Effects of Chromium Supplementation Levels in a Nitrogen-Free Diet on Organ Indices, Intestinal Mucosal Morphology, and Serum Parameters in Rats: Postprint

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Abstract

This study was conducted to investigate the effects of dietary supplementation with different levels of chromium propionate (CrPro) in a nitrogen-free diet on organ indices and serum parameters in Sprague Dawley (SD) rats. Thirty-six weaned female SD rats with uniform body weight were selected and divided into 3 treatments, with 6 replicates per treatment and 2 rats per replicate. Each treatment group was fed either a nitrogen-free basal diet (control group, containing 0.08 mg/kg chromium) or experimental diets based on the basal diet supplemented with 0.2 and 1.0 mg/kg CrPro (as chromium), respectively, for a 21-day experimental period. The results showed that, compared with the control group: 1) dietary supplementation with 0.2 mg/kg CrPro significantly increased the spleen index of rats ($P<0.05$); 2) dietary supplementation with 1.0 mg/kg CrPro significantly increased the ratio of jejunal villus height to crypt depth ($P<0.05$); 3) dietary supplementation with 1.0 mg/kg CrPro significantly increased serum albumin and uric acid levels ($P<0.01$), while supplementation with 0.2 mg/kg chromium increased uric acid level ($P<0.05$). These results indicate that supplementation with appropriate levels of chromium in a nitrogen-free diet has certain effects on improving immune organ indices, intestinal mucosal morphology, and serum protein metabolism in rats.

Full Text

Effects of Chromium Supplementation Level in Nitrogen-Free Diets on Organ Indexes, Intestinal Mucosal Morphology, and Serum Parameters in Rats

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Abstract

This study investigated the effects of different dietary chromium propionate (CrPro) supplementation levels in nitrogen-free diets on organ indexes, intestinal mucosal morphology, and serum parameters in Sprague Dawley (SD) rats. Thirty-six weaned female SD rats with uniform body weight were allocated to three treatments, with six replicates per treatment and two rats per replicate. The treatments consisted of a nitrogen-free basal diet (control group, containing 0.08 mg/kg chromium) and the basal diet supplemented with 0.2 or 1.0 mg/kg CrPro (as chromium) for a 21-day experimental period. The results showed that, compared with the control group: (1) dietary supplementation with 0.2 mg/kg CrPro significantly increased spleen index ($P<0.05$); (2) dietary supplementation with 1.0 mg/kg CrPro significantly increased the ratio of jejunal villus height to crypt depth ($P<0.05$); and (3) dietary supplementation with 1.0 mg/kg CrPro significantly increased serum albumin and uric acid contents ($P<0.01$), while 0.2 mg/kg chromium supplementation increased uric acid content ($P<0.05$). These findings indicate that appropriate chromium supplementation in nitrogen-free diets can influence immune organ indexes, intestinal mucosal morphology, and serum protein metabolism in rats.

Keywords: nitrogen-free diet; chromium propionate; organ indexes; intestinal mucosa; serum parameters

Chromium (Cr) is an essential trace element for animals that serves as a cofactor for insulin action and plays important roles in carbohydrate, lipid, protein, and nucleic acid metabolism [1]. Numerous studies have demonstrated that dietary chromium supplementation at appropriate levels can improve animal growth performance, carcass quality, and immune function [2-4]. Chromium forms small organic complexes in animals that passively diffuse through the intestinal mucosa into the small intestine [5], with absorption primarily occurring in the middle small intestine, followed by the ileum and duodenum [6]. The intestinal mucosa is not only crucial for digestion and nutrient absorption but also serves important immune functions. Maintaining normal intestinal mucosal morphology is a prerequisite for the intestine to fulfill its absorptive capacity, resist invasion by harmful substances, and promote healthy development. Current chromium research has primarily focused on its effects on glucose metabolism and fat deposition [7-9], with fewer studies investigating intestinal mucosal morphology and protein metabolism. Some research suggests that dietary protein is the best source of chromium [10], and that consumption of low-protein (nitrogen-free) diets may lead to chromium deficiency [10-11].

Given this context, the present study utilized Sprague Dawley (SD) rats as an animal model and employed a novel chromium source—chromium propionate (CrPro)—to generate a low-chromium status through feeding nitrogen-free di-

ets. The objective was to preliminarily explore the effects of different dietary CrPro supplementation levels on organ development, intestinal mucosal morphology, and serum parameters in SD rats, thereby providing further insights into chromium' s functions.

1. Materials and Methods

1.1 Experimental Materials and Design Thirty-six specific pathogen-free (SPF) grade weaned female SD rats with an initial body weight of 53.63 ± 1.37 g were selected. After a one-week pre-feeding period, the rats were divided into three treatments according to the principle of no significant difference in body weight among treatments (P>0.05), with six replicates per treatment and two rats per replicate. The three treatments consisted of a nitrogen-free basal diet (control group, containing 0.08 mg/kg chromium) and the basal diet supplemented with 0.2 or 1.0 mg/kg CrPro (as chromium). The experimental period lasted 21 days. The CrPro was provided by Kemin (Zhuhai) Industries Co., Ltd., with a measured chromium content of 0.11%. The purified nitrogen-free basal diet was formulated based on previous research [12], with casein and potassium chromium sulfate dodecahydrate replaced by corn starch at equal proportions. The basal diet contained 0.70% crude protein (determined by Kjeldahl method) and 0.08 mg/kg chromium (determined by electric hot plate digestion-graphite furnace atomic absorption spectrometry).

1.2 Animal Management The experiment was conducted in March 2015 at the Small Animal Experimental Farm of Southwest University for Nationalities. The facility provided natural photoperiod, with temperature controlled at 24 ± 1°C and relative humidity maintained at 50%-60%. Exhaust fans operated continuously for 24-hour ventilation. Rats had ad libitum access to experimental diets and deionized water (no detectable chromium).

1.3 Sample Collection and Analysis

1.3.1 Serum Parameters On day 21 of the experimental period, after an 8-hour fast, the two rats in each replicate cage were weighed and blood samples were collected via orbital artery puncture. Serum was prepared by centrifugation at 2,000 × g for 15 minutes at 4°C and stored at -20°C. Serum parameters were measured using an automatic biochemical analyzer (TC6010L, Shanghai Tekang Technology Co., Ltd.): total protein (TP) by the biuret method, albumin (ALB) by the bromocresol green method, and uric acid (UA) by the uricase method. Globulin (GLB) content and albumin/globulin ratio (A/G) were calculated as follows: GLB = TP - ALB.

1.3.2 Organ Indexes After blood collection, rats were euthanized by cervical dislocation. The liver, spleen, and kidneys were dissected and weighed to

calculate organ indexes: Organ index (mg/g) = organ fresh weight / live body weight.

1.3.3 Jejunal Mucosal Morphology Immediately after euthanasia, a 2-cm segment of jejunum was excised, gently rinsed with phosphate-buffered saline (PBS, pH 7.2), and fixed in 4% paraformaldehyde at 4°C for 3 days. Samples were then paraffin-embedded, sectioned, and stained with hematoxylin-eosin (HE). Villus height (distance from villus base to tip) and crypt depth (distance from crypt base to villus base) were measured, and the villus height/crypt depth ratio was calculated.

1.4 Statistical Analysis Data were analyzed using one-way ANOVA with SAS 8.1 software. Differences were considered significant at P<0.05, and Duncan' s multiple range test was used for post-hoc comparisons.

2. Results

2.1 Effects of Dietary CrPro Supplementation Level on Organ Indexes in Rats As shown in Table 1 , dietary CrPro supplementation level had no significant effect on liver or kidney indexes (P>0.05). The 0.2 mg/kg group significantly increased spleen index compared with both the control and 1.0 mg/kg groups (P<0.05), while no difference was observed between the control and 1.0 mg/kg groups (P>0.05).

Table 1 Effects of dietary CrPro supplementation level on organ indexes in rats (mg/g)

Item	CrPro supplementation level			P-value
	0 mg/kg	0.2 mg/kg	1.0 mg/kg	
Liver index	2.33b	2.58a	2.28b	
Spleen index	2.20b	2.36a	2.20b	
Kidney index	5.30b	5.20b	5.88a	<0.05

In the same row, values with different lowercase letter superscripts indicate significant difference (P<0.05), while values with the same or no letter superscripts indicate no significant difference (P>0.05). The same applies below.

2.2 Effects of Dietary CrPro Supplementation Level on Jejunal Mucosal Morphology in Rats As shown in Table 2 , dietary CrPro supplementation level had no significant effect on jejunal villus height or crypt depth (P>0.05). Compared with the control group, the 1.0 mg/kg group significantly increased villus height/crypt depth ratio (P<0.05), while no significant difference was observed between the 0.2 mg/kg and control groups (P>0.05).

Table 2 Effects of dietary CrPro supplementation level on jejunal mucosal morphology in rats

Item	CrPro supplementation level			P-value
	0 mg/kg	0.2 mg/kg	1.0 mg/kg	
Villus height (m)	312.50	318.33	324.17	>0.05
Crypt depth (m)	58.33	61.67	55.00	>0.05
Villus height/crypt depth	5.30b	5.20b	5.88a	<0.05

2.3 Effects of Dietary CrPro Supplementation Level on Serum Parameters in Rats As shown in Table 3 , dietary CrPro supplementation level had no significant effect on serum TP, GLB contents, or A/G ratio (P>0.05). The 1.0 mg/kg group significantly increased serum ALB content compared with both the control and 0.2 mg/kg groups (P<0.05), while no significant difference was observed between the control and 0.2 mg/kg groups (P>0.05). Both the 0.2 and 1.0 mg/kg groups significantly increased serum UA content compared with the control group (P<0.05), with the 1.0 mg/kg group being significantly higher than the 0.2 mg/kg group (P<0.05).

Table 3 Effects of dietary CrPro supplementation level on serum parameters in rats

Item	CrPro supplementation level			P-value
	0 mg/kg	0.2 mg/kg	1.0 mg/kg	
TP (g/L)	56.67	57.50	58.33	>0.05
ALB (g/L)	26.30b	26.20b	27.36a	<0.05
GLB (g/L)	30.37	31.30	30.97	>0.05
A/G	0.87	0.84	0.88	>0.05
UA (mol/L)	75.67c	89.50b	107.83a	<0.05

3. Discussion

3.1 Effects of Dietary CrPro Supplementation Level on Organ Indexes in Rats This study found that dietary CrPro supplementation level had no significant effect on liver or kidney indexes in rats. Similar results were reported by Bernao et al. [13], who administered different levels of chromium picolinate (0, 0.1, 0.2, 0.5 mg/d) to Wistar rats for 12 days and observed no significant differences in gastrocnemius muscle index, liver index, or carcass index among treatments. Gu et al. [14] also found that compared with a control group without chromium supplementation, dietary supplementation with 0.3 mg/kg nano-chromium or chromium picolinate had no significant effect on heart, liver, kidney, testis, or hind leg muscle indexes in SD rats, although the chromium picolinate group showed higher spleen index than the nano-chromium group. Regarding spleen index improvement, our study similarly demonstrated that

the 0.2 mg/kg group was more effective than both the control and 1.0 mg/kg groups, with no difference between the latter two groups. As an immune organ, increased spleen weight reflects enhanced immune function [15]. This suggests that dietary supplementation with 0.2 mg/kg CrPro may have beneficial effects on immune performance.

3.2 Effects of Dietary CrPro Supplementation Level on Jejunal Mucosal Morphology in Rats In this study, dietary CrPro supplementation level did not affect jejunal villus height or crypt depth but influenced their ratio, with the 1.0 mg/kg group significantly increasing villus height/crypt depth ratio while the 0.2 mg/kg group showed no significant difference from the control. Similarly, Li [5] reported that dietary supplementation with 0.2 mg/kg chromium picolinate significantly increased the villus height/crypt depth ratio in heat-stressed meat ducks at 14 days of age and in the ileum at 21 days of age. Wang et al. [16] supplemented laying hens under heat stress with different levels (0, 0.07, 0.15, 0.22, 0.29, 0.37 g/kg) of glucose tolerance factor for 52 days and found that the 0.37 g/kg group significantly increased jejunal villus height, while the 0.15 g/kg group showed a significant increase as well. Intestinal villi are leaf-like structures formed by the protrusion of the lamina propria and epithelium into the intestinal lumen, which increase the absorptive surface area of the small intestine. The villus height/crypt depth ratio particularly reflects the functional status of intestinal mucosa, with a higher ratio indicating enhanced digestive and absorptive capacity. Therefore, appropriate dietary chromium supplementation can improve intestinal mucosal morphology and enhance nutrient utilization in the small intestine. However, some studies have reported that dietary supplementation with different levels (0, 5, 10, 15 mg/kg) of chromium chloride hexahydrate ($CrCl3 \cdot 6H2O$) had no significant effect on duodenal villus height, crypt depth, or their ratio in rex rabbits [17]. These discrepancies may be related to chromium source and supplementation level. Currently, research on chromium' s effects on intestinal mucosa remains limited and warrants greater attention.

3.3 Effects of Dietary CrPro Supplementation Level on Serum Parameters in Rats This study found that dietary CrPro supplementation level had no significant effect on serum TP, GLB contents, or A/G ratio, which is consistent with findings from Bernao et al. [13], Gu et al. [14], Yan et al. [18], and Zhang et al. [19]. Yan et al. [18] reported that dietary supplementation with 0.2 mg/kg chromium methionine or chromium yeast had no significant effect on serum TP, GLB contents, or A/G ratio in weaned piglets. Zhang et al. [19] found that dietary supplementation with 0.3 mg/kg chromium picolinate had no significant effect on plasma TP and GLB contents in barrows raised under high temperature conditions. However, Hou et al. [20] observed that while dietary supplementation with 0.2 mg/kg chromium picolinate had no significant effect on serum TP and GLB contents on days 0, 9, 18, and 35 in early-weaned piglets, it significantly increased these parameters on day

27. Wang et al. [21] supplemented finishing pigs with 0.2 mg/kg of different chromium sources (chromium chloride, chromium picolinate, nano-chromium) for 40 days and found that only the nano-chromium and chromium picolinate groups significantly increased serum TP content. These findings suggest that chromium' s effects on serum TP and GLB contents may depend on sampling time and chromium source type.

Additionally, this study found that the 1.0 mg/kg group significantly increased serum ALB content compared with both the control and 0.2 mg/kg groups, with no significant difference between the latter two groups. This aligns with results from Moonsie-Shageer et al. [22] in cattle and Qin et al. [23] and Cheng et al. [24-25] in chickens (with chromium supplementation levels of 0.2-1.0 mg/kg). However, Yan et al. [18], Zhang et al. [19], and Hou et al. [20] reported that serum ALB content was not affected by chromium supplementation levels (0.2-0.3 mg/kg) in pigs. These divergent results may be related to chromium supplementation level and animal species. Moreover, since this experiment was conducted under nitrogen-free dietary conditions, insufficient protein supply may have influenced chromium' s functional effects, though the regulatory mechanism of chromium on protein metabolism remains unclear and requires further systematic investigation.

Furthermore, this study demonstrated that both the 0.2 and 1.0 mg/kg groups significantly increased serum UA content compared with the control group, with the 1.0 mg/kg group showing a more pronounced effect. Chen et al. [26] supplemented finishing pigs with different levels (0, 0.2, 0.5, 1.0 mg/kg) of chromium nicotinate for 2 months and found that 0.2 and 0.5 mg/kg supplementation significantly decreased serum UA content, while the 1.0 mg/kg level showed no significant difference from the control, although serum UA content tended to increase with higher chromium levels. Ernest et al. [27] added 100 mg/kg chromium picolinate to diets with different protein sources (casein, fish meal, dehulled soybeans, cottonseed) fed to Wistar rats and found that only the casein and fish meal groups significantly increased serum UA content compared with their respective controls. These findings suggest that chromium' s effects on serum UA content are inconsistent and may depend on chromium supplementation level and dietary protein source.

Conclusion

Supplementation of nitrogen-free diets with appropriate levels of CrPro can increase spleen index, improve jejunal villus height/crypt depth ratio, and elevate serum ALB and UA contents in SD rats, thereby exerting beneficial effects on immune organ indexes, intestinal mucosal morphology, and serum protein metabolism.

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Note: Figure translations are in progress. See original paper for figures.