EFFECTS OF SUPPLEMENTAL CHROMIUM PICOLINATE ON GROWTH PERFORMANCE, CARCASS COMPOSITION AND SERUM TRAITS OF BROILERS FED DIETS VARYING IN PROTEIN AND LYSINE

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Summary

Arbor Acres broiler chickens (N=288) with an average initial weight of 59.4 g were fed diets varying in protein and lysine (80, 100, 120% of NRC; 100, 120% of NRC, 1984) in order to investigate the effects of supplemental chromium picolinate on growth performance, nutrient utilizability, carcass composition, serum traits and *in vitro* protein synthesis. Six replicates of eight chicks were grouped into one treatment. Six chicks were sacrificed from each treatment for carcass analysis, and six additional chicks were chosen and dissected for *in vitro* culture of liver tissue.

Body weight gain, feed intake, feed conversion, mortality, carcass composition and serum glucose, HDL/cholesterol ratio, serum triglyceride and serum nonesterified fatty acid appeared to be affected by either the level of dietary crude protein or lysine when supplemented with 200 ppb chromium picolinate ($p < 0.05$). Retained and secreted proteins in liver acinar cell cultured *in vitro* were not affected by dietary lysine level but affected by dietary protein level when added with 200 ppb chromium picolinate.

(**Key Words**: Broiler Chicks, Chromium Picolinate, Lysine, Crude Protein, Growth Performance, Nutrient Utilizabiltiy, Carcass Composition, Serum Traits)

Introduction

Since chromium was first introduced to be essential for animals by Schwartz and Mertz in 1959, nutritional status of chromium in poultry has been investigated. Jensen et al. (1978) determined that chromium was biologically active in the laying hen and Polansky et al. (1989) reported decreases in tissue chromium during turkey egg production. Hill and Matrone (1970) and Hafez and Kratzer (1976) reported that chromium reduced the toxicity of vanadium in growing chicks. While, Jensen and Maurice (1980) and Benabdeljelil and Jensen (1990) reported that chromium was not effective in reversing the adverse effects of vanadium on albumen quality. Growth rate of turkey poults was improved by chromium (Rosebrough and Steele, 1981) and supplemental chromium increased the percentage of turkey breast (Anderson et al., 1989).

Chromium is considered to be essential for maintenance of normal glucose tolerance (Schwartz and Mertz, 1959) and to play a role as a cofactor for insulin (Schwartz and Mertz, 1957; Mertz, 1969). Steele et al. (1977) indicated that the glucose tolerance factor was "biologically active" in swine by potentiating the action of insulin. Glucose independent effects of chromium on amino acid transport and utilization for protein synthesis also have been shown (Weser and Koolman, 1969; Okada et al., 1983, 1984).

Although several investigations were conducted to evaluate the effects of chromium picolinate supplementation, it was rare about interaction of chromium picolinate with other nutrients. Therefore this study was to evaluate the effects of feeding diets different in crude protein and lysine with addition of chromium picolinate (200 ppb) to broiler chicks.

Materials and Methods

1) Experimental Design

Experimental diets contained three levels of crude

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protein [80%, 100% and 120% of the requirement suggested by NRC (1984)], two levels of lysine [100 and 120% of the requirement suggested by NRC (1984)] and were supplemented with 200 ppb of chromium picolinate. All treatments had 6 replicates of 8 birds in each replicate.

Animal subjects were broiler chicks of Arbor Acres produced by Han Il Breeding Farm. At 3 days of age, experimental animals (a total of 288) with similar body weight were fed experimental diets for 6 weeks. Feeding trial, metabolic trial and chemical analysis of experimental feed and excreta were conducted in the Animal Nutrition Laboratory, Department of Animal Science and Technology, College of Agriculture and Life Sciences, Seoul National University located in Suweon, Korea. Feeding trials were initiated in May 26, 1993 and were terminated in July 7, 1993.

2) Experimental Diets

Birds were fed a commercial diet (CP : 23%, 3,200 kcal ME/kg) for a preliminary period of three days. Six basal isocaloric diets (3,200 kcal/kg) were formulated to contain three different levels of dietary crude protein (80, 100, 120%) and two different levels of lysine (100, 120%). The formula and chemical composition of basal diets for starting and finishing period are presented in Table 1. Each basal diet used in each period was supplemented with 200 ppb of chromium picolinate. All the nutrients except protein and lysine were formulated to meet the National Research Council requirement (NRC, 1984).

3) Methods of Experiment

TABLE 1. FORMULA AND CHEMICAL COMPOSITION OF THE BASAL DIETS FOR EXPERIMENT

Treatments	Starter						Finisher					
Chromium picolinate	200 ppb						200 ppb					
Levels of lysine[1](%)	100			120			100			120		
Levels of protein[2](%)	80	100	120	80	100	120	80	100	120	80	100	120
Ingredient (%) :												
Corn, yellow	71.28	56.95	42.95	71.70	54.50	42.12	73.50	60.00	48.94	73.50	60.00	49.89
Soybean meal	8.90	24.28	30.44	8.50	26.00	34.10	6.80	18.00	26.59	7.00	18.00	28.00
Fish meal	2.40	2.41	2.30	2.00	1.40	3.50	1.00	0.60	0.40	1.00	0.40	1.05
Cron gluten meal	9.50	9.20	12.99	9.60	9.60	10.00	7.60	9.00	11.50	7.50	8.99	10.00
Wheat bran	2.40	0.00	2.40	2.40	0.00	1.00	5.00	4.40	3.94	4.50	4.40	2.40
Tallow	2.00	4.01	5.92	2.00	4.80	6.41	2.50	4.70	5.62	2.60	4.70	5.55
Limestone	2.15	2.20	2.20	2.14	2.50	2.00	2.30	2.30	2.21	2.40	2.30	2.21
Salt	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Vit.-min. mix.[4]	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45
Lysine	0.57	0.15	0.00	0.86	0.40	0.07	0.50	0.20	0.00	0.70	0.41	0.10
Antibiotics	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Chemical composition :												
ME (kcal/kg)	3,209	3,200	3,200	3,206	3,210	3,200	3,202	3,220	3,201	3,205	3,214	3,200
Crude protein (%)	18.45	23.00	27.00	18.41	23.42	27.25	16.04	20.02	24.00	16.17	20.10	24.02
Lysine (%)	1.20	1.20	1.25	1.44	1.44	1.44	1.00	1.01	1.05	1.20	1.20	1.20
Methionine (%)	0.43	0.49	0.58	0.42	0.49	0.56	0.36	0.43	0.50	0.36	0.42	0.50
Calcium (%)	0.90	0.95	0.97	0.87	1.01	0.97	0.87	0.88	0.86	0.91	0.87	0.90
Avail. phosphorus (%)	0.40	0.43	0.48	0.39	0.41	0.50	0.37	0.40	0.42	0.37	0.39	0.43

[1] All treatments are added 200 ppb of chromium picolinate.

[2] 80, 100 and 120% of protein levels suggested by NRC (1984).

[3] 100 and 120% of lysine levels suggested by NRC (1984).

[4] Vit.- min. mixture contains followings in 1 kg: vitamin A, 10,000 IU; vitamin D_3, 1,500 IU; vitamin K_3, 5 mg; vitamin E, 15 mg; vitamin B_2, 8 mg; vitamin B_{12}, 0.008 mg; Ca-d-pantothenate, 8 mg; niacin, 25 mg; folic acid, 0.4 mg; biotin, 0.2 mg; choline, 500 mg; pyridoxine, 1 mg; B.H.T. 125 mg; Co, 0.85 mg; I, 1.29 mg; Zn, 100 mg; Mn, 110 mg; Cu, 8.75 mg; Se, 0.15 mg; Fe, 35 mg.

(1) Feeding Trial

All the birds were raised in battery cages made of steel wire and housed in a room with constant light and air ventilation. During the pre-experimental period of 3 days, broiler chicks were fed a commercial diet. Experimental diets and water were provided *ad libitum* during an entire period of 6 weeks. Chicks were grouped to have uniform mean body weight and allocated into the respective experimental group. Body weight and feed intake were recorded weekly on replication basis. Body weight gain was calculated by the difference between the initial body weight and final body weight. Feed conversion was calculated by dividing the amount of feed consumed with the corresponding body weight gain. During the feeding trial, mortality was also recorded.

(2) Metabolic Trial

To investigate the nutrient utilizability of the experimental diets, the metabolizability coefficient was calculated by total fecal collection during 7 days at the end of each feeding trial. Three chicks were housed in metabolic cages individually and experimental diets and water were fed *ad libitum*. After four days of adaptation, total excreta were collected four times a day for the three consecutive days to avoid the contamination of foreign materials such as feed, feathers and scales. Total excreta were pooled and dried in an air-forced drying oven at 60℃ for 72 hours (Nongyao, 1990) to gain constant dry weight. All the samples prepared in this way were ground with 1 mm mesh Wiley mill for chemical analysis.

(3) Carcass Collection

To evaluate the carcass composition, at 42th day of the experimental period, six chicks were sacrificed from each treatment by cervical dislocation and were frozen for determination of final carcass composition. Carcass sample was freeze dried (ISE, Korea), ground and analyzed by AOAC (1990) methods.

(4) Blood Collection

Blood samples were collected from cervical artery after decapitation.

Samples were centrifuged (Hanil, Korea) at 3,000 rpm for 20 minutes and supernatants (serum) were collected and used for analysis of glucose, insulin, non esterified fatty acid (NEFA), triglyceride (TG), total cholesterol and high density lipoprotein (HDL).

(5) Chemical Analysis

Approximate composition of experimental diets and excreta were analyzed according to the methods of AOAC (1990). Following hydrolysis with 6N HCl at 110℃ for 16 hours (Mason et al., 1984), amino acid contents present in diet and feces were measured using amino acid analyzer (LKB, 4150, Pharmacia Instrument Co., England).

Analysis of glucose, triglyceride (TG), total cholesterol and high density lipoprotein (HDL) in serum were done by enzymatic kits (Asan Co., Korea), insulin analysis was conducted by a RIA kit (Diagnostic Products Co., USA), and serum nonesterified fatty acid (NEFA) was measured by an enzymatic kit (Ilsu Co., Japan).

(6) Protein Synthesis Activity

A) Preparation of Acinar Cell

Immediately after liver tissues were taken, the tissues were immersed in sterile Balanced Salts Solution (BSS) containing antibiotics and calf serum. The cell dispersion was accomplished according to the method devised by Choi et al. (1987). The liver tissues were trimmed free of large pieces of connective, lymph, adipose tissues and blood vessels, and minced with sterile scissors to less than 5 mm^3 and were dissociated with the collagenase hyaluronidase solution for 3 hours at 37℃ with continuous stirring. The cells were washed twice in BSS and resuspended in 1 X MEM. The cell suspension was filtered through four layers of cheese cloth. The cells were plated on plastic tissue culture dishes (about 106 cells/dish).

B) Culture Medium

The basic medium used was Eagle's MEM (Eagle, 1959) as modified by Smith et al. (1982). Glucose and bovine serum were added to 1 X MEM to final concentrations of 0.2% (W/V) and 5% (V/V), respectively. Antibiotics (Penicillin 10,000 IU, Amphotericin-B 250 μg, Streptomycin 10,000 IU per 100 ml media) were added to all media. The pH of the media was adjusted to 7.4 by addition of 7.5% sodium bicarbonate. The isotope used for labelling the cells was [^{3}H] lysine. Routinely, 0.5 μCi of the tracer was added to 1 ml media for determining *in vitro* synthetic activity of protein.

C) Protein Synthesis and Amino Acid Uptake Activity

At the end of the 18-h incubation, cells were collected, pooled (four dishes per treatment), and centrifuged at 1,000 × g at 4℃ for 10 minutes. The activity of protein

synthesis was measured as described in figure 1. The equation used for calculation of synthesized protein is as follow:

$$P = [C/E]/A$$

where P = specific activity of protein synthesis (dpm/mg protein)
C = cpm incorporated into protein (cpm)
E = isotope counting efficiency
A = the amount of protein (mg)

The specific activity of [^{3}H] lysine incorporated into protein or specific activity of [^{14}C] cycloleucine uptake by acini was counted in Insta gel by a liquid scintillation counter (LS 100C, Beckman).

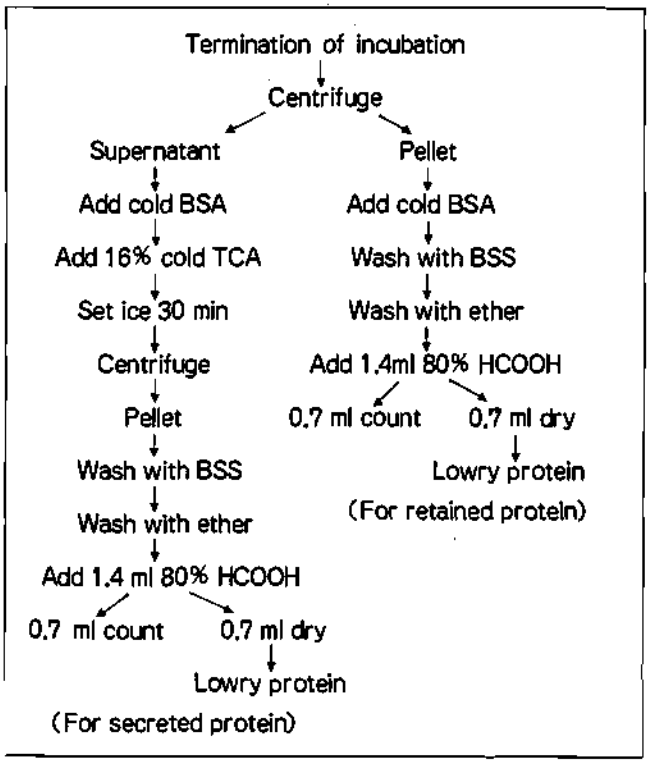

Figure 1. Experimental steps for analysis of secreted protein and retained protein

(7) Statistical Analysis

Statistical analyses for the present data were carried out by comparing means according to Duncan's multiple range test (Duncan, 1955), using General Linear Model (GLM) Procedure of SAS (1985) package program.

Results and Discussion

1) Growth Performance and Nutrient Utilizability

The data obtained from the feeding trial (1-6 weeks) were summarized in table 2. The highest weight gain was obtained at the 120% CP diet with 120% lysine and the lowest weight gain was obtained at the 80% CP diet with 120% lysine ($p < 0.05$). However, when chromium picolinate was added to the low protein diets, weight gain was higher than when chromium picolinate was not added.

The best feed conversion was obtained at 120% CP and 120% lysine group and 100% CP and 120% lysine group, respectively ($p < 0.05$). Weight gain, feed intake and feed conversion was not significantly different between lysine levels. As crude protein increased, weight gain, feed intake and feed conversion also increased ($p < 0.05$). Mortality was lowest at 100% CP diet with 100% lysine. Interaction between dietary crude protein and lysine was found in feed intake and feed conversion ($p < 0.05$).

The utilizability of crude protein and crude fat was significantly lower at 80% CP diet with 120% lysine than other groups ($p < 0.05$).

There was no significant difference between lysine levels in dry matter, crude protein, crude fat and crude ash utilizability.

Among crude protein levels, crude fat utilizability was significantly different ($p < 0.05$). Chicks fed diets containing 100% and 120% CP were higher in crude fat utilizability than those fed 80% CP diet.

Interaction between dietary crude protein and lysine was found in crude fat utilizability.

From these data, it can be concluded that additional lysine has no effect on nutrient utilizability even though 200 ppb chromium picolinate was supplemented. This is similar to the results by Lindemann et al. (1993).

Broilers fed 80% CP diet with 100% lysine was lowest in essential amino acid utilizability ($p < 0.05$) (figure 2).

Meanwhile, the utilizability of non-essential amino acids was not significantly different among treatments.

2) Carcass Composition

The effects of feeding the experimental diets varying in crude protein and lysine with 200 ppb chromium picolinate supplementation on carcass composition are summarized in table 3.

Crude protein content was significantly ($p < 0.05$) influenced by the levels of dietary crude protein and lysine. It was highest at 120% CP diet with 100% lysine

TABLE 2. GROWTH PERFORMANCES OF BROILERS FED DIETS VARYING IN PROTEIN AND LYSINE WITH ADDITION OF CHROMIUM PICOLINATE (1-6 WEEKS)

Chromium picolinate	200 ppb						SE[3]
Lysine[1]	100			120			
Protein[2]	80	100	120	80	100	120	
Weight gain (g)	1,855^{bc}	2,031^{ab}	1,993^{ab}	1,753^{c}	1,967^{ab}	2,045^{a}	27.87
Feed intake (g)	3,827^{a}	3,788^{a}	3,674^{ab}	3,447^{b}	3,897^{a}	3,703^{ab}	41.50
Feed/gain	2.06^{a}	1.87^{cd}	1.84^{d}	1.97^{bc}	1.98^{ab}	1.81^{d}	0.02
Mortality (%)	2.08^{b}	0.00^{b}	2.08^{b}	16.67^{a}	6.25^{b}	5.00^{b}	1.04
Nutrient utilizability (%)							
Crude protein	74.6	72.5	72.7	69.1	73.9	73.1	0.87
Crude fat	95.4	96.1	95.0	92.7	96.5	96.1	0.32
Crude ash	35.3^{ab}	41.0^{a}	38.1^{ab}	34.1^{ab}	30.4^{ab}	29.3^{b}	1.47
Essential amino acid	86.1^{b}	88.6^{ab}	88.7^{ab}	88.3^{ab}	88.5^{ab}	91.2^{a}	0.53

Between lysine		Weight gain	Feed intake	Feed/ gain	Mor- tality	Crude protein	Crude fat	Crude ash	Essential amino acid
	100	1,960	3,763	1.92	1.39^{b}	73.3	95.4	38.0	87.9
	120	1,921	3,682	1.92	9.56^{a}	72.0	95.1	31.3	89.4
Among protein	80	1,804^{b}	3,637^{b}	2.02^{a}	9.38^{a}	71.9	94.1^{b}	34.7	87.2
	100	1,999^{a}	3,843^{a}	1.92^{b}	3.13^{b}	73.2	96.3^{a}	35.7	88.5
	120	2,019^{a}	3,689^{ab}	1.83^{c}	3.41^{b}	72.9	95.6^{a}	33.7	90.0
Interaction (P value)									
Lysine × protein		0.3901	0.0219	0.0074	0.0974	0.2826	0.0171	0.5462	0.5796

[1] 100 and 120% of lysine levels suggested by NRC (1984).
[2] 80, 100 and 120% of protein levels suggested by NRC (1984).
[3] Pooled standard error.
[a,b,c] Mean values with different superscripts within the same column or row are significantly different ($p < 0.05$).

or 120% lysine and lowest at 80% CP diet with 120% lysine ($p < 0.05$).

Crude fat content was lowest at 120% CP diet with 100% lysine or 120% lysine groups ($p < 0.05$).

As dietary crude protein increased from 80% to 120%, crude protein content of carcass also increased ($p < 0.05$), whereas crude fat content decreased ($p < 0.05$).

The crude ash content was highest at 120% CP diet with 120% lysine and lowest at 80% CP diet with 100% lysine ($p < 0.05$).

It was found that crude protein content and crude ash content increased as dietary crude protein level was increased from 80 to 120% ($p < 0.05$), whereas crude fat decreased ($p < 0.05$).

Overall, broilers fed diets containing 120% CP and 100% lysine or 120% lysine showed the highest crude protein content in carcass composition and the lowest crude fat content ($p < 0.05$), whereas those fed 80% CP diet with 100% lysine showed the lowest crude protein content and the highest crude fat content ($p < 0.05$).

3) Serum Traits

Effects of feeding diets varying in crude protein and lysine with supplementation of 200 ppb chromium picolinate on the ratio of total cholesterol versus HDL in serum are presented in figure 3.

The HDL/total cholesterol ratio was highest at 120% CP with 100% lysine, 80% CP with 120% lysine and 100% CP with 120% lysine ($p < 0.05$) and was lowest at 100% CP with 100% lysine ($p < 0.05$). There was no significant difference among crude protein levels.

As revealed in table 3, the amount of serum insulin in broilers fed 100% CP diet with 100% lysine was largest and smallest at 80% CP diet with 100% lysine ($p < 0.05$).

Content of serum glucose was not affected by dietary crude protein and lysine levels. Between lysine levels, 120% lysine added group showed higher glucose content

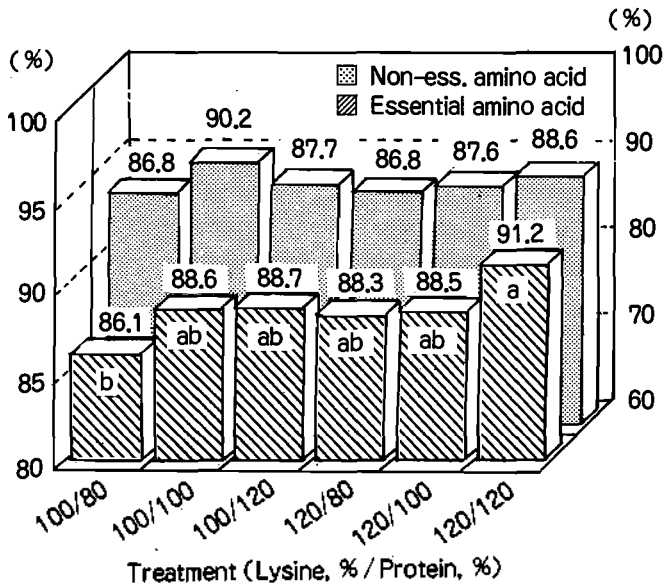

Figure 2. Effects of dietary protein and lysine on amino acid utilizability of broilers at 6 weeks old when added 200 ppb chromium picolinate

[ab] Mean values with different superscripts within the same row are significantly different ($p < 0.05$)

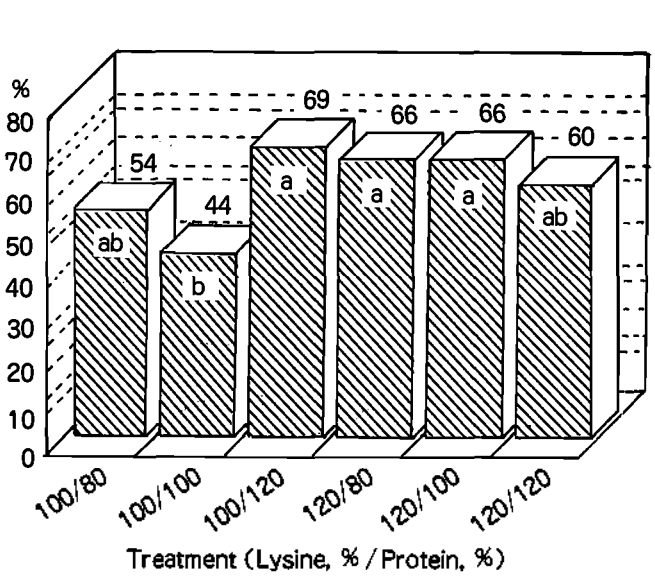

Figure 3. Effects of different levels of crude protein and lysine on the HDL / total cholesterol ratio when added 200 ppb chromium picolinate

[ab] Mean values with different superscripts within the same row are significantly different ($p < 0.05$)

TABLE 3. CARCASS COMPOSITION OF BROILERS FED DIETS VARYING IN PROTEIN AND LYSINE WITH ADDITION OF CHROMIUM PICOLINATE

Chromium picolinate	200 ppb						
Lysine[1]	100			120			SE[3]
Protein[2]	80	100	120	80	100	120	
Carcass composition (%)							
Crude protein	38.7[b]	43.2[ab]	46.7[a]	42.1[ab]	40.0[b]	46.8[a]	0.87
Crude fat	51.8[a]	46.5[ab]	42.8[b]	50.6[a]	52.1[a]	42.9[b]	1.02
Crude ash	5.6[c]	6.4[bc]	7.0[ab]	6.3[bc]	6.0[c]	7.8[a]	0.16
Serum trait							
Insulin (μIU/ml)	6.7[b]	14.3[a]	12.2[ab]	11.7[ab]	9.1[ab]	11.1[ab]	0.81
Glucose (mg/dl)	236	236	239	225	232	224	2.30
Triglyceride (mg/dl)	80.7[a]	60.8[cd]	53.0[cd]	74.7[ab]	64.2[bc]	50.0[d]	2.05
NEFA (μEq/l)	845[a]	682[b]	626[b]	929[a]	715[b]	617[b]	21.69

Between lysine		Crude protein	Crude fat	Crude ash	Insulin	Glucose	Triglyceride	NEFA
	100	42.9	47.0	6.4	11.1	237[a]	64.9	714
	120	43.0	48.5	6.7	10.6	227[b]	63.0	753
Among protein	80	40.4[b]	51.2[a]	5.9[b]	9.2	231	77.7[a]	889[a]
	100	41.6[b]	49.3[a]	6.2[b]	11.7	234	62.5[b]	698[b]
	120	46.8[a]	42.8[b]	7.4[a]	11.7	232	51.5[c]	621[b]
Interaction (P value)								
Lysine × protein		0.2308	0.2188	0.0552	0.0362	0.5626	0.4892	0.5660

[1] 100 and 120% of lysine levels suggested by NRC (1984).
[2] 80, 100 and 120% of protein levels suggested by NRC (1984).
[3] Pooled standard error.
[a,b,c] Mean values with different superscripts within the same column or row are significantly different ($p < 0.05$).

than 100% lysine group ($p < 0.05$).

Broilers received the diets containing 80% CP and 100% lysine showed the highest content of serum triglyceride ($p < 0.05$), and those fed diets containing 120% CP and 120% lysine presented the lowest serum triglyceride content ($p < 0.05$). Among dietary crude protein levels, serum triglyceride content decreased as dietary crude protein levels increased ($p < 0.05$).

An increase in NEFA would be indicated by an increase in the process of fat degradation or lipolysis in adipose tissue (Mersmann and MacNeil, 1985). 80% CP diet with 100% lysine or 120% lysine showed higher NEFA content than other treatments ($p < 0.05$). When fed a diet containing low CP, NEFA content in serum was higher than high CP ($p < 0.05$).

4) *In vitro* Protein Synthesis

The effects of different levels of dietary crude protein and lysine when added with 200 ppb chromium picolinate on retained protein and secreted protein in liver acinar cell cultured *in vitro* are summarized in table 4. Secreted and retained protein by liver tissue was not significantly different among lysine treatments. Retained protein by liver tissue were higher at 100% and 120% CP treatments than 80% CP treatment ($p < 0.05$).

TABLE 4. SECRETED AND RETAINED PROTEIN BY LIVER TISSUE CELL CULTURED (DPM/MG)

Chromium picolinate	200 ppb						
Lysine[1]	100			120			SE[3]
Protein[2]	80	100	120	80	100	120	
Secreted protein[4]	3,130.6	3,669.9	3,417.4	3,625.8	3,632.8	3,417.5	187.5
Retained protein[5]	3,906.7	4,584.5	4,590.4	4,085.0	4,783.5	4,964.2	186.2
Between lysine	Secreted protein			Retained protein			
100	3,405.9			4,360.5			
120	3,558.7			4,610.9			
Among protein							
80	3,378.2			3,995.8[b]			
100	3,651.3			4,683.9[a]			
120	3,417.4			4,777.3[a]			
Interaction (P value)							
Lysine × protein	0.4555			0.8963			

[1] 100 and 120% of lysine levels suggested by NRC (1984).
[2] 80, 100 and 120% of protein levels suggested by NRC (1984).
[3] Pooled standard error.
[4] The amount of secreted protein was determined by the incorporation of [^{3}H]-lysine (0.5 μCi/ml) into TCA-insoluble material.
[5] The amount of retained protein was determined by the incorporation of [^{3}H]-lysine (0.5 μCi/ml) into TCA-insoluble material.
[a,b] Mean values with different superscripts within the same column are significantly different ($p < 0.05$).

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