ISSN 110-2047. ALEX. J. VET.SCIENCE. 2001 , VOL 17 NO.1 : 17-28

ALTERATIONS IN LIPID AND LIPOPROTEINS METABOLISM IN CHICKEN KEPT ON HIGH DIETARY METHIONINE

A. Mandour
Department of Biochemistry, Physiology and Pharmacology
Faculty of Veterinary Medicine, Alexandria University

ABSTRACT ρ This study was carried out to clarify the effect of high dietary methionine (1 and 2% for one and two months) on serum lipids and lipoprotein patterns, total hepatic lipids, cholesterol and glycogen, as well serum lipase and lecithin cholesterol acyl transferase (LCAT) activities, besides blood glucose, triiodothyronine (T_3) and thyroxine (T_4) concentrations. The obtained results revealed that the high dietary methionine resulted in a significant increase in values of total serum lipids, cholesterol, triacylglycerols (TAG), phospholipids, low density lipoprotein-cholesterol (LDL-C), very low density lipoprotein-cholesterol (VLDL-C) and blood glucose throughout the experimental periods. On the other hand, the concentrations of both T_3 and T_4 were increased significantly in birds supplemented with 2% DL- methionine for two months.

Furthermore, this study showed that feeding of methionine led to a significant decrease in values of non-esterified fatty acids (NEFAs), lipase enzyme activity, liver total lipids, cholesterol and glycogen during the experimental periods. While, the activity of LCAT and level of high density lipoprotein-cholesterol (HDL-C) were significantly decreased only in birds received 2% methionine for two months.

INTRODUCTION

Dietary proteins and their constituent amino acids have variable effects on plasma lipids especially cholesterol concentration in many animal species. Animal proteins cause hypercholesterolemia, while, plant proteins maintain normal serum concentration of cholesterol **(Sugano, 1983)**.

In rabbits, serum cholesterol concentration is elevated by feeding casein rich diets but not soy-bean protein **(Kurowska et al., 1989)**. Also, **Carroll (1991)** reported that replacing dietary animal protein with soy-bean proteins reduces both total and LDL cholesterol especially in hypercholesterolemic subjects.

The hypercholesterolemic response of casein (animal protein) was similar when casein was replaced with an equivalent level of its corresponding amino acids mixture, whereas soy-bean protein amino acids moderately elevated plasma cholesterol compared with intact soybean protein **(Barth et al., 1990)**. This indicates that, the plasma cholesterol concentration can be altered by changing the proportions of amino acids in the diet and supported an earlier hypothesis that the hypercholesterolemic effect of animal proteins can be attributed to their generally higher content of essential amino acids compared with plant protein **(Carroll, 1981)**.

Several authors have shown that all essential amino acids except arginine had hypercholesterolemic potential, although methionine and lysine seemed to be most effective **(Sugiyama and Muramatsu, 1990 and Kurowska and Carroll, 1992)**.

It is well known that lipotropic factors which prevent and cure fatty liver include choline and methionine. In this concern, methionine is one of sulfur containing amino acids which is nutritionally essential, its molecule is primarily required for protein biosynthesis, prevent the relative deficiency of choline, and secondary it plays an important role for furnishing labile methyl group as a S-adenosyl methionine and as a source of inorganic sulfur **(Murray et al., 1991)**.

El-Masry and Aboulnaga (1991) found that the 2% supplementation of methionine increased the concentrations of serum total lipids, cholesterol and phospholipids in lambs. Nearly similar observations were noticed by **El-Ghannam et al. (1995)** in chickens. Accordingly, the present investigation was planned to explore the effect of high dietary methionine on serum and hepatic lipids metabolism with special reference to its effect on T_3 and T_4 concentrations and liver glycogen of chickens.

MATERIAL AND METHODS

The present study was carried out on 150 one day old Hubbard chicks reared under the same housing and environmental conditions, provided with fresh distilled water and fed for one week on broiler starter ration contained the necessary ingredients for optimal growth. Then they were weighed and randomly classified into 3 groups each one included 50 chicks. The first one served as a control group and received a commercial maintaining (broiler finisher) ration until the end of the experiment, this ration composed of necessary ingredients for fattening, the second group was supplemented with 1% DL- methionine (Sigma Chemicals yCo. St Louis Mo from Adwia, Egypt) and the third group received 2% DL-methionine added to the ration replaced with an equivalent amount of corn starch.

All chicks were kept under good hygienic conditions throughout the experiment. After one month, of the experiment, half the number of each group were slaughtered after overnight fasting for collection of blood and liver samples, the remaining chicks were slaughtered after 2 months.

Blood samples were collected rapidly during slaughtering in clean, dry and sterile centrifuge tubes. Small portion of blood was used for rapid determination of plasma glucose **(Trinder, 1969)**. The remaining blood was allowed to clot then centrifuged to separate serum for estimation of total lipids **(Frings et al., 1972),** total cholesterol **(Richmond, 1973)**, phospholipids **(Zilversmit and Davis, 1956)**, non-esterified fatty acids **(Duncombe, 1964),** HDL-C **(Lopes-Virella et al., 1977)**, LDL-C and VLDL-C were calculated as described in **Bauer (1982)**, TAG **(Fassotip, 1982)**, lipase activity **(Tietz, 1976)**, LCAT **(Kostner and Diephinger, 1980)**, T_3 and T_4 concentrations **(Tietz, 1987)** and liver glycogen **(Van Handel, 1965)**.

The lipids were extracted from the liver according to the method of **Folch et al. (1957)**, then the total liver lipids and cholesterol were determined in the extracted lipids by the same methods used for serum. The obtained data were statistically analyzed following the method of **Snedecor and Cochran (1982)**.

RESULTS

The obtained data presented in Table (1) showed that the values of total serum lipids, cholesterol, TAG and VLDL-C were significantly (P<0.05) increased in birds kept on ration supplemented with 1% methionine for one month and highly significantly (P<0.01) increased in birds kept on ration supplemented with 2% methionine. Also, serum phospholipids and LDL-C concentrations and the previously mentioned parameters were highly increased in birds fed ration supplemented with 2% methionine for one month. The non-esterified fatty acids were significantly (P<0.01) decreased in the same groups.

On the other hand, Table (2) revealed that the values of total serum lipids, cholesterol, TAG, VLDL-C, LDL-C and phospholipids were highly increased in groups kept on 1 and 2% methionine for two months while the values of NEFAs were significantly (P<0.01) decreased in methionine treated groups for 2 months. The concentration of HDL-C was significantly diminished in birds kept on 2% methionine.

Results in Table (3) proved that the activity of serum lipase was highly significantly reduced (P<0.01) in groups treated with methionine throughout the experimental periods while the activity of LCAT was significantly decreased only in group given 2% methionine for two months. The concentrations of both T_3 and T_4 were significantly (P<0.05) augmented in birds kept on 2% methionine for tow months.

Finally, Table (4) demonstrated that the hepatic total lipids, cholesterol and glycogen were significantly decreased in all methionine treated groups allover the experimental periods when compared with those in control (non-treated) groups, meanwhile, the values of blood glucose exhibited a significant increase in groups administered DL-methionine for one and two months.

DISCUSSION

Lipid metabolism in poultry was previously studied by many authors, who concluded that the lipids and lipoprotein pattern are influenced by dietary nutrient quantity **(Bragg et al., 1973)**, estrogen and ACTH injections **(Harms et al., 1977** and **Gould and Siegel, 1985)**, lipotropic factors **(Scott, 1982)** and plant oils **(Mandour et al., 1999)**. The effect of high dietary protein in reducing fat deposition in broiler-type birds has been reported by **Jackson et al. (1982)** and **Koratum (2000)**. Lipid synthesis in the liver is enhanced in birds fed low protein diets **(Collado and Tasaki, 1981)**. Therefore, this study was planned to throw light on the metabolism of lipids and lipoprotein patterns as well as lipids and glycogen of liver and T_3 and T_4 concentrations in chickens kept on 1 or 2% methionine for one and two months.

The obtained data in Tables (1 and 2) showed that the mean values of total lipids, cholesterol, TAG, phospholipids, LDL-C and VLDL-C increased significantly in the sera of birds kept on ration supplemented with 1 and 2% methionine for one and two months. Meanwhile, the values of NEFAs and lipase activity were significantly decreased in birds kept on the same ration throughout the experimental periods. The concentration of HDL-C was significantly decreased in chickens treated with 2% methionine for two months. The LCAT activity was significantly diminished in birds kept on ration supplemented with 2% methionine for two months (Table 3).

The mechanisms by which hypercholesterolemia is induced by methionine rich protein involves a reduction in bile acids and fecal steroid excretion. Furtherly, it enhances the conversion of VLDL to LDL and also it increases the direct synthesis of LDL by the liver **(Beynen et al., 1986** and **Daley et al., 1994)**.
Moreover, the hyperlipidemic effect of high doses of methionine could be related
to the decreased hepatic fat due to its lipotropic effect **(El-Ghannam et al., 1995)**.

The noticed increased values of serum phospholipids (Tables 1 and 2) might occur due to the supplementation of the lipotropic factor methionine which increased the synthesis of phospholipids by the liver **(Brown and Goldstein, 1981)**.

On the other hand, the feasible reason for the significant decrement of NEFAs (Tables 1 and 2) is the significant decrease in serum lipase activity recorded in the same groups kept on high dietary methionine, while the significant elevations of TAG and cholesterol may be the cause of decreased lipase activity **(Emara, 1999)**.

The obtained data presented in Tables (1, 2 and 3) demonstrated that the high dietary methionine resulted in a non-significant decrease in the values of HDL-C and LCAT activity.

They are significantly decreased ($P<0.05$) in birds received 2% methionine for 2 months. The recorded decrease in LCAT activity may be attributed to the increase in the esterified cholesterol levels as reported by **Forsythe et al. (1980)**, who suggested that the increased esterified cholesterol level was responsible for decreased molar LCAT activity, which positively correlated with changes in unesterified cholesterol, triacylglycerols and phospholipids levels. It is well known that LCAT prevents the accumulation of serum unesterified cholesterol derived from the surface of chylomicrons and very low density lipoprotein, also it plays a role in transport of unesterified cholesterol from peripheral tissues to the liver **(Heller et al., 1981)**. Furthermore, the decrement of LCAT might be related to the decreased HDL concentrations where LCAT was highly correlated with increased cholesteryl ester content of plasma HDL **(Fernandez et al., 1995)**.

Dietary fat and cholesterol alter serum lipoprotein concentrations and affect specific classes of lipoprotein of LDL and HDL and increase the content of cholesterol in VLDL, the observed changes of serum LDL-C and HDL-C in methionine fed birds might be related to the increased saturated fats **(Shepherd et al., 1980)**.

Moreover, the elevated level of LDL appearing in the circulation upon feeding the hyperlipidemic diet is mainly derived from VLDL. LDL is uptaken by the LDL receptors in the liver and extrahepatic tissues **(Goldstein and Brown, 1977)**, the production of LDL exceeds the capacity of LDL receptors i.e. efflux of cholesterol from the liver is more than influx, this could explain the recorded elevated serum LDL level.

The data listed in Table (4) revealed a significant decrease in concentrations of hepatic total lipids and cholesterol in groups supplemented with high dietary methionine for 1 or 2 months. The obtained data came in accordance with those reported by **Collado and Tasaki (1981)** and **Jackson et al. (1982)**. The lipotropic effect of methionine as well as the role of high protein or amino acids in reducing fat deposition in liver and tissues may be the cause of decrement of hepatic total lipids and cholesterol.

On the other hand, the present study gives an evidence that the high dietary methionine (2% for two months) lead to significant increase in T_3 and T_4 concentrations (Table 3). Moreover, the values of blood glucose were augmented significantly while the glycogen values were significantly decreased in liver of methionine treated birds.

The increased serum glucose level under the effect of methionine supplementation accompanied by significant decrease in liver glycogen and increased serum T_3 and T_4 which act as anti-insulin leading to increase in the level of glucose via stimulation of gluconeogensis, lipolysis and glycogenolysis as well as the inhibition of glycogenesis and lipogenesis **(Gannon and Nuttall, 1987)**.

The recorded decrease in liver glycogen could be related to increased glycogenolysis following the administration of methionine rich protein which is probably due to activation of phosphorylase enzyme which is the rate limiting enzyme in glycogen degradation **(Gannon, 1993)**.

In conclusion, it becomes evident that the high dietary methionine in chickens alters lipid and lipoproteins metabolism, since it has hyperlipidemic and hyperglycemic effect through influencing the lipase and LCAT activities, hepatic lipids, glycogen and thyroid hormones concentrations which need further studies.

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Table (1): Serum lipids and lipoprotein patterns in birds kept on high dietary methionine (1 and 2%) for one month.

Parameters	Groups		
	Control	Treated with methionine	
		1 %	2 %
Total serum lipids (mg/dl)	422.88 ± 25.51	540.18 ± 37.06 *	667.07 ± 38.63 **
TAG (mg/dl)	90.31 ± 6.70	110.65 ± 7.17 *	155.27 ± 9.08 **
Total serum cholesterol (mg/dl)	100.97 ± 6.48	121.60 ± 5.88 *	140.29 ± 7.22 **
Phospholipids (mg/dl)	109.73 ± 5.71	151.16 ± 7.38 **	188.11 ± 6.26 **
NEFAs (mg/dl)	22.54 ± 0.95	15.38 ± 0.68 **	13.09 ± 0.59 **
LDL-C (mg/dl)	41.76 ± 1.80	60.40 ± 2.07 **	72.88 ± 3.17 **
HDL-C (mg/dl)	38.97 ± 2.10	36.00 ± 1.82	34.99 ± 2.09
VLDL-C (mg/dl)	18.09 ± 1.06	22.47 ± 1.09 *	31.22 ± 2.10 **

Values represented means ± S.E.
* Significant at P<0.05. ** Significant at P<0.01.

Table (2): Serum lipids and lipoprotein patterns in birds kept on high dietary methionine (1 and 2%) for two months.

Parameters	Groups		
	Control	Treated with methionine	
		1 %	2 %
Total serum lipids (mg/dl)	458.89 ± 29.51	602.18 ± 36.80**	669.07 ± 40.67**
TAG (mg/dl)	100.31 ± 5.72	138.65 ± 6.01**	168.26 ± 5.89**
Total serum cholesterol (mg/dl)	108.93 ± 4.46	146.65 ± 4.89**	126.71 ± 5.22*
Phospholipids (mg/dl)	118.77 ± 4.35	152.19 ± 6.71**	209.24 ± 7.66**
NEFAs (mg/dl)	22.54 ± 0.91	15.38 ± 0.69**	13.09 ± 0.71**
LDL-C (mg/dl)	47.76 ± 1.80	82.28 ± 2.19**	56.88 ± 2.60*
HDL-C (mg/dl)	41.37 ± 2.77	36.37 ± 1.90	33.69 ± 2.91*
VLDL-C (mg/dl)	20.73 ± 1.02	27.99 ± 1.13**	33.81 ± 2.07**

Values represented means ± S.E.
* Significant at P<0.05. ** Significant at P<0.01.

Table (3): Lipase and LCAT activities; T_3 and T_4 concentrations in birds kept on high dietary DL-methionine (1 and 2%) for 1 and 2 months.

Parameters	Groups (one month treatment)			Groups (two months treatment)		
	Control	Treated with methionine		Control	Treated with methionine	
		1%	2%		1%	2%
Serum lipase E (IU/L)	116.58±4.37	84.25±3.98**	76.08±3.17**	110.55±6.31	70.22±5.98**	63.11±4.83**
LCAT (nmol/ml/hr)	30.28±2.66	27.05±1.93	26.86±1.81	28.33±1.99	25.22±1.73	20.76±1.67*
Triiodothyronine (T_3) (ng/dl)	174.30±6.89	187.10±7.34	199.22±8.22	161.30±8.08	178.38±8.11	189.30±9.28*
Thyroxine (T_4) (µg/dl)	0.37±0.019	0.42±0.009	0.44±0.02	0.50±0.022	0.55±0.03	0.60±0.029*

Values represented means ± S.E.

* Significant at P<0.05.

** Significant at P<0.01.

Table (4): Hepatic total lipids, cholesterol, glycogen and blood glucose in birds kept on high dietary DL-methionine (1 and 2%) for 1 and 2 months.

Parameters	Groups (one month treatment)			Groups (two months treatment)		
	Control	Treated with methionine		Control	Treated with methionine	
		1%	2%		1%	2%
Hepatic total lipids (gm/100gm)	6.50±0.25	5.64±0.17*	4.47±0.16**	6.81±0.29	5.34±0.17**	4.47±0.164**
Hepatic total cholesterol (gm/100 gm)	0.76±0.017	0.69±0.21*	0.388±0.013**	0.85±0.027	0.51±0.014**	0.39±0.016**
Hepatic glycogen (gm/100g)	5.94±0.14	4.80±0.21*	4.08±0.19	11.71±0.91	8.12±0.71**	7.33±0.66**
Blood glucose (mg/dl)	188.90±6.27	210.00±5.38	236.71±7.91**	196.11±6.23	249.66±8.71**	266.00±7.55**

Values represented means ± S.E.

* Significant at P<0.05.

** Significant at P<0.01.