

## Effects of Cod Liver Oil and Chromium Picolinate Supplements on the Serum Traits, Egg Yolk Fatty Acids and Cholesterol Content in Laying Hens

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**ABSTRACT :** This study investigated the effects of cod liver oil and chromium picolinate on the serum traits and egg yolk fatty acids and cholesterol content in laying hens. One hundred 45-week old single comb white Leghorn laying hens were assigned randomly to four groups. These groups were: (1) control (soybean oil), (2) 1,000 ppb ( $\mu\text{g/kg}$ ) chromium (organic form chromium picolinate) (Crpic), (3) 3% cod liver oil (CLO), and (4) 1,000 ppb chromium with 3% cod liver oil (CLO+Crpic). The experiment was conducted for 40 days. Results indicated that serum triacylglycerol (TG) and cholesterol contents in the CLO group and the serum glucose content in the Crpic group were significantly lower than those in the control group ( $p < 0.05$ - $0.01$ ). The yolk cholesterol content in the CLO and Crpic groups were also lower than the control group ( $p < 0.01$ ). The lipoprotein profile displayed that in the Crpic group, high-density lipoprotein (HDL) and HDL-cholesterol (HDL-C) were significantly higher ( $P < 0.05$ ) than the control group. Meanwhile, low-density lipoprotein+very low-density lipoprotein (LDL+VLDL) and LDL-C+VLDL-C were significantly lower ( $p < 0.05$ ) than the control group. Notably, of all four groups, the CLO group displayed a more profound effect on serum traits and lipoprotein ( $p < 0.05$ - $0.001$ ). Furthermore, the fatty acid composition of the egg yolks presented that C18:2 in the CLO and Crpic groups was significantly lower ( $p < 0.05$ - $0.001$ ) compare to the control. However, only in the CLO group, C18:3, C20:5 and C22:6 were significantly higher ( $p < 0.001$ ) than the control. Only serum glucose and LDL+VLDL showed the CLO $\times$ Crpic interaction ( $p < 0.05$ ), most parameters did not. Therefore, supplemented chromium picolinate or cod liver oil in the diet of laying hens had beneficial effects. However, when these two factors were combined, there was no interaction with most parameters. (*Asian-Aust. J. Anim. Sci.* 2003, Vol 16, No. 8 : 1177-1181)

**Key Words :** Cod Liver Oil, Chromium Picolinate, Serum Traits, Egg Yolk Cholesterol, Laying Hens

### INTRODUCTION

Several studies have reported that feeding laying hens with a ration rich in  $\omega$ -3 polyunsaturated fatty acids (PUFA) produces eggs that contain high quality  $\omega$ -3 PUFA (Hargis et al., 1991; Jiang and Sim, 1992; Oh et al., 1994; Van-Elsky et al., 1994). Consuming  $\omega$ -3 PUFA eggs could reduce serum triacylglycerol (TG), cholesterol and blood pressure (Jiang and Sim, 1992; Oh et al., 1994).  $\omega$ -3 PUFA eggs are best suited for infants and the elderly because they have a limited ability to elongate and desaturate fatty acids (Simopoulos and Salem, 1992). Many researchers indicated that enriched  $\omega$ -3 PUFA reduces plasma TG and LDL-cholesterol levels markedly (Sanders et al., 1981, 1985; Sanders and Roshani, 1983; Kim et al., 1991).

The high cholesterol level is the main shortcoming of fresh eggs. Evans (1989) and Page et al. (1993) indicated that an organic form of trivalent chromium supplement could reduce blood cholesterol levels. Our previous study indicated that a diet supplemented with chromium picolinate could markedly reduce the cholesterol contents in serum LDL and that in the egg yolk of laying hens (Lien et al., 1997). Trivalent chromium has been recognized as a cofactor of insulin, and plays a prominent physiological role in humans (Evan, 1989, 1992; Press et al., 1990) and

domestic animals (Lien et al., 1996, 1997, 1998, 1999, 2001). It is involved in carbohydrate, lipid, amino acid and nucleic acid metabolism (Okada et al., 1984; Press et al., 1990; McCarty, 1991; Xi et al., 2001) and is considered as an essential animal trace element. In this study, cod liver oil (enrich with  $\omega$ -3 PUFA) and chromium picolinate were added to a laying hen diet to further investigate their effects on serum traits and yolk cholesterol.

### MATERIALS AND METHODS

#### Animals and treatment

One hundred 45 week-old single comb white Leghorn laying hens were randomly divided into four groups. The groups were (1) control (supplemented with soybean oil), (2) Crpic (supplemented with chromium picolinate), (3) CLO (supplemented with cod liver oil containing 0.5% linolenic acid, 3.6% eicosapentaenoic acid and 3.6% docosahexaenoic acid) and (4) CLO+Crpic (supplemented with cod liver oil and chromium picolinate). The supplemented oil and chromium were 3% and 1,000 ppb ( $\mu\text{g/kg}$ ), respectively. Table 1 presents the basal diet composition. During this 40 day experiment, the birds were individually caged and had unlimited access to feed and water. The animals used in this experiment were cared for under the guidelines stated in the Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching. Blood samples were extracted from the wing vein.

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**Table 1.** Composition of basal diet

Ingredients	Percentage
Yellow corn meal	58.85
Soybean meal, 44%	24.00
Fish meal	3.00
Soybean oil	3.00
Dicalcium phosphate	1.30
Limestone, pulverized	4.25
Salt	0.30
Vitamin premix <sup>a</sup>	0.05
Mineral premix <sup>b</sup>	0.20
Oyster shells	4.00
DL-Methionine	0.05
Choline chloride, 50%	1.00
Total	100.00
Calculated value :	
Crude protein, %	17.80
ME, kcal kg <sup>-1</sup>	2,840.00
Calcium, %	3.40
Available phosphorous, %	0.47
Lysine, %	0.98
Methionine+cystine, %	0.62

<sup>a</sup> Vitamin premix supplied the followings per kilogram of diet: Vitamin A, 25,000 IU; Vitamin D<sub>3</sub>, 3,125 IU; Vitamin E, 37.5 IU; Vitamin K<sub>3</sub>, 6.25 mg; Vitamin B<sub>1</sub>, 3.75 mg; Vitamin B<sub>2</sub>, 12.5 mg; Vitamin B<sub>6</sub>, 10.0 mg; Pantothenate, 18.8 mg; Niacin, 50 mg; Biotin, 0.06 mg; Folic acid, 1.25 mg; Vitamin B<sub>12</sub>, 0.05 mg.

<sup>b</sup> Mineral premix supplied the following per kilogram of diet: Cu (CuSO<sub>4</sub>·5H<sub>2</sub>O, 25.45% Cu) 6 mg; Fe (FeSO<sub>4</sub>·7H<sub>2</sub>O, 20.29% Fe) 50 mg; Mn (MnSO<sub>4</sub>·H<sub>2</sub>O, 32.49% Mn) 40 mg; Zn (ZnO, 80.35% Zn) 60 mg; Se (NaSeO<sub>3</sub>, 45.56% Se) 0.175 mg.

Eggs were collected from each bird at the beginning and the end of the experiment.

An enzyme kit with a serum autoanalyzer (Roche, Co., Switzerland) was employed to measure the serum glucose, cholesterol and TG concentrations. Electrophoresis was used for determining the serum lipoprotein profiles and cholesterol components (Houstmuller, 1969; Cobb and Sanders, 1978). A densitometer (Helena, Co., USA) was employed to examine each gel band concentration. Yolk cholesterol was extracted according to the method reported by Beyer and Jansen (1989). An enzymatic kit (Roche Co. No. 07-36635) was used to determine the yolk cholesterol level. Flame ionization detector (FID) gas chromatography (Fisons GC 8160-00, Italy) was used to determine the fatty acid compositions. The initial capillary column (Supelco wax-10, 30 m, 0.25 mm ID) temperature was 160°C. The

temperature was increased to 225°C at a rate of 2°C/min and maintained for 2 minutes. The FID temperature was 260°C, and the carrier gas (nitrogen air) flow rate was 50 mL/min.

### Statistical analysis

The variance among groups was identified and the significance determined using Tukey's test via a SAS system (SAS, 1990). Cr and CLO were the main effects, and their interaction according to the model

$$Y = \mu + Cr_i + CLO_j + (Cr \times CLO)_{ij} + e_{ijk}$$

Where Y is the dependent variable,  $\mu$  is the mean and e is the random residual error term.

## RESULTS

Table 2 displays the cod liver oil and chromium picolinate supplement effects on both the serum traits and cholesterol content of egg yolk in laying hens. This table indicates that the serum TG and cholesterol levels in the CLO group were significantly lower than those in the control group ( $p < 0.05$ ). The Crpic group serum glucose content was less than that in the control ( $p < 0.001$ ), and showed CLO×Crpic interaction ( $p < 0.05$ ). As compared to the control, the yolk cholesterol content was significantly reduced in the CLO ( $p < 0.001$ ) and Crpic ( $p < 0.05$ ) groups. There had the trend of CLO×Crpic interaction ( $p = 0.06$ ).

Table 3 shows the cod liver oil and chromium picolinate supplement effect on the lipoprotein profile and cholesterol component in the serum of laying hens. This table displays that HDL, HDL-C were significantly increased and LDL+VLDL, LDL-C+VLDL-C were decreased in the Crpic group ( $p < 0.05$ ). The CLO group displayed a more profound effect than the Crpic group ( $p < 0.05-0.001$ ). The CLO×Crpic interaction also occurred in LDL-C+VLDL-C ( $p < 0.05$ ).

Table 4 lists the cod liver oil and chromium picolinate supplement effects on the yolk fatty acid composition. Compared to the control group, C16:1 in the CLO group ( $p < 0.05$ ) and C18:2 in both the CLO and Crpic groups ( $p < 0.05-0.001$ ) were significantly lower. However, C18:3,

**Table 2.** The effects of supplement of chromium picolinate and cod liver oil on the serum traits and egg yolk cholesterol content of laying hens

	Control	Crpic	CLO	CLO+Crpic	SEM	Significant		
						Crpic	CLO	CLO×Crpic
Cholesterol, mg/dL	126.40	113.05	96.90	93.20	6.96	NS	**	NS
Triacylglycerol, mg/dL	1,320.85	1,219.95	1,097.80	999.45	80.23	NS	*	NS
Glucose, mg/dL	140.80	111.30	137.10	129.21	3.98	***	0.08	*
Yolk cholesterol, mg/g	15.21	14.40	13.74	11.51	0.55	*	***	0.06

n=25. \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ . NS: Non-significant ( $p > 0.05$ ).

**Table 3.** The effects of supplement of chromium picolinate and cod liver oil on the lipoprotein profile and cholesterol component in serum of laying hens

	Control (%)	Crpic (%)	CLO (%)	CLO+Crpic (%)	SEM (%)	Significant		
						Crpic	CLO	CLO×Crpic
HDL	7.98	9.24	11.70	15.03	0.83	*	***	NS
LDL+VLDL	92.02	90.94	88.26	84.49	0.89	*	***	NS
HDL-C	40.27	45.87	46.92	47.94	1.44	*	*	NS
LDL-C+VLDL-C	59.74	54.12	53.03	51.96	1.13	*	**	*

n=25. \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ . NS: Non-significant ( $p > 0.05$ ).

**Table 4.** The effects of supplement of chromium picolinate and cod liver oil on the fatty acid composition of egg yolk of laying hens

	Control (%)	Crpic (%)	CLO (%)	CLO+Crpic (%)	SEM (%)	Significant		
						Crpic	CLO	CLO×Crpic
C16:0	24.71	25.23	24.16	26.37	0.84	NS	NS	NS
C16:1	2.28	2.53	1.90	2.03	0.43	NS	*	NS
C18:0	9.43	10.43	9.29	9.24	0.58	NS	NS	NS
C18:1	49.04	47.49	48.79	48.62	0.99	NS	NS	NS
C18:2	12.14	11.39	10.91	8.31	0.52	*	***	NS
C18:3	0.61	0.61	0.82	0.87	0.05	NS	***	NS
C20:5	0.24	0.24	0.47	0.43	0.02	NS	***	NS
C22:6	0.49	0.50	1.95	1.97	0.10	NS	***	NS

n=25. \*  $p < 0.05$ , \*\*\*  $p < 0.001$ . NS: Non-significant ( $p > 0.05$ ).

C20:5 and C22:6 were significantly higher in the CLO group ( $p < 0.001$ ) than in the control. No CLO×Crpic interaction ( $p > 0.05$ ) was observed.

## DISCUSSION

The study indicated that the serum cholesterol in the cod liver oil supplemented group was lower than that in the control group. Kim et al. (1991) and Van-Elswyk et al. (1994) also reported that a marine oil supplement resulted in significant plasma cholesterol level reduction. Sanders et al. (1981, 1985) and Weintraub (1988) reported that a diet supplemented with  $\omega$ -3 PUFA reduced serum TG and LDL-C levels. This agreed with our result that a 3% cod liver oil supplement reduced serum TG, cholesterol and LDL-C+VLDL-C. Consequently, the egg yolk cholesterol was reduced.

It is well known that the body fatty acid composition could occur in response to dietary fatty acids. Vilchez et al. (1991) and Van-Elswyk et al. (1994) reported that feeding hens varying fatty acids was reflected in the yolk lipid. Herein, the yolk fatty acid composition in the cod liver oil supplemented group, which was rich in  $\omega$ -3 PUFA, also resulted in a significant increase in C18:3, C20:5 and C22:6. Furthermore, as cod liver oil contains abundant C20:5 and C22:6 levels, these levels were higher in the CLO group than they were in the control. Hargis et al. (1991) and Oh et al. (1994) also indicated that a 3% supplement of marine oil increased the C20:5 and C22:6 levels in yolk lipid.

A few reports indicated chromium reduced serum glucose that is consistent with the results of the present study (Evock-Clover et al., 1993; Amoikon et al., 1995;

Lindemann et al., 1995). It has been recognized that chromium facilitates insulin membrane receptor binding and thereby increases biological insulin activity (Anderson et al., 1985, 1987, 1991; McCarty, 1991; Morris et al., 1993). Elevated insulin activity from chromium picolinate supplementation could stimulate anabolism and inhibited catabolism. Glucose utilization is thereby increased (Rosebrough and Steele, 1981; Cupo and Donaldson, 1987; Mirsky, 1993).

The lipoprotein profile results showed that chromium reduced VLDL+LDL and increased HDL. This is consistent with a previous study on broilers (Lien et al., 1999). Howard et al. (1993) indicated that chromium stimulated lipoprotein lipase activity could increase VLDL metabolism. Brindley and Salter (1991) demonstrated that insulin increased liver LDL receptor numbers, and thus, the LDL+VLDL content were reduced and HDL was increased. McCarty (1991) revealed that a dietary chromium picolinate supplement could increase human HDL content. Several reports indicated that chromium supplementation elevated HDL-cholesterol and depressed the LDL-cholesterol content in humans and other animals (Anderson, 1986; Lefavi et al., 1993; Kim et al., 1996; Lien et al., 1998; Wang et al., 1989). A similar result was observed in this study. This phenomenon might be due to lecithin-cholesterol acyltransferase (LCAT) activity that was increased by chromium supplementation (Lien et al., 1998). This in turn, accelerated cholesterol esterification and excretion.

A previous study revealed that a hens diet supplemented with 800 ppb chromium picolinate reduced egg yolk cholesterol (Lien et al., 1997). This study further confirms

that result, and showed a trend for CLO×Crpic interaction ( $p=0.06$ ). However, this study indicated that the egg yolk fatty acid compositions in the supplemented chromium group had no beneficial effect. Except for the serum glucose, serum VLDL-C+LDL-C, most parameters failed to interaction between cod liver oil and chromium supplementation.

## CONCLUSION

The study showed that both chromium picolinate and cod liver oil supplemented was beneficial to hens. However, when these two factors were combined, there was no interaction with most parameters.

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