

## Effects of Dietary Manganese Proteinate or Chromium Picolinate Supplementation on Plasma Insulin, Glucagon, Glucose and Serum Lipids in Broiler Chickens Reared Under Thermoneutral or Heat Stress Conditions.

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**Abstract:** A study was conducted to investigate the effects of supplemental chromium picolinate (CrPic) or manganese proteinate (MnPro) on heat-distressed broiler chickens. In a completely randomized design, diets were supplemented with either 200 or 400 Fg Cr/kg as CrPic or 0, 60 and 240 mg Mn/kg as MnPro and fed to broilers under heat stress (HS) or thermoneutral (TN) conditions. Commercial broilers were reared in brooder pens and fed the experimental diets from Day 1 to 21 and then assigned the same dietary treatments in one of two environmentally controlled chambers. One chamber was maintained at 23.9 °C, whereas birds in the second chamber were exposed to 8-h of 23.9 °C, 4-h of 23.9 to 35 °C, 4-h of 35 °C and 8-h of 35 to 23.9 °C. At 9 wks, plasma concentration of insulin (I) was lower ( $p < 0.01$ ) in birds supplemented with 240 mg Mn /kg compared with 60 mg Mn/Kg. However, neither glucagon (G) concentration, I:G ratio, or glucose were affected ( $p > 0.05$ ) by dietary treatment regimen. Heat stress lowered insulin ( $p < 0.004$ ), increased glucagon ( $p < 0.002$ ) and lowered I:G ratio ( $p < 0.02$ ). Serum concentrations of non-esterified fatty acids were lower in high Mn supplemented group, but triglycerides (TG), total cholesterol (CHOL), high-density lipoprotein (HDL) cholesterol, and HDL: CHOL ratio were not affected ( $p > 0.05$ ) by dietary treatment. Heat stressed birds receiving no Mn supplementation had lower HDL: CHOL ratios ( $p < 0.02$ ) while HS reduced serum TG concentrations ( $p < 0.04$ ). Data suggest that under conditions of this experiment, Cr and Mn may play a part in lipid and/or carbohydrate metabolism in broilers.

**Key words:** Chromium picolinate, manganese proteinate, heat stress, plasma metabolites, broiler

### Introduction

Chromium and Manganese are both intimately involved in lipid and carbohydrate metabolism. Chromium is generally accepted to be the active component in glucose tolerance factor (GTF), which increases the sensitivity of tissue receptors to insulin, resulting in increased glucose uptake by cells (Schwarz and Mertz, 1959; Mertz, 1967, 1969). The effect of insulin in chickens differs from its effect in mammals (Leville *et al.*, 1975). Insulin induces hypoglycemia in chickens and tends to elevate circulating free fatty acid levels. Insulin may also stimulate glucagon release in chickens. Manganese is an activator or co-factor of many enzymes that are directly or indirectly involved in carbohydrate metabolism. Manganese has been shown to cause increased synthesis of insulin from pancreatic beta cells in rats, while Mn-deficient rats and guinea pigs exhibit impaired glucose tolerance (Baly *et al.*, 1984, 1985; Everson and Shrader, 1968 a,b).

Although Cr is not currently considered an essential trace element for poultry, this micronutrient may play a nutritional and physiological role. Moreover, the National Research Council (NRC) has recommended 300 ug Cr /kg diet for laboratory animals (NRC, 1995). Currently there are no NRC recommendations for Cr in poultry

diets (NRC, 1994). Supplementation with an organic source of Cr such as chromium picolinate (Kim *et al.*, 1996), if it has greater biological availability, may prove to be beneficial to birds under heat stress because birds may obtain more Cr despite lowered feed consumption. In addition, it is generally necessary to supplement corn-soybean-based broiler diets with manganese because of the relatively low Mn content of such diets. Interaction with other nutrients in the gastrointestinal tract can decrease the availability of dietary manganese (Halpin and Baker, 1986a,b; Southern *et al.*, 1987). Under environmental conditions that lead to heat stress, the availability of manganese is reduced (Belay *et al.*, 1992). Compared with inorganic sources, proteinated manganese had greater biological availability when supplemented under thermoneutral and heat stress conditions, (Smith *et al.*, 1995). Increasing Mn supplementation of broilers may help to alleviate some of the detrimental effects of heat stress.

This study was designed to assess the effects of dietary supplementation with manganese proteinate or chromium picolinate on plasma insulin, glucagon, glucose and serum lipid concentrations of broilers reared under thermoneutral or heat stress environments.

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**Materials and Methods**

**Animals and diets:** Three hundred, 1-day old commercial male broilers (Arbor Acres X Arbor Acres) were individually weighed, wing-banded, and randomly assigned to one of 25 floor pens in a single brooder house. Five replicate groups of twelve chicks were assigned to each of five dietary treatments. Dietary treatments were prepared from a corn-soybean meal-based common starter diet, formulated to contain 3133 kcal of metabolizable energy and 23.17% crude protein (Table 1). Treatments were three levels, 0, 60 and 240 mg/kg of supplemental manganese from manganese proteinate (MnPro) and two levels, 200 and 400 µg/kg of supplemental chromium from chromium picolinate (CrPic). The diet containing 60 mg/kg supplemental Mn served as a control for both the Mn and Cr supplemented diets. Chicks were allowed ad libitum access to feed and water. Body weights for individual chicks were determined weekly, while feed and water consumption was calculated weekly for each pen.

**Growth Environment:** On day 22 of the experiment, birds from replicate groups, arranged by treatment, were placed together. Twenty birds per treatment were then randomly selected and assigned to individual cages fitted with feed and water dispensing equipment in two separate environmental chambers. Dietary treatments for the last four weeks consisted of a corn-soybean meal-based grower diet (Table 1), maintaining the same source and level of supplemental Mn and Cr as in the starter diet used in the first three weeks of the experiment. One chamber was allowed to cycle between 18.3 and 23.9 °C (thermoneutral) and the other between 23.9 and 35 °C (heat-stressed). The thermoneutral chamber was held at 18.3 °C for 10 hours and then gradually increased to 23.9 °C, and maintain as such for 2 hours, and then gradually decreased to 18.3 °C. The heat-stress chamber was held at 23 °C for 8 hours, then increased gradually over 4 hours from 23.9 to 35 °C, kept at 35 °C for 4 hours and then decreased gradually over 8 hours from 35 to 23.9 °C. Body weights as well as feed and water consumption were determined weekly.

**Blood Samples:** After 9 weeks on the experimental diets, six birds per treatment from each chamber were fasted for 12 hours and blood samples collected by brachial venipuncture using EDTA as an anticoagulant. The birds were then re-fed for 24 hr before a second blood sample was collected using the same method. Following collection, samples were centrifuged at 3000x g for 15 minutes to separate supernatant (plasma), from red blood cells. Each sample was divided into three aliquots by decanting into sterile polypropylene tubes for storage. One hundred µL of Aprotinin (protease inhibitor, Sigma Chemical Co., St. Louis, MO), per ml of

**Table 1: Composition of experimental diets**

Ingredient	Starter	Grower
	----- (%)-----	
Corn	55.65	61.00
Soybean meal 49%	35.00	30.00
Fat <sup>a</sup>	3.50	4.40
Fish meal (menhaden)	2.00	0.95
Dicalcium phosphate	1.50	1.40
Limestone	1.00	1.00
Vitamin-trace mineral mix <sup>b</sup>	0.50	0.50
Salt	0.40	0.35
DL-methionine	0.15	0.10
Sand <sup>c</sup>	0.20	0.20
Coccidiostat	0.10	0.10

<sup>a</sup>A mixture of corn oil and soybean oil; Tennessee Farmers Cooperative, La Vergne, TN 37806

<sup>b</sup>Supplied per kilogram of diet: copper, 8 mg; iodine, 0.4 mg; iron, 100 mg; selenium, 0.3 mg; zinc, 75 mg; vitamin A (retinyl acetate), 4540 IU; vitamin D3, 1543 ICU; vitamin E, 15 IU; choline, 284 mg; niacin, 34 mg; d-panthothenic acid, 5.7 mg; menadione, 0.85 mg; vitamin B12, 0.01 mg; biotin, 0.1 mg; folic acid, 0.5 mg; thiamine, 0.6 mg.

<sup>c</sup>Chromium (200 and 400 ug/kg) as chromium picolinate or manganese (0, 60, and 240 mg/kg) as manganese proteinate was added at the expense of equivalent percentages of sand.

plasma was added to the tube designated for analysis of glucagon. Samples for plasma insulin and glucose analysis were stored at -20 °C while those for plasma glucagon analysis were stored at -72 °C.

Following a 12 h fast, blood samples for serum lipid determination were collected (6 birds per treatment) by cardiac puncture in non-heparinized needles and syringes without an anticoagulant. Samples were allowed to clot before being centrifuged at 3000x g for 15 minutes to separate supernatant (serum) from red blood cells. Serum samples were then decanted into three aliquots and stored at -20 °C for later analysis of non-esterified fatty acids (NEFA), triglycerides (TG), total cholesterol (CHOL) and high density lipoprotein (HDL) cholesterol.

**Chemical Analyses:** Plasma insulin and glucagon were analyzed using commercial RIA kits (insulin kit, Diagnostic Products Corp., Los Angeles, CA; glucagon kit, Linco Research Inc., St. Charles, MO) used in previous studies (Johnson, *et al.*, 1986; Davis and Vasilatos-Younken, 1995). Serum NEFA, TG, CHOL, HDL cholesterol and plasma glucose was analyzed using commercial enzymatic kits (Wako Pure Chemicals Industries, Ltd., Richmond, VA). All samples were analyzed in duplicate within each assay. The intra- and interassay coefficients of variation in the insulin radioimmunoassay were 3 and 6%, respectively, while for the glucagon radioimmunoassay, they were 3 and 8% respectively.

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Table 2: Plasma insulin, glucagon, glucose and ratio of insulin to glucagon (I:G) concentration of broilers supplemented with chromium and manganese under thermoneutral (TN) or heat stress (HS) conditions<sup>a</sup>

Dietary supplement <sup>b</sup>	Temp <sup>c</sup>	Insulin <sup>c</sup> (ng/mL)	Glucagon <sup>c</sup> (pg/mL)	Glucose (mg/dL)	I:G ratio <sup>c</sup>
60 mg/kg Mn	TN	0.290	218	210	1.963
	HS	0.176	231	207	0.926
200 ug/kg Cr	TN	0.227	245	208	1.455
	HS	0.200	293	221	0.845
400 ug/kg Cr	TN	0.230	192	229	1.424
	HS	0.192	257	208	0.858
0 mg/kg Mn	TN	0.235	204	206	1.489
	HS	0.160	235	192	0.725
240 mg/kg Mn	TN	0.176	196	216	1.196
	HS	0.121	224	223	0.717
SEM <sup>d</sup>		0.30	17.6	18.4	0.47

<sup>a</sup>Values are least square treatment means. Samples from 6 birds per treatment. <sup>b</sup>Diets supplemented with Cr at 200 and 400 ug/kg also contained Mn at 60 mg/kg. Diets supplemented with Mn at 0, 60 (control) and 240 mg/kg contained no added Cr. <sup>c</sup>The effect of temperature was significant (P<.02) <sup>d</sup>Standard error of the mean.

Table 3: Serum non-esterified fatty acids (NEFA), triglycerides (TG), high-density lipoprotein cholesterol (HDL), total cholesterol (CHOL) and ratio of HDL to CHOL concentration of broilers supplemented with chromium or manganese under thermoneutral or heat stress conditions<sup>a</sup>

Dietary Supplement <sup>b</sup>	Temp	NEFA (mEq/L)	Tg <sup>c</sup> (mg/dL)	HDL (mg/dL)	CHOL (mg/dL)	HDL:CHOL ratio
60 mg/kg Mn	TN	0.47	28.2	98.5	137.9	0.72
	HS	0.51	30.8	101.4	143.1	0.74
200 ug/kg Cr	TN	0.41	29.9	101.2	136.7	0.75
	HS	0.47	30.9	102.0	134.8	0.77
400 ug/kg Cr	TN	0.44	28.6	100.3	135.3	0.74
	HS	0.43	29.4	103.1	132.6	0.75
0 mg/kg Mn	TN	0.52	27.1	103.4	141.9	0.72
	HS	0.43	30.8	100.1	119.1	0.79
240 mg/kg Mn	TN	0.41	28.8	103.0	136.2	0.76
	HS	0.43	30.1	94.2	127.4	0.74
SEM <sup>d</sup>		0.03	1.43	4.02	5.9	0.02

<sup>a</sup>Values are least square treatment means. Samples from 6 birds per treatment. <sup>b</sup>Diets supplemented with Cr at 200 and 400 ug/kg also contained Mn at 60 mg/kg. Diets supplemented with Mn at 0, 60 (control) and 240 mg/kg contained no added Cr. <sup>c</sup>The effect of temperature was significant (P<.04). <sup>d</sup>Standard error of the mean

**Statistical Analysis:** Data were analyzed using the mixed model procedure of SAS (SAS, 1996) with meaningful single-degree-of-freedom comparisons made. Comparisons evaluated were: effect of Mn supplementation (0 versus 60 mg/kg supplemented Mn); effect of high level of Mn supplementation (60 versus 240 mg/kg Mn); effect of Cr supplementation (60 mg/kg Mn versus 200 and 400 ug/kg Cr); effect of Cr dose (200 versus 400 ug/kg Cr); effect of temperature (thermoneutral versus heat stress); and the interaction of temperature with the above diet effects.

**Results**

**Effect of Diet and Environment on Plasma Constituents:** The effect of dietary CrPic and MnPro supplementation on plasma concentration of insulin (I), glucagon (G), I:G ratio and glucose is shown in Table 2. Plasma insulin concentration in birds fed high Mn diets was significantly (p<0.01) lower compared with those fed control diets. The effect of Cr dosage was significant

(p<0.02) for plasma glucagon concentrations. Plasma glucagon levels were higher in birds supplemented with 200 ug/kg Cr than those receiving 400 ug/kg. Kim *et al.* (1995) reported increased insulin and glucose in CrPic supplemented broilers but these effects were not observed in the present study. The effect of temperature was significant for plasma glucagon (p<0.002) insulin (p<0.004) and I: G ratio (p<0.02). Heat stressed birds had lower plasma insulin, increased plasma glucagon and decreased I: G ratio. Examination of bird alimentation status (Fig. 1) indicates that plasma insulin and glucose were higher in the fed state while plasma glucagon was higher in the fasted state.

**Effects of Diet and Environment on Serum Constituents:** Dietary supplementation of Mn at high levels significantly reduced (p<0.05) serum non-esterified fatty acid (NEFA) concentration (Table 3). Serum NEFA concentrations were not affected by Cr supplementation or temperature (p>0.05). There was

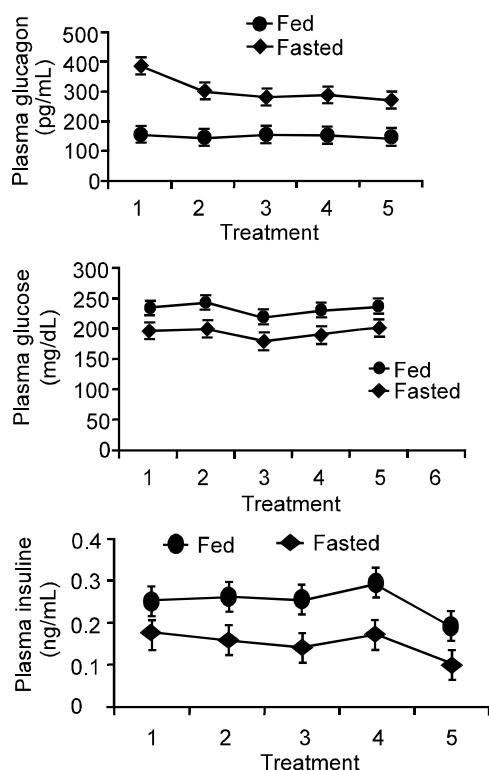


Fig. 1: Changes in plasma insulin, glucose and glucagon during the fasted and fed state in broiler chickens. Treatment 1= diet supplemented with 60 mg/kg Mn only; treatment 2 = 60 mg/kg Mn + 200 ug/kg Cr; treatment 3= 60 mg/kg Mn + 400 ug/kg Cr; treatment 4 = no supplemental Mn or Cr; treatment 5 = 240 mg/kg Mn.

a significant ( $p < 0.04$ ) effect of temperature on serum triglyceride (TG) levels due to higher concentrations under heat stress conditions regardless of dietary treatment (Table 3). Serum TG, high density lipoprotein (HDL) cholesterol and total cholesterol (CHOL) were not significantly affected ( $p > 0.05$ ) by treatment (Table 3). However, birds under heat stress with no Mn supplementation had increased HDL: CHOL ratio ( $p < 0.02$ ).

### Discussion

Chromium is generally accepted as the active component in the glucose tolerance factor (GTF), which increases the sensitivity of tissue receptors to insulin, resulting in increased glucose uptake by cells. Research suggests Cr involvement in carbohydrate metabolism including glucose uptake, glucose utilization for lipogenesis, and glycogen formation (Anderson *et al.*, 1991). It was hypothesized that increased glucose uptake should increase oxidation of

glucose which would be otherwise converted to fatty acids and stored as triglycerides in adipose tissues. There were no differences ( $p > 0.05$ ) in plasma insulin or glucagon concentrations in response to Cr supplementation. Interestingly, there was a dose response due to higher plasma glucagon levels for birds supplemented at 400  $\mu\text{g}/\text{kg}$  Cr compared with those at 200  $\mu\text{g}/\text{kg}$  but this response was not different from the control group. There were also no significant differences in serum concentrations of NEFA, TG, HDL cholesterol or total cholesterol as a result of Cr supplementation but NEFA concentrations tended to be lower ( $p = 0.07$ ). In contrast to the present study, Kim *et al.* (1995) reported increased HDL cholesterol, decreased total cholesterol and higher ratios of HDL: CHOL in CrPic supplemented broilers.

Manganese has been reported to increase the secretion of insulin from the  $\beta$  cells of the pancreas in rats, (Baly *et al.*, 1984). Adipose cells from Mn deficient rats *in vitro* was also reported to have lower glucose uptake and insulin-stimulated glucose oxidation to  $\text{CO}_2$  and conversion to triglycerides (Baly *et al.*, 1990). In the current study, high Mn supplementation (240 mg Mn/kg) to broiler chickens resulted in significantly lower insulin concentrations under thermoneutral conditions. There was also reduced serum NEFA concentrations in response to high Mn supplementation. These findings suggest Mn is involved in carbohydrate and lipid metabolism in chickens. Dietary Mn supplementation above NRC (1994) recommended levels may be beneficial for lean growth; therefore, further investigation is warranted.

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