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BIOCHEMICAL EFFECT OF SOME LIPOTROPIC FACTORS ON LIPID PATTERN IN BLOOD SERUM OF CHICKENS

Abdel- Rehim A. El-Ghannam, Mohammed. T. Ibrahim*; Samy A. Husseln **, Mohy A. Abdel- Atty * and Zeinb D. Ghanem**

Department of Physiology and Biochemistry,
Faculty of Veterinary Medicine, Suez Canal University

* Department of Hygeine, Preventive Medicine, and Animal feeding, Faculty Veterinary Medicine, Suez Canal University

**Department of Physiology, Biochemistry and Pharmacology, Faculty of Veterinary Medicine, Moshtohor (Benha branch), Zagazig University.

***Department of Biochemistry, Faculty of Science, Suez Canal University.

ABSTRACT

One hundred and eighty Hubbard chicks of one day old were used for this investigation. The chicks were divided into three equal, groups. Group I (control groups), group II (choline supplemented groups) and group (III) (Methionine supplemented groups). The amounts of choline and methionine supplemented were double to the amount added to the basal ration. Blood samples for serum separation were collected from 20 chicks of each group periodically every two weeks until the end of the experimental periods (6 weeks). All sera used for determination of total lipids, total cholesterol, phospholipids, triglycerides and free fatty acids. The obtained results revealed that, total, lipids, total cholesterol, phospholipids, triglycerides and free fatty acids levels were highly significant higher for both choline and methionine supplemented chicks in comparison with the control values. So we recommends a supply of good and adequate amount of choline and methionine to diets of chicks as a lipotropic factors during starting and growing age in order to obtain a maximum efficiency and utilization of food and prevention of fatty liver.

INTRODUCTION

Body lipid are distributed in all organisms as well as a-store in certain depots of specialized connective and adipose tissues, they include triglycerides (Neutral fat), phospholipids and steroids in addition to products of their metabolism such as free fatty acids, glycerol and ketone bodies.

Liver is the major site of lipid synthesis and lipoproteins serve to transport these lipids to visceral and subcutaneous tissues or other extrahepatic tissues **(Cantarow and Schepartz, 1967).**

Lipotropic factors which prevent and cure fatty liver include choline and methionine in this concern, methionine is one of the sulphur containing amino acids which is nutritionally essential, its molecules are primarily required for protein synthesis **(Stryer, 1981)** and to prevent the relative deficiency of choline, flavin and vitamin B12 in the dietary furmulation, and secondly, it plays an important role for furnishing labile methyl groups as S-adenosyl methionine and as a source of inorganic sulpur **(Almquist, 1964)** labile methyl groups used for choline synthesis also methionine renders choline available for incorporation in erythrocyte membrane as phosphatidyl choline.

Both choline and methionine showed improved feed efficiency and increased average gain in poultry **(El- Masry and AbouInaga, 1991)** .

Concerning the effect of choline and methionine on the liver fat, **Schexnailder and Griffith** (1973) stated that, choline supplementation or combination of choline and vitamin B12 caused a decrease in liver fat, but this decrease was not as large as that obtained by feeding choline.

El-Masry and Aboulnaga (1991) found that, the choline or the combined effect of 0.3% choline and 0.2% methionine dietary supplementation increase the concentration of serum total lipids, phospholipids and cholesterol in lambs.

There are several mechanisms have been suggested to explain the role of choline as a lipotropic agent including its absences causing an impairment in synthesis of lipoprotein phospholipids **(Murray et al., 1991).**

Accordingly, this investigation was planned to throw some light on the effect of some lipotropic factors as choline and methionine on lipid pattern in blood serum of chickens, and its relation to body lipids, including total lipids, triglycerides, total cholesterol, phospholipids and free fatty acids.

MATERIAL AND METHODS

One hundred and eighty Hubbard chicks of one day old were allowed in three identical built-up liter poultry house and divided into three groups, each one contain 60 chicks. Water was supplied ad-libitum. The house was provided with source of heat to give a starting temperature of 35°C, reduced gradually to a constant temperature of 24 °C at the end of the third week. The chicks were fed on broiler starter ration up to three weeks of age. This ration contained the necessary ingredients for optimum growth.

After the first three weeks chickens received a commercial maintaining ration (broiler finisher ration) until the end of the experiment (6 weeks), this ration was composed of necessary ingredients for complete fattening. Chicks were kept throughout the experiment under good hygienic control.

The amounts of DL-methionine and choline supplemented were double to the amount added to the balanced rations. Chickens were classified into three main groups as follows:-

Group I. (Control groups). Comprised 60 chicks, used as control for all experimental groups.

Group II. (Choline supplemented groups) included 60 chicks, where choline supplement, were added by double amount to the basal ration (1400 mg/Kg).

Group III (Methionine, supplemented groups) contained 60 chicks where the methionine supplements were added by double amount to the basal ration. (0.25% and 0.50%) for starter and grower finisher ration, respectively.

Blood samples were collected from 20 chicks of each group periodically every two weeks, until the end of the experimental periods (6 weeks).

The blood samples were collected rapidly after slaughtering the chicks into clean, dry and sterile centrifuge tubes and allowed to clot at 37 °C for 2 hours, the serum samples were separated by centrifugation at 3000 r. p.m. for 15 minutes, and kept frozen at-20 °C till analysed.

All sera used, for colorimetric determination of total lipids, total cholesterol, phospolipids, triglycerides and non-estrified fatty acids **(NEFA)** according to the methods described by **Frings et al.,(1970), Zak et al.(1954), Zilversmit and Davis (1956), Foster and Dunn (1973) and Duncombe (1964),** respectively. Statistical analysis of the obtained results were carried out

using the method of **Snedecor (1956) and Kempthorn, (1969)**.

RESULTS AND DISCUSSION

The obtained results concerning the levels of various serum lipid constituents in broiler chicks supplemented with choline and methionine in their ration have been statistically summarized in table (1) and (2). The mean values of serum total lipids, total cholesterol, triglycerides, and phospholipids showed a highly significant increase for both choline and methionine supplemented chicks in comparison with control values. On the other hand , the values of free fatty acids, showed a significant increase only for both choline and methionine supplemented chicks.

The increased levels of total serum lipids with choline and methionine supplemented chicks as shown in table (1.2) is in accordance with the results of **El-Masry and Aboulnaga (1991)** who found that the choline or the combined effect of 0.3 % choline and 0.2 % methionine dietary supplementation increase the concentration of serum total lipids, phospholipids and cholesterol in lambs. In this respect **Parker and Peterson (1965)** stated that blood phospholipids increased with choline administration and this increase may be due to high synthesis of lipoproteins by the liver, beside the biosynthesis of erythrocytes as its cell membrane requires excessive amount of phospholipids.

Furtherly, this increase in the value of serum total lipids, may be due primary to an increased ability of liver to synthesize triglycerides or secondry to an increased concentration of plasma free fatty acids **Hawkins and Heald (1966)**.

Moreover, the liver fat was significantly decreased by adding choline or a combination of choline, methionine and vitamin B12 to the basal diet **(Griffith et al., 1969)** and according to the fact that the stored fat in liver is taken up by the blood so the lipid serum levels increase (tissue translocation).

The highly significant increase of serum total cholesterol in choline and methionine supplemented chicks is in accordance with the results of **El-Masry and Aboulnaga (1991) and Aziza (1987)**. On the other hand **Weiss et al., (1967)** reported that there was a considerable variation in the blood cholesterol levels among hens of the same flock, this variability may be the result of differences in the rate of fat metabolism.

The effect of lipotropic factors on serum phospholipids in chicks, revealed that, there were a highly significant increase in both choline and methionine supplemented chicks, which may be attributed to increase, in blood phospholipids with choline administration, such raised level could be due to increase synthesis of lipoproteins by the liver **(Parker and Peterson, 1965 and Skipski et al., 1964)**.

Moreover, such increase in the serum value of phospholipids may be due to their increasing synthesis by the liver, since there is evidence that synthesis of phospholipids in birds occurs mainly if not entirely in the liver, **Vanstone et al., (1955)**.

The data showed highly significant increase in serum triglycerides for choline and methionine supplemented chicks and these increased level were in accordance with **Wagner et al., (1976)** who noticed that the rise in liver lipid concentrations was accompanied with increased levels of plasma triglycerides. Methionine and thyroprotein contributed to the prevention of fatty liver by lowering liver and abdominal fat **(Roberson et al., 1970)** and the liver fat released into the blood stream leading to increase serum triglycerides.

The obtained results agree with **Kelley et al., (1976)** who attributed the increasing of plasma triglycerides to decrease in plasma or adipose tissue lipoprotein lipase activity.

Our results showed that free fatty acids was significantly increased for choline and methionine supplemented chicks and such significant changes agree with the result of **(Griffith et al., 1969)** who stated that, liver fat was significantly decreased by adding choline and methionine to the basal diet accordingly, serum free fatty acids increase.

From the obtained data it could be concluded that, there was mostly, an increase in all serum lipid fractions including total lipids, cholesterol, triglycerides, phospholipids and free fatty acids during supplementation with choline and methionine compounds to the basal diets. Accordingly, we recommended a supply of good and adequate amount of choline and methionine to diets of chicks as a lipotropic factors during starting and growing age in order to obtain a maximum efficiency and utilization of food and prevention of fatty liver.

REFERENCES

Almiquist, H.J.(1964): Feed. Stuffs 36 : 60-63 .

Aziza, S.A.(1987): Lipid metabolism in egg laying hens M.V.Sc. Thesis, Fac.of Vet. Med. Zagazig Univ. (Benha branch).

Cantarow, A. and Schepartz, B. (1967): Biochemistry 4 th Edition P.450 Sounders, W.B. Company Philadelphia and London.

Duncombe, W.G. (1964) : Clin. Chem Acta. 9: 122-125.

El-Masry, A.K. and Aboulnaga, I.A. (1991): Zagazig Vet. J. Vol. 19 No. 2: 324-334.

Foster, L.B. and Dunn, R.T. (1973): Clin. Chem. 19: 338-340 .

Frings,Christopher,S. and DumRalph T. (1970):Am. J. Clin. Pathol. 53: 89-91 .

Griffith,M.;Clinde,A.J.Schexnailder , R.; Davenport, R.F. and Mcknight,W.F.(1969): Poultry Sci.; 48: 2160-2172.

Hawkins, R.A. and Heald, P.J. (1966): Biochem: Biophys. Acta. 116: 41-55 .

Kelley, J.L.; Ganeson, D.; Bass, H.B.; Thayer, R.H. and Alqupovie, P. (1976): FEBS letters, 67: 28-31 .

Kempthorn, O. (1969): The design and analysis of experiments; John wiley and sonine (editor). New York, 47:96 .

Murray,R.K.;Granner, D.K.; Mayers, P.A.and Rodwell, V.W. (1991): Harper'seview of biochemistry 22 th Ed. P.O. Box. 945. Beirut Lebanon.

Parker,F.and Peterson, N.F. (1965) :Journal of lipid Research 6: 455-461.

Roberson, R.H.; Trujilio, V. and Francis, D.W. (1970): Poultry Sci. 49:1431 .

Schexnailder, R. and Griffith, M. (1973):Poultry Sci. 52: 118-119.

Skipski, V.P. Peterson, R.F. and Barclay, M. (1964): Biochem. J. 90: 374-380.

Snedecor, G.M. (1956): Statistical methods 4 th ed. Thoma, state colligue press Ames. Iowa.

Stryer, L. (1981): Biochemistry W.H. freeman. and Co. San-Francisco, CA.

Vanstone, W.E.; Maw, W.A. and Common, R.M. (1955): Canad. J. Biochem. Physiol. 33: 891-903.

Wagner,M.S.;Kelley, J.L.; Nelson, E.C.; Aioupovic, P,. and Thayer, R. (1976): Poultry Sci., 55: 2103.

Weiss, J.F.; Johnson, R.M. and Naber, E.C. (1967): J. Nutri 91: 119-128.

Zak, B.;Dickenman, R., White, E.; Burnett, H. and Cherney, P. (1954):Am. J. Clin. Pathol. 24: 1307-1315.

Table (1): The effect of choline supplementation on serum lipid pattern in broiler chicks (mg/dl).

Experimental group / Parameters	Control chicks			Choline supplemented Chicks		
	Group I (After 15 days)	Group II (After 30 days)	Group III (After 45 days)	Group I (After 15 days)	Group I (After 30 days)	Group I (After 45 days)
Total lipids	523.6 ± 38.94	433.9 ± 26.26	409.9 ± 39.02	536.0 ± 26.5	481.2 ± 31.2**	539.9 ± 27.8**
Total cholesterol	163.00 ± 3.5	177.17 ± 9.4	160.6 ± 2.72	170.4 ± 4.1	189.15 ± 7.07	204.08 ± 3.9**
Phospholipids	156.9 ± 6.95	177.1 ± 8.1	162.0 ± 10.8	198.6 ± 4.74**	215.1 ± 9.1**	193.9 ± 3.58**
Triglycerides	113.0 ± 1.3	116.0 ± 2.1	120.0 ± 3.1	152.0 ± 3.1**	135.0 ± 2.5**	137.0 ± 3.2**
Free fatty acids	28.27 ± 5.4	15.21 ± 1.6	15.83 ± 2.8	24.9 ± 2.8	17.97 ± 2.4	29.8 ± 1.2**

Data are presented as means ± S.E S.E. : Standard error. ** High significant (P < 0.001).

Table (2): The effect of methionine supplementation on serum lipid pattern in broiler chicks (mg/dl).

Experimental group / Parameters	Control chicks			methionine supplemented Chicks		
	Group I (After 15 days)	Group II (After 30 days)	Group III (After 45 days)	Group I (After 15 days)	Group I (After 30 days)	Group I (After 45 days)
Total lipids	523.6 ± 38.94	433.9 ± 26.26	409.9 ± 39.02	533.2 ± 38.6	589.5 ± 39**	468.6 ± 23.3**
Total cholesterol	163.0 ± 2.5	177.17± 9.4	160.6 ± 2.72	173.1 ± 4.3	202.0 ± 12.5**	196.2 ± 4.7**
Phospholipids	156.9 ± 6.95	177.1 ± 8.1	162.0 ± 10.8	196.6 ± 4.44**	206.3 ± 4.6**	195.61 ± 3.33**
Triglycerides	113.0 ± 1.3	116.0 ± 2.1	120.0 ± 3.1	144.0 ± 3.2**	175.0 ± 3.4**	140.0 ± 2.1**
Free fatty acids	28.27 ± 5.4	15.21 ± 1.6	15.83 ± 2.8	31.38 ± 1.7	20.77 ± 1.4**	23.07 ± 3.2**

Data are presented as means ± S.E S.E. : Standard error. ** High significant (P < 0.001).