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## EFFECT OF CHROMIUM AND PROTEINS DIETS IN RATS

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**ABSTRACT:** A study was conducted to improve the effects of chromium tripicolinate (CTP) on the growth, organs weight and serum metabolites in rats fed on different proteins sources. Four groups of six growing male rats were fed on control diets containing casein, fish powder, soybean meal, cotton seeds meal. Four other groups of six growing male rats were fed on the control diets supplemented with 100 ppm of chromium as chromium tripicolinate (CTP). The diets were formulated to provide 14.76 % of crude proteins and 4417.6 kcal of brute energy per kg of dry matter. Results showed that chromium in association with proteins did not improve growth characteristics in rats. Cr+casein increased average weight of liver and kidneys, decreased ( $P \leq 0.05$ ) average level of aspartate transaminase, alanine transaminase and total bilirubin. Both supplements increased average level of glucose and uric acid, and that of serum ions ( $\text{Na}^+$ ,  $\text{Fe}^{2+}$ ,  $\text{Mg}^{2+}$ ). Cr+fish powder decreased average spleen weight, average level of serum triglycerides,  $\text{Na}^+$  and  $\text{Mg}^{2+}$ , and increased average level of serum uric acid. Cr+soybean meal reduced average level of serum urea, creatinine, and  $\text{Fe}^{2+}$ . Cr+cotton seeds meal reduced average weight and serum level of total bilirubin. All these changes in average level of serum metabolites indicate that chromium and casein are more likely to affect liver functions. Moreover, fish, soybean and cotton seeds proteins affect level of very few metabolites.

**Key words:** Rats, chromium, proteins, growth, serum metabolites

## INTRODUCTION

Chromium, as a trace mineral, has minimal or equivocal effects on growth, meat qualities and level of blood metabolites in humans and animals [14, 2, 21, 18, 19]. In order to increase its influence, some authors have combined chromium with other supplements, like porcine somatotropin [6], minerals [15], vitamins [12]. Page [13] tested the effect of the environment, while Sahin *et al.* [15], Kim *et al.* [9], and Sahin *et al.* [16] modified room temperature to stress animals during the experiments. In addition, Gammelgaard *et al.* [7] reported that the availability of different chromium compounds, depends on their metabolism in the gastrointestinal environment, and on their ability to penetrate into the intestinal tissue. In this study, four sources of proteins were used in the diet to prospect the effect of 100 ppm of chromium tripicolinate (CTP) on growth and metabolites level of growing male rats.

## MATERIALS AND METHODS

### Materials

During this experiment, animals were penned in Plexiglas cages covered with wire covering on which feed was laid down. Water bottles containing tap water were adapted to the grills. Rats and feed were weighed on a precision scale (Sartorius). Dry matter percentage of feed was determined after drying five (5) g of feed sample in an oven set at 100°C, for four hours. Blood samples were centrifuged in a refrigerated centrifuge (4°C). Dosages of serum metabolites and electrolytes were determined by an autoanalyzer, (HITACHI, 902-Roche) at the University Hospital of Cocody, Abidjan.

## Methods

### Experimental conditions

The experiment was conducted on male Wistar rats, born and raised at the animals barn of the UFR-Bioscience of the University of Cocody-Abidjan. The average temperature of the room was 25°C, and the percentage of humidity was 70 %, with 12 hours of daylight and 12 hours of darkness. At the beginning of the experiment, rats were aged 60±3 days, weighing 60 g. They were penned in cages with six (6) rats per cage, and were allowed to feed *ad libitum*, for three weeks. Every morning, the animals were fed between 7 a.m. and 8 a.m., and were weighed once a week. Each feed offered was weighed every day, and the following day, the refused feed was also weighed, in order to determine the amount of feed intake.

### Diets composition

Animals feed was made up in a metal dish, by mixing different ingredients according to the proportions mentioned in Table 1. Then, the whole mixture was cooked on an electrical stove. The hot meal was then cooled before keeping it in a refrigerator. To determine the dry matter, five (5) g of every feed cooked was kept in an oven for four (4) hours, at 100 °C. After weighing it, the dry matter was calculated following formulas described in Table 2. A total of eight (8) groups of rats was used. Four groups of rats were submitted to four (4) control diets containing casein, fish powder, and dehulled flour of soybean and cotton seeds. Four other groups of rats were submitted to the control diets supplemented with 100 ppm of chromium, as 833 mg of chromium tripicolinate (CTP) (CHROMAX II™, lot n° 910179 ; Nutrition 21, La Jolla, CA, USA) per kg. Each diet contained 14.76 % of crude proteins and 4417.6 kcal of brute energy per kg of dry matter.

**Table 1 : Composition of control diets**

Ingredients	Diets (1 kg of mixed ingredients)			
	Casein	Fish	Soybean	Cotton
Proteins (g)	180	207	307.5	360
Corn starch (g)	400	373	272.5	220
Sugar (g)	395	395	395	395
Salts mixture (g)	40	40	40	40
vitamins mixture (g)	10	10	10	10
Agar Agar (g)	5	5	5	5
Corn oil (ml)	50	50	50	50
Water (l)	1	1	1	1

Calculated values : Crude proteins : 14.76 % ; Total energy : 4417.6 kcal

**Table 2 : Expression of values of nutritional parameters**

Parameters	Mathematical expressions
Feed intake (FI)	Feed given- Feed refused
Dry matter (DM)	$100 - [(Wet\ weight - Dry\ weight) / Wet\ weight] \times 100$
Dry matter intake (DMI)	FI x DM
Average weight gain (AWG)	AWG = Final weight - Initial weight
Average daily weight gain (ADWG)	AWG/21 days
Feed efficiency (FE)	FE = AWG / DMI
Total protein intake (TPI)	TPI = DMI x % Protein of diet
Protein efficiency (PE)	PE=AWG / TPI

## Collection of blood samples, organs, and dosage of serum metabolites

At the end of the experiment, all the rats were submitted to fast for 16 hours. Then, they were sacrificed after anesthesia with ethyl urethan (20 %), between 7 a.m. and 9 a.m. Blood collected was kept in "venoject" tubes. After centrifugation of samples at 3000 tours per minutes (tpm), for 10 min, in a refrigerated centrifuge (4°C), the serum was collected and kept in hemolysis tubes. The dosage of the metabolites was done on serum samples by the mean of an autoanalyzer (HITACHI, 902-Roche). On sacrificed animals, a longitudinal laparotomy was performed in order to remove organs such as the heart, the two kidneys, the liver, the spleen and the abdominal fat pad. These organs, after determination of their weight, were kept in a freezer.

## Expression of the results and statistical analyses

All the results in this work were presented as tables, using a computer. "Word 2007" was used for typing the manuscript, and drawing tables. "Excell 2007" was used to perform the different calculations. "Statistica version 7.1" was used for the statistical analyses. The contrasts between two means were realized using the Student test, 5 % significance. Two means were significantly different if the calculated probability was less or equal to 5 % ( $p \leq 0.05$ ). Otherwise, they were not significantly different ( $p > 0.05$ ). All the means were followed by their standard deviations.

## RESULTS

### Effect of chromium on the average value of growth characteristics

Results of this experiment (Table 3) showed that chromium associated to proteins (casein, fish powder, soybean and cotton seeds) had no effect ( $p > 0.05$ ) on growth characteristics of rats. Except that the average daily weight gain (ADWG) of rats fed on chromium (CTP) and cotton seeds proteins was lower ( $2.17 \pm 0.36$ ) than that of the control group ( $2.87 \pm 0.72$ ).

**Table 3 : Average value of growth characteristics**

Criteria	Treatments							
	Casein (n=6)	Casein + CTP (n=6)	Fish (n=6)	Fish+ CTP (n=6)	Soybean (n=6)	soybean + CTP (n=6)	Cotton (n=6)	Cotton + CTP (n=6)
IW (g)	94.87±27.46 <sup>a</sup>	99.48±12.24 <sup>a</sup>	99.77±17.66 <sup>a</sup>	97.61±8.85 <sup>a</sup>	96.05±13.05 <sup>a</sup>	98.00±23.70 <sup>a</sup>	98.94±26.77 <sup>a</sup>	95.50±19.28 <sup>a</sup>
FW (g)	146.25±33.70 <sup>b</sup>	144.00±7.34 <sup>b</sup>	141.75±12.50 <sup>b</sup>	149.25±31.95 <sup>b</sup>	122.34±13.62 <sup>b</sup>	134.32±16.10 <sup>b</sup>	156.50±35.14 <sup>b</sup>	139.00±18.45 <sup>b</sup>
DM (%)	77.12	75.71	70.01	72.57	80.03	82.93	80.24	74.69
DMI/D (g)	14.83	14.10	13.93	14.96	10.88	14.91	19.45	16.04
TPID (g)	2.18	2.08	2.05	2.20	1.60	2.20	2.87	2.36
ADWG (g)	2.56±0.48 <sup>c</sup>	2.22±0.41 <sup>c</sup>	2.09±0.88 <sup>c</sup>	2.58±1.20 <sup>c</sup>	1.31±0.24 <sup>c</sup>	1.81±0.86 <sup>c</sup>	2.87±0.72 <sup>c</sup>	2.17±0.36 <sup>d</sup>
FE	0.17±0.03 <sup>d</sup>	0.15±0.02 <sup>d</sup>	0.15±0.06 <sup>d</sup>	0.17±0.08 <sup>d</sup>	0.12±0.02 <sup>d</sup>	0.12±0.05 <sup>d</sup>	0.14±0.03 <sup>e</sup>	0.13±0.02 <sup>e</sup>
PE	1.17±0.22 <sup>e</sup>	1.06±0.20 <sup>e</sup>	1.02±0.42 <sup>e</sup>	1.16±0.54 <sup>e</sup>	0.81±0.15 <sup>e</sup>	0.82±0.39 <sup>e</sup>	1.00±0.25 <sup>f</sup>	0.91±0.15 <sup>f</sup>

(n) : Number of rats ; Each mean is followed by its standard deviation ; Student test, 5 % ; For the same protein in two columns, and in the same row, means followed by different superscript alphabet letters are significantly different ; CTP: chromium tripicolinate; IW : initial weight ; FW : final weight.

### Effect of chromium on the average organs weight

Table 4 showed that the average weight of the liver of rats consuming casein diet supplemented with chromium ( $3.33 \pm 0.20$ ) was higher than that of the control group ( $3.15 \pm 0.09$ ). The same animals had an average weight of kidneys ( $0.84 \pm 0.25$ ) higher than that of the control ones ( $0.62 \pm 0.02$ ). The average weight of the spleen of the rats treated with chromium and consuming fish powder ( $0.30 \pm 0.03$ ) was lower than that of the control ones ( $0.36 \pm 0.05$ ) (Table 4). Results reported in Table 4 indicated that the average weight of organs (liver, kidneys, heart, spleen, abdominal fat) of rats fed on soybean and cotton seeds proteins was not statistically different ( $p > 0.05$ ) in both groups.

Table 4: Average weight of organs

Criteria (% of body weight)	Treatments							
	Casein (n=6)	Casein + CTP (n=6)	Fish (n=6)	Fish + CTP (n=6)	Soybean (n=6)	Soybean + CTP (n=6)	Cotton (n=6)	Cotton + CTP (n=6)
Liver	3.15±0.09 <sup>b</sup>	3.33±0.20 <sup>a</sup>	3.27±0.35 <sup>a</sup>	3.31±0.28 <sup>a</sup>	2.66±0.39 <sup>a</sup>	2.67±0.73 <sup>a</sup>	3.23±0.59 <sup>a</sup>	3.18±0.32 <sup>a</sup>
Kidneys	0.62±0.02 <sup>d</sup>	0.84±0.25 <sup>c</sup>	0.67±0.06 <sup>b</sup>	0.63±0.07 <sup>b</sup>	0.52±0.07 <sup>b</sup>	0.58±0.11 <sup>b</sup>	0.64±0.06 <sup>b</sup>	0.63±0.04 <sup>b</sup>
Heart	0.40±0.03 <sup>e</sup>	0.43±0.02 <sup>e</sup>	0.41±0.02 <sup>c</sup>	0.40±0.05 <sup>c</sup>	0.31±0.06 <sup>c</sup>	0.36±0.06 <sup>c</sup>	0.45±0.05 <sup>c</sup>	0.42±0.04 <sup>c</sup>
Spleen	0.34±0.06 <sup>f</sup>	0.31±0.04 <sup>f</sup>	0.36±0.05 <sup>d</sup>	0.30±0.03 <sup>e</sup>	0.20±0.03 <sup>d</sup>	0.22±0.06 <sup>d</sup>	0.31±0.10 <sup>d</sup>	0.31±0.03 <sup>d</sup>
Abd. fat	2.70±0.67 <sup>e</sup>	2.17±1.11 <sup>e</sup>	2.14±0.83 <sup>f</sup>	2.69±0.40 <sup>f</sup>	1.54±0.8 <sup>e</sup>	2.27±0.82 <sup>e</sup>	2.23±0.67 <sup>e</sup>	2.85±1.16 <sup>e</sup>

(n) : Number of rats ; Each mean is followed by its standard deviation ; Student test , 5 % ; For the same protein in two columns, and in the same row, means followed by different superscript alphabet letters are significantly different ; CTP: chromium tripicolinate; Abd. fat : abdominal fat.

### Effect of chromium on the average value of serum metabolites

According to the results mentioned in Table 5, in rats fed on chromium and casein, the average value of glucose (0.78±0.10) was higher than that of the control group (0.70±0.02) (p<0.05). The average value of serum uric acid (27.50±4.20) was higher than that of the control group (23.75±2.87), and the average value of serum total bilirubin (16.00±2.16) was lower than that of the control group (18.00±1.41). In rats fed on chromium and fish powder, the average value of serum triglycerides (0.42±0.27) was lower than that of the control group (0.88±0.19) (Table 5). However, the average value of uric acid (29.50±3.87) was higher than that of the control group (26.25±1.70). In animals fed on chromium and soybean meal, the average value of urea was lower (0.11±0.00) than that (0.13±0.02) of the control group (Table 5). Likewise, the average value of creatinine (4.25±0.50) was lower than that of the control group (5.00±0.81). The effect of chromium combined with cotton seeds proteins indicated that the average value of total bilirubin of rats treated with chromium (15.00±1.41) was lower (p<0.05) than that (16.75±1.70) of the control group (Table 5).

Table 5: Average value of serum metabolites

Criteria	Treatments							
	Casein (n=6)	Casein + CTP (n=6)	Fish (n=6)	Fish + CTP (n=6)	Soybean (n=6)	Soybean + CTP (n=6)	Cotton (n=6)	Cotton + CTP (n=6)
Glucose (G/l)	0.70±0.02 <sup>b</sup>	0.78±0.10 <sup>a</sup>	0.75±0.10 <sup>a</sup>	0.78±0.05 <sup>a</sup>	0.76±0.08 <sup>a</sup>	0.77±0.08 <sup>a</sup>	0.81±0.08 <sup>a</sup>	0.82±0.07 <sup>a</sup>
T.G. (g/l)	0.55±0.27 <sup>c</sup>	0.74±0.32 <sup>c</sup>	0.88±0.19 <sup>b</sup>	0.42±0.27 <sup>c</sup>	0.45±0.20 <sup>b</sup>	0.60±0.38 <sup>b</sup>	0.65±0.16 <sup>b</sup>	0.52±0.29 <sup>b</sup>
T. Prot. (g/l)	68.00±3.16 <sup>d</sup>	67.00±2.44 <sup>d</sup>	68.75±2.21 <sup>d</sup>	68.00±2.58 <sup>d</sup>	66.75±3.39 <sup>c</sup>	66.87±2.57 <sup>c</sup>	66.00±4.24 <sup>c</sup>	65.75±2.21 <sup>c</sup>
T. Chol. (g/l)	0.96±0.12 <sup>e</sup>	0.95±0.05 <sup>e</sup>	0.99±0.16 <sup>e</sup>	0.87±0.14 <sup>e</sup>	0.91±0.04 <sup>e</sup>	0.88±0.15 <sup>e</sup>	0.85±0.15 <sup>e</sup>	0.89±0.07 <sup>e</sup>
Urea (g/l)	0.13±0.02 <sup>f</sup>	0.13±0.02 <sup>f</sup>	0.12±0.01 <sup>f</sup>	0.11±0.01 <sup>f</sup>	0.13±0.02 <sup>f</sup>	0.11±0.00 <sup>f</sup>	0.11±0.01 <sup>e</sup>	0.11±0.01 <sup>e</sup>
Creat. (mg/l)	4.75±0.50 <sup>g</sup>	4.75±0.50 <sup>g</sup>	4.75±0.50 <sup>g</sup>	4.50±0.57 <sup>g</sup>	5.00±0.81 <sup>g</sup>	4.25±0.50 <sup>h</sup>	5.00±0.81 <sup>f</sup>	4.50±1.00 <sup>f</sup>
U. ac. (mg/l)	23.75±2.87 <sup>i</sup>	27.50±4.20 <sup>h</sup>	26.25±1.70 <sup>i</sup>	29.50±3.87 <sup>h</sup>	29.50±3.87 <sup>i</sup>	27.00±2.44 <sup>i</sup>	27.00±2.94 <sup>e</sup>	27.00±3.16 <sup>e</sup>
T. Bili. (mg/l)	18.00±1.41 <sup>j</sup>	16.00±2.16 <sup>k</sup>	14.50±2.38 <sup>j</sup>	16.00±2.58 <sup>j</sup>	15.50±2.08 <sup>j</sup>	16.25±2.50 <sup>j</sup>	16.75±1.70 <sup>h</sup>	15.00±1.41 <sup>i</sup>
C. bili. (mg/l)	12.50±1.29 <sup>l</sup>	11.75±1.70 <sup>l</sup>	11.00±1.63 <sup>k</sup>	12.25±1.25 <sup>k</sup>	11.75±1.70 <sup>k</sup>	12.00±1.63 <sup>k</sup>	11.00±1.41 <sup>j</sup>	11.50±1.29 <sup>j</sup>

(n) : Number of rats ; Each mean is followed by its standard deviation ; Student test 5% ; For the same protein in two columns, and in the same row, means followed by different superscript alphabet letters are significantly different ; CTP: chromium tripicolinate; T.G. : triglycerides ; T. Prot.: total proteins ; T. Chol.: total cholesterol ; Creat. : creatinine ; U. ac. : uric acid ; T. bili.: total bilirubin ; C. bili.: conjugated bilirubin.

### Effect of chromium on the average activity of serum enzymes

The chromium tripicolinate (CTP) combined to casein provoked a significant decrease (p<0.05) of the average activity of serum aspartate transaminase (TGO) (73.50±6.40) and that (63.50±5.56) of the alanine transaminase (TGP) (Table 6). The average values of TGO and TGP of the control group were respectively 92.50±8.88 and 74.75±13.20. The average value of TGO, TGP, and alkaline phosphatase (alk. Ph.) was not affected by the supplementation of the chromium (p>0.05) in the diets containing fish, soybean and cotton seeds proteins (Table 6).

**Table 6: Average activity of serum enzymes**

Criteria (IU/l)	Treatments							
	Casein (n=6)	Casein + CTP (n=6)	Fish (n=6)	Fish + CTP (n=6)	Soybean (n=6)	Soybean + CTP (n=6)	Cotton (n=6)	Cotton + CTP (n=6)
TGO	92.50±8.88 <sup>a</sup>	73.50±6.40 <sup>o</sup>	81.25±8.34 <sup>a</sup>	87.75±22.55 <sup>a</sup>	96.25±26.72 <sup>a</sup>	112.75±31.71 <sup>a</sup>	88.75±13.42 <sup>a</sup>	90.25±12.71 <sup>a</sup>
TGP	74.75±13.20 <sup>c</sup>	63.50±5.56 <sup>d</sup>	78.50±10.47 <sup>b</sup>	77.00±7.61 <sup>b</sup>	82.75±19.06 <sup>b</sup>	94.00±28.36 <sup>b</sup>	83.25±8.22 <sup>b</sup>	80.00±15.09 <sup>b</sup>
Alk.ph.	71.75±7.27 <sup>e</sup>	70.25±2.98 <sup>e</sup>	69.75±3.77 <sup>e</sup>	73.00±5.94 <sup>e</sup>	73.25±6.70 <sup>e</sup>	73.25±5.12 <sup>e</sup>	72.50±6.55 <sup>e</sup>	73.75±5.31 <sup>e</sup>

(n) : Number of rats ; Each mean is followed by its standard deviation ; Student test 5% ; For the same protein in two columns, and in the same row, means followed by different superscript alphabet letters are significantly different ; CTP: chromium tripicolinate; TGO : Aspartate transaminase ; TGP : Alanin transaminase; alk. ph.: alkaline Phosphatase.

### Effect of chromium on the average value of serum electrolytes

The chromium tripicolinate (CTP) associated to casein provoked in rats, a significant increase ( $p \leq 0.05$ ) of the average value of serum electrolytes like  $\text{Na}^+$  ( $141.50 \pm 1.29$ ),  $\text{Fe}^{2+}$  ( $1.15 \pm 0.08$ ), and  $\text{Mg}^{2+}$  ( $20.87 \pm 0.51$ ) (Table 7). The average values of these ions in the control rats were respectively  $140.00 \pm 0.81$ ,  $1.02 \pm 0.14$ , and  $19.72 \pm 0.85$ . The chromium tripicolinate (CTP) associated to fish proteins (Table 7), provoked a significant ( $p \leq 0.05$ ) decrease of the average value of serum ions like  $\text{Na}^+$  ( $140.00 \pm 0.81$ ) and  $\text{Mg}^{2+}$  ( $19.42 \pm 1.23$ ). The average values of serum ions  $\text{Na}^+$  and  $\text{Mg}^{2+}$  of the control group were respectively  $141.25 \pm 1.50$  and  $20.80 \pm 1.12$ . The average value of  $\text{Fe}^{2+}$  ions ( $0.98 \pm 0.08$ ) of rats fed on soybean meal and supplemented with CTP was significantly reduced ( $p \leq 0.05$ ), compared to that of the control group ( $1.22 \pm 0.07$ ) (Table 7). The average value of the serum electrolytes of both groups of rats fed on cotton seeds proteins was not statistically different ( $p > 0.05$ ) (Table 7).

**Table 7 : Average value of serum electrolytes**

Criteria	Treatments							
	Casein (n=6)	Casein + CTP (n=6)	Fish (n=6)	Fish + CTP (n=6)	Soybean (n=6)	Soybean + CTP (n=6)	Cotton (n=6)	Cotton + CTP (n=6)
$\text{Ca}^{2+}$ (mg/l)	81.00±7.25 <sup>a</sup>	82.75±2.75 <sup>a</sup>	83.00±9.62 <sup>a</sup>	82.25±7.13 <sup>a</sup>	88.50±9.11 <sup>a</sup>	88.00±3.16 <sup>a</sup>	79.50±3.69 <sup>a</sup>	83.00±9.30 <sup>a</sup>
$\text{Na}^+$ (mEq/l)	140.00±0.81 <sup>c</sup>	141.50±1.29 <sup>b</sup>	141.25±1.50 <sup>b</sup>	140.00±0.81 <sup>c</sup>	139.75±1.70 <sup>b</sup>	140.00±0.81 <sup>b</sup>	141.75±2.06 <sup>b</sup>	140.50±1.73 <sup>b</sup>
$\text{K}^+$ (mEq/l)	4.00±0.24 <sup>d</sup>	3.92±0.32 <sup>d</sup>	4.12±0.17 <sup>d</sup>	4.00±0.18 <sup>d</sup>	3.92±0.26 <sup>c</sup>	4.07±0.20 <sup>c</sup>	4.20±0.11 <sup>c</sup>	4.10±0.14 <sup>c</sup>
$\text{P}^{3+}$ (mg/l)	78.50±5.19 <sup>a</sup>	79.00±3.55 <sup>a</sup>	72.00±6.97 <sup>a</sup>	72.50±1.91 <sup>a</sup>	71.75±6.39 <sup>d</sup>	72.25±6.13 <sup>d</sup>	78.75±5.90 <sup>d</sup>	80.75±2.75 <sup>d</sup>
$\text{Fe}^{2+}$ (mg/l)	1.02±0.14 <sup>e</sup>	1.15±0.08 <sup>e</sup>	1.13±0.25 <sup>e</sup>	1.05±0.14 <sup>e</sup>	1.22±0.07 <sup>e</sup>	0.98±0.08 <sup>e</sup>	1.02±0.03 <sup>e</sup>	1.01±0.14 <sup>e</sup>
$\text{Mg}^{2+}$ (mg/l)	19.72±0.85 <sup>i</sup>	20.87±0.51 <sup>h</sup>	20.80±1.12 <sup>e</sup>	19.42±1.23 <sup>h</sup>	20.40±0.65 <sup>e</sup>	20.07±1.03 <sup>e</sup>	20.45±0.99 <sup>i</sup>	19.77±1.06 <sup>d</sup>

(n) : Number of rats ; Each mean is followed by its standard deviation ; Student test 5% ; for

the same protein in two columns, and in the same row, means followed by different superscript alphabet letters are significantly different ; CTP: chromium tripicolinate.

## DISCUSSION

Results of this experiment indicated that rats submitted to diet containing cotton seeds proteins, and supplemented with chromium had an average daily weight gain lower than that of the control group ( $p \leq 0.05$ ). However, chromium associated to casein, to fish powder and soybean meal does not have significant effect on the growth of the rats. The absence of effect of chromium on the characteristics of growth of rats fed on casein, fish and soybean meal proteins are corroborated by Bernao *et al.* [3], Krol *et al.* [10] and Staniek *et al.* [19]. The decrease of the average body weight of rats fed on cotton seeds proteins may be associated to the quality and the amount of the amino-acids contents in this source of proteins. As a fact, cotton seeds proteins are poor in essential amino acids like lysine, methionine, tryptophan and threonine. The evaluation of organs biometry showed that rats fed on chromium and casein had the liver and kidneys weight higher than that of the control group. Rats consuming the fish powder had the spleen average weight reduced by dietary chromium. No significant difference was observed about organs weight of rats consuming proteins from soybean and cotton seeds. The increase of the liver and the kidneys weight of rats treated with chromium and consuming casein is probably due to the intrinsic quality of casein, an animal protein. Liver and kidneys are organs involved in the metabolism of nutrients.

The spleen, on the other hand, is the main producer of lymphocytes involved in immunity. Changing in the weight of these organs constitutes a way to explore them indirectly in nutrition study [1]. Rats fed on casein supplemented with chromium had a significant increase of the average serum values of glucose, uric acid, and a significant decrease of the average value of total bilirubin. The average value of serum aspartate transaminase and alanine transaminase was reduced by the chromium in the casein diet. The average value of serum electrolytes like  $\text{Na}^+$ ,  $\text{Fe}^{2+}$  and  $\text{Mg}^{2+}$  was increased by chromium supplementation.

The increment of glycemia in rats fed on chromium has been reported by Cefalu *et al.* [4] and Sreejayan *et al.* [17] in animals artificially or naturally obese and/or diabetic. The uric acid is the product of the degradation of the purine which is a base present in the chain of the nucleic acids. The increase of its level in the blood means that chromium is capable to activate the catabolism of the nucleic acids, as reported by Stearns *et al.* [20]. The reduction of the average value of serum total bilirubin would be the expression of a good status of the liver and the red blood cells in animals under chromium and casein diet. The reduction of the average value of aspartate and alanine transaminase could corroborate the good status of the liver and the kidneys, as stated before. The augmentation of the average value of the electrolytes ( $\text{Na}^+$ ,  $\text{Fe}^{2+}$ ,  $\text{Mg}^{2+}$ ) by the presence of chromium in the diet can provoke physiological disturbances. Ions concentrations in the organism must remain constant, so should be the relationship between each other. Generally, these factors are modified in a situation of serious infections or illness. Rats with diet composed of chromium associated to fish powder had the average value of serum triglycerides reduced, and the average value of the uric acid increased. Average value of serum ions like  $\text{Na}^+$  and  $\text{Mg}^{2+}$  were lowered by the chromium supplementation. The diminution of the average value of serum triglycerides can be explained by an increased utilization of fatty acids for the energetic purpose. In fact, It has been demonstrated that in pigs, chromium has a trend to reduce fat proportion in the body, while it tends to increase muscle percentage [14, 11, 8]. The augmentation of the average value of serum uric acid in rats fed with chromium and fish powder is due to the degradation of purine base in nucleic acids [20]. Only the average value of  $\text{Na}^+$  and  $\text{Mg}^{2+}$  was affected by chromium. These results showed that 100 ppm of chromium is not toxic for the experimental rats. Chromium reduced the average value of serum urea, and creatinine of animals fed on soybean meal. The average value of  $\text{Fe}^{2+}$  ions was also lowered. The reduction of the average value of urea and creatinine by chromium can be explained by a reduction of the catabolism of proteins or amino acids and creatine, since urea is the product of the degradation of creatine. Chromium and iron ions ( $\text{Fe}^{2+}$ ) have an interaction on the iron transport protein, the transferrin [5]. Therefore the level of  $\text{Fe}^{2+}$  ions which was reduced can be explained by the supplementation of chromium in the diet.

Rats submitted to a diet of cotton seeds proteins had an average value of total bilirubin reduced by chromium. This result can be the expression of a good status of the liver, and the integrity of the red blood cells. The average activity of the enzymes aspartate transaminase, alanine transaminase and alkaline phosphatase is not affected by chromium.

## CONCLUSION

The association of chromium tripicolinate and different proteins sources (casein, fish powder, soybean meal, cotton seed meal) had no positive effect on the growth performance of rats. The association between chromium and casein increased the activity of the liver and kidneys of rats, through a significant augmentation of their average weight. In these rats, a reduction of the average activity of the enzymes aspartate transaminase and alanine transaminase was noticed. Likewise, an increase of the average level of the electrolytes  $\text{Na}^+$ ,  $\text{Fe}^{2+}$ ,  $\text{Mg}^{2+}$ , glucose, uric acid, and a decrease of the average value of total bilirubin were observed. However, Chromium associated to fish powder, soybean meal and cotton seed meal had little effects on the same parameters. More investigations should be undertaken in order to determine the effect of chromium associated to limiting amino acids like lysine, methionine, threonine, and tryptophan.

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