Original Research Article

Biochemical role of experimentally induced malnutrition on depressed brain and moderate mood with blood picture in chicken

Alaa Mohammed[1], Afaf Desouky[1], Raafat R. Mohammed[2], Hussein Abdel-Maksoud[1,*]

[1] *Biochemistry Department, Faculty of Veterinary Medicine, Benha University, Kalyobiya 13637, Egypt*

[2] *Clinical Pathology Department, Benha University Hospital, Benha 6470031, Egypt*

*** Corresponding author:** Hussein Abdel-Maksoud, husseinabdelmaksoud2@gmail.com

Abstract: **Background:** Nutritional deficiency and malnutrition induce cyclothymia, depressed mood, and anemia. Therefore, in the present study, we experimentally induced malnutrition in chickens to follow up on brain functions and anemia profiles (blood indices) in chickens. **Material and methods:** The study was conducted on 60 one-day-old chicks that were equally divided into two groups and fed two different diets for six weeks. The control group was fed commercial grower and finisher rations during the growing and finishing periods, while the second group, which was the food deficiency test group, was fed yellow corn constantly during the growing and finishing periods. All chicks were weighed weekly to record weight differences. Whole blood samples and brain homogenates were collected from 10 chicks in each group every two weeks to evaluate brain tissue homogenate parameters, differential leukocyte count, complete blood count, and blood indices. **Results:** In the food deficiency group, acetylcholinesterase (ACHE) and total antioxidant capacity (TAC) consistently increased throughout the study period. Tumor necrosis factor-alpha (TNF-α) increased after the second and fourth weeks but showed no significant difference after the sixth week. Glutathione peroxidase (GPx) and superoxide dismutase (SOD) increased significantly in the control group during the entire experiment. In terms of blood counts, the white blood cells (WBCs) were consistently higher in the food deficiency group. Eosinophils were significantly elevated after two and four weeks but not after six weeks. Lymphocytes were elevated in the control group after the second week and in the food deficiency group after six weeks. Band neutrophils increased significantly in the food deficiency group after six weeks, while monocytes increased after two weeks in the food deficiency group and after six weeks in the control group. Basophils and segmented neutrophils increased after two weeks in the food deficiency group but showed no significant differences after four and six weeks in either group. Furthermore, hemoglobin (Hb), red blood cells (RBCs), packed cell volume (PCV), mean corpuscular volume (MCV), and platelet count increased significantly in the control group throughout the study. Mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) increased significantly after four and six weeks in the control group. **Conclusion:** Malnutrition can have high effects on brain functions and blood parameters.

Keywords: chicken; malnutrition; brain functions; blood indices; anemia; CBC

1. Introduction

Malnutrition can significantly impact brain functions and blood indices, leading to a range of negative consequences. When an individual does not receive enough essential nutrients, their brain may not develop properly thus leading to cognitive impairments and reduced intellectual capacity.

Malnutrition can have an impact on the body's hematopoietic system, resulting in reduced generation of all blood cell lines. People who are malnourished and have hematological abnormalities, such as anemia, are at an increased risk of death[1]. Leukopenia and leukocytosis are caused by an infection in PEM (protein-energy malnutrition) by forming a malnutrition-infection connection, in which inadequate nutritional status affects leucopoiesis[2]. Adequate dietary nutrient consumption is essential for normal brain functions. There is

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substantial evidence that dietary nutrition aids in the treatment and prevention of many mental and neurological illnesses. There is an impact of macronutrients (such as fatty acids and amino acids) and micronutrients (such as vitamins and minerals) on various brain functions, such as neuronal functioning, synaptic plasticity, memory, neuroinflammation, and neuronal signaling networks. It is important to examine the underlying neuroprotective mechanisms of dietary nutrients. Antioxidant and anti-inflammatory actions, as well as hypothalamic-pituitary-adrenal (HPA) axis modulation, neurotransmitter production, and neurotrophic actions, are regulated. The highlighted relationships of dietary nutrients on brain functions in health and psychiatric diseases have revealed some of the critical mechanisms underpinning a diet's effect on brain health, which will aid in controlling how best to use dietary nutrients to boost neuronal resistance to injuries and promote mental health[3].

Following decades of research on the relationships between nutrition vs. cognition and mood, several nutrients that can be obtained through food or dietary supplements have been advocated as a means of boosting mental performance and brain health. They can influence neuronal plasticity and function. This type of product is referred to as a nutraceutical. This term refers to any nutritional product that has a health and medical advantage, such as the prevention or treatment of certain ailments. Nutraceuticals enter the brain via the blood-brain barrier via the choroid plexus, primary cerebrospinal fluid transport locus, and various other mechanisms, such as active transport or assisted diffusion. Throughout the history of psychology, the term "cognition" and its definition have been a source of contention[4].

Emotions have the ability to either improve or weaken cognitive processes. For example, emotionally salient stimuli, such as seeing spiders or snakes, can have a strong effect on attention, and worry can disturb working memory. Anxiety, on the other hand, enhances alertness and potentiates cortical responses to safe environmental cues, which aids in the detection of emotionally salient data[5].

The primary objective of the present research was to investigate the biochemical connections that underlie the relationship between malnutrition and brain functions.

2. Materials and methods

2.1. Experimental animals

In this study, two groups of chicks were used in the investigation. In order to study malnutrition, 60 one-day-old chicks were treated for six weeks in special rooms for all control and experimental chicks in the poultry research units in the Faculty of Veterinary Medicine, Benha University, Egypt.

2.2. Experimental design

The chicks were equally divided into two groups, with equal water supply, lighting time, and temperature, with the only difference being the ration. The first control group was fed a standard grower ration and then a finisher ration, while the second group, which was the food deficiency group, was fed yellow floury maize throughout the whole experimental duration. On the 12th day, there was one mortality in the food deficiency group, and on the 38th day, there was one mortality in the control group.

2.3. Blood samples

For collecting sterile blood, a 25-gauge 1-in needle was inserted into a chick's brachial wing vein at a shallow angle (about 10°–20°) with the level facing up. Withdrawing blood must be done very gradually. The risk of hematoma can also be decreased by using a winged infusion set with a 25-gauge needle. If a hematoma develops, blood should not be drawn from the area, since it will probably clot before it can be transferred to a blood collection tube. Ethylenediaminetetraacetic acid (EDTA) is used to prevent clotting by chelating calcium,

an essential component of coagulation. In this study, samples were collected from 10 chicks of each group every two weeks for the biochemical evaluation of blood indices.

2.4. Brain tissue homogenate preparation

A fresh brain tissue was first obtained and made sure to be free from any contaminants or damage. The brain tissue was placed in a petri dish or a clean container and rinsed with an ice-cold saline solution to remove any blood or debris. It was then cut into small pieces using a sharp knife or scissors. The size of the pieces depended on the method of homogenization, as smaller pieces are generally easier to homogenize. The brain tissue pieces were transferred into a homogenization vessel appropriate for the chosen method of homogenization. Common vessels include tissue grinder tubes or Dounce homogenizers. A suitable buffer solution was added to the tissue, such as phosphate-buffered saline (PBS), RIPA buffer, or Tris-HCl buffer. The buffer should be kept cold, preferably on ice, to maintain the integrity of the proteins. The tissue was homogenized using a suitable homogenizer or tissue grinder. The homogenization process disrupted the tissue and released the cellular components. The number of homogenization cycles depended on the desired degree of homogenization and the sensitivity of the proteins being extracted. After homogenization, the homogenate was centrifuged at a low speed of around 1000–3000 rpm for 10–15 min to remove any large debris or unbroken cells. This step yielded a supernatant containing the desired tissue extract. The supernatant was transferred to a clean tube and further centrifuged at a higher speed of around 10,000–15,000 rpm for 30 min to remove any remaining cellular debris or aggregates. The resulting supernatant was now a brain tissue homogenate and can be used for further analysis or stored at −80 °C for future use[6].

2.5. Assessment of brain tissue homogenate parameters

Acetylcholinesterase (AChE) activity was determined using the colorimetric assay of Ellman et al.[7]. Antioxidant enzyme activity was determined as in Azhar et al.[8]. Tumor necrosis factor-alpha (TNF-α) concentrations were assayed using ELISA kits based on TNF-α monoclonal antibodies and converted to the TNF-α levels expressed as picograms per milligram tissue according to the study by Petrovas et al.[9]. A spectrophotometric assay was performed using the superoxide dismutase method[10]. The determination of glutathione peroxidase was according to the method in Nelly et al.[11].

3. Statistical analysis

The statistical analysis was conducted using a two-way analysis of variance (ANOVA) in SPSS ver. 25 (IBMCorp., 2013). As according to Steel et al.[12], the data were regarded as a complete randomization design. The Duncun test was applied to carry off multiple comparisons. Significance was fixed at <0.05.

4. Results

4.1. Brain acetylcholinesterase (AChE)

The obtained result (**Table 1** and **Figure 1**) showed that in the food deficiency group, there was a non-significant difference between the AChE mean values after the second and fourth weeks, but a significant increase existed after the sixth week compared with those after the second and fourth weeks. When comparing the two groups, a significant increase existed in the food deficiency group in comparison with the control group throughout the whole duration.

Table 1. Mean values (±S.E.) of brain acetylcholinesterase (ng/mL) in control and food deficiency groups.

Duration	Control	Food deficiency
After two weeks	30.82 ± 2.71^{aB}	40.56 ± 2.31^{bA}
After four weeks	28.14 ± 2.38^{abB}	42.22 ± 0.87^{bA}
After six weeks	23.40 ± 2.50^{bB}	71.92 ± 4.41^{aA}

Note: a, b, and c: no significant difference ($p > 0.05$) between any two means with same superscript letter inside same column.
A and B: no significant difference ($p > 0.05$) between any two means with same superscript letter inside same row.

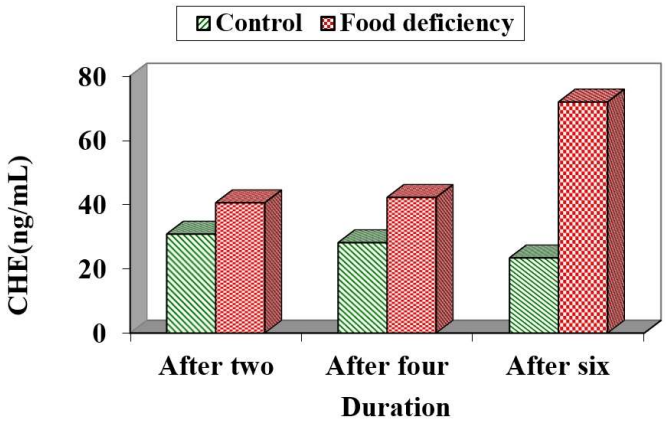

Figure 1. Mean values of brain acetylcholinesterase in food deficiency group and control group.

4.2. Tumor necrosis factor-alpha (TNF-α)

The obtained result (**Table 2** and **Figure 2**) showed that in the food deficiency group, there was a non-significant difference between the TNF-α mean values after the second and fourth weeks, but a significant increase existed after the sixth week when compared with those after the second and fourth weeks. When comparing the two groups, a significant increase existed in the food deficiency group in comparison with the control group throughout the second and fourth weeks, while a non-significant difference existed after the sixth week.

Table 2. Mean values (±S.E.) of brain tumor necrosis factor-alpha (pg/mL) in control and food deficiency groups.

Duration	Control	Food deficiency
After two weeks	15.22 ± 1.38^{bB}	21.33 ± 2.67^{bA}
After four weeks	15.07 ± 1.68^{bB}	19.15 ± 2.16^{bA}
After six weeks	26.43 ± 1.93^{aA}	26.01 ± 2.62^{aA}

Note: a, b, and c: no significant difference ($p > 0.05$) between any two means with same superscript letter inside same column.
A and B: no significant difference ($p > 0.05$) between any two means with same superscript letter inside same row.

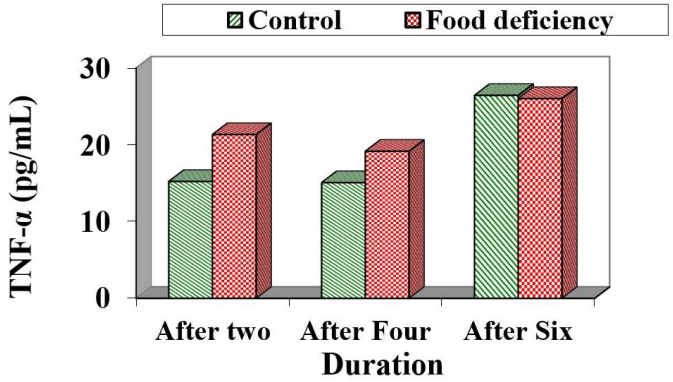

Figure 2. Mean values of brain tumor necrosis factor-alpha in food deficiency group and control group.

4.3. Brain total antioxidant capacity (TAC)

The obtained result (**Table 3** and **Figure 3**) showed that in the food deficiency group, there was a non-significant difference between the TAC values after the second and fourth weeks, while a significant increase existed after the sixth week when compared with those after the second and fourth weeks. When comparing the two groups, a significant increase existed in the food deficiency group in comparison with the control group throughout the whole duration.

Table 3. Mean values (±S.E.) of brain total antioxidant capacity (%) in control and food deficiency groups.

Duration	Control	Food deficiency
After two weeks	0.50 ± 0.13^{bB}	1.52 ± 0.28^{bA}
After four weeks	1.07 ± 0.15^{bB}	1.85 ± 0.21^{bA}
After six weeks	1.80 ± 0.20^{aB}	2.66 ± 0.54^{aA}

Note: a, b, and c: no significant difference ($p > 0.05$) between any two means with same superscript letter inside same column.
A and B: no significant difference ($p > 0.05$) between any two means with same superscript letter inside same row.

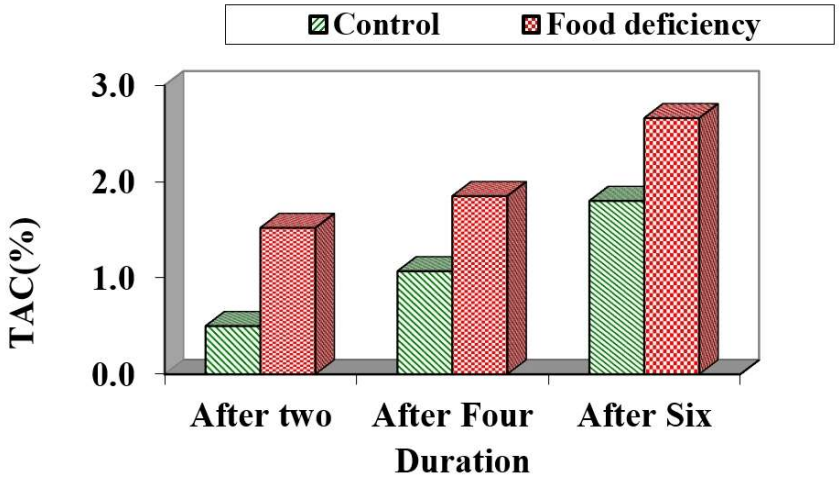

Figure 3. Mean values of brain total antioxidant capacity in food deficiency group and control group.

4.4. Brain glutathione peroxidase (GPx)

The obtained result (**Table 4** and **Figure 4**) showed that in the food deficiency group, there was a significant increase in the GPx mean value after the second week when compared with those after the fourth and sixth weeks, while there was a non-significant difference between those after the fourth and sixth weeks. When comparing the two groups, a significant decrease existed in the food deficiency group in comparison with the control group throughout the whole duration.

Table 4. Mean values (±S.E.) of brain glutathione peroxidase (U/L) in control and food deficiency groups.

Duration	Control	Food deficiency
After two weeks	14.03 ± 1.15^{bA}	10.11 ± 0.42^{aB}
After four weeks	13.56 ± 0.82^{bA}	8.09 ± 0.36^{bB}
After six weeks	18.47 ± 1.42^{aA}	8.50 ± 0.69^{bB}

Note: a, b, and c: no significant difference ($p > 0.05$) between any two means with same superscript letter inside same column.
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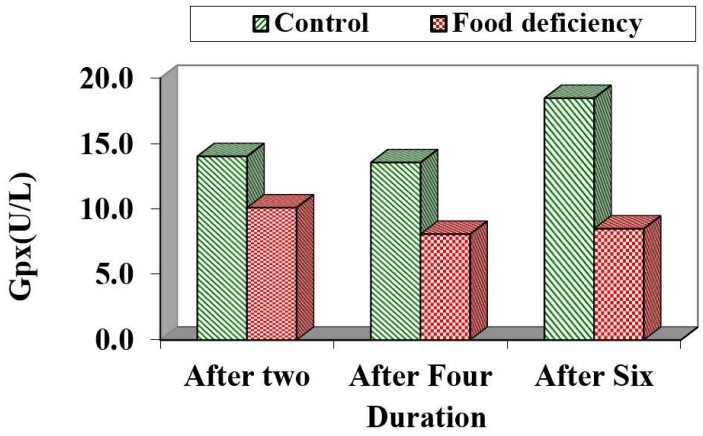

Figure 4. Mean values of glutathione peroxidase in food deficiency group and control group.

4.5. Brain superoxide dismutase (SOD)

The obtained result (**Table 5** and **Figure 5**) showed that in the food deficiency group, there was a non-significant difference between the SOD mean values after the second, fourth, and sixth weeks. When comparing the two groups, a significant decrease existed in the food deficiency group in comparison with the control group throughout the whole duration.

Table 5. Mean values (±S.E.) of brain superoxide dismutase (IU/mL) concentration in control and food deficiency groups.

Duration	Control	Food deficiency
After two weeks	7.26 ± 0.83^{bA}	4.70 ± 0.39^{aB}
After four weeks	7.87 ± 0.77^{abA}	3.86 ± 0.31^{aB}
After six weeks	8.79 ± 0.54^{aA}	3.52 ± 0.36^{aB}

Note: a, b, and c: no significant difference (p >0.05) between any two means with same superscript letter inside same column.
A and B: no significant difference (p >0.05) between any two means with same superscript letter inside same row.

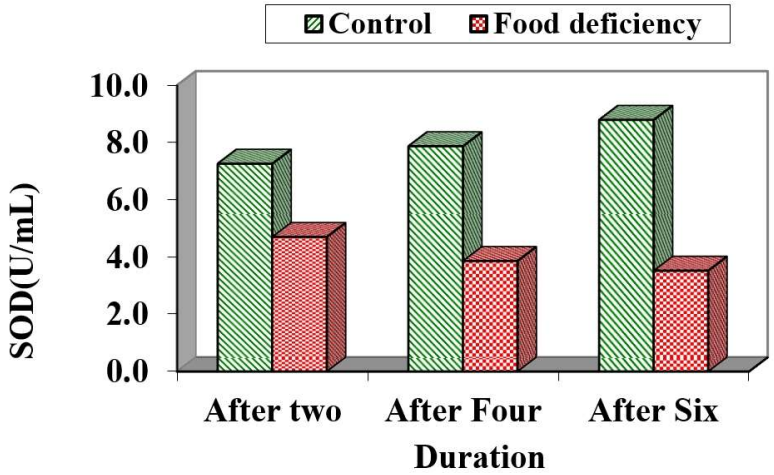

Figure 5. Mean values of brain superoxide dismutase in food deficiency group and control group.

4.6. Red blood cell (RBC) count

The obtained result (**Table 6** and **Figure 6**) showed that in the food deficiency group, there was a non-significant difference between the mean values of RBC count after the second and fourth weeks and a non-significant difference between those after the fourth and sixth weeks, but there was a significant decrease between those after the second and sixth weeks. When comparing the two groups, a significant decrease existed in the food deficiency group in comparison with the control group throughout the whole duration.

Table 6. Mean values (±S.E.) of red blood cell count ($\times 10^6$/mL) in control and food deficiency groups.

Duration	Control	Food deficiency
After two weeks	4.47 ± 0.11^{bA}	3.78 ± 0.08^{aB}
After four weeks	5.06 ± 0.21^{aA}	3.51 ± 0.18^{abB}
After six weeks	5.29 ± 0.19^{aA}	3.37 ± 0.07^{bB}

Note: a, b, and c: no significant difference ($p > 0.05$) between any two means with same superscript letter inside same column.
A and B: no significant difference ($p > 0.05$) between any two means with same superscript letter inside same row.

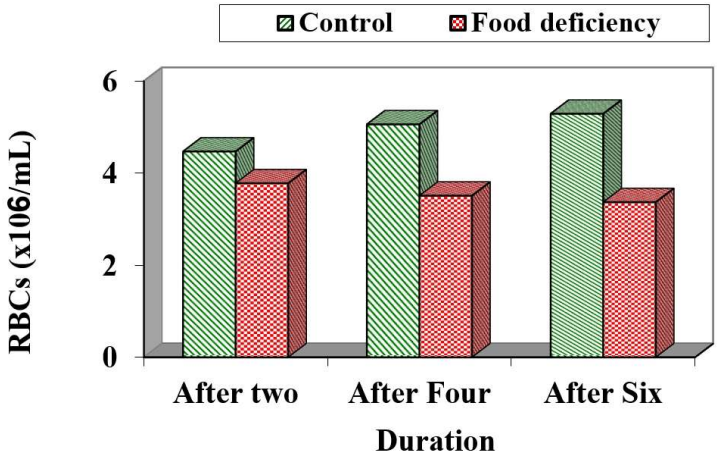

Figure 6. Mean values of red blood cell count in food deficiency group and control group.

4.7. Hemoglobin (Hb) concentration

The obtained result (**Table 7** and **Figure 7**) showed that in the food deficiency group, there was a non-significant difference between the HB mean values after the second and fourth weeks, while a significant decrease existed after the sixth week compared with those after the second and fourth weeks. When comparing the two groups, a significant decrease existed in the food deficiency group throughout the whole duration.

Table 7. Mean values (±S.E.) of hemoglobin concentration (g/dL) in control and food deficiency groups.

Duration	Control	Food deficiency
After two weeks	12.78 ± 0.31^{cA}	10.70 ± 0.22^{aB}
After four weeks	13.44 ± 0.35^{bA}	10.51 ± 0.19^{aB}
After six weeks	14.13 ± 0.14^{aA}	9.02 ± 0.13^{bB}

Note: a, b, and c: no significant difference ($p > 0.05$) between any two means with same superscript letter inside same column.
A and B: no significant difference ($p > 0.05$) between any two means with same superscript letter inside same row.

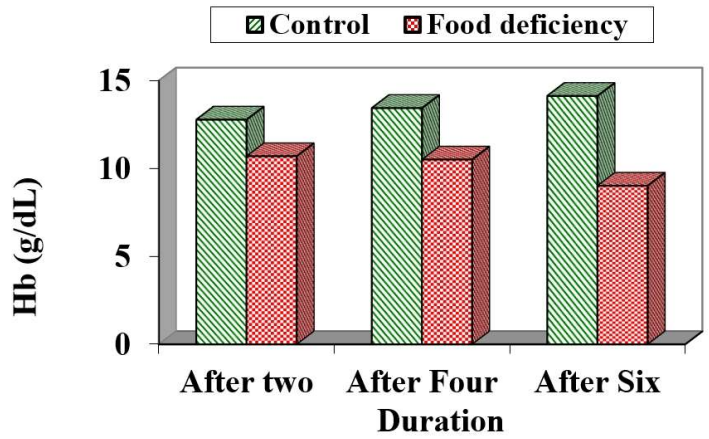

Figure 7. Mean values of hemoglobin concentration in food deficiency group and control group.

4.8. Packed cell volume (PCV)

The obtained result (**Table 8** and **Figure 8**) showed that in the food deficiency group, there was a non-significant difference between the PCV mean values after the second and fourth weeks and a non-significant difference between those after the fourth and sixth weeks, while there was a significant decrease between those after the second and sixth weeks. When comparing the two groups, a significant decrease existed in the food deficiency group in comparison with the control group throughout the whole duration.

Table 8. Mean values (±S.E.) of packed cell volume (%) in control and food deficiency groups.

Duration	Control	Food deficiency
After two weeks	42.61 ± 1.11^{cA}	35.15 ± 1.31^{aB}
After four weeks	49.13 ± 1.96^{bA}	33.88 ± 1.2^{abB}
After six weeks	52.99 ± 2.05^{aA}	31.84 ± 1.34^{bB}

Note: a, b, and c: no significant difference ($p > 0.05$) between any two means with same superscript letter inside same column.
A and B: no significant difference ($p > 0.05$) between any two means with same superscript letter inside same row.

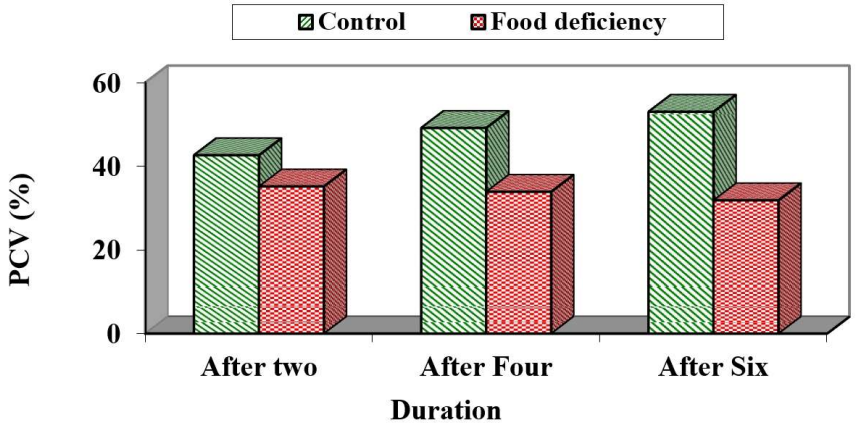

Figure 8. Mean values of packed cell volume in food deficiency group and control group.

4.9. Mean corpuscular volume (MCV)

The obtained result (**Table 9** and **Figure 9**) showed that in the food deficiency group, there was a non-significant difference between the MCV mean values after the second and fourth weeks, while a significant decrease existed after the sixth week when compared with those after the second and fourth weeks. When comparing the two groups, a significant decrease in the food deficiency group existed throughout the whole duration.

Table 9. Mean values (±S.E.) of mean corpuscular volume (fL) in control and food deficiency groups.

Duration	Control	Food deficiency
After two weeks	97.28 ± 2.34^{aA}	88.02 ± 2.91^{Ab}
After four weeks	98.32 ± 2.60^{aA}	85.89 ± 3.45^{aB}
After six weeks	97.26 ± 2.03^{aA}	72.92 ± 1.97^{bB}

Note: a, b, and c: no significant difference ($p > 0.05$) between any two means with same superscript letter inside same column.
A and B: no significant difference ($p > 0.05$) between any two means with same superscript letter inside same row.

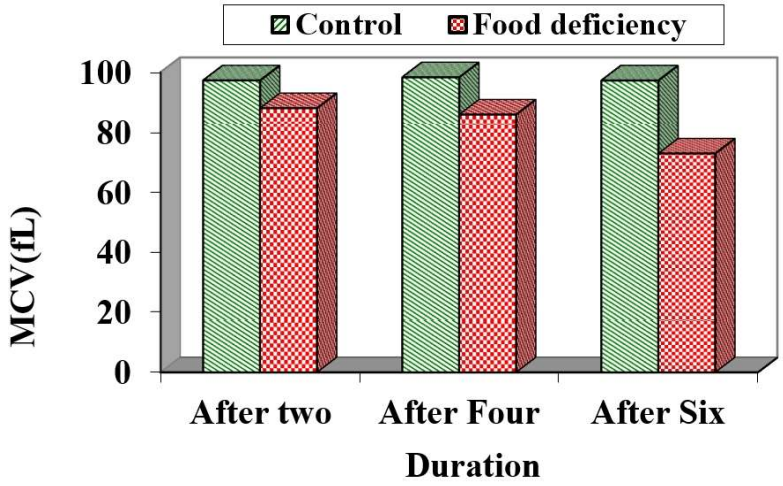

Figure 9. Mean values of mean corpuscular volume in food deficiency group and control group.

4.10. Mean corpuscular hemoglobin (MCH)

The obtained result (**Table 10** and **Figure 10**) showed that in the food deficiency group, there was a non-significant difference among the MCH mean values after the second, fourth, and sixth weeks. When comparing the two groups, a non-significant difference existed after the second week, while there were significant decreases after the fourth and sixth weeks in the food deficiency group.

Table 10. Mean values (±S.E.) of mean corpuscular hemoglobin (pg) in control and food deficiency groups.

Duration	Control	Food deficiency
After two weeks	30.23 ± 1.32^{aA}	28.56 ± 0.52^{aA}
After four weeks	30.83 ± 1.93^{aA}	28.28 ± 0.56^{aB}
After six weeks	30.60 ± 1.93^{aA}	26.67 ± 0.92^{aB}

Note: a, b, and c: no significant difference ($p > 0.05$) between any two means with same superscript letter inside same column.
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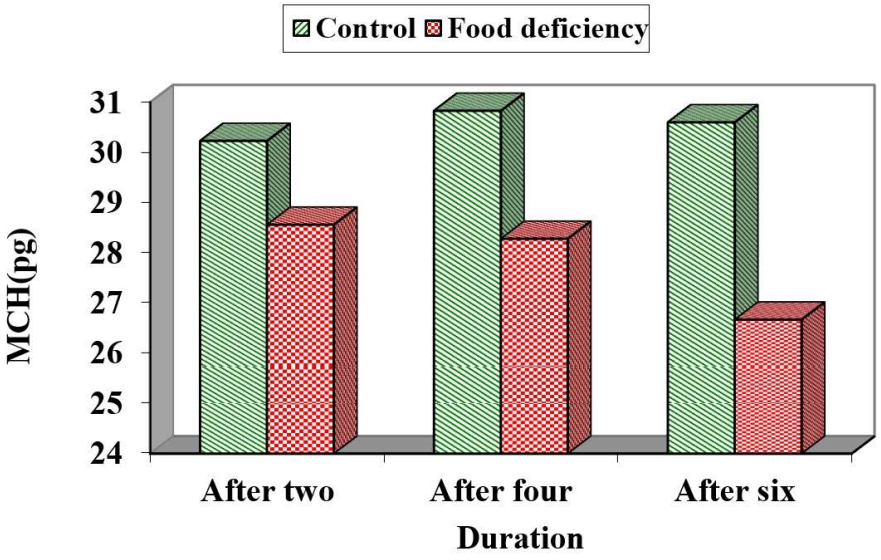

Figure 10. Mean values of mean corpuscular hemoglobin in food deficiency group and control group.

4.11. Mean corpuscular hemoglobin concentration (MCHC)

The obtained result (**Table 11** and **Figure 11**) showed that in the food deficiency group, there was a non-significant difference between the MCHC mean values after the second and fourth weeks and a non-significant difference existed between those after the fourth and sixth weeks, but there was a significant decrease between the fourth and sixth weeks. When comparing the two groups, a non-significant difference existed in the second week, while there was a significant decrease after the fourth and sixth weeks in the food deficiency group.

Table 11. Mean values (±S.E.) of mean corpuscular hemoglobin concentration (g/dL) in control and food deficiency groups.

Duration	Control	Food deficiency
After two weeks	30.77 ± 0.76^{aA}	30.00 ± 0.54^{aA}
After four weeks	32.30 ± 1.89^{aA}	29.46 ± 0.73^{abB}
After six weeks	31.90 ± 1.62^{aA}	27.45 ± 0.88^{bB}

Note: a, b, and c: no significant difference ($p > 0.05$) between any two means with same superscript letter inside same column.
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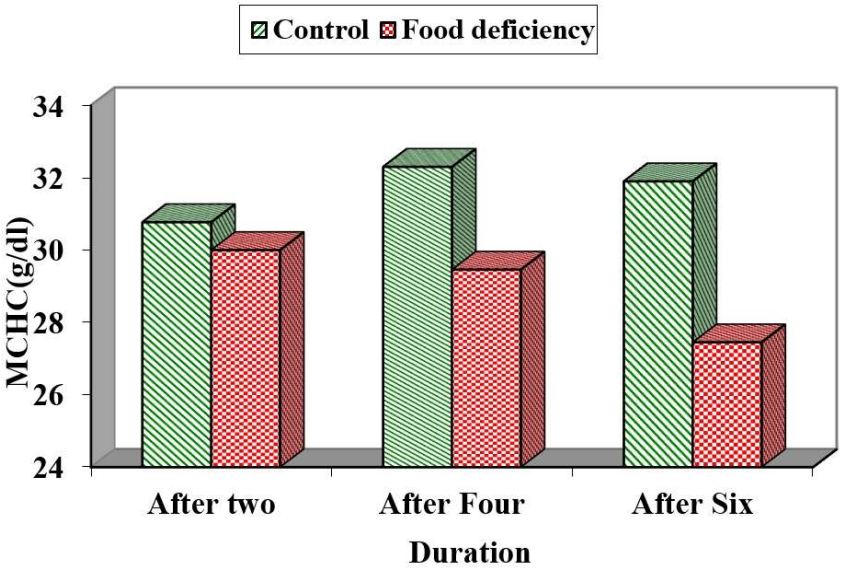

Figure 11. Mean values of mean corpuscular hemoglobin concentration in food deficiency group and control group.

4.12. White blood cell (WBC) count

The obtained result (**Table 12** and **Figure 12**) showed that in the food deficiency group, there was a significant increase in the mean values of WBC count after the second, fourth, and sixth weeks, where the highest was in the sixth week compared with those after the second and fourth weeks. When comparing the two groups, a significant increase existed in the food deficiency group in comparison with the control group throughout the whole duration.

Table 12. Mean values (±S.E.) of white blood cell count ($\times 10^3$) in control and food deficiency groups.

Duration	Control	Food deficiency
After two weeks	9.83 ± 0.64^{bB}	10.85 ± 0.47^{cA}
After four weeks	10.29 ± 0.33^{abB}	13.19 ± 0.48^{bA}
After six weeks	11.15 ± 0.35^{aB}	14.82 ± 0.28^{aA}

Note: a, b, and c: no significant difference ($p > 0.05$) between any two means with same superscript letter inside same column.
A and B: no significant difference ($p > 0.05$) between any two means with same superscript letter inside same row.

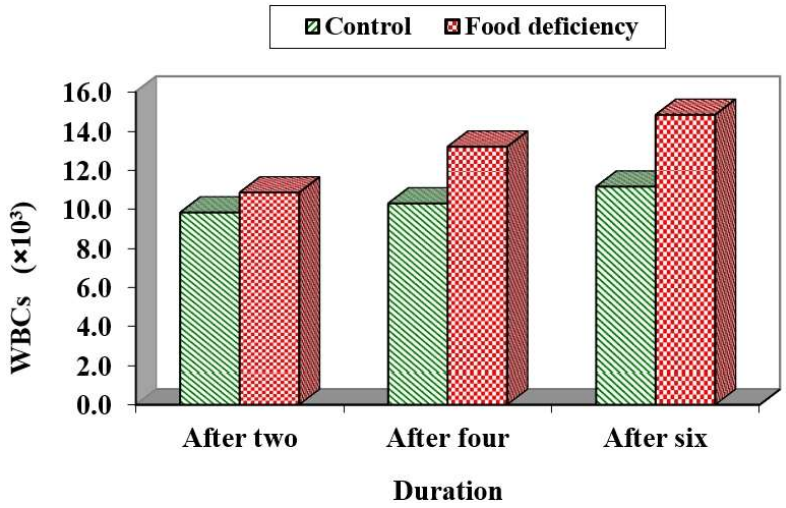

Figure 12. Mean values of white blood cell count in food deficiency group and control group.

4.13. Platelet count

The obtained result (**Table 13** and **Figure 13**) showed that in the food deficiency group, there was a significant decrease in the mean values of platelet count after the fourth and sixth weeks compared with that after the second week, while there was a non-significant difference between those after the fourth and sixth weeks. When comparing the two groups, a significant decrease existed in the food deficiency group in comparison with the control group throughout the whole duration.

Table 13. Mean values (±S.E.) of platelet count ($\times 10^9$/L) in control and food deficiency groups.

Duration	Control	Food deficiency
After two weeks	8.07 ± 0.61^{bA}	6.56 ± 0.34^{aB}
After four weeks	8.94 ± 0.27^{aA}	5.57 ± 0.35^{bB}
After six weeks	9.13 ± 0.23^{aA}	4.89 ± 0.37^{bB}

Note: a, b, and c: no significant difference ($p > 0.05$) between any two means with same superscript letter inside same column.
A and B: no significant difference ($p > 0.05$) between any two means with same superscript letter inside same row.

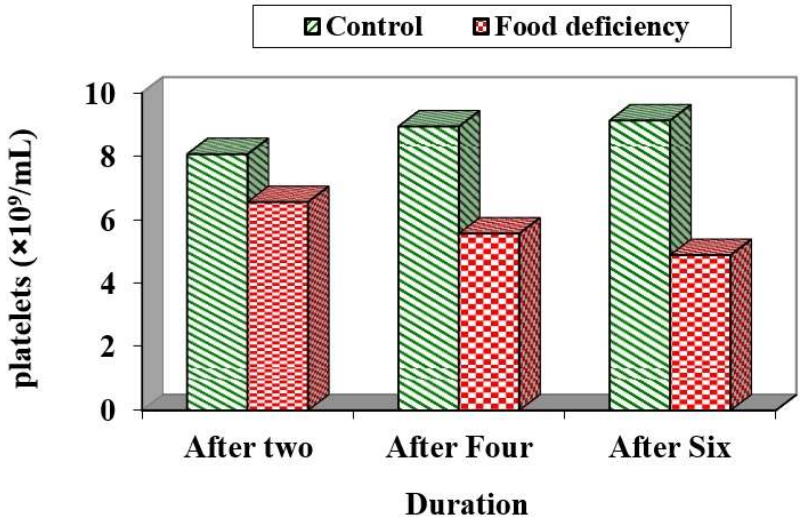

Figure 13. Mean values of platelet count in food deficiency group and control group.

4.14. Segmented neutrophils

The obtained result (**Table 14** and **Figure 14**) showed that in the food deficiency group, there was a significant increase in the mean values of segmented neutrophils after the fourth and sixth weeks compared with that after the second week, while there was a non-significant difference between those after the fourth and sixth weeks. When comparing the two groups, a significant increase existed in the food deficiency group after the second week in comparison with that in the control group, while there was a non-significant difference after the fourth and sixth weeks.

Table 14. Mean values (±S.E.) of segmented neutrophils (%) in control and food deficiency groups.

Duration	Control	Food deficiency
After two weeks	52.68 ± 1.52^{bB}	58.11 ± 0.90^{bA}
After four weeks	62.64 ± 1.38^{aA}	62.55 ± 1.34^{aA}
After six weeks	63.51 ± 2.35^{aA}	61.26 ± 1.23^{aA}

Note: a, b, and c: no significant difference ($p > 0.05$) between any two means with same superscript letter inside same column.
A and B: no significant difference ($p > 0.05$) between any two means with same superscript letter inside same row.

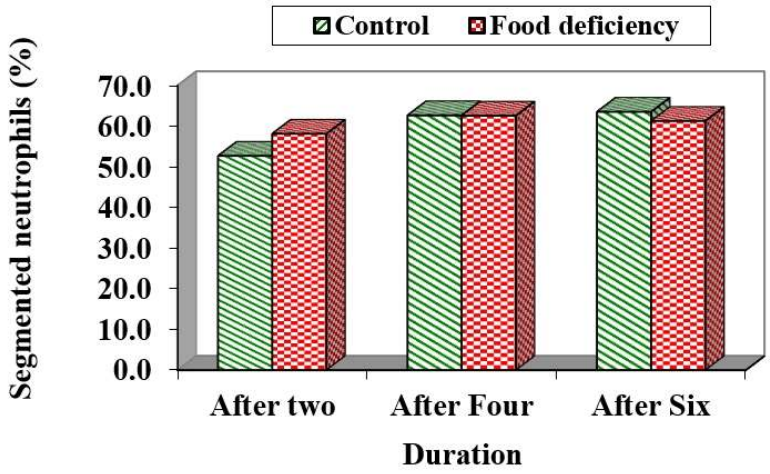

Figure 14. Mean values of segmented neutrophils in food deficiency group and control group.

4.15. Blood band neutrophils

The obtained result (**Table 15** and **Figure 15**) showed that in the food deficiency group, there was no significant difference between the mean values of blood band neutrophils after the second and fourth weeks, while a significant increase existed after the sixth week compared with those after the second and fourth weeks. When comparing the two groups, a non-significant difference existed after the second and fourth weeks, while a significant increase existed in the food deficiency group after the sixth week in comparison with that of the control group.

Table 15. Mean values (±S.E.) of blood band neutrophils (%) in control and food deficiency groups.

Duration	Control	Food deficiency
After two weeks	1.60 ± 0.24^{aA}	1.40 ± 0.24^{bA}
After four weeks	1.80 ± 0.20^{aA}	1.80 ± 0.20^{bA}
After six weeks	1.60 ± 0.24^{aB}	2.40 ± 0.24^{aA}

Note: a, b, and c: no significant difference ($p > 0.05$) between any two means with same superscript letter inside same column.
A and B: no significant difference ($p > 0.05$) between any two means with same superscript letter inside same row.

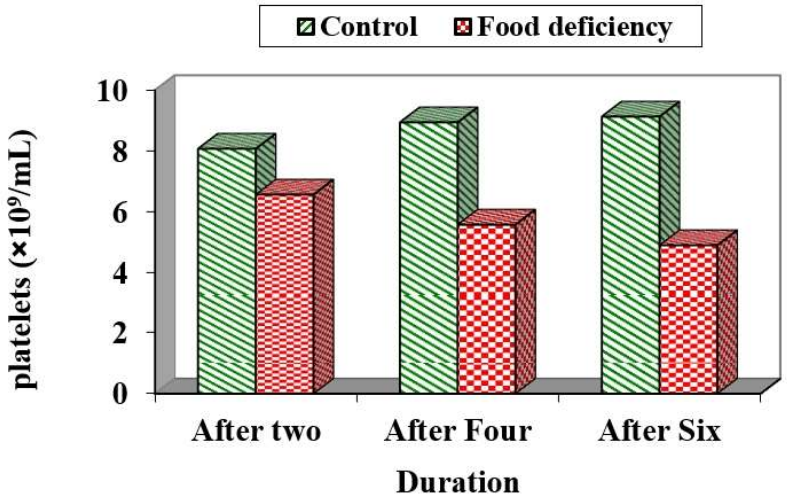

Figure 15. Mean values of blood band neutrophils in food deficiency group and control group.

4.16. Blood basophils

The obtained result (**Table 16** and **Figure 16**) showed that in the control group, that there was a significant increase in the mean values of blood basophils after the fourth and sixth weeks compared with that after the second week. Also, in the food deficiency group, there was a significant increase after the fourth and sixth weeks when compared with that after the second week, while a non-significant difference existed between those after the fourth and sixth weeks.

When comparing the two groups, a significant increase existed in the control group in comparison with the food deficiency group after the second week, while there were non-significant differences between the two groups after the fourth and sixth weeks.

Table 16. Mean values (±S.E.) of blood basophils (%) in control and food deficiency groups.

Duration	Control	Food deficiency
After two weeks	0 ± 0^{bB}	1.00 ± 0.45^{bA}
After four weeks	2.20 ± 0.37^{aA}	1.80 ± 0.37^{aA}
After six weeks	2.00 ± 0.32^{aA}	2.00 ± 0.00^{aA}

Note: a, b, and c: no significant difference ($p > 0.05$) between any two means with same superscript letter inside same column.
A and B: no significant difference ($p > 0.05$) between any two means with same superscript letter inside same row.

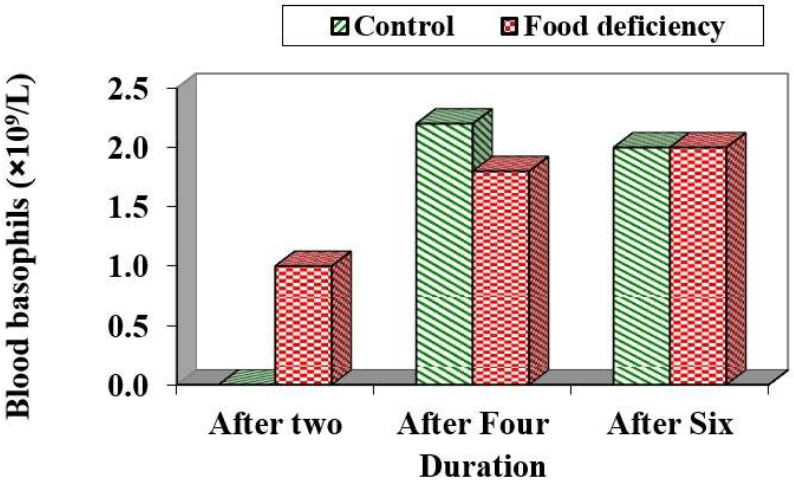

Figure 16. Mean values of blood basophils in food deficiency group and control group.

4.17. Blood eosinophils

The obtained result (**Table 17** and **Figure 17**) showed that in the food deficiency group, there was a significant increase in the mean value of blood eosinophils after the fourth week compared with that after the second week and a significant decrease existed after the sixth week compared with that after the fourth week, while a non-significant difference existed between those after the second and sixth weeks. When comparing the two groups, significant increases existed in the food deficiency group in comparison with the control group after the second and fourth weeks, while a non-significant difference existed after the sixth week.

Table 17. Mean values (±S.E.) of blood eosinophils (%) in control and food deficiency groups.

Duration	Control	Food deficiency
After two weeks	4.95 ± 0.36^{bB}	6.45 ± 0.41^{bA}
After four weeks	7.28 ± 0.28^{aB}	7.90 ± 0.23^{aA}
After six weeks	6.70 ± 0.21^{aA}	7.09 ± 0.44^{bA}

Note: a, b, and c: no significant difference ($p > 0.05$) between any two means with same superscript letter inside same column.
A and B: no significant difference ($p > 0.05$) between any two means with the same superscript letter inside same row.

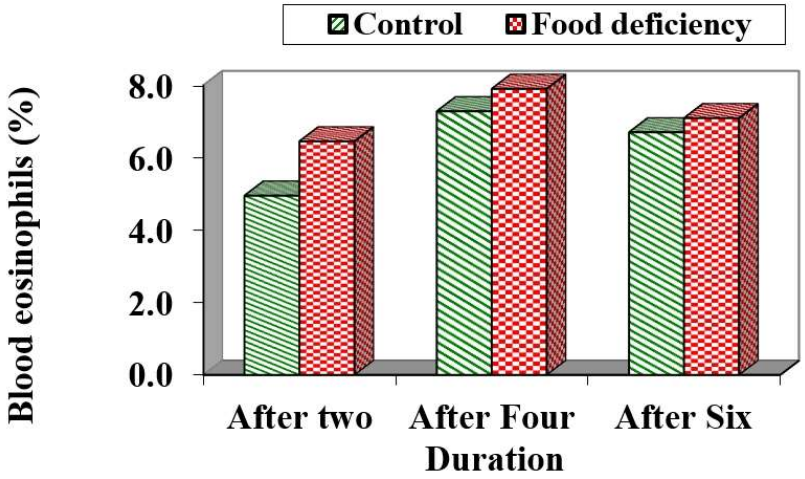

Figure 17. Mean values of blood eosinophils in food deficiency group and control group.

4.18. Blood monocytes

The obtained result (**Table 18** and **Figure 18**) showed that in the food deficiency group, there was a non-significant difference between the mean values of blood monocytes after the second and fourth weeks, while a significant decrease existed after the sixth week compared with those after the second and fourth weeks. When comparing the two groups, a significant increase existed in the food deficiency group in comparison with the control group after the second week, a non-significant difference existed after the fourth week, and a significant decrease existed after the sixth week.

Table 18. Mean values (±S.E.) of blood monocytes (%) in control and food deficiency groups.

Duration	Control	Food deficiency
After two weeks	4.86 ± 0.21^{cB}	6.13 ± 0.48^{aA}
After four weeks	6.15 ± 0.44^{bA}	6.52 ± 0.24^{aA}
After six weeks	7.48 ± 0.64^{aA}	5.03 ± 0.35^{bB}

Note: a, b, and c: no significant difference ($p > 0.05$) between any two means with same superscript letter inside same column.
A and B: no significant difference ($p > 0.05$) between any two means with same superscript letter inside same row.

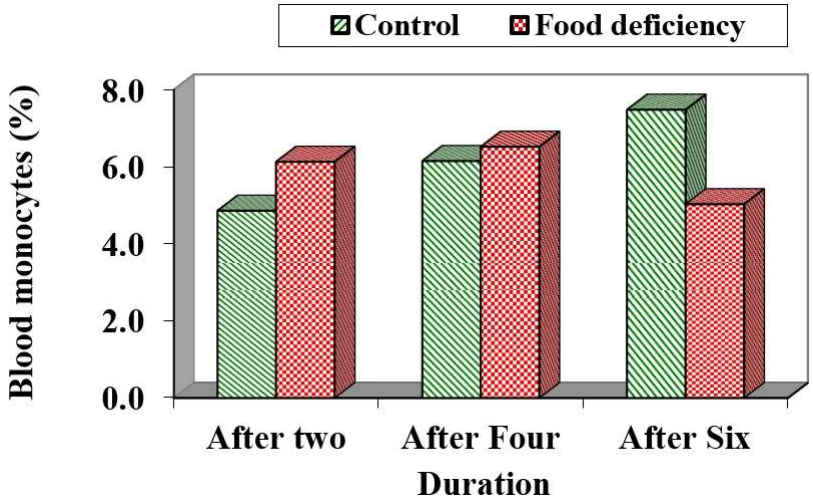

Figure 18. Mean values of blood monocytes in food deficiency group and control group.

4.19. Blood lymphocytes

The obtained result (**Table 19** and **Figure 19**) showed that in the food deficiency group, there was a significant decrease in the mean values of blood lymphocytes after the fourth and sixth weeks compared with that after the second week, while a non-significant difference existed between those after the fourth and sixth weeks. When comparing the two groups, a significant decrease existed in the second week in the food deficiency group in comparison with that in the control group, while a non-significant difference existed after the fourth week and a significant increase existed after the sixth week.

Table 19. Mean values (±S.E.) of blood lymphocytes (%) in control and food deficiency groups.

Duration	Control	Food deficiency
After two weeks	35.91 ± 1.49^{aA}	27.29 ± 0.84^{aB}
After four weeks	19.93 ± 2.14^{bA}	19.43 ± 1.97^{bA}
After six weeks	18.70 ± 3.11^{bB}	22.34 ± 1.64^{bA}

Note: a, b, and c: no significant difference ($p > 0.05$) between any two means with same superscript letter inside same column.
A and B: no significant difference ($p > 0.05$) between any two means with same superscript letter inside same row.

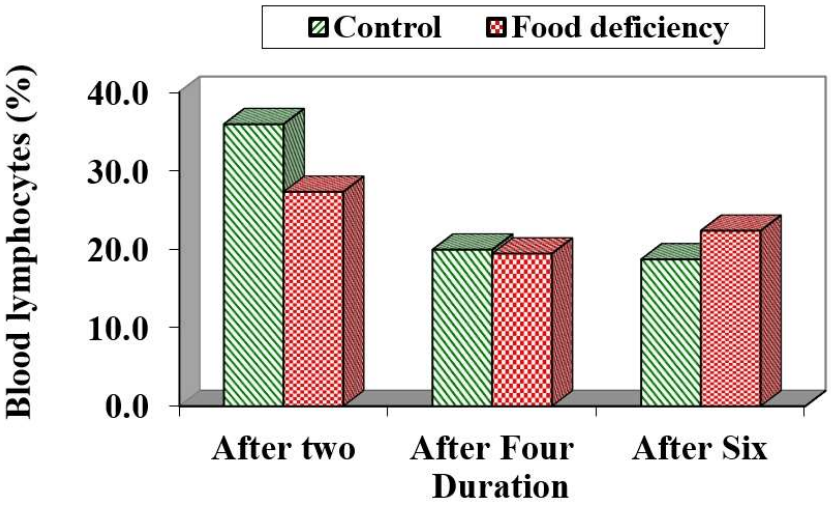

Figure 19. Mean values of blood lymphocytes in food deficiency group and control groups.

5. Discussion

Depression is one of the most common mental illnesses[13]. Although existing research is very limited, potential correlations between nutritional inadequacies and mental health are indicated to have some importance to general societal well-being on a worldwide scale. Consideration of their role in depression can provide new clues to the mechanisms underlying this disease and may help to design primary and secondary preventative strategies. It should be mentioned, however, that scientific evidence on this topic is now very limited[14]. The primary goal of this study was to investigate the influence of experimental undernutrition on the activity of brain tissue homogenate parameters (AChE, TAC, SOD, TNF-α, and GPx), blood CBC, and differential leukocyte count.

Our result showed that chicks reared only on yellow corn for six weeks exhibited an increase in acetylcholinesterase activity. The AChE activity may be linked to behavioral impairments in the chicks after food deprivation during critical stages of brain development. This finding is comparable to those of Winick and Noble[15] and Crnic[16], who found that undernourished rats showed increased AChE-specific activity in the cerebellum, hypothalamus, and striatum. It had been proposed that the increase in activity was due to a decrease in the brain water content. However, the water contents in the brain areas of rats from both dietary groups were similar. Furthermore, an increase in activity may be caused by a decrease in protein concentration in brain locations. In undernourished rats, the drop in protein content was proportionate to the decrease in brain area weight.

Also, our finding agrees with that of Zivkovic et al.[17], who found that a sustained increase in AChE activity correlated with injury severity and supported the proinflammatory phase of the early immune response. The increase in AChE-specific activity could be attributed to a decrease in protein concentration in the brain area. The observed increase continued throughout the observation period and was directly proportional to the degree of damage[18–20].

The observed result showed that after the chicks were reared on yellow corn for six weeks, the activity of total antioxidant capacity (TAC) showed increased activity in the malnutrition group, Protein deficiency was found to increase oxidative damage to lipids and proteins in the examined brain regions. TAC, also known as non-enzymatic TAC, is the sum of all antioxidants' synergistic interaction effects in a particular matrix[21]. This result disagrees with that of Feoli et al.[22], who stated that protein deficiency changed oxidative stress measurements, particularly the damage to macromolecules. Furthermore, in the cerebral cortex of protein-malnourished rats, there was a substantial drop in total antioxidant reactivity ($p < 0.05$). The quantity of thiobarbituric acid-reactive compounds, an indicator of lipid peroxidation, increased. These findings could point to an important mechanism for alterations in brain development induced by protein deficiency.

Our result demonstrated that after six weeks, the chicks' brain tumor necrosis factor-alpha (TNF-α) had a non-significant difference between those in malnutrition and nutrition groups. The result agrees with that of Azevedo et al.[23]. There was no statistically significant difference in the malnutrition group. The highest levels of TNF-α production were not related to high leukocyte numbers. This suggests that tumor necrosis factor-alpha is a pleiotropic cytokine that appears to play a role in the blood-brain barrier and the inflammatory, thrombogenic, and vascular alterations associated with brain damage[24–26]. These are disagreed upon by Postal et al.[27], who stated that TNF-α levels were elevated in systemic lupus erythematosus patients with mood and anxiety disorders, and the investigations revealed a clear link between immune system activation, peripheral proinflammatory cytokines, and mental health symptoms.

The tumor necrosis factor-alpha is a pleiotropic cytokine that produces different stimuli in various physiological and pathological conditions. TNF-α exerts its biological effect mainly by binding to tumor

necrosis factor receptor 1 and receptor 2, causing the activation of complex signaling cascades that mediate different intracellular effects in the brain. TNF-α may contribute to depression by activating the hypothalamic-pituitary-adrenocortical axis and neuronal serotonin transporters and stimulating indoleamine 2,3-dioxygenase, which leads to tryptophan depletion. This finding was validated by that of Himmerich[28].

Our result demonstrated that after six weeks, the chicks' brain glutathione peroxidase (GPx) was lower in the food deficiency group than in the control group. Glutamine is the primary nitrogen source for biosynthesis and has a strong link with intracellular redox status[29]. As a substrate, glutamine may increase amino acid, nucleotide, and protein synthesis, as well as glutathione synthesis, via glutamate delivery. A glial phosphate-activated glutaminase is required for this[30]. This result agrees with that of Feoli et al.[22]. They discovered that alterations in a rat brain caused by protein malnutrition (PMN) during the first postnatal week, including decreases in glutathione content and glutamate uptake, as well as an increase in glutamine synthetase, indicating specific changes in astrocyte metabolism and resulting in a higher vulnerability to excitotoxic/oxidative damage. The key change that causes the oxidative imbalance appears to be the loss in antioxidant defense rather than the increase in reactive oxygen species.

Our result demonstrated that after six weeks, brain superoxide dismutase decreased in the food deficiency group, which indicated that protein malnutrition interfered with the synthesis of enzymes that served as antioxidants. Our result agrees with that of Feoli et al.[22], who stated that protein malnutrition was shown to cause oxidative stress, an imbalance between the free radical content and tested scavenging systems, in the cerebral cortex and cerebellum. Furthermore, lower amino acid residue levels appeared to be important in all studied brain locations, indicating a malfunction of the repair processes of protein damage. The result also agrees with that of Halliwell[31], who discovered that severe protein deficiency caused long-term oxidative damage to macromolecules by increasing lipid peroxidation levels and drastically decreasing tyrosine and tryptophan concentrations. Changes in membrane structure, biochemical parameters, and functional activities, such as membrane fluidity and mitochondrial dysfunction, may be connected with oxidative damage to lipids and proteins.

The result demonstrated that after six weeks, blood indices (HB%, HB g/dL, MCV, MCHC, PCV, MCH, and RBC count) decreased in the food deficiency group. This indicated that the hematopoietic system was one of the systems that might be harmed by starvation, as according to Arya et al.[32], who concluded that anemia was a prevalent co-morbid condition in virtually all individuals with severe acute malnutrition. The majority of the individuals had moderate to severe anemia. According to Getawa et al.[1], hematopoietic tissues have a high rate of cell renewal and proliferation, which requires a proper supply of nutrients and may thus be affected by a nutrient deficit.

Also, the result demonstrated that after six weeks, blood platelet count significantly decreased in the food deficiency group. The result agrees with that of Khan et al.[33], who showed that anemia was also more prevalent among malnourished children than in the overall population. The majority of SAM (severe acute malnutrition) children had significantly lower red blood cell count, white blood cell count, and platelet count, as well as cytopenia and pancytopenia.

The result demonstrated that after six weeks, the white blood cell count increased in the malnutrition group, indicating that protein-energy malnutrition is usually connected with immunological dysfunctions and plays a major role in vulnerability to infectious illnesses. According to Gohain et al.[34], the mean value of WBC count was greater in undernourished children than in their counterparts. Infections, such as gastroenteritis and respiratory tract infections, may be the cause of the increase in leukocyte count.

Also, the result demonstrated that after six weeks, blood monocytes decreased at the end of the experimental period in the malnutrition group. The result was similar to the finding of Jordan et al.[35], who stated that reduced circulating monocyte count in fasting mice could be attributed to increased monocyte cell death, decreased bone marrow (BM) myelopoiesis, or decreased BM egress to the periphery. These results disagree with that of Corware et al.[36], who stated that monocytes and macrophages greatly enhanced in the bone marrow and blood of mice fed with a low-protein diet.

The result demonstrated that after six weeks, blood segmented neutrophils showed a non-significant difference in the food deficiency group. This indicated that malnutrition did not affect segmented neutrophil count. The result agrees with that of Takele et al.[37], who found that there were no significant changes in neutrophils between people with a normal body mass index and those who were moderately malnourished and severely malnourished. Neutrophils are highly flexible cells that play critical roles in the creation and resolution of inflammation, immune response control, and pathogen elimination via processes such as phagocytosis and toxic chemical synthesis. However, their involvement in human adult malnutrition is unknown. The ability of malnourished neutrophils to create reactive oxygen species is greatly decreased, implying that malnourished neutrophils are less capable of killing infections.

The finding showed that after six weeks, the malnourished group's blood band neutrophils and lymphocytes increased. These outcomes are consistent with that of Franceschi and Campisi[38], who stated that older persons who were malnourished or at risk of being malnourished had a higher blood neutrophil-lymphocyte ratio when compared to those with a normal nutritional status, but this was not found to be substantially connected with multivariate analyses. Inflammation is a chronic low-grade inflammation characterized by elevated pro-inflammatory cytokines.

Moreover, the result showed that after six weeks, blood basophils and eosinophils in chicks were unaffected by starvation. These results are consistent with that of Tigner et al.[39], who stated that although malnutrition can have far-reaching impacts on several components of the body's immune system, including white blood cells, there have been few investigations into the association between malnutrition and blood basophils and eosinophils. Basophils and eosinophils are white blood cells that help the body's immune system fight parasites, allergies, and infections. During allergic reactions, basophils release histamine and other chemicals, whereas eosinophils fight parasitic infections and modulate immunological responses.

6. Conclusion

The findings of the present study demonstrated that malnutrition can have high effects on brain functions and blood parameters. Our study represents a step towards a deeper understanding of the complex interplay between nutrition and brain functions, which holds significant implications for public health and clinical practice.

Author contributions

Conceptualization, HAM; methodology, AM and AD; software, RRM and AD; validation, HAM and AM; formal analysis, AM and RRM; investigation, RRM and HAM; resources, RRM and AM; data curation, HAM and RRM; writing—original draft preparation, AM and AD; writing—review and editing, HAM and RRM; visualization, RRM and HAM; supervision, HAM; project administration, RRM and AD; funding acquisition, AM and RRM. All authors have read and agreed to the published version of the manuscript.

Conflict of interest

The authors declare no conflict of interest.

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