The immune system can be grouped into two main categories: the innate immune system and the

adaptive immune system. The first line of defense is the innate immune system, which is the immediate and non-specific response by the body to pathogens, including physical barriers such as the skin and cellular components like white blood cells. The innate immune system is non-specific and does not have the ability to 'remember' previous exposures to pathogens, making it relatively slower and less efficient when compared to the adaptive immune system (Hillion et al., 2020). The immune system is one of the most essential parts of the body that is responsible for the body's defense against diseases and infections. An essential feature of the immune response in relation to food is its capacity to discern between beneficial compounds, such as nutrients, and potential dangers. This is especially important since the gut-associated lymphoid tissue (GALT) is in charge of detecting and reacting to substances ingested through the digestive system (Alator, 2024).

Consumption of nutrients is crucial in maintaining a strong immune response. For instance, vitamins C, D, and E and minerals like zinc and selenium, as well as other bioactive compounds, are crucial in the support of immune health (Calder et al., 2020). Lack of these nutrients weakens the body's immune system and results in vulnerability to diseases. Specific nutrients like antioxidants and anti-inflammatory substances present in fruits, vegetables, and other plant products can have a positive impact on immunity (Childs et al., 2019). Thus, good bacteria or probiotics, which are found in foods such as yogurt, may also help maintain healthy gut bacteria, boosting the immune system (La Fata et al., 2018; Yeşilyurt et al., 2021). The immune system is a complex structure that consists of different tissues and is sensitive to the foods we eat (Munteanu & Schwartz, 2022). In this regard, it is crucial to focus on nutrition, including the consumption of diverse and nutrient-dense foods, to support the immune system and general health (Mitra et al., 2022). Some vertebrates are more tolerant to starvation than others. For instance, some small birds and mammals may survive with food for just one day, while some snakes and frogs can go up to about 723 days of starvation". This study aims to evaluate the effect of starvation on rats through the evaluation of the concentrations of antioxidants (catalase, glutathione peroxidase, superoxide dismutase, and malondialdehyde) and immune markers (interleukin-4 and interferon-γ) serologically using the quantitative ELISA.

2. Methodology

Animals and Study Design

In total, 32 female rats of 4 weeks of age at most were bought from the local market and were transported to the Lab Animal House, "College of Veterinary Medicine, University of Wasit". Firstly, all the study animals were acclimatized for one week, where they were fed with a pellet, given access to tap water, and had a light/dark cycle of 12/12 hours (Hussen et al., 2024). Then, the study rats were divided equally into NG (negative group) and EG (experimental group) groups. In NG, rats were fed and drank normally, while rats of the EG group received half the quantity of feed throughout the study period that extended for 28 days.

Samples

After ending of the study period, all study rats were anesthetized and subjected to direct sampling of blood from the heart into labeled free-anticoagulant gel tubes that were cooled to the laboratory and centrifuged at 5000rpm for 5 minutes. The obtained sera were transferred into 1.5 ml Eppendorf tubes and saved at -4°C in the dark bag until be tested serologically using the ELISA (Gharban & Al-Shaeli, 2021).

Measurement of Antioxidants and Immune Markers

According to manufacturer instructions of quantitative ELISA Kits "(SunLong Biotech, China), the concentrations of antioxidants (catalase [SL1084Ra], glutathione peroxidase [SL1033Ra], superoxide dismutase [SL1341Ra], and malondialdehyde [SL0475Ra]) and immune markers (interleukin-4 [SL0409Ra], and interferon-γ [SL0786Ra]) were measured. For each marker, the contents of each kit and the sera were prepared, processed, and the optical density (OD) of Standards and sera were calculated at 450nm using a Microplate ELISA Reader (BioTek, USA). After setting the Blank well to zero, the ODs and concentrations of Standards, as well as the ODs of sera, were plotted on a diagram to measure the concentration of each marker in sera.

Statistical analysis

The results were analyzed using GraphPad Prism version 8.0.2 (GraphPad Software, USA). Two-way ANOVA and the t-test were applied to detect significant variation between values of different study groups at $p<0.05$ (*), $p<0.01$ (**), $p<0.001$ (***), and $p<0.0001$ (****). Values were represented as mean ± standard errors (M±SE)", (Gharban, 2023).

3. Results

The findings of catalase showed a significant reduction (p<0.0001) in values of EG (126.88 ± 8.11 pg/ml) when compared to those of NC (369.81 ± 21.28 pg/ml) "(Table 1, Figure 1).

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Table 1. Statistical analysis for values of catalase concentration in rats of both study NG and EG Group.

Value of NC	Value of EG	Difference between means	SE of difference	95% CI of difference	
369.81 ± 21.28	126.88 ± 8.11	242.93	20.91	198.1 to 287.8	
Source of Variation	% of total variation	P value	P value summary	Significant?	
Row Factor	11.42	0.2808	NS	No	
Column Factor	80.26	<0.0001	****	Yes	
ANOVA Table	SS	DF	MS	F (DFn, DFd)	P value
Row Factor	62990	14	4499	F (14, 14) = 1.372	P=0.2808
Column Factor	442600	1	442600	F (1, 14) = 135.0	P<0.0001
Residual	45902	14	3279	-	-

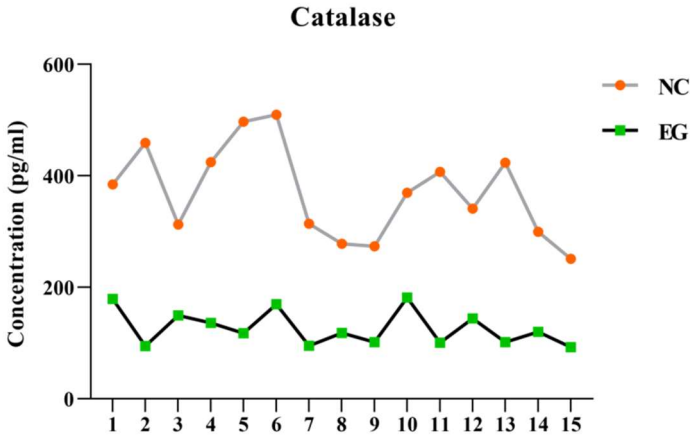

Figure 1. Concentration of catalase among the sera of study rats in both the NG and EG study groups.

Concerning values of the glutathione peroxidase, there were significant decreases (p<0.0001) in values of EG (0.776 ± 0.03 IU/ml) when compared to those of NC (2.985 ± 01 IU/ml) (Table 2, Figure 2).

Table 2. Statistical analysis for values of glutathione peroxidase concentration in rats of both study NG and EG goups.

Value of NC	Value of EG	Difference between means	SE of difference	95% CI of difference	
2.985 ± 01	0.776 ± 0.03	2.209	0.108	1.977 to 2.440	
Source of Variation	% of total variation	P value	P value summary	Significant?	
Row Factor	2.946	0.5469	NS	No	
Column Factor	93.91	<0.0001	****	Yes	
ANOVA Table	SS	DF	MS	F (DFn, DFd)	P value
Row Factor	1.148	14	0.08197	F (14, 14) = 0.9379	P=0.5469
Column Factor	36.59	1	36.59	F (1, 14) = 418.6	P<0.0001
Residual	1.224	14	0.08741	-	-

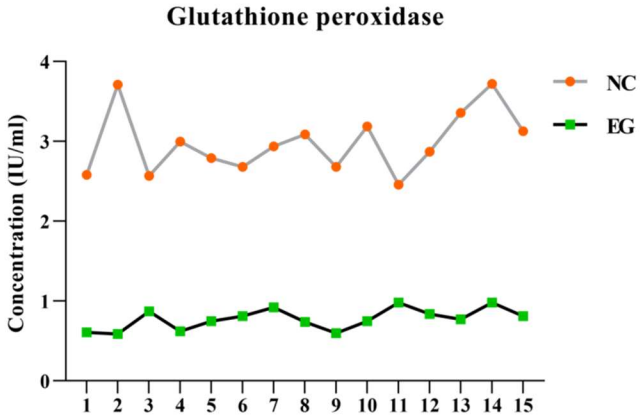

Figure 2. Concentration of glutathione peroxidase among the sera of study rats in both the NG and EG study groups.

Regarding the levels of superoxide dismutase, the findings of EG (1.195 ± 0.07 U/ml) were decreased significantly (p<0.0001) in comparison with those of the NC (6.255 ± 0.38 U/ml) (Table 3, Figure 3).

Table 3. Statistical analysis for values of superoxide dismutase concentration in rats of both study NG and EG groups.

Value of NC	Value of EG	Difference between means	SE of difference	95% CI of difference	
6.255 ± 0.38	1.195 ± 0.07	5.06	0.3961	4.210 to 5.909	
Source of Variation	% of total variation	P value	P value summary	Significant?	
Row Factor	6.601	0.5814	NS	No	
Column Factor	86.02	<0.0001	****	Yes	
ANOVA Table	SS	DF	MS	F (DFn, DFd)	P value
Row Factor	14.73	14	1.052	F (14,14) = 0.8941	P=0.5814
Column Factor	192	1	192	F (1, 14) = 163.1	P<0.0001
Residual	16.48	14	1.177	-	-

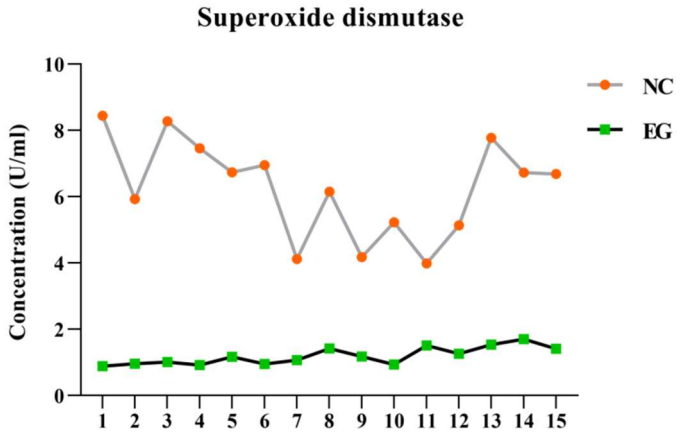

Figure 3. Concentration of superoxide dismutase among the sera of study rats in both the NG and EG study groups.

The concentration of malondialdehyde revealed a significant elevation ($p<0.0001$) in values of EG (197.87 ± 7.71 ng/ml) when compared to values of NC (50.47 ± 4.06 ng/ml) (Table 4, Figure 4).

Table 4 Statistical analysis for values of malondialdehyde concentration in rats of both study NG and EG groups.

Value of NC	Value of EG	Difference between means	SE of difference	95% CI of difference	
50.47 ± 4.06	197.87 ± 7.71	-147.4	10.34	-169.6 to -125.2	
Source of Variation	% of total variation	P value	P value summary	Significant?	
Row Factor	2.636	0.9419	NS	No	
Column Factor	91.09	<0.0001	****	Yes	
ANOVA Table	SS	DF	MS	F (DFn, DFd)	P value
Row Factor	4716	14	336.8	F (14,14) = 0.4199	P=0.9419
Column Factor	162951	1	162951	F (1, 14) = 203.1	P<0.0001
Residual	11230	14	802.1	-	-

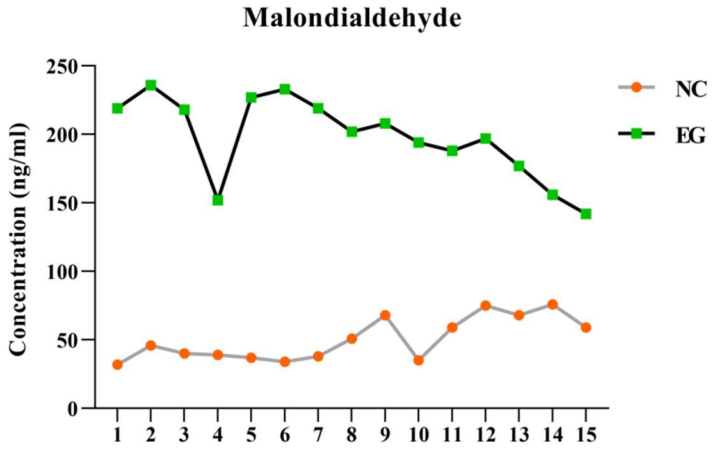

Figure 4. Concentration of malondialdehyde among the sera of study rats in both the NG and EG study groups.

For immune markers, the findings of interleukin-4 were increased significantly ($p<0.0001$) in EG (57.11 ± 2.44 pg/ml) when compared to those of NC (15.51 ± 1 pg/ml) (Table 5, Figure 5).

Table 5. Statistical analysis for values of interleukin-4 concentration in rats of both study NG and EG groups.

Value of NC	Value of EG	Difference between means	SE of difference	95% CI of difference	
15.51 ± 1	57.11 ± 2.44	-41.6	2.424	-46.80 to -36.40	
Source of Variation	% of total variation	P value	P value summary	Significant?	
Row Factor	5.878	0.2793	NS	No	
Column Factor	89.85	<0.0001	****	Yes	
ANOVA Table	SS	DF	MS	F (DFn, DFd)	P value
Row Factor	849	14	60.65	F (14, 14) = 1.376	P=0.2793
Column Factor	12979	1	12979	F (1, 14) = 294.4	P<0.0001
Residual	617.1	14	44.08	-	-

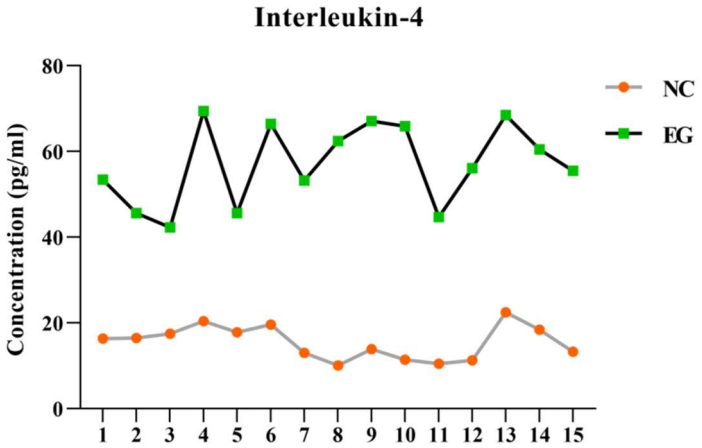

Figure 5. Concentration of interleukin-4 among the sera of study rats in both the NG and EG study groups.

In relation to values of interferon-γ, significantly lower values ($p<0.0003$) were observed in EG (20.11 ± 0.84 pg/ml) than those of NC (69.21 ± 10.19 pg/ml) (Table 6, Figure 6).

Table 6. Statistical analysis for values of interferon-γ concentration in rats of both study NG and EG groups.

Value of NC	Value of EG	Difference between means	SE of difference	95% CI of difference	
69.21 ± 10.19	20.11 ± 0.84	49.09	10.2	27.23 to 70.96	
Source of Variation	% of total variation	P value	P value summary	Significant?	
Row Factor	27.53	0.4934	NS	No	
Column Factor	45.18	0.0003	***	Yes	
ANOVA Table	SS	DF	MS	F (DFn, DFd)	P value
Row Factor	11016	14	786.9	F (14,14)= 1.009	P=0.4934
Column Factor	18077	1	18077	F (1, 14) = 23.18	P=0.0003
Residual	10917	14	779.8	-	-

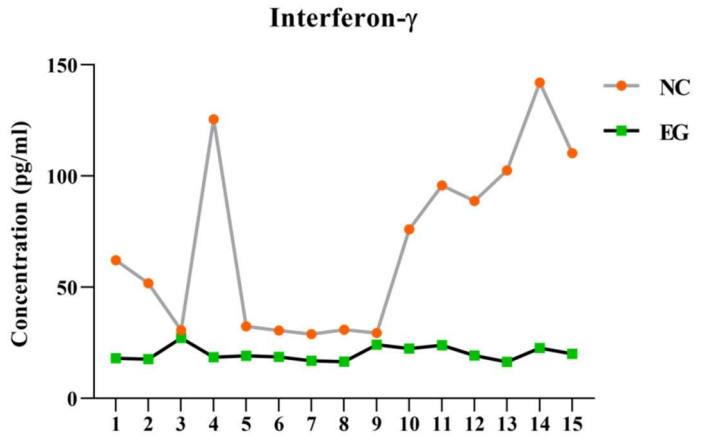

Figure6. Concentration of interferon-γ among the sera of study rats in both the NG and EG study groups.

3. Discussion

Our results in this study suggested the detrimental effects of starvation on the body through the assessment of antioxidant enzymes, lipid peroxidation, and immune system markers. These changes are in concordance with the observations of Pascual et al. (2003) in gilt-head bream (Sparus aurata), where there was an increase in malondialdehyde and a gradual decline in glutathione peroxidase level in the liver during starvation. Similarly, Bayir et al. (2011) reported the elevation of malondialdehyde concentration in the liver tissue of brown trout (Salmo trutta) under long-term fasting conditions. The initial phase markers of these processes in the liver increased significantly only on the 6th week of the fasting period, while the metastable end product of the lipid peroxidation processes was significantly higher on the 3rd and the 6th weeks of the starvation period. This may be due to differences in the fatty acid mobilisation during starvation, for mostly the saturated and monounsaturated fatty acids are mobilised, as was found in carp (Csengeri, 1996; Zajic et al., 2012). However, polyunsaturated fatty acids were mobilised later, and those are more prone to peroxidation (Houten et al., 2016). The diminished activity of the enzymatic antioxidants, particularly glutathione peroxidase, and the extent of its substrate was found to have enhanced the intensity of the free radicals generated lipid peroxidation in the liver of the starved fish (Hidalgo et al., 2017). According to Varju et al. (2018), the concentration of malondialdehyde, as a marker of lipid peroxidation processes, increased notably after the sixth week of starvation. As a result of starvation, significantly lower reduced glutathione peroxidase concentration was reduced significantly in the 6th week.

On the other hand, (2021) has estimated experimentally the effects of feeding and starvation concerning oxidative stress and enzymatic antioxidant activities in the whole body of 4 cm rainbow trout fry (Oncorhynchus mykiss) for 28 days. The author also discovered that the biomarkers catalase, glutathione peroxidase, and superoxide dismutase were significantly elevated on the 14th and 28th day of starvation. This, he said, could be attributed to the higher H2O2 production due to the free radicals that came from the oxidation of lipids. Further, the higher activities imply that the fries were subjected to higher oxidative stress in 14-day starved fry. Chen et al. (2022) showed that the counts of glutathione and superoxide dismutase were significantly higher in the experimental group after 3–7 days of starvation but reduced again to the normal baseline at the end of the study. In another study to estimate the effects of starvation and re-feeding on growth, digestion, nonspecific immunity, and lipid-metabolism-related genes, Gou et al. (2023) reported that the level of lysozyme, acid phosphate, alkaline phosphate, superoxide dismutase, glutathione peroxidase and catalase in hepatopancreas was significantly higher in second week starved–re-fed group compared to the control group.

However, it is well known that hunger-induced oxidative stress / ROS (reactive oxygen species) generation can affect the antioxidant capability and other physiological functions through lipid peroxidation in different organisms, which is reflected in the form of an increase in the level of malondialdehyde in the metabolically active tissues of some starved teleost. However, the malondialdehyde levels seemed to be reduced by the end of the starvation period, and this variation might be attributed to the physiologic characteristics of muscular tissue (Karatas, 2018). Similar results were observed in other cases where lipid peroxidation remained unchanged, while the activity of antioxidant enzymes increased in the muscle after 70 days of fasting (Furné et al., 2009; Furne & Sanz, 2018). In this study, the total antioxidative capacity was also determined as a marker of the overall antioxidant state in muscles, and we obtained data that are generally in agreement with the changes in both enzymatic and nonenzymatic types of antioxidants (Chen et al., 2022). Altogether, levels of the total antioxidative capacity were either unchanged or slightly enhanced during the early and middle parts of food deprivation, and a reduction of total antioxidative capacity was observed at the end of the fasting trial only (Huseynova et al., 2012; Moyankova et al., 2014; Bayliak et al., 2021). This implies that fasting for more than 14 days reduces the nutrient stores, such as different antioxidants, and negatively affects the metabolic functionality of the antioxidant capacity. Hence, it can be concluded that muscle could effectively combat ROS and oxidative stress due to short-term starvation through multiple antioxidant mechanisms, though the ability may fade away with prolonged fasting.

4. Conclusion

The outcome of this study revealed that starvation negatively impacted the rats in terms of oxidative stress by reducing the level of antioxidants (catalase, glutathione peroxidase, and superoxide dismutase) and increasing the level of lipid peroxidation (malondialdehyde). The feed restriction also affected the immune system by decreasing the level of interferon-γ and increasing the level of interleukin-4. This study recommended that further studies are of great importance to indicate the effect of starvation on other physiological and immunological markers as well as on the health status of body tissues/organs.

References

[1]. Alator, N. (2024). Building a Resilient Immune System: The Role of Nutrition and a Healthy Lifestyle. Journal of Nutrition and Health Care, 29-34.

[2]. Bar, N. (2014). Physiological and hormonal changes during prolonged starvation in fish. Canadian Journal of Fisheries and Aquatic Sciences, 71(10), 1447-1458.

[3]. Bayir, A., Sirkecioglu, A. N., Bayir, M., Haliloglu, H. I., Kocaman, E. M., and Aras, N. M. (2011). Metabolic responses to prolonged starvation, food restriction, and refeeding in the brown trout, Salmo trutta: oxidative stress and antioxidant defenses. Comparative biochemistry and physiology part B: Biochemistry and molecular biology, 159(4), 191-196.
[4]. Bayliak, M. M., Sorochynska, O. M., Kuzniak, O. V., Gospodaryov, D. V., Demianchuk, I., Vasylyk, Y. V., and Lushchak, V. I. (2021). Middle age as a turning point in mouse cerebral cortex energy and redox metabolism: modulation by every-other-day fasting. Experimental Gerontology, 145, 111182.
[5]. Calder, P. C., Carr, A. C., Gombart, A. F., and Eggersdorfer, M. (2020). Optimal nutritional status for a well-functioning immune system is an important factor to protect against viral infections. Nutrients, 12(4), 1181.
[6]. Chen, X., Xu, Y., Cui, X., Zhang, S., Zhong, X., Ke, J., and Cheng, H. (2022). Starvation Affects the Muscular Morphology, Antioxidant Enzyme Activity, Expression of Lipid Metabolism-Related Genes, and Transcriptomic Profile of Javelin Goby (Synechogobius hasta). Aquaculture Nutrition, 2022(1), 7057571.
[7]. Childs, C. E., Calder, P. C., and Miles, E. A. (2019). Diet and immune function. Nutrients, 11(8), 1933.
[8]. Csengeri, I. (1996). Dietary effects on fatty acid metabolism of common carp. Archives of Animal Nutrition, 49(1), 73-92.
[9]. Dogan, A., Severcan, F., Tuzlaci, A., and Guvenc, B. H. (2024). Comparison of human breast milk vs commercial formula-induced early trophic enteral nutrition during postoperative prolonged starvation in an animal model. Scientific Reports, 14(1), 21610.
[10]. Furné, M., García-Gallego, M., Hidalgo, M. C., Morales, A. E., Domezain, A., Domezain, J., and Sanz, A. (2009). Oxidative stress parameters during starvation and refeeding periods in Adriatic sturgeon (Acipenser naccarii) and rainbow trout (Oncorhynchus mykiss). Aquaculture Nutrition, 15(6), 587-595.
[11]. Furné, M., and Sanz, A. (2018). Starvation in fish–sturgeon and rainbow trout as examples. Handbook of Famine, Starvation, and Nutrient Deprivation; Preedy, V., Patel, V., Eds, 1-16.
[12]. Galef, B. G. (2013). Imitation in animals: History, definition, and interpretation of data from the psychological laboratory. In Social learning. Psychology Press. Pp: 3-28.
[13]. Gharban, H. A., and Al-Shaeli, S. J. (2021). Clinical and serum biochemical evaluation of goats with hypomagnesemia. Biochemical and Cellular Archives, 21(1), 587-592.
[14]. Gharban, H. A. (2023). Molecular prevalence and phylogenetic confirmation of bovine trichomoniasis in aborted cows in Iraq. Veterinary world, 16(3), 580-587.
[15]. Gombart, A. F., Pierre, A., and Maggini, S. (2020). A review of micronutrients and the immune system–working in harmony to reduce the risk of infection. Nutrients, 12(1), 236.
[16]. Gou, N., Wang, K., Jin, T., and Yang, B. (2023). Effects of starvation and refeeding on growth, digestion, nonspecific immunity and lipid-metabolism-related genes in Onychostoma macrolepis. Animals, 13(7), 1168.
[17]. Hidalgo, M. C., Morales, A. E., Arizcun, M., Abellán, E., and Cardenete, G. (2017). Regional asymmetry of metabolic and antioxidant profile in the sciaenid fish shi drum (Umbrina cirrosa) white muscle. Response to starvation and refeeding. Redox Biology, 11, 682-687.
[18]. Hillion, S., Arleevskaya, M. I., Blanco, P., Bordron, A., Brooks, W. H., Cesbron, J. Y., and Renaudineau, Y. (2020). The innate part of the adaptive immune system. Clinical reviews in allergy and immunology, 58, 151-154.
[19]. Houten, S. M., Violante, S., Ventura, F. V., and Wanders, R. J. (2016). The biochemistry and physiology of mitochondrial fatty acid β-oxidation and its genetic disorders. Annual review of physiology, 78(1), 23-44.
[20]. Huseynova, I. M. (2012). Photosynthetic characteristics and enzymatic antioxidant capacity of leaves from wheat cultivars exposed to drought. Biochimica et Biophysica Acta (BBA)-Bioenergetics, 1817(8), 1516-1523.
[21]. Hussen, T. J., Al-Shaeli, S. J. J., Al-Mahna, B. H. R., and Gharban, H. A. J. (2024). Biochemical and histological effects of long-term administration of estrogen on female mice. Adv. Anim. Vet. Sci, 12(8), 1563-1572.
[22]. Karatas, T. (2018). Effect of short-term starvation on serum metabolites, antioxidant enzymes and endogenous reserves of rainbow trout, Oncorhynchus mykiss. Pakistan Journal of Zoology, 50(5).
[23]. La Fata, G., Weber, P., and Mohajeri, M. H. (2018). Probiotics and the gut immune system: indirect regulation. Probiotics and antimicrobial proteins, 10, 11-21.
[24]. McCue, M. D., Lillywhite, H. B., and Beaupre, S. J. (2012). Physiological responses to starvation in snakes: low energy specialists. Comparative physiology of fasting, starvation, and food limitation, 103-131.
[25]. McCue, M. D., Terblanche, J. S., and Benoit, J. B. (2017). Learning to starve: impacts of food limitation beyond the stress period. Journal of Experimental Biology, 220(23), 4330-4338.

[26]. Mitra, S., Paul, S., Roy, S., Sutradhar, H., Bin Emran, T., Nainu, F., and Mubarak, M. S. (2022). Exploring the immune-boosting functions of vitamins and minerals as nutritional food bioactive compounds: A comprehensive review. Molecules, 27(2), 555.
[27]. Moyankova, D., Mladenov, P., Berkov, S., Peshev, D., Georgieva, D., and Djilianov, D. (2014). Metabolic profiling of the resurrection plant Haberlea rhodopensis during desiccation and recovery. Physiologia Plantarum, 152(4), 675-687.
[28]. Munteanu, C., and Schwartz, B. (2022). The relationship between nutrition and the immune system. Frontiers in nutrition, 9, 1082500.
[29]. Pascual, P., Pedrajas, J. R., Toribio, F., López-Barea, J., and Peinado, J. (2003). Effect of food deprivation on oxidative stress biomarkers in fish (Sparus aurata). Chemico-biological interactions, 145(2), 191-199.
[30]. Pirkmajer, S., and Chibalin, A. V. (2011). Serum starvation: caveat emptor. American Journal of Physiology-Cell Physiology, 301(2), C272-C279.
[31]. Sergio, F., Blas, J., and Hiraldo, F. (2018). Animal responses to natural disturbance and climate extremes: a review. Global and Planetary Change, 161, 28-40.
[32]. Subramanian, N., Torabi-Parizi, P., Gottschalk, R. A., Germain, R. N., and Dutta, B. (2015). Network representations of immune system complexity. Wiley Interdisciplinary Reviews: Systems Biology and Medicine, 7(1), 13-38.
[33]. Varju, M., Müller, T., Bokor, Z., Żarski, D., Mézes, M., and Balogh, K. (2018). The effects of excessive starvation on antioxidant defence and lipid peroxidation in intensively reared, commercial-size pikeperch (Sander lucioperca L.). The Egyptian Journal of Aquatic Research, 44(4), 349-352.
[34]. Yeşilyurt, N., Yılmaz, B., Ağagündüz, D., and Capasso, R. (2021). Involvement of probiotics and postbiotics in the immune system modulation. Biologics, 1(2), 89-110.
[35]. Zajic, T., Mraz, J., Kozak, P., and Pickova, J. (2012). Effect of pugging on lipid quality of common carp Cyprinus carpio L. flesh. AQUA 2012 Global Aquaculture. Prague. p. 1197.
[36]. Zengin, H. (2021). The effects of feeding and starvation on antioxidant defence, fatty acid composition and lipid peroxidation in reared Oncorhynchus mykiss fry. Scientific Reports, 11(1), 16716