

Effects of Dietary Chromium Picolinate on Performance, Egg Quality, Serum Traits and Mortality Rate of Brown Layers

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ABSTRACT: This experiment was conducted with total 960 brown layers, consisted of 8 treatment to investigate the effects of dietary chromium as chromium picolinate on egg production, egg quality, nutrient utilizability, serum traits and mortality in brown layers. Layers were fed diets with two levels of dietary protein (14% and 16%) and supplemented with 0, 200, 400, 800 ppb/kg of chromium as chromium picolinate, respectively.

The highest egg production, egg weight and egg mass were found in 800 ppb chromium picolinate supplementation group with high protein level (16%) ($p < 0.05$). Although there was no significant difference, layers receiving 400 ppb of chromium picolinate with high protein (16%) represented the lowest broken eggs. The utilization of energy, dry matter and crude protein of 400 ppb chromium picolinate group with low protein level (14%) were significantly higher than those of control or other chromium picolinate group ($p < 0.05$). 400 ppb

chromium picolinate with low protein level (14%) showed the lowest serum glucose concentration. But serum glucose concentrations in all treatments showed no significant differences. Present data revealed that the lowest serum cholesterol concentration of layers was found at 400 ppb chromium picolinate group with high protein level (16%) ($p < 0.05$). Crude protein content in yolk was significantly higher in eggs of layers received 800 ppb chromium picolinate and the lowest in eggs from layers received 400 ppb chromium picolinate among chromium picolinate levels ($p < 0.05$). Mortality was remarkably decreased by chromium picolinate supplementation and the lowest mortality value was found in layers receiving 800 ppb chromium picolinate with high protein level.

(Key Words: Chromium Picolinate, Layers, Egg Quality, Serum Traits, Cholesterol, Glucose, Mortality)

INTRODUCTION

Since the mammalian need for dietary chromium was first recognized by Schwarz and Mertz in 1957, chromium regarded as an essential element for mammals. Today, the major roles of chromium is known as follow; ① maintain normal glucose tolerance ② reduce lipid accumulation in tissues ③ be involved in protein and several amino acids synthesis and nucleic acid metabolism, and ④ have a potential power in immune system.

In human, chromium is known as activating insulin internalization into cell membrane as glucose metabolism can be maintained normal. Generally chromium is regarded as GTF (glucose tolerance factor). The major function of the GTF is stimulation of the action of insulin in chromium deficient tissue (Mertz, 1969). Insulin promotes anabolic processes in tissue and stimulates the

active transport of glucose and amino acids into muscle cells and thus protein synthesis is enhanced. Anderson et al. (1983) reported that chromium supplementation decreased serum glucose level of human with more than or equal to 100 mg/dl 90 minutes after a glucose challenge of 1 g glucose per kg of body weight.

Chromium has long been linked to lipid metabolism. Schroeder et al. (1970) firstly established the hypothesis that chromium deficiency may be a risk factor in atherosclerotic disease. Schroeder et al. (1970) also reported an average 12.2% decline in the serum cholesterol concentrations in seven patients supplemented with 2 mg of chromium/day.

Until now, the nutritional status and function of chromium in various animal species also have been investigated. In 1980, Abraham et al. (1980) reported that chromium reduced cholesterol-induced atherosclerotic plaques in rabbits. And chromium has been shown to increase the growth rate of turkey poults (Steele and

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Rosebrough, 1979). Glismann and Mertz (1966) reported that chromium supply was associated with decreased mortality and greater longevity. Study conducted by Page et al. (1993), chromium picolinate decreased backfat thickness and serum cholesterol in pigs. Jensen and Maurice (1980) reported that, in the laying hen, the Haugh unit value was significantly improved by adding 10 ppm chromium to the basal diet. Page (unpublished) reported recently that Haugh units, specific gravity and egg cholesterol were not affected by chromium picolinate supplementation, but chromium picolinate reduced percentage of egg fat and crude protein. And egg production was incrementally increased by 50, 100 and 200 ppb chromium picolinate to the diets, but they could not explain the increase of egg production.

Until now, little studies conducted to investigate the effects of chromium picolinate in laying hen. Therefore, this study conducted to investigate the effects of chromium picolinate on performance, nutrient utilizability, yolk and serum traits and mortality of layers when fed diets containing different levels of dietary crude protein and chromium picolinate.

MATERIALS AND METHODS

1) Experimental design and animal

Experimental diets based on corn-soybean diet containing two levels of crude protein (14% and 16%) with four levels of chromium as a chromium picolinate (0, 200, 400, 800 ppb/kg) were used in this study. Each treatment had eight replicates and consisted of twenty layers per replicate.

Total 960 brown layers of Shaver breed (thirty-six-weeks-old) were used in this feeding trial. Layers were blocked by egg production and egg weight and a randomized completed design was used in assigning layers to treatments.

Feeding trial was conducted in Iee-chen, Korea and was initiated on July 8 and terminated on August 25, 1994. Before the feeding trial began, layers were fed commercial diets for seven days. And during the entire feeding trial, experimental diets and drinking water were provided *ad libitum*. Upon termination of feeding trial four layers per treatment were transferred to Animal Nutrition Laboratory for metabolic trial.

2) Experimental diets

Commercial diet (CP; 15%, 2,900 ME/kcal) were offered for seven days prior to feeding trial. During feeding trial, layers were fed corn-soy meal based diets with two different levels of dietary crude protein (14%

and 16%) and four levels of chromium picolinate (0, 200, 400, 800 ppb/kg). Chromium picolinate used in this experiment was produced by Prince Agri Products, Inc. (One Prince Plaza, Quincy, IL 62301, USA and it consisted of calcium carbonate, chromium picolinate and iron oxide. The mixture contained 0.04% chromium picolinate and 35-40% calcium. Except for crude protein, other nutrients were formulated to meet or exceed the nutrient requirement suggested by NRC (1994). The formula and chemical composition of basal diets for this experiment are presented in table 1.

Table 1. Formula and chemical composition of experimental diets

Protein levels (%)	14	16
Ingredients (%):		
Corn	70.38	66.00
Soybean meal	16.00	17.80
Corn gluten meal	2.00	4.70
Tallow	1.40	1.40
Limestone	8.70	8.70
TCP (18%)	0.65	0.65
Salt	0.30	0.30
Lysine · HCl	0.07	—
DL-Methionine	0.06	0.01
Vit.-Min. mix. ¹	0.44	0.44
Total	100.00	100.00
Chemical Compositions ² (%):		
ME (kcal/kg)	2,907.93	2,901.78
C. Protein	14.03	16.03
Lysine	0.69	0.69
Methionine	0.30	0.30
Ca	3.27	3.27
P, available	0.26	0.27

¹ Vitamin and mineral mixture (unit/kg): vitamin A, 1,600,000 IU; vitamin D₂, 300,000 IU; vitamin K₃, 130 mg; vitamin B₂, 1,000 mg; Choline chloride, 35,000 mg; Niacin, 2,000 mg; Ca-pantothenate, 800 mg; folic acid, 60 mg; DL-methionine, 6,000 mg; Mn, 12,000 mg; Zn, 9,000 mg; Fe, 4,000 mg; Cu, 500 mg; I, 250 mg; Co, 100 mg; Ca, 7,140 mg; B.H.T., 6,000 mg.

² Calculated values.

3) Methods of experiment

(1) Feeding trial

Thirty-six-weeks-old brown layers used in this experiment were raised in adult wire cages. This adult cage can hold two layers in one cage. Experimental diets were offered twice in a day according to treatments and replicates at 04:00 AM and 14:00 PM. And diets were

flatted by hand at 08:00 AM and 15:00 PM for the purpose of feeding evenly. Eggs were collected twice daily to investigate egg production, egg weight, broken eggs and egg mass at 09:00 AM and 13:00 PM. During the entire experimental period, light was fixed 17 hour every day and air ventilation system was also used. To investigate mortality, dead layers were also recorded.

Feed intake was recorded weekly on replicates unit. And feed/kg egg was calculated by dividing the amounts of feed consumed by the corresponding egg weight.

(2) Metabolic trial and blood collection

To investigate the nutrient utilizability of the experimental diets, the metabolizability coefficient was calculated by total fecal collection method during seven days after feeding trial termination. Four layers per treatment, a total thirty-two layers were randomly selected and moved to Animal Nutrition Laboratory and housed in metabolic cage individually for metabolic trial. After five days of preliminary period for adaptation, total excreta were collected four times a day. The collected total excreta were pooled and dried in an air-forced drying

oven (Dong Yang Co, Korea) at 60°C for 72 hours to gain constant dry weight. All the samples prepared were grounded with 1 mm mesh Wiley mill for further chemical analysis.

Blood samples were collected from axillary artery of layers. All blood samples were centrifuged (Hanil, Korea) at 3,000 rpm for 20 minutes and the supernatants (serum) were kept frozen at -20°C for analysis of glucose and cholesterol.

(3) Egg production, egg weight, broken eggs and egg mass

During feeding trial, eggs were collected twice a day at 09:00 AM and 14:00 PM. Egg production was calculated dividing total produced eggs by total layers per replicate and to investigate egg weight, total normal eggs were weighed and divided by total number of normal eggs. Broken egg rate was recorded by dividing the number of broken eggs collected everyday with total number of produced eggs. Egg mass was calculated by multiplying total egg weight by the number of produced eggs per replicate and treatment

Table 2. Effects of dietary chromium picolinate on the performance of brown layers (37-43 weeks)

Protein (%)	CrP ¹ (ppb)	Egg production (%)	Egg weight (g)	Egg mass (g/hen/d)	Broken egg (%)	Feed intake (g/hen/d)	Feed/kg egg
16	0	72.40	59.27 ^{dce}	42.94 ^{ab}	1.17	96.91 ^a	2.20
	200	72.34	59.49 ^{bc}	43.19 ^a	1.13	93.48 ^{ab}	2.21
	400	72.63	60.13 ^a	43.66 ^a	1.01	94.66 ^{ab}	2.18
	800	72.85	60.47 ^a	43.92 ^a	1.10	94.77 ^{ab}	2.18
14	0	72.27	59.42 ^{dc}	42.94 ^{ab}	0.98	96.03 ^a	2.19
	200	72.64	58.89 ^{de}	42.76 ^{ab}	1.06	93.57 ^{ab}	2.18
	400	70.42	60.01 ^{ab}	42.40 ^{ab}	1.35	93.90 ^{ab}	2.31
	800	70.71	58.83 ^c	41.61 ^b	0.94	90.71 ^b	2.27
Between protein levels (%)							
16		72.55	59.84 ^a	43.43 ^a	1.10	94.95	2.19
14		71.51	59.29 ^b	42.43 ^b	1.08	93.55	2.23
Among CrP levels (ppb)							
	0	72.34	59.34 ^{bc}	42.94	1.08	96.47 ^a	2.19
	200	72.49	59.19 ^c	42.97	1.10	93.52 ^{ab}	2.19
	400	71.52	60.07 ^a	43.03	1.18	94.28 ^{ab}	2.24
	800	71.78	59.65 ^b	42.76	1.02	92.74 ^b	2.22
Probability (P):							
Protein		0.1563	0.0001	0.0029	0.8942	0.1776	0.1576
CrP		0.5958	0.0001	0.9505	0.8588	0.0667	0.5958
Protein & CrP		0.1775	0.0001	0.0761	0.4679	0.5079	0.1775

¹Chromium picolinate.

^{ab,c,d,e}: Values with different superscripts within the same column are significantly different ($p < 0.05$).

(4) Egg collection for analysis

Eggs were collected on last day of each weeks for ether extract and crude protein analysis. To determine yolk protein and ether extract, eggs were hardly cooked by immersion in boiling water for 10 minutes. Yolk was isolated from whole egg and dried in freeze dryer (Il Sin Engineering Co., Korea). Finally, yolk was kept frozen in refrigerator for further analysis.

(5) Chemical analysis

Proximate analysis of experimental diets and excreta were conducted according to the method of AOAC (1990). Serum glucose and total cholesterol were analyzed by an enzymatic kit (Asan Co., Korea).

(6) Statistical analysis

Statistical analysis for the presented data were subjected to analysis of variance for a 2×4 factorial design and significant differences among treatment means were compared by Duncan's multiple range test using General Linear Model (GLM) procedure of SAS (1985)

package program.

RESULTS AND DISCUSSION

1) Total performance

Table 2 summarizes the effects of dietary chromium picolinate on performance - egg production, egg weight, egg mass, broken eggs, feed intake and feed/gain - of brown layers during the entire experimental period (37-43 weeks).

The highest egg production, egg weight and egg mass were found in 800 ppb chromium picolinate with high protein level ($p < 0.05$). Layers received 400 ppb chromium picolinate with high protein level represented the lowest broken eggs. But there was no significant difference. The layers fed high protein basal diet recorded the higher feed intake ($p < 0.05$). Though there was no significant difference, the best feed/kg egg was found in 400 and 800 ppb chromium picolinate groups with high protein level and 200 ppb chromium picolinate group with low protein level.

Table 3. Effects of dietary chromium picolinate on the nutrient utilization of brown layers (37-43 weeks)

Protein (%)	CrP ¹ (ppb)	Energy	Dry matter	Crude protein	Crude fat	Crude ash
16	0	87.34 ^{abc}	82.43 ^b	79.91 ^b	91.75	60.48
	200	86.95 ^c	82.69 ^b	82.49 ^{ab}	90.75	60.35
	400	87.16 ^{bc}	83.63 ^b	82.87 ^{ab}	90.45	63.97
	800	87.55 ^{abc}	82.90 ^b	84.08 ^{ab}	91.03	60.99
14	0	88.19 ^{abc}	82.81 ^b	80.13 ^b	92.69	61.23
	200	88.10 ^{abc}	84.81 ^{ab}	84.38 ^{ab}	92.28	61.31
	400	89.84 ^a	86.91 ^a	86.09 ^a	92.08	63.68
	800	89.23 ^{ab}	86.39 ^a	85.41 ^a	91.91	61.92
Between protein levels (%)						
16		87.22 ^b	82.91 ^b	82.34	91.00	61.34
14		88.84 ^a	85.23 ^a	84.00	92.24	62.34
Among CrP levels (ppb)						
	0	87.83	82.62 ^b	80.02 ^a	92.21	60.91
	200	87.46	83.75 ^{ab}	83.43 ^b	91.51	60.83
	400	88.50	85.27 ^a	84.48 ^b	91.27	63.80
	800	88.39	84.65 ^a	84.75 ^b	91.47	61.46
Probability (P);						
	Protein	0.0045	0.0005	0.1440	0.2083	0.7811
	CrP	0.4334	0.0207	0.0212	0.9041	0.8229
	Protein & CrP	0.6730	0.2379	0.8088	0.9887	0.9991

¹ Chromium picolinate.

² Pooled standard error.

^{abc}: Values with different superscripts within the same column are significantly different ($p < 0.05$).

From these results, it can be concluded that supplementation of chromium picolinate has no effect on feed intake. But during the entire experimental period chromium picolinate addition slightly improved the performance i.e. egg production, egg weight, egg mass and broken egg of brown layers.

Several studies related with animal productivity have been reported previously and these results correspond with our present results. Steele and Rosebrough (1979) reported that chromium from $\text{CrCl}_3 \cdot 6\text{H}_2\text{O}$ improved the growth rate of turkey poults. Chromium from $\text{CrCl}_3 \cdot 6\text{H}_2\text{O}$ also has been shown to stimulate the growth of rats fed a low protein diet (Mertz and Roginski, 1969). In regards to laying hen, Page (Unpublished) reported that chromium picolinate addition to the diets increased the egg production. But, until now, not so many studies were conducted about the laying hen performance. Accordingly, to investigate the performance of laying hen further studies are needed.

2) Nutrient availability and excretion

The effects of dietary chromium picolinate on the utilization of energy, dry matter, crude protein, crude fat and crude ash are summarized in table 3.

At level of 400 ppb chromium picolinate with low protein, utilization of energy, dry matter and crude protein was significantly higher than control or other dietary chromium picolinate groups.

The utilization of crude fat was the highest in control group and that of crude ash was the highest in 400 ppb chromium picolinate group with low protein level. But utilizability of crude fat and crude ash did not show any statistical difference among treatment groups ($p < 0.05$).

Between protein levels, the utilization of energy, dry matter, crude protein, crude fat and crude ash were higher in high protein.

Table 4 shows the effects of chromium picolinate on the excretion of dry matter and nitrogen of brown layers. The excretion of dry matter and nitrogen was the significantly lower in layers received 400 ppb chromium picolinate in the diets ($p < 0.05$). Especially, the layers fed 400 ppb chromium picolinate supplementation with low protein level reduced the excretion of nitrogen by 25.5% compare to the high protein groups and control groups.

In the study conducted by Kim (1995), broilers were fed 200, 400 and 1,600 ppb of chromium picolinate and chromium picolinate did not affect greatly growth rate, but reduced the excretion of dry matter and nitrogen by 3.3-5.4 and 2-2.8 %, respectively. Chromium picolinate also increased growing performance and reduced the nitrogen

excretion in rat (Min, 1995).

These results are agree with present data, and from these results one can conclude that chromium picolinate not only improves growth rate but decreases nutrient excretion, so it can make a contribution to reduce pollution.

Table 4. Effects of different levels of dietary chromium picolinate on dry matter and nitrogen excretion per kg diet of brown layers (37-47 weeks)

Protein (%)	CrP ¹ (ppb)	Dry matter (g)	Nitrogen (g)
16	0	178.68 ^a	7.87 ^a
	200	173.15 ^a	7.76 ^a
	400	163.70 ^a	7.33 ^a
	800	171.03 ^a	7.66 ^a
14	0	171.88 ^a	7.70 ^a
	200	151.95 ^{ab}	6.81 ^{ab}
	400	130.87 ^b	5.86 ^b
	800	136.07 ^b	6.10 ^b
Between protein levels (%)			
16		170.89 ^a	7.66 ^a
14		147.69 ^b	6.62 ^b
Among CrP levels (ppb)			
	0	173.78 ^a	7.79 ^a
	200	162.55 ^{ab}	7.28 ^{ab}
	400	147.29 ^b	6.60 ^b
	800	153.55 ^b	6.88 ^b
Probability (P) ;			
	Protein	0.0005	0.0004
	CrP	0.0207	0.0210
	Protein & CrP	0.2379	0.2368

¹ Chromium picolinate.

^{a,b}: Values with different superscripts within the same column are significantly different ($p < 0.05$).

3) Glucose and total cholesterol in serum

The level of glucose and total cholesterol in serum of layers fed chromium picolinate are presented in table 5.

Serum glucose content was the lowest in layers receiving 400 ppb chromium picolinate in the diet. Serum glucose was higher in low protein group than high protein group. But all treatments showed no significant difference.

Anderson et al. (1983) reported that chromium picolinate supplementation decreased serum glucose level of human. From these previously conducted studies, one can find consistent results with present data that the content of serum glucose was lowered in 400 ppb

chromium picolinate supplementation.

Total cholesterol content in serum showed similar results to glucose. In all treatments, the lowest cholesterol content was presented in 400 ppb chromium picolinate group with high protein level ($p < 0.05$). Diet, supplemented with 400 ppb chromium picolinate showed the lowest total serum cholesterol content ($p < 0.05$).

Several studies have already shown that chromium supplementation decreased total cholesterol and increased HDL cholesterol (Riales and Albrink, 1981; Mossop, 1983). Mertz (1993) reported that, in monogastric animals supplemented with chromium, reduced total cholesterol concentration in serum is one of the most frequently reported responses in lipid metabolism associated with chromium. Abraham et al. (1980) provided an evidence that chromium not only decreased cholesterol accumulation in rabbits, but also increased the removal rate of cholesterol deposited in the aorta.

Present data also show the same results that the

Table 5. Effects of different levels of dietary chromium picolinate on serum glucose and total cholesterol concentrations of brown layers (37-43 weeks)

Protein (%)	CrP ¹ (ppb)	Glucose (mg/dl)	Total cholesterol (mg/dl)
16	0	152.46	183.16 ^a
	200	143.76	166.17 ^{ab}
	400	148.48	128.56 ^b
	800	137.83	154.29 ^{ab}
14	0	141.76	152.92 ^{ab}
	200	138.73	152.06 ^{ab}
	400	123.89	132.98 ^b
	800	162.77	138.90 ^{ab}
Between protein levels (%)			
16		145.28	156.44
14		139.79	144.60
Among CrP levels (ppb)			
	0	146.04	168.04 ^a
	200	141.75	159.12 ^{ab}
	400	136.19	130.52 ^b
	800	150.30	146.59 ^{ab}
Probability (P) ;			
Protein		0.4848	0.2323
CrP		0.6891	0.0466
Protein & CrP		0.2801	0.5577

¹ Chromium picolinate.

^{ab}: Values with different superscripts within the same column are significantly different ($p < 0.05$).

chromium picolinate supplement lowered the content of serum cholesterol.

4) Crude protein and ether extract in yolk

Table 6 summarizes the effects of dietary chromium picolinate on crude protein and ether extract in egg yolk. Percentage of egg protein in yolk was the highest in eggs of layers received 800 ppb chromium picolinate and the lowest in eggs of layers received 400 ppb chromium picolinate regardless of protein levels ($p < 0.05$).

The lowest percentage of ether extract in egg yolk was recorded in 200 ppb chromium picolinate group and the layers supplemented 400 ppb chromium picolinate showed the highest ether extract in egg yolk but there is no significant difference between treatments.

5) Mortality

Figure 1 shows the effects of dietary chromium picolinate on mortality. Control groups, regardless of protein levels, showed higher mortality than any other group which were supplemented with various levels of chromium picolinate.

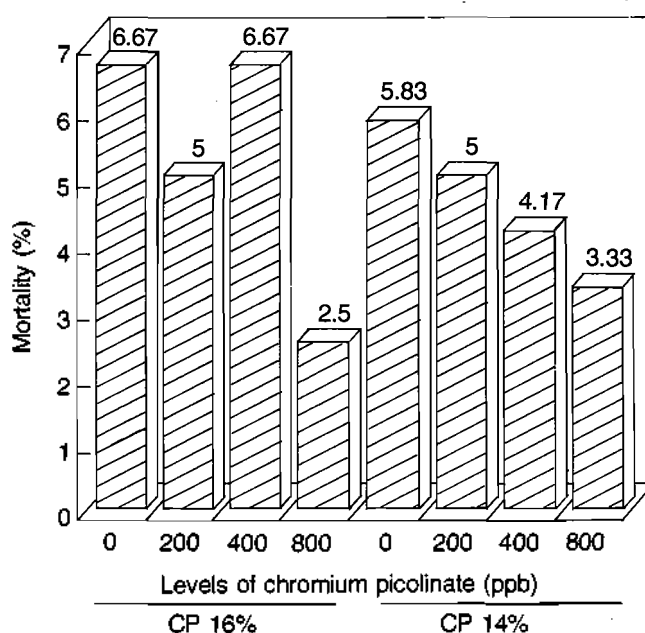


Figure 1. Effects of chromium picolinate on mortality rate of brown layers fed different levels of dietary protein levels.

The effects of dietary chromium on animal mortality have also been investigated in a few previous studies. Schroeder et al. (1965) reported that chromium reduced mortality in female rats on epidemic pneumonia. Mertz and Roginsky (1969) also found that chromium

supplementation reduced mortality in stressed rats subjected to the additional stress of acute haemorrhage. Mowat et al. (1993) reported that Cr⁺³ (as a high-chromium yeast supplement) reduced circulating cortisol and alleviated shipping stress morbidity of calves during a post-stress feeding trial. This result is suggesting that chromium may be a limiting mineral in corn silage. Such studies are shown the similar results with the present data.

Decreased mortality caused by chromium picolinate supplementation provides a economic benefits than just temporarily improved performance of layers.

Table 6. Effects of different levels of dietary chromium picolinate on yolk crude protein and ether extract contents in eggs of brown layers (37-43 weeks)

Protein (%)	CrP ¹ (ppb)	Crude protein (%)	Ether extract (%)
	0	31.50	59.43
16	200	31.79	59.38
	400	31.17	59.67
	800	31.53	59.27
14	0	31.62	59.55
	200	31.28	58.08
	400	31.36	59.83
	800	31.94	59.07
Between protein levels (%)			
16		31.50	59.44
14		31.55	59.13
Among CrP levels (ppb)			
	0	31.56 ^{ab}	59.49
	200	31.53 ^{ab}	58.73
	400	31.26 ^b	59.75
	800	31.73 ^a	59.17
Probability (P) ;			
Protein		0.7260	0.4514
CrP		0.1947	0.3167
Protein & CrP		0.1837	0.5362

¹ Chromium picolinate.

^{ab}: Values with different superscripts within the same column are significantly different (p < 0.05).

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