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IMPORTANT EFFECTS OF FISH OIL AND/OR ANIMAL FAT ON PLASMA LEPTIN AND CHOLESTEROL IN MALE BROILER CHICKENS

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ABSTRACT

The present study was undertaken to evaluate the influence of animal fat and fish oil rich in polyunsaturated fatty acids, on plasma leptin, total cholesterol, high density lipoprotein-cholesterol (HDL-C), low density lipoprotein-cholesterol (LDL-C), very low density lipoprotein-cholesterol (VLDL-C) and triglycerides (TG). A total number of 60 male broiler chickens was used in this study. The birds were divided into four groups. The first group served as a control. The second group was treated with fish oil, while the third one was treated with animal fat and the fourth group was treated with a mixture of animal fat and fish oil. The doses were given orally and daily for ten days. Ten birds of every group were slaughtered, while the remained numbers were kept as recovery groups for other seven days. Blood samples were collected from all groups. The results indicated that the plasma leptin in the group treated with fish oil revealed a highly significant decrease in plasma leptin, where it returned again to the control level 7 days after stopping the treatment. At the same time, there was non significant effect of fish oil on plasma triglycerides and lipoprotein-cholesterol, while treatment of male chickens with animal fat caused a highly significant elevation in plasma leptin. The best results of all experimental groups were a combination of fish oil and animal fat which presented medium levels of plasma leptin; it also had a lowering significant effect on TG and VLDL-C; insignificant effect on LDL-C and elevating influence on HDL-C.

It is suggested that supplementation of a mixture of fish oil and animal fat revealed a significant moderate level of leptin which plays an important physiological role in improving plasma lipoprotein-cholesterol and triglycerides levels in male broiler chickens.

Key words: *animal fat , cholesterol & broilers, fish oil, leptin .*

1. INTRODUCTION

Leptin was discovered in 1994 (Zhang *et al.*, 1994). Leptin (from the Greek leptos, meaning thin) is a polypeptide hormone with important effects in regulating body weight, metabolism and reproduction in mammals (Friedman and Halaas 1998). There was a relationship between leptin and lipid metabolism in broiler chickens (Ashwell *et al.*, 1998 and Taouis *et al.*, 1998) and liver lipogenesis in avian species (Cassy *et al.*, 2003).

Most obese humans have highly circulating concentration of leptin which is even raised in proportion to fat mass (Maffei *et al.*, 1995). Furthermore several studies indicated that the fatty acid composition of the diet might have an impact on serum leptin levels (Cha and Jones, 1998 and Backus *et al.*, 2000). The circulating leptin in sheep and cattle is increased by fatness and levels of nutrition that is consistent with the results in human and rodents (Ehrhardt *et al.*, 2000).

Leptin is primarily secreted by adipose tissue; smaller amount of leptin is also secreted by cells in the epithelium of the stomach and placenta. Leptin receptors are highly expressed in areas of the hypothalamus resulting in an important role in regulating body weight (Friedman and Halaas, 1998) and other tissues (lungs, kidney, gonads, liver and skeletal muscles). In birds there is surprisingly few data related to leptin with its receptors found in the liver and yolk sac of chickens (Lamasova and Zeman, 2001). The mechanism of leptin control of food intake in chicken hypothalamus was provided by Dridi *et al.* (2005).

Takahashi and Ide (2000) examined the effect of dietary fats rich in 3-polyunsaturated fatty acids (n-3 PUFA) and suggested that the alterations in gene expression in adipose tissue may be due to the physiological activities of n-3 PUFA in preventing body fat accumulation.

The beneficial effect of fish intake has been attributed to long chain polyunsaturated n-3 fatty acids, especially eicosapentaenoic acid and decosahexaenoic acid. Although fish oil exerts its effects primarily on serum lipids, lipoproteins and platelet function (Steerenberg *et al.*, 2002), and it may be used for preventing and treatment of acute and chronic inflammatory conditions (Das *et al.*, 2003).

Omega-3 polyunsaturated fatty acids are known to influence crucial membrane functions, eicosanoid metabolism and gene expression mechanisms (Jayasooriya *et al.*, 2004).

Dietary lipid quantity and quality have been shown to affect serum leptin levels in adult rats (Korotkova *et al.*, 2001). Park and Harris (2003) indicated that omegas-3 fatty acids accelerate the clearance of chylomicron triglycerides in human's fetus effectively reducing triacylglycerol concentration in the serum. Understanding the regulation of leptin levels by (n-3), PUFA might be a tool for preventing adiposity and consequently the enhanced risk of developing diabetes and cardiovascular diseases (Peyron-Caso *et al.*, 2002).

It has been demonstrated that omega-3 fatty acids are negative regulators of hepatic lipogenesis and can also modulate the inflammatory response in mice (Ian *et al.*, 2005). The first article showing the effect of leptin on cholesterol and triglyceride level in birds was introduced by Lohmus *et al.* (2006). Since there were no more studies on broiler chicken leptin, lipoproteins and nutrition fats; so, the aim of this study was to investigate the relationships between the intake of animal fat and/or fish oil and both on plasma leptin and lipoprotein-cholesterol in broiler chickens.

2. MATERIALS AND METHODS

Sixty healthy male broiler chickens, weighing 800-1000 g, aged 35 days were housed in laboratory cages in a temperature ranged from 25-30 °C, and controlled light system (12 h light : 12 h dark cycle).

Chickens were given a regular special standard diet for broiler (200-300 g/daily) with free access to water *adlibitum*. The chickens were acclimated to their environment for five days before the initiation of each experiment. Chickens were randomly divided into four groups.

Group (1) served as a control group, while groups (2), (3) and (4) were experimental groups and treated daily with different types of

fats ,orally as shown in Table (1), at morning for 10 days with regular daily diet. At 11^{th} day, ten broiler chickens from each group were sacrificed after fasting for 8 lasted hours. The remaining chickens of every group were kept as recovery groups (without any treatment) for another one week.

It is worth mentioning that the fish oil used in this investigation is pure code liver oil which is rich in omega-3 polyunsaturated fatty acids; this oil is purchased from Seven Seas LTD, Hull, England and the used dose is the equivalent dose of human per kilogram of body weight. On the other hand, butter of cow was used as the saturated animal fat.

Table (1): Schematic design of the experiment.

Groups	Total (n)	Dose ml/kg b. wt (oil or fat)	Control and treated groups	Recovery groups
(1) Control	N=15	----	N=10	N=5
(2) Treated with fish oil	N=15	0.25	N=10	N=5
(3)Treated with saturated animal fat	N=15	0.25	N=10	N=5
(4) Treated with fish oil+ saturated animal fat mixture (1:1)	N=15	0.25+0.25	N=10	N=5
Total	60		n=40	n=20

N=number of broiler chickens/group.

Blood was collected and plasma was separated immediately using EDTA coated tubes and then stored at -20 °C until subsequent analyses.

Plasma leptin concentrations were measured according to Maffei *et al.* (1995) using radioimmunoassay kit (multispecies leptin RIA kit, Linco, St. Charles, MO, USA), according to manufacturer specification. Inter and intra assay coefficient of variation were 3% (n=10) and 4% (n=10), respectively.

Plasma total cholesterol, HDL-C, triglycerides concentrations were analyzed by colorimetric methods (Biosystem S. A. Costa Brava, 30 Barcelona (Spain) on a M501 single beam scanning UV/visible spectrophotometer. Plasma total cholesterol was estimated according to Allain (1974); triglycerides determination was done according to Wahlefeld (1974) and high denisty lipoprotein-cholesterol according to Warnick *et al.* (1983); while LDL-cholesterol was calculated by the difference between total cholesterol and that of HDL-C. VLDL-C was estimated as one-fifth of the concentration of triglycerides (Friedwald *et al.*, 1972).

Statistical Analysis

All data were expressed as means±SE, and analyzed by ANOVA to detect intergroup differences. Comparisons between two groups were performed by least significant difference test (LSD). Statistical significance was set at $P< 0.05$. The correlation coefficient analysis was adopted among all resultant tested parameters values. All statistical procedures were carried out according to SPSS 8.0 for windows computer statistical program.

3. RESULTS

The data presented in Table (2) reveal that the level of leptin hormone in the control group is 2.185 ±0.0044 ng/ml, while the group treated with fish oil showed a highly significant reduction in leptin level compared with the control group (1.36±0.0878 ng/ml), it is returned to the control level in the recovery group.

Nevertheless, chickens supplemented with animal fat showed a significantly increased level in leptin (3.08±0.0719 ng/ml) when compared with the other treated groups with either fish oil alone (1.36±0.0878 ng/ml) or mixed with animal fat (2.47±0.0289 ng/ml). This value was highly significantly increased over the control group and its level continued at the same high level although after stopping the treatment (recovery group).

Male chickens treated with fish oil and animal fat together showed a medium value between the two other groups, it showed a highly significant increase when compared with fish oil-treated group but it showed a high decrease when compared with the animal fat-treated group alone, this value indicated also a highly significant increase on comparison with the control group. The same trend was also observed in the recovery groups.

The same table shows that cholesterol levels in all groups did not give any significant differences in both treated and recovery groups.

Plasma triglyceride measurements showed that in chickens administered with fish oil plus animal fat the plasma triglycerides were significantly decreased when compared with both control group and fat-treated chickens.

In the recovery group which was treated with fish oil, plasma triglycerides showed a significant increase in comparison with other experimental groups (79.3±17.63 mg/dl).

Plasma HDL-C in all treated and recovery groups did not show any significant differences with the control group, but when chickens had been given fish oil alone or with animal fat, HDL-C was increased significantly when compared with chickens treated with fat alone and the recovery control group. Plasma HDL-C in all recovery groups showed no significant variation comparing with different treatments.

Table (2): Effect of fish oil and/or animal fat on plasma leptin and lipoproteins in male broiler chickens.

Groups	Measurements					
	Leptin ng/ml	**Total cholesterol mg/dl**	**Triglyceride mg/dl**	**HDL-C mg/dl**	**LDL-C mg/dl**	**VLDL-C mg/dl**
1-Control	$2.185^{a,b,c}$ ± 0.0044	113.56 ± 9.02	$55.1^{a,f}$ ± 6.79	67.56 ± 16.49	46 ± 13.22	$11.01^{a,f}$ ± 1.35
2-Treated with fish oil (n-3 PUFA)	$1.36^{a,c,h}$ ± 0.0878	176 ± 40.79	35.68^{g} ± 6.18	$68.95^{a,b}$ ± 12.8	113.27 ± 47.27	7.13^{g} ± 1.23
3-Treated with saturated animal fat	$3.08^{b,c,d}$ ± 0.0719	120.6 ± 26.16	$44.08^{b,h}$ ± 3.43	$38.09^{a,c}$ ± 8.74	93.35 ± 27.1	$8.81^{b,h}$ ± 0.68
4-Treated with fish oil+ saturated animal fat	$2.47^{d,h,i}$ ± 0.0289	92.97 ± 11.81	$20.85^{a,b,i}$ ± 2.36	$80.85^{c,d}$ ± 8.13	27.79 ± 7.5	$4.22^{a,b,i}$ ± 0.46
Recovery groups	Leptin ng/ml	Total cholesterol mg/dl	Triglycerides mg/dl	HDL-C mg/dl	LDL-C mg/dl	VLDL-C mg/dl
R-control	$2.14^{e,j}$ ± 0.022	133.15 ± 22.59	34.53^{c} ± 8.4	$34.28^{b,d}$ ± 12.16	97.76 ± 18.47	6.91^{c} ± 1.68
R-fish oil rich in (n-3 PUFA)	$2.11^{f,k}$ ± 0.017	153.15 ± 44.35	$79.3^{c,d,e,f,g,h,i}$ ± 17.63	53.42 ± 14.69	108.19 ± 54.75	$15.86^{c,d,e,f,g,h,i}$ ± 3.52
R-saturated animal fat	$3.03^{e,f,g}$ ± 0.0772	79.64 ± 19.83	34.12^{d} ± 9	59.72 ± 14.54	39.37 ± 17.96	6.82^{d} ± 1.8
R-fish oil+ saturated animal fat	$2.24^{g,j,k}$ ± 0.0399	134.59 ± 21.72	39.23^{e} ± 7.89	67.12 ± 7.88	67.46 ± 20.93	7.84^{e} ± 1.58
F	96.095	1.56	3.843	2.221	1.15	3.813
Significance	0.000	No. sig.	0.000	0.022	No. sig.	0.000

All results are expressed as Means ±standard error

Values having the same letter in the same column are significantly different

LDL-C measurements in chickens' plasma revealed non significant differences among groups.

Plasma VLDL-C level in Table (2) shows the same behavior of plasma triglycerides in the treated and recovery groups.

Also, TG and VLDL-C levels in the recovery fish oil group were increased significantly when compared with their levels in fish oil and other treated groups.

Table (3): Correlation coefficients between the studied parameters.

	Total cholesterol	HDL-C	LDL-C	Triglycerides	VLDL-C	Leptin
Total cholesterol	1					
HDL-C	-0.102 .175	1				
LDL-C	.884** .000	-.477** .000	1			
Triglycerides	0.07 .262	.007 .473	.064 .282	1		
VLDL-C	.069 .266	.004 .486	.064 .281	1** .000	1	
Leptin	-.277** .005	-.156 .074	-.172 .059	-.184** .045	-.185* .044	1

**Correlation is significant at the 0.01 level (1-tailed)

*Correlation is significant at the 0.05 level (1-tailed)

Table (3) shows that there are positive and negative correlations between cholesterol and both LDL-C and leptin, respectively. Significantly negative correlation between LDL-C and HDL-C, significantly positive correlation between triglycerides and VLDL-C and significant negative correlation between leptin and both cholesterol, triglycerides and VLDL -C were also recorded.

4. DISCUSSION

The results showed that the mean value of plasma leptin in the control broiler chickens was 2.185±0.0044 ng/ml. This result is in agreement with that reported by Backus *et al.* (2000) in domestic cats, and Kauter *et al.* (2000) in ewes, male castrate animals and rams.

Mean of plasma cholesterol level in the control male broiler chickens was (113.56±9.02 mg/dl) in agreement with the data published by Sturkie (2000) who found that cholesterol concentration was 116-134 mg/dl in unsexed White Leghorn chicks, from 1 to 15 weeks of age. Circulating lipids in the blood was derived from intestinal absorption, synthesis (mainly in the liver) or mobilization of

fat depots. Lipid concentration in birds is influenced by species, age, sex, nutrition, state of health and energy needs including climate conditions and other factors Sturkie (2000).

Supplementation of fish oil rich in n-3 PUFA to male broiler chickens showed significantly lowered level of plasma leptin and non significant decrease in triglycerides and VLDL-C. This is in agreement with findings of Park and Harris (2003) and Ukropec *et al.* (2003). They found in male rats that dietary fish oil stimulates fatty acid oxidation in both liver and skeletal muscle, probably playing an important role in lowering plasma lipid concentration which was accompanied by low plasma leptin concentration and increased hepatic leptin receptor in RNA. The lowering effect of unsaturated fats (linolenic acid) on leptin levels was found also by Koba *et al.* (2002).

Animal fat-treated chickens showed a highly significant increase in plasma leptin (3.08±0.0719 ng/ml) in comparison with the control group; this result is in agreement with that obtained by Wang *et al.* (2002). This high level of plasma leptin was accompanied with a slight decrease in HDL-C and an increase in LDL-C. The same results were obtained by Ehrhardt *et al.* (2000).

In contrast, Cha and Jones (1998) observed, in rats, that a diet rich in n-3 PUFA led to higher serum leptin levels than that found in a diet rich in saturated fatty acids.

Group of male chickens treated with animal fat mixed with fish oil had no effect on plasma cholesterol level. The same results have been shown by Sturkie (2000) when he had added 1% dietary cholesterol or 10% corn oil to young chickens.

A mixture of animal fat and fish oil was given to chickens resulted in a moderate mean in plasma leptin level which lies between the mean levels of both animal fat and fish oil groups indicating that fish oil had a primary lowering effect on plasma leptin level. This result is in agreement with Pieke *et al.* (2000) who observed that serum leptin level was decreased in hypertriglyceridemic patient after dietary saturated fatty acids had been replaced by n-3 PUFA. This moderate level of leptin in this study was accompanied with lower levels of total cholesterol, LDL-C and VLDL-C. In contrast, HDL-C level had the highest value comparing to the all groups.

The lowering effect of fish oil on triglycerides and VLDL-C in male broiler chickens was associated with reduced plasma leptin. This result is in agreement with the results of Ukropec *et al.* (2003).

This effect of fish oil could be ascribed to its content of very long chain n-3 PUFA such as EPA (eicosapentaenoic acid) and DHA (decosahexaenoic acid) (Steerenberg *et al.*, 2002); these long chain n-3 PUFA have a lowering influence on blood triglycerides concentration (Sebokova and Klimes, 1997).

The present results showed that plasma leptin was decreased when male broiler chickens had been given fish oil alone or mixed with animal fat indicating that fish oil is more effective in regulating plasma leptin level in broilers and this conclusion is in agreement with that reported by Peyron-Caso *et al.* (2002).

In the recovery groups of fish oil-treated chickens alone or combined with animal fat, the plasma leptin levels returned to the basal control level. On the other hand, the lower leptin value in fish oil group was increased again after removing the fish oil; while the higher leptin value in animal fat group was not decreased after removing the animal fat. These results may point to the longer effects of animal fat on leptin levels in broiler chickens (7 days after stopping the treatment) than that happened in the chicken groups treated with fish oil alone or combined with animal fat.

It is concluded that the combination of animal fat (saturated fat) and fish oil (polyunsaturated fatty acids) was a good method to modify plasma leptin level and to improve the lipoprotein-cholesterol levels to the best.

Because of the clear significant effect of fish oil on leptin level in different groups. It is believed that leptin may play an important physiological role in the control of lipoprotein-cholesterol and TG levels. In addition, the effect of fish oil on leptin was temporary because this effect was abolished after the end of the treatment.

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التأثيرات الهامة لزيت السمك الغنى بالأحماض الدهنيه عديدة عدم التشبع و/ أو الدهن الحيواني المشبع على مستويات البلازما من اللبتين والكولسترول في ذكور الدجاج اللاحم

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ملخص

تهدف الدراسة الحالية الى معرفة تأثير الدهن المشبع و زيت السمك الغنى بالأحماض الدهنيه عديدة عدم التشبع على مستويات البلازما من هرمون اللبتين والليبوبروتينات. تم استخدام عدد ٦٠ من ذكور الدجاج اللاحم لهذا الغرض وقد تم تقسيمها لأربع مجموعات استخدمت الأولى كمجموعة شاهد بينما الثلاث الأخرى تجريبية.

عوملت المجموعة الثانية بزيت السمك بينما عوملت الثالثه بالدهن الحيواني المشبع أما المجموعة الرابعة فقد عوملت بخليط من الدهن المشبع وزيت السمك وقد أعطيت الجرعات يوميا عن طريق الفم لمدة ١٠ أيام، ثم ذبح عشرة من كل مجموعة وترك العدد المتبقي من كل منها كمجموعة استشفاء لمدة ٧ أيام ثم ذبحت.

جمعت عينات الدم من كل المجاميع الشاهد والتجريبية والاستشفاء ودلت النتائج على أن مستوى اللبتين في مجموعة زيت السمك أوضح انخفاضا شديد المعنوية ثم عاد إلى مستواه قبل المعاملة بعد ٧ أيام من وقف المعالجة. وفي نفس الوقت كان هناك تأثير غير معنوي على الجلسريدات الثلاثية والليبوبروتينات في البلازما بينما اتضح أن معالجة ذكور الدجاج بالدهن المشبع أدى إلى ارتفاع شديد المعنوية في مستوى اللبتين في البلازما

وقد كانت أفضل النتائج عند إعطاء الدجاج اللاحم خليط من زيت السمك والدهن الحيواني المشبع الذي أعطى مستويات متوسطة من اللبتين في البلازما وفي نفس الوقت أدت إلى خفض معنوي للجلسريدات الثلاثية والكولسترول منخفض الكثافة والكولسترول منخفض الكثافة جدا كما أدت إلى ارتفاع مستوى الكولسترول عالي الكثافة.

ويستنتج من هذه الدراسة أن إعطاء جرعة مشتركة من زيت السمك الغنى بالأحماض الدهنيه عديدة عدم التشبع مع الدهن الحيواني أعطت مستوى معتدل من اللبتين و قد لعبت دورا وظيفيا هاما في تحسين مستويات البلازما من الجلسريدات الثلاثية والليبوبروتينات في ذكور الدجاج اللاحم.

المجلة العلمية لكلية الزراعة – جامعة القاهرة – المجلد (٥٧) العدد الثالث (يوليو ٢٠٠٦) :٤٤٩—٤٦٢ .