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LIST OF ABBREVIATIONS

A/G ratio	Albumen/Globulin ratio
AOS	Antioxidant defense system
B.W	Body weight
CAT	Catalase
CP	Crude protein
CTC	Copper tartarate/carbonate
GCS	γ-glutamylcysteine synthetase
GPx	glutathione peroxidase
γ-GT	Gamma glutamyl transferase
GR	Glutathione Reductase
GSH	Reduced glutathione
GSH-Px	Glutathione peroxidase
GSSG	Oxidized glutathione
GST	Glutathione S-Transferase
H_2O_2	Hydrogen peroxide
Hb	Hemoglobin
KCl	Potassium chloride
L-MDA	L- Malondialdehyde
LPO	Lipid Peroxidation
NADPH	Necotenamide Adnine Dinuclutoide Phosphate
NRC	Nutritional Research Council
O^{-2}	Superoxide
PUFA	Polyunsaturated fatty acids
RBC	Red Blood Cells
ROS	Reactive oxygen species
SOD	superoxide dismutase
TBARS	Thiobarbituric Acid Reactive Substances

SUMMARY

This study investigated the harmful effects of low protein ration on several biochemical blood parameters. Lipid peroxidation and evaluation of antioxidant enzymes activities in broiler chicken fed on low protein ration. Lipid peroxidation were evaleuated in plasma and tissues. Also antioxidant enzymes, glutathione peroxidase, glutathione reductase, catalase and superoxide dismutase activities in hemolyste and tissue homogenate. In addition, reduced glutathione were estimated.

Plasma were analyzed for determination of the following parameters: total protein, albumin, globulins, albumin/globulin ratio and L-Malondialdhyde concentration.

blood samples were used directly for determination of hemoglobin. The study was conducted with two hundred broiler Hubbard chickens one day old. The chickens were fed on a starter balanced ration containing 23% crude protein essential for optimum growth rate and fresh water ad-libitum up to two weeks. After two weeks the chickens were divided into two main equal groups; the chickens were reared under strict hygienic conditions in isolated constant lightened rooms all over the period of the experiment as follows:

Group I (Normal protein ration): One hundred Hubbard chickens fed on normal protein ration. Starter ration 23% protein for 2.5 weeks, growing ration 20% protein for 2.5 weeks and finisher ration 18% protein up to the end of the experiment according to NRC used as control group.

Group II (Protein Deficient ration): chickens fed on low protein (11%) ration.

The rations were supplemented and fresh water ad-libitum up to the end of the period of the experiment.

Sampling:

Blood samples were collected randomly from 15 chickens(fasting over night) belonging to control and experimentally deficient groups at 2^{nd}, 4^{th},and 6^{th} week from the onset of the deficient diets supplementation at two weeks of chicken's age**.**

Samples preparation:

Approximately 5ml of heparinzed blood were collected from the wing vein, of 15 chickens from each group, in sterile closed tubes then centrifuged at 3000 r.p.m for 10 minutes. The clear clean plasma was separated and kept in dry sterile screw caped tubes using sterile pipette. Another sample of blood collected from he wing vein in clean heparinzed tube to get PCV for heamolysate preparation. After sampling small portion of heamolysate which used directly for determination of glutathione peroxidase, glutathione reductse, catalase and superoxide dismutase activities. Also used for determination of reduced glutathione in red blood cells.

All plasma were analyzed for the following biochemical parameters: total protein, albumin, globulin, albumin/globulin ratio and estimation of lipid peroxidation.

Tissue specimens:

After six weeks from the onset of the experiment the chickens were fastened for 24 hours and then slaughtered. The brain, liver, heart, kidneys, and muscle specimen were rapidly removed, washed with saline weighed to avoid drying and processed directly for determination of lipid peroxidation. Antioxidant enzymes activities (glutathione peroxidase, glutathione reductse, catalase and superoxide dismutase activities in addition to reduced glutsthione and lipid per oxidation).

The obtained results summarize the following:

1- Plasma L- Malondialdhyde (MDA):

Feeding of broiler chickens on low protein ration resulted in a non-significant increase in plasma L- malondialdhyde, after two weeks of the experiment this increase became high significant after four meanwhile, became very high significant six weeks as compared to control group.

The highest L-MDA concentration was observed after six weeks in the plasma of broiler chickens fed on low protein ration.

2- L-Malondialdehyde (MDA) concentration in brain, heart, liver, kidney and muscles tissues of broiler chickens:

Feeding of low protein ration in broiler chickens resulted in a very high significant increase in L- malondialdhyde concentration in the brain tissue. Moreover, a high significant increase recorded in the heart and kidney tissues. In addition, a significant increase observed in the liver and muscle tissues of broiler chickens fed on low protein ration after six weeks of the experiment in comparison with control group.

The highest concentration of L-MDA was recorded in the brain tissue.

3- Erythrocyte reduced glutathione concentration (GSH) in broiler chickens fed on low protein ration:

A high significant decrease in erythrocyte glutathione concentration in broiler chickens fed on low protein ration was observed after two and four weeks this decrease became very high significant after six weeks of experiment.

The lowest erythrocyte glutathione concentration was observed after six weeks of the experiment.

4- Reduced glutathione concentration (GSH) in brain, heart, liver, kidney and muscles tissues of broiler chickens fed on low protein ration:

The broiler chickens fed on low protein ration revealed a very significant decrease in GSH concentration the brain, heart, liver, kidney and muscles tissues at the end of the experiment in broiler chickens fed on low protein ration in comparison with control group.

The lowest glutathione (GSH) concentration were observed in the brain tissue.

5- Erythrocyte Glutathione Peroxidase (GPx) Activity of broiler chickens fed on low protein ration:

The broiler chickens fed on low protein ration revealed a very high significant increase in erythrocyte glutathione peroxidase activity (GPx) after two, four and six weeks of the experiment in comparison with the control group. The highest activity was observed after six weeks of the experiment.

6- Glutathione Peroxidase (GPx) Activity in Brain, Heart, Liver, Kidney and Muscles tissues of broiler chickens fed on low protein ration:

A very high significant increase in activity of cytosolic glutathione peroxidase activity in the tissues of the brain, heart and muscle tissues. Moreover, Moreover, the liver and kidney tissues of broiler chickens fed on low protein ration recorded a significant in when compared with the control group.

The highest activity was recorded in the liver tissue at the end of the experiment.

7- Erythrocyte glutathione reductase (Gr) activity in broiler chickens fed on low protein ration:

The activity of Glutathione reductase in the erythrocyte of broiler chickens fed on low protein ration showed a very high significant increase after two, four and six weeks of the experiment in comparison with the control group.

The highest activity was recorded after six weeks of the experiment.

8- Glutathione Reductase (GR) Activity in Brain, Heart, Liver, Kidney and Muscles tissues of Broiler Chickens fed on low protein ration:

The activity of glutathione reductase showed a significant increase in the brain, and heart tissues of broiler chickens fed on low protein ration, furthermore, a high significant increase in the activity was showed in the liver and kidney while the muscle tissue revealed a non significant increase after six weeks of the experiment in comparison with the control group.

The lowest activity was recorded in muscle tissue of Broiler Chickens fed on low protein ration.

9- Erythrocyte catalase activity in broiler chickens fed on low protein ration:

A very high significant increase in activity of erythrocyte catalase activity was observed in broiler chickens fed on low protein ration along the periods of the experiment.

The highest activity was recorded after six weeks of the experiment.

10- Catalase activity in brain, heart, liver, kidney and muscles tissues of broiler chickens fed on low protein ration:

There is a significant increase in the activity of catalase in the brain, heart, liver and kidney of the broiler chickens fed on low protein ration which. Moreover, the results revealed a non significant increase in catalase activity in the muscle tissue of broiler chickens fed on low protein ration at the end of the periods of the experiment.

The highest activity of catalase enzymes was observed in the liver tissues.

11- Erythrocyte superoxide dismutase (SOD) activity in broiler chickens fed on low protein ration:

A significant decrease the erythrocyte superoxide dismutase activity in of broiler chickens fed on low protein ration after two, four and six weeks of the experiment in comparison with the control group.

The lowest superoxide dismutase enzyme activity observed after six weeks of the experiment.

12- Superoxide dismutase (SOD) activity in the brain, heart, liver, kidney and muscles tissues in broiler chickens fed on low protein ration:

A very high significant decrease the activity of superoxide dismutase in the brain and heart tissues. Meanwhile, the liver, kidney and muscle tissue recorded a high significant decrease of superoxide dismutase activity in broiler chickens fed on low protein ration after six weeks of the experiment in comparison with the control group.

The lowest activity of this enzyme was observed in the brain tissues.

13- Plasma total protein concentration in broiler chickens fed on low protein ration:

There is a significant decrease in plasma total protein of broiler chickens fed on low protein ration was observed after two and four of the experiment. This decrease became non significant after six weeks when compared with the control group.

14- Plasma Albumen Concentration in broiler chickens fed on low protein ration:

There is a high significant decrease in plasma albumen level in broiler chickens fed on low protein ration after two, four and six weeks of the experiment when compared with the control group.

15- Plasma Globulin Concentration in broiler chickens fed on low protein ration:

There is a non significant decrease in plasma total globulin level observed in broiler chickens fed on low protein ration after two, four and six weeks of the experiment when compared with the control group.

16- Plasma Albumen/Globulin Ratio:

The value of albumin/globulin ratio in broiler chickens fed on low protein ration revealed a non significant increase after two, four and six weeks of the experiment when compared with the control group.

17- Tissue protein in broiler chickens fed on low protein ration:

There is a significant decrease in protein concentration of the brain, liver, kidney and muscle tissue. Furthermore, a high significant decrease in the heart tissue protein concentration was recorded in broiler chickens fed on low protein ration at the end of the experiment when compared with the control group.

18- Hemoglobin Concentration in Broiler Chickens fed on low protein ration:

A high significant decrease in hemoglobin concentration of broiler chickens fed on low protein ration after two weeks, this decrease became very high significant at four and six weeks of the experiment in comparison with the control group.

19- Body weight:

A very high significant decrease in Body weight of broiler chickens fed on low protein ration after two, four and six weeks when compared with the control group.

20- Correlations coefficient for significant relationships between plasma total proteins and oxidative stress in RBCs of broiler chickens fed on low protein ration.

Table (20) and figure (20) shows that the plasma total proteins concentration was revealed negative correlation with L-MDA concentration after two, four and six weeks of the experiment.

Moreover, the plasma total proteins positively correlated with GSH concentration after two, four and six weeks of age.

On the other hand the data demonstrates a negative correlation with activities of GPx, after two, four and six weeks.

Furthermore, GR activity revealed, a negative correlation after two, four and six weeks.

Meanwhile, CAT activity demonstrates negative correlation after two, four and six weeks.

Furthermore, plasma total proteins concentration was positively correlated with SOD activities after two, four and six weeks.

21- Correlations coefficient between L-malondialdehyde and antioxidant enzymes activities in RBCs of broiler chickens fed on low protein ration:

Analysis of data shows that the plasma L-malondialdehyde concentration revealed a non significant negative correlation with GSH after two weeks, while a strongly significant negative correlation observed after four and six weeks of the experiment.

Moreover, GPx, showed a non significant positive correlation after two weeks these correlation became strongly significant after four and six weeks of the experiment.

Furthermore, L-malondialdehyde concentration demonstrated a non significant positive correlation with the activity of GR after two weeks this correlation became strongly positive after four and six weeks of the experiment.

In addition CAT activity showed a strong significant positive correlation with plasma L-malondialdehyde concentration all over the period of the experiment.

On the other hand, SOD activity revealed a non significant positive correlation with L-malondialdehyde concentration all over the period of the experiment.

22- Correlations coefficient between tissue protein, and corresponding L-MDA and antioxidant enzymes activities in brain, heart, liver, kidney and muscles tissues of broiler chickens fed on low protein ration:

Table (22) and figure (22) analysis of data showed that brain tissue protein revealed significant negative correlation with the tissue L-MDA concentration, GPx, GR and CAT activities in brain tissue of broiler

chickens fed on low protein ration. On the other hand, GSH concentration and SOD activity showed positive correlation with brain tissue protein cocentration.

The data revealed negative correlation between the heart tissue protein and L-MDA concentration, GPx, GR and CAT activities. Moreover, GSH concentration and SOD activity showed positive correlation with the heart tissue protein.

The liver tissue protein showed negative correlation with the tissue L-MDA concentration, GPx, GR and CAT activities. On the other hand, GSH concentration and SOD activity showed positive correlation with the liver tissue protein at the end of the experiment.

Moreover, Kidney tissue protein shows negative correlation with L-MDA concentration, GPx, GR and CAT activities. On the other hand, GSH concentration and SOD activity shows positive correlation after six weeks of the experiment.

The analysis of the data illustrated negative correlation between muscle protein concentration from one side and L-MDA concentration, GPx, GR and CAT activities from the other side. On the other hand, GSH concentration and SOD activities revealed positive correlation with muscles tissue protein.

23- Correlations coefficient between L-malondialdehyde concentration and antioxidant enzymes activities in the brain, heart, liver, kidney and muscles tissues of broiler chickens fed on low protein ration:

Table (23) and figure (23) analysis of data showed that L-MDA concentration revealed negative correlation with GSH concentration and SOD activity. On the other hand, L-MDA concentration showed a

positive correlation GPx, GR and CAT activities in the brain tissues of broiler chickens fed on low protein ration.

A negative correlation demonstrated between GSH concentration and SOD activity with the heart L-MDA concentration Moreover, GPx, GR and CAT activities revealed positive correlation.

Liver L-MDA concentration shows a negative correlation with GSH concentration and SOD activity. Moreover, GPx, GR and CAT activities illustrated a positive correlation.

Moreover, the kidney tissue L-MDA concentration shows a negative correlation with GSH concentration and SOD activity. On the other hand, GPx, GR and CAT activities showed positive correlation in the kidney tissue of broiler chickens fed on low protein ration.

The analysis of the data illustrated a negative correlation between muscle tissue L-MDA concentration from one side and GSH concentration and SOD activity from the other side. On the other hand, GPx, GR and CAT activities revealed a positive correlation.

Conclusion

This study demonstrated that, the alteration in the activity of the antioxidant enzymes as well as enhanced lipid peroxidation could be early markers of protein malnutrition. When there is an inadequate protein levels in diet the increased level of L-Mlondialdhyde reflecting the accumulation of free radicals in the blood and tissues.

The accumulation of free radicals will stimulate the defense system to promote the regulation and expression of antioxidant enzymes. The biological system responds by increasing the activity of the antioxidant enzymes in order to combat the increasing free radicals.

This study demonstrated that, the important role of adequate dietary protein in the maintenance of the balance between the antioxidative enzyme activities and tissue lipid peroxidation.

It could be concluded that, the extent of the alteration of the antioxidant enzyme activities and the enhancement of tissue lipid peroxidation were generally correlated with the degree of protein deficiency.

Recommendation

- More studies should be excreted to clarify the actual role of balanced protein in the protection of antioxidant defense system and prevention of lipid peroxidation.
- A team work must be established to evaluate the exact dangerousity of protein deficiency on the activity of antioxidant defense system and lipid peroxidation.
- An adequate amounts of the essential amino acids necessary for syntheses of antioxidant enzymes and the circulating GSH which is foremost the cellular protective mechanisms must be supplied.
- Dietary protein important for the maintenance of the balance between the antioxidative enzyme activities and tissue lipid peroxidation should be provided.