# Clinico-pathological studies of the relationship between methionine and thyroid disorders in broiler

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#### **Abstract**

This work was carried out on 140 one day old chicks for 6 weeks to study the effect of different dietary supplementation of methionine on the growth performance and the thyroid function in broilers. Chickens were divided into 4 equal groups. Group I served as control group fed on balanced commercial ration. Group (II, III and IV) fed on rations containing 1, 0.3 and 0.2 % methionine. In clean sterile tubes for serum separation five random samples were collected from all experimental groups at 4<sup>th</sup> and 6<sup>th</sup> weeks for investigation of some biochemical parameters.

The results revealed significant increase in the body weight and body weight gain, significant decrease in total feed intake in the treated group fed 1% methionine, meanwhile, significant decrease in groups fed 0.3 and 0.2% methionine.

Biochemical investigations revealed that the levels of ALT, AST and albumin were non-significantly changed in all groups when compared to the control group. Total proteins, globulin and glucose were significantly decreased while cholesterol was significantly increased in the treated groups fed 0.3 and 0.2% methionine. The results of renal function tests revealed significant increase in the level of uric acid in the treated groups fed 0.3 and 0.2% met at 4 weeks, while creatinine level showed non significant changes in all groups allover the experimental period There were significant decrease in the catalase, SOD, GSH, NO, T3 and T4 in the groups fed 0.3 and 0.2% methionine.

From the present study, we could be conclude that, dietary methionine at level 1% has a growth promoter effect in broilers while requirement deficiency of dietary methionine, depressed the growth performance and thyroid hormone levels.

# Introduction

To optimize production cost, the hen's diets must contain adequate quantities of all nutrients needed, particularly protein. Limiting protein consumption and adding synthetic dl-methionine and l-lysine can improve the protein utilization and production perfor-

mance of the hens (Bunchasak and Silapasorn, 2005 and Narva'ez et al., 2005).

The supplementation of methionine (Met) increases the protein synthesis capacity of tissues, regulation and helps proper nutrient utilization as well as reducing reactive

oxygen species. Methionine also acts as a methyl and sulfur donor for transmethylation and trans-sulfuration reactions, is known to counter the deleterious influence of copper sulfate. It was suggested that its supplementation in diets improved dietary amino acid balance and promoted greater protein build-up and less fat deposition in broilers (Rana et al., 2010 and Kalbande et al., 2009). However excess methionine was toxic for poultry, and growth depression was observed in broilers and turkeys when this amino acid was above 1% (Carew et al., 1998: Scherer and Baker, 2000 and Acar et al., 2001).

The thyroid gland and its hormones comprise an endocrine system in birds and mammals that has important regulatory effects on growth, energy utilization, and other physiological responses (May, 1979; Elkin et al., 1980 and Carew et al., 1997). Several studies have shown that low intakes of dietary protein influence thyroid function and circulating levels of thyroid hormones. Plasma triiodothyronine (T3) and thyroxine (T4) are often elevated in protein-deficient chicks (Alster and Carew, and 1984; Keagy et al., 1987 and Buyse et al., 1992).

The present study aims to investigate the effect of dietary supplementation of different levels of methionine on the growth performance, oxidative stress parameters and thyroid function in broilers.

# Material & Methods

# 1) Experimental chickens:

One hundred forty one-day old, apparent healthy chicks, Cobb breed were obtained from Ismailia- Misr Poultry Company Serapium, Ismailia, Egypt. Chickens were reared in litter under

standard environmental and hygienic conditions. All chickens were subjected to the following vaccination schedule, Hitchner at 5<sup>th</sup> day of age, Gumboro at 14<sup>th</sup> and 24<sup>th</sup> day of age and Lasota at 18<sup>th</sup> day of age.

#### 2) Methionine:

Commercial product of methionine (Methionine 99%) manufactured by Begussa co., U.S.A, was used as a dietary supplementation to broilers.

#### 3) Experimental design:

One hundred and forty, one-day old, apparent healthy chickens were classified into 4 equal groups (35) dietary supplemented with methonine for 6 weeks. Group I served as control fed on commercial balanced ration. Group II, III and IV fed on ration containing 1%, 0.3% and 0.2% respectively.

Five random blood samples in duplicate were taken from all experimental groups at the end of 4<sup>th</sup> and 6<sup>th</sup> week for serum separation to investigate some biochemical parameters.

# 4.1- Growth performance parameters:

# a) Body weight:

Each chick was weighed at the beginning of the experiment (one-day old) and at the end of 2<sup>nd</sup>, 4<sup>th</sup>, 6<sup>th</sup> week of the experiment. Individual live body weight was summed and divided by the number of chickens of each group to obtain the average live body weight. Also Body weight gain, Feed consumption & Feed conversion ratio (FCR) were calculated according to **Brady (1968).** 

# 4.2-Biochemical parameters:

The biochemical tests were performed using commercial test kits to determine ALT and AST (Randox Co. UK) according to the method of *Reitman* 

and Frankel, (1957), albumin (STANBIO kits, Texas) according to Dumas and Biggs (1972), globulin and A/G ratio (Kaneko et al., 1997), creatinine (Human, Germany) according to Henry (1974), total proteins, glucose, uric acid and cholesterol (SPINREACT, Spain) according to according to Young (2001).

### 4.3- Oxidative stress parameters:

They were performed using commercial test kits (Biodiagnostic Co., Egypt). Serum catalase according to Aebi (1984), superoxide dismutase according to Maral et al., (1977), reduced glutathione enzymes according to Beutler et al., (1963) and nitric oxide levels according to Montgomery and Dymock (1961).

# 4.4- Hormonal assay:

T3, T4 and TSH parameters were estimated using ELISA Kits (Bio-Supply Co. UK) according to (Britton et al., 1975).

## 5-Statistical analysis:

Data collected were statistically analyzed using statistical software program (SPSS for Windows, version 15, USA). Differences between means of different groups were carried out using one way ANOVA with Duncan multiple comparison tests.

## **Result & Discussion**

The present results show increase in body weight, weight gain, decreased total feed intake and insignificant change in feed conversion ratio in treated groups fed 1% methionine in comparison with control. Fasuyl and Alertor (2005) concluded that performance can still be obtained with adequate supplementation of essential amino acids especially methionine which has been identified to be in

marginal quantities in most poultry diets. Dietary addition of methonine improves growth performance in broiler Hesabi et al. (2006) and Meirelles et al. (2003) reported no significant effects were seen for different methionine levels on feed conversion, however, they observed numerical improvement with the increasing ratio. On the other hand, Maroufvan et al.. (2010) reported that body weight gain. feed intake and feed conversion ratio were significantly decreased by the dietary treatments of methionine in starter and grower phase at the highest levels 1ppn in broiler chickens. Decreasing feed intake may be related to influence of amino acid on appetite control. Hypothalamus is an area of the brain that plays a critical role to control appetite in poultry. Excess methionine may stimulate and increase hypothalamus activity to decrease feed intake. These observed results are consistent with those Harper et al. (1970), Peng et al. (1973) and Edmonds and Baker (1987) who found that excessive dietary amino tend to attenuate feed intake. While, treated groups fed 0.3% and 0.2% methionine showed decreased body weight. weight gain, increased total feed intake and feed conversion ratio compared to the control group. Our results agreed with Carew et al., (2003) who reported that the methionine deficiencies of 0.3 and 0.2% caused graded reductions in feed intake and weight gain. On the contrary, Osman and Mona (2007) found that chicks fed the diet supplemented with 0.3 g Zn methionine/kg diet had the lowest feed intake and the best feed conversion. However, Potter et al., (1974) found

no effect of Zn-Met addition in broilers.

Regarding the results of biochemical investigation, the levels of ALT and AST were non-significantly changed in all methionine dietary supplemeted groups. Our results came in agreement with Osman and Mona (2007) and Kalbande et al., (2009) who reported that the deficiency of methionine in broilers diet did not increase liver enzymes.

Total proteins and globulin were significantly decreased in the treated groups fed 0.3 and 0.2% methionine at 4 and 6 weeks, while albumin was non-significantly changed. These results were similar to Ali (2006) who found that the total serum protein, albumin and globulin were highest but did not vary significantly from the control group. On the other hand, these results differed from those obtained by Adeyemo et al., (2010) where there was no significant differences (p>0.05) in total protein of broilers fed the different levels of methionine inclusion (0.19%, 0.31%, 0.53% and 0.64%).

Glucose is supplied by intestinal absorption of dietary glucose or by hepatic glucose production from its precursors (Kaneko et al., 1997). The present results of glucose level showed hypoglycemia in the treated groups fed 0.3 and 0.2% methionine. Adeyemo et al., (2010) observed wide variations in the glucose concentration of the birds; the highest glucose concentration was in birds on diet containing 0.19% methionine. Robert and David (1975) recorded the consumption of a diet containing 1.25% excess methionine resulted in a significant reduction in plasma glucose.

Dietary cholesterol is present in both free and esterified forms, but only non-esterified cholesterol is absorbed (Kaneko et al., 1997). The present results revealed hypercholesterolemia in the treated groups fed 0.3 and 0.2% methionine. Methionine deficiency in ration may lead to fatty liver syndrome in poultry (Riddell, 1997). The findings of current experiment are well correlated to those reported by Roth and Milstein, (1957) who reported that dietary methionine insufficiency may induce fatty liver thereby increasing total serum cholesterol.

Concerning the results of renal function tests, which revealed significant increase in the level of uric acid in the treated groups fed 0.3 and 0.2% mehionine at 4 weeks, while the creatinine level was not significantly changed in all groups all over the experimental period. Chaiyapoom et al., (2006) found that plasma uric acid of chicks fed with diet sufficient in methionine was higher but not significantly differed from the insufficient methionine consumption groups. Machin et al. (2004) and Amubode and Fetuga (1984) found that plasma uric acid of broiler chickens decreased when broilers fed on initial feed containing dietary methionine at level 0.60% and also decreased in broilers fed on finishers between 0.26 and 0.50% dietary methionine. Ford et al., (1989) reported significant decreases in the mean plasma concentrations of urate and significant increases in creatinine in hyperthyroid patients. The mean concentration of urea did not change significantly in patients when hyperthyroid and again after they had been euthyroid for at least 4 months.

The biological effects of oxidative stress are controlled in vivo by enzymatic and non enzymatic defense mechanisms. The defense mechanisms include antioxidant enzymes, such as catalase, glutathione peroxidase and glutathione-S-transferase, while non enzymatic may be achieved by cellular antioxidant such as reduced glutathione (Oztiirk and Giimiislii, 2004). Antioxidant enzyme levels are sensitive markers of oxidative stress which are implied in the inactivation or transformation of oxidants (Saeed et al., 2009). Our results showed significant decrease in the catalase activity in the groups fed 0.3 and 0.2% met. While, the treated group fed 1% met showed significant increase. Patra et al., (2001) reported that the brain catalase activities in rats exposed to lead and treated with methionine (100 mg/kg b.wt) in the 5th week were higher than in controls.

Superoxide dismutase is metalloenzyme catalyses the O2 into O2 and H<sub>2</sub>O<sub>2</sub>. It is important in the antioxidative defense against lipid peroxidation. Superoxide is a reactive molecule, but it can be converted to hydrogen peroxide superoxide dismutase and then to oxygen and water by catalase. The present results revealed that there were significant increase in the SOD of the group fed 1% methionine and significant decrease in the groups fed 0.3 and 0.2% methionine when compared with the control one. Francieli et al., (2009) stated that SOD activity was not affected by hypermethioninemia in rats injected s/c with met. Sophie et al., (2004) stated that SOD activity was decreased in rats after methionine supplementation.

The reduced glutathione activity revealed significant decrease in the groups fed 0.3 and 0.2% met, while the treated group fed 1% met showed significant increase in the GSH level at 6 weeks. Wang et al., (2010) who found that methionine supplemented at 3.2 and 4.0 g/kg significantly increased glutathione concentration in the egg yolk. Sophie et al., (2004) found decreases in liver GSH levels were observed in rats fed with methionine enriched diet.

The nitric oxide level showed significant decrease in the NO level in the groups fed 0.3 and 0.2% met at 6 weeks. Rats supplemented with an additional 8g/kg of D-L methionine showed no change in nitrates and nitrites levels (known to be nitric oxidederived metabolites) (Robin et al., 2004).

The fact that thyroid hormones had a major role in regulating oxidative metabolism of birds has been established (Sturkie, 1986). Triiodothyronine (T3). Any pronounced alteration in thyroid function (hyperthyroidism or hypothyroidism) is reflected in an altered metabolic rate. Our results TSH revealed that was nonsignificantly changed in all treated groups allover the experimental period. T3 significantly decreased in groups treated with 0.3 and 0.2% methionine allover the period of the experiment. This result disagree with Carew et al., (1997) and Carew et al., (2003) who found that Plasma T3 in the methonine deficient chicks showed significant elevation compared to the control one. Also our results disagree with Chaiyapoom et al., (2006) who stated that deficiency in methionine intake significantly enhanced level of T3. While T4 results at 2 and 4 weeks agree with Carew et al., (1998) who reported that excess of methionine did not significantly change plasma T4 levels. Also Carew et al., (1997) recorded that deficiency of methionine did not alter level of plasma T4. On the other hand Carew et al., (2003) reported that plasma thyroxine (T4) was lower in all deficient groups at 22

days, which was significant only with 0.2% methionine.

In conclusion from the results of the present study, the dietary methionine at level 1% has a growth promoter effect in broilers, while deficiency dietary methonine requirement depressed the growth performance and thyroid hormone levels.

Table (1): Some growth performance parameters (Mean  $\pm$  S.E.) in chicken dietary supplemented with different levels of methionine for 6weeks.

Group	Control	Met. 1%	Met. 0.3%	Met. 0.2%
BW 2nd week/gm	262.8 <sup>b</sup> ±7.01	300.0° ±3.93	199.14° ±9.09	195.29° ±6.32
B W 4th week /gm	862.9° ±20.4	840.1°±24.5	750.5 <sup>b</sup> ±33.7	635.1°±10.3
B W 6th week /gm	1545ª ±29.3	1495°±60.6	1352°±54.2	1252°±44.16
WG 2-4 week/gm	215.7°±6.7	250.3° ±3.9	152.1 <sup>d</sup> ±9.8	155.6°±6.2
WG 4-6 week/gm	600.2° ±18.8	540.1 <sup>b</sup> ±22.2	551.2 b ±35.3	439.8°±17.6
WG 4-6 week/gm	682.4°±21.2	655.1 <sup>ab</sup> ±25.2	602.2 <sup>b</sup> ±24.2	617.1 <sup>b</sup> ±21.75
FCR 2nd week	2.67 <sup>b</sup> ±0.19	2.46 <sup>b</sup> ±0.14	$3.87^a \pm 0.28$	3.81°±0.14
FCR 4th week	2.23b ±0.07	2.41 <sup>b</sup> ±0.10	2.45 <sup>b</sup> ±0.27	2.78°±0.14
FCR 6th week	2.93b±0.38	2.98 <sup>b</sup> ±0.41	3.71°±0.58	3.73°±0.46

BW; body weight-WG; weight gain-FCR; food conversion rate.

Means with the same letter in the same row are insignificant at P<0.05

Table (2): Some biochemical parameters (Mean  $\pm$  S.E.) in chickens supplemented with methionine at different levels for 4 weeks.

Group	ALT	AST	T.P	Alb.	Glob.	A/G	Glue.	Choles	U.A	Creatin
	IU/I	IU/I	gm/dl	gm/dl	gm/dl	ratio	mg/dl	mg/dl	mg/dl	mg/dl
Control	5.92°	63.66°	5.29°	1.98"	3.30*	0.61*	305.6°	112.4 <sup>b</sup>	4,20 <sup>h</sup>	0.47*
	±0.55	±10.63	±0.10	±0.14	±0.18	±0.06	±7.79	±6.21	±0,31	±0.03
Met.	5.76*	48.87	5.11"	1.82*	3.29*	0.55°	329.6"	115.6 <sup>b</sup>	5.09 <sup>h</sup>	0.43"
1%	±0.55	±9.13	±0.17	±0.19	±0.12	±0.07	±8.62	±4.91	±0.60	±0.05
Met.	5.66°	46.59°	4.25 <sup>b</sup>	1.75°	2.50°	0.70 <sup>b</sup>	251.6b	124.8°	6.99*	0.42°
0.3%	±0.43	±1.48	±0.23	±0.18	±0.10	±0.06	±9.29	±3.56	±0.56	±0.03
Met.	5.40°	62.37*	4.01 <sup>b</sup>	1.71°	2.31 <sup>b</sup>	0.73 <sup>b</sup>	248 <sup>b</sup>	131.2ª	7.95°	0.46"
0.2%	±0.41	±8.48	±0.18	±0.17	±0.14	±0.05	±8.45	±4.98	±0.39	±0.04

Means with the same letter in the same column are non significant at P<0.05

Table (3): Some biochemical parameters (Mean  $\pm$  S.E.) in chickens supplemented with methionine at different levels for 6 weeks.

Group	ALT	AST	T.P	Alb.	Glob.	A/G	Gluc.	Choles	U.A	Creatin
	IU/I	IU/I	gm/dl	gm/dl	gm/dl	ratio	mg/dl	mg/dl	mg/di	mg/dl
Control	6.37°	60.86*	5.35°	2.08"	3.26 <sup>4</sup>	0.64*	280.2*	108.0 <sup>b</sup>	4.89 <sup>ab</sup>	0.43"
	±0.97	±9.34	±0.25	±0.21	±0.16	±0.05	±11.02	±5.81	±0.53	±0.02
Met.	5.85°	59.19°	5.28°	1.91*	3.35°	0.58°	256.2°b	104.6 <sup>b</sup>	4.24 <sup>b</sup>	0.38°
1%	±0,81	±4.51	±0.34	±0.18	±0.18	±0.04	±10.28	±5.43	±0.50	±0.02
Met.	5.98"	62.36*	4.10°	1.78 <sup>a</sup>	2.30 <sup>b</sup>	0.77°	239.4 <sup>™</sup>	125.2°	6.06**	0.37
0.3%	±0.66	±9.18	±0.25	±0.16	±0.12	±0.06	±9.64	±5.63	±0.57	±0.03
Met.	5.78*	60.12*	3.91 <sup>b</sup>	1.74°	2.15 <sup>b</sup>	0.80 <sup>5</sup>	223.0°	134.2°	6.96"	0.36°
0.2%	±0.66	±5.21	±0.29	±0.19	±0.15	±0.07	±5.87	±4.29	±1.09	±0.03

Means with the same letter in the same column are non significant at P<0.0

Table (4): Some serum oxidative stress parameters (Mean  $\pm$  S.E.) in chickens supplemented with methionine at different levels for 4 weeks.

Group	Catalase U/L	Superoxide dismu- tase	Reduced gluta- thione	Nitric oxide µmol/l
	OIL	U/ml	mg/dl	PHION
Control	611.80°	685 <sup>b</sup>	0.59 <sup>ah</sup>	3.52°
	±15.24	±13.6	±0.04	±1.31
Met. 1%	569,78°	745ª	0.53 <sup>b</sup>	2.98ª
	±24.33	±10.4	±0.09	±0.82
Met. 0.3%	405.60 <sup>b</sup>	671 <sup>6</sup>	0.73 <sup>ab</sup>	3.11 <sup>a</sup>
	$\pm 18.20$	±15.2	±0.07	±0.59
Met. 0.2%	372.99 <sup>b</sup>	662 <sup>b</sup>	0.77 <sup>4</sup>	3.19 <sup>8</sup>
ii	±12.64	±14.7	±0.04	±0.85

Means with the same letter in the same column are non significant at P<0.05

Table (5): Some serum oxidative stress parameters (Mean ± S.E.) in chickens supplemented with methionine at different levels for 6 weeks

Group	Catalase U/L	Superoxide dismutase U/ml	Reduced glutathione mg/dl	Nitric oxide µmol/l
Control	611.92 <sup>b</sup>	705 <sup>h</sup>	0.81 <sup>8</sup>	2.48°
	±5.93	±16.3	±0.09	±0.64
Met. 1%	681.50ª	825ª	1.27 <sup>a</sup>	2.22*
	±17.85	±15.9	±0.33	±0.29
Met. 0.3%	425.50°	615°	0.39°	1.20 <sup>b</sup>
	±12.85	±10.6	±0.04	±0.21
Met. 0.2%	402.20°	601°	0.33 <sup>c</sup>	1.37 <sup>b</sup>
	$\pm 12.40$	±9.9	±0.08	±0.15

Means with the same letter in the same column are non significant at P<0.05

Table (6): Serum thyroid hormones levels (Mean  $\pm$  S.E.) in chickens supplemented with different levels of dietary methionine.

			V1 W100W1, 111001110#	
Group	Control	Met. 1%	Met. 0.3%	Met. 0.2%
TSH (mIU/L)	0.0013 <sup>a</sup>	0.0030*	0.0017*	0.0017*
4th week	±0.0003	±0.0010	±0.0003	±0.0003
T4 (ug/dl)	3.80ª	3.20	3.50*	3.28 <sup>a</sup>
4th week	±0.32	±1.16	±0.15	±0.13
T3 (ng/ml)	2.15ª	2.01*	0.716	0.58°
4th week	±0.14	±0.16	±0.10	±0.96
TSH (mIU/L)	0.002 <sup>ab</sup>	0.001	0.0013*5	0.0023ª
6th week	±0.0006	±0.00i	±0.0003	±0.0003
T4 (μg/αl)	3.09ª	3.033	1.75 <sup>b</sup>	1.25°
6th week	±0.21	±0.31	±0.13	±0.15
T3 (ng/ml)	2.05	1.95*	0.60 <sup>6</sup>	0.56 <sup>b</sup>
6th week	±0.28	±0.18	±0.07	±0.05

Means with the same letter in the same column are non significant at P<0.05

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# الملخص العربي

دراسات باثولوجية اكلينيكية على العلاقة بين المثيونين واضطرابات الغدة الدرقية في بداري التسمين

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أجريت هذه الدراسة على ١٤٠ كتكوت عمر يوم و قسموا الى ٤ مجموعات كالآتى: المجموعة رقم ١٤ تمت معالجتهم باضافة المجموعة رقم ١٤ ٣ ٣٠ تمت معالجتهم باضافة المثيونين في العلف بنسبة ١ %٣ ٣٠٠% ٣ ١٠٠% على التوالى لمدة ٦ أسابيع. وقد تم دراسة مظاهر النمو في الفراخ عند عمر ٢٣٤ ١٤ أسابيع .وقد تم أخذ عينات دم لدراسة بعض التحاليل الكيميائية وذلك عند عمر ٤ و ٦ أسابيع

وقد أسفرت الدراسة عن النتائج التالية: زيادة ملحوظة في وزن الطيور والوزن المكتسب وقلة كمية العلف المستهلكة في المجموعة المعالجة بالمثيونين ١ % بينما وجد نقص ملحوظ في وزن الطيور و كمية العلف المستهلكة وأيضا زيادة ملحوظة في معدل التحويل الغذاني بالنسبة المجموعات المعالجة بالمثيونين ١، و ١،٠% و بالفحص الكيميائي, لم يتغير مستوى انزيمات الكبد والزلال في جميع المجاميع. أما البروتين الكلي الجلوبيولين والسكر فقد وجد بهم نقص معنوى في المجموعات المعالجة ب ١، و ١،٠% مثيونين بينما وجدت زيادة في الكولستيرول. مع زيادة معنوية في مستوى حمض البوليك في المجموعات التي أخذت ١، و ١،٠% متيونين بينما لم يتغير الكيرياتينين في جميع المجموعات على مدار مدة التجربة. لوحظ نقص معنوى في انزيم الكتالاز السوبر أوكسيد ديسميوتاز والجلوتائيون المختزل وكسيد النيتريك معنوى في المجموعات التي أخذت ١،٠ و ١،٠% مثيونين. ومما سبق نستنتج أن اضافة المثيونين بنسبة ١% لعلائق بدارى التسمين يحفز من النمو، بينما اضافته بنسب أقل من النسبة المثيونين بلعر المدوقة الدرقية.