R. Bras. Zootec., 50:e20200173, 2021
https://doi.org/10.37496/rbz5020200173

Non-ruminants
Full-length research article

Revista Brasileira de Zootecnia

Brazilian Journal of Animal Science
e-ISSN 1806-9290
www.rbz.org.br

Effects of chromium yeast supplementation on productive and metabolic responses of laying hens fed diets containing different energy levels

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Received: July 22, 2020
Accepted: December 21, 2020

How to cite: Siloto, E. V.; Sartori, J. R.; Santos, T. S.; Fascina, V. B.; Centenaro, L. P.; Miranda, C. C.; Pinke Testa, C. A. E. and Sartori, D. R. S. 2021. Effects of chromium yeast supplementation on productive and metabolic responses of laying hens fed diets containing different energy levels. Revista Brasileira de Zootecnia 50:e20200173. https://doi.org/10.37496/rbz5020200173

ABSTRACT - This study evaluated the effects of chromium yeast (Cr yeast) and two apparent metabolizable energy (AME) levels on productive performance, egg quality, and lipid and carbohydrate metabolism in laying hens. A total of 192 Bovans White laying hens at 47 weeks of age were randomly assigned to eight dietary treatments (six replicates each) in a 4 × 2 factorial arrangement: four levels of supplemental Cr (0, 0.2, 0.4, and 0.8 ppm as Cr yeast) and two AME levels (2,780 and 2,900 kcal AME kg^{-1}). No significant effect of Cr yeast supplementation on feed intake, egg weight, egg production, intact eggs, egg mass, feed conversion ratio, or egg quality was observed. Egg quality parameters and Cr content in the yolk were not affected by dietary treatments. Plasma glucose and lipid levels were not influenced by either Cr yeast or AME levels used in this study. However, Cr yeast supplementation improved yolk percentage and hepatic glycogen content. The inclusion of Cr yeast at 0.2 and 0.4 ppm induced the highest hepatic glycogen content with the energy levels 2,900 and 2,780 kcal AME kg^{-1}, respectively. Laying hens fed 2,900 kcal AME kg^{-1} showed the highest abdominal fat. The results observed in the present study support the hypothesis that the lack of positive effects of Cr yeast supplementation on production performance and egg quality may be related to good management practices, as the birds were not subjected to stressful conditions.

Keywords: egg quality, laying hen, lipid metabolism, mineral supplementation, serum parameters

1. Introduction

Increasing evidence has supported that supplemental chromium (Cr) in diets for poultry may show multiple beneficial effects on their health and production (Khan et al., 2014). Chromium is involved in carbohydrate, lipid, protein, and nucleic acid metabolism, and its interaction with DNA provides the formation of a low-molecular-weight Cr-binding protein (chromodulin), which binds with the insulin receptor (Vincent, 2000; Sreejayan et al., 2008; Vincent, 2010). Vincent (2015) proposed that Cr may act as a second messenger, amplifying insulin signaling.

The most common Cr forms used as a dietary supplement to poultry and animal diets are Cr picolinate (CrPic), Cr nicotinate (CrNic), Cr propionate (CrPro), Cr bound to yeast (Cr yeast), and Cr methionine

(CrMet) (Farag et al., 2017; Valera et al., 2019). Chromium picolinate and Cr yeast are the most used sources, and beneficial effects on productive indicators, as well as metabolic and immune factors were observed in broilers and laying hens (Kani, 2015; Farag et al., 2017; Valera et al., 2019). Research on laying hens has shown positive effects on egg production, feed efficiency, and egg quality when CrPic was added to the diets (Lien et al., 1996; Sahin et al., 2001a; Sahin et al., 2002b). According to Ma et al. (2014), CrPro supplementation improved egg production and eggshell thickness in late-phase laying hens.

Several studies have shown that CrPic supplementation improves growth rate and feed efficiency in broilers (Amatya et al., 2004; Jackson et al., 2008). Still in broilers, Toghyani et al. (2006) observed that CrPic may reduce abdominal fat. Body fat reduction induced by Cr supplementation can be accounted for the activation of sympathetic nervous system-mediated hypothalamic insulin, which cause increased thermogenesis, as observed in a study by Brief and Davis (1984), wherein insulin led to thermogenesis and decreased feed intake in rats. Thus, Cr supplementation can reduce body fat by either increasing energy expenditure or reducing calorie intake (Hasten, 1997). For poultry production, excessive fat can impair egg production (Costa et al., 2009). Striffler et al. (1995) found that rats were more responsive to diets with high levels of energy supplemented with Cr. Nevertheless, there are few studies reporting the association of dietary Cr supplementation and energy levels in poultry.

Therefore, this study aimed to evaluate the effects of Cr yeast supplementation in diets with two energy levels on productive and metabolic responses of laying hens.

2. Material and Methods

2.1. Birds and management

All procedures with animals used in this research were performed according to local Ethical Committee for Animal Use (case number 06/2011). This study was carried out in Botucatu, SP, Brazil (latitude 22°55'28" S, longitude 48°25'38" W, and elevation of 760 m). A total of 192 47-week-old commercial Bovans White hens with initial body weight of 1.491±0.077 kg was randomly distributed into eight dietary treatments with four levels of supplemental Cr yeast (0, 0.2, 0.4, and 0.8 ppm) and two metabolizable energy levels (2,780 and 2,900 kcal of AME kg^{-1}). Six replicates of four hens were assigned to each treatment. The birds were housed in 42 metal cages (40 × 45 × 45 cm) with individual galvanized trough feeders and nipple drinkers. Water and feed were supplied *ad libitum* throughout the experimental period (112 days or four 28-d cycles). The hens were provided with artificial illumination of 16 h light/day. The average temperature in this period was 22.8±6 °C.

2.2. Diet preparation

The layers were fed a corn-soybean meal-based diet to meet their nutritional requirements according to recommendations of Rostagno et al. (2005) for lightweight hens, except for apparent metabolizable energy (AME), which was supplied according to experimental treatments (Table 1). The Cr source used in this study was derived from yeast (Co-Factor III, Alltech Inc., Curitiba, PR, Brazil), and the different Cr levels were supplemented as suggested by the manufacturer. The percentages of Cr included in the experimental diets were 0, 0.02, 0.04, and 0.08% Co-Factor III® to obtain four levels of supplemental Cr yeast (0, 0.2, 0.4, and 0.8 ppm), respectively. Chromium concentrations in four treatments with 2,780 kcal AME kg^{-1} were 0.01, 0.25, 0.45, and 0.75 ppm, and the concentrations in the other four treatments with 2,900 kcal AME kg^{-1} were 0.01, 0.18, 0.44, and 0.77 ppm, according to measurements using the atomic absorption spectrometer (Shimadzu AA-6800, Kyoto, Japan) with a graphite furnace (Silva et al., 2006). Results indicated that Cr levels in diets without the addition of Cr (control diets) were very low (0.01 ppm).

Table 1 - Ingredients and nutrient composition of the diets (basal diets)

Item	AME (kcal kg^{-1})	
	2,780	2,900
Ingredient		
Corn	61.18	59.45
Soybean meal (45%)	24.74	25.39
Wheat bran	1.00	0.00
Soybean oil	1.46	3.54
Salt	0.30	0.30
Limestone	9.37	9.35
Dicalcium phosphate	1.43	1.45
DL-methionine 99%	0.14	0.14
Vitamin mix[1]	0.15	0.15
Mineral mix[2]	0.15	0.15
Carrier[3] or chromium yeast[4]	0.08	0.08
Calculated composition		
AME (kcal kg^{-1})	2,780	2,900
Crude protein (%)	16.5	16.5
Calcium (%)	4.03	4.02
Available phosphorus (%)	0.36	0.36
Methionine (%)	0.40	0.40
Methionine + cysteine (%)	0.68	0.68
Lysine (%)	0.85	0.84
Sodium (%)	0.22	0.22
Chlorine (%)	0.20	0.20

AME - apparent metabolizable energy.
[1] Vitamin suplement (Postura C, Multimix) per kg feed: vitamin A, 10,500 IU; vitamin D, 3,000 IU; vitamin E, 7.5 mg; vitamin K, 2.7 mg; thiamin, 1.0 mg; niacin, 30 mg; riboflavin, 4.5 mg; pyridoxine, 1.0 mg; vitamin B12, 12 µg; pantothenic acid, 7.5 mg; folic acid, 0.4 mg; biotin, 0.015 mg; antioxidant, 22.5 mg.
[2] Mineral supplement (Multimineral aves, Multimix®) per kg feed: copper, 12 mg; iron, 75 mg; manganese, 105 mg; zinc, 75 mg; iodine, 1.8 mg; selenium, 0.3 mg.
[3] Inert filler used to complete diet formulations to 100%.
[4] For diets with 0.2, 0.4, and 0.8 ppm Cr, 0.02, 0.04, and 0.08% Co-Factor III® Alltech were included, respectively.

2.3. Performance and egg quality parameters

Feed intake and egg weight were recorded weekly (to calculate egg mass and feed conversion ratio per dozen eggs), whereas egg production and percentage of intact eggs were determined daily. Feed conversion was calculated as the ratio between feed intake and egg mass.

At the end of each experimental period (28-d period), two eggs per replicate (cage) were collected daily for three consecutive days – a total of 36 eggs per treatment – to evaluate the following parameters: specific egg gravity, eggshell thickness, yolk, albumen and shell percentages, yolk index, and Haugh unit. Specific egg gravity was determined using the method of egg immersion in saline solution. Solutions were prepared with densities between 1.060 to 1.100 with 0.005 graded variation between each one. Specific gravities were determined using a densitometer (Incoterm Mod 5582, Porto Alegre, Brazil). Eggs were then broken, and their eggshell, albumen, and yolk were separated and weighed. Eggshells were dried in a forced-ventilation oven at 60 °C for three days to calculate eggshell percentage, and their thickness was determined in three points using a precision micrometer (Mitutoyo Mod PK0510, Kawasaki, Japan). Albumen height was measured using the same micrometer. Haugh unit was calculated through the equation suggested by Card and Nesheim (1966): $HU = 100 \log (H + 7.57 - 1.7 W^{0.37})$, in which H = albumen height (mm), W = egg weight (g), 7.57 = correction factor for albumen height, and 1.7 = correction factor for egg weight. Chromium content in the yolk was determined by using the graphite furnace atomic absorption spectroscopy technique (Silva et al., 2006).

2.4. Sample collection and analytical determination

At the end of each 28-d cycle, blood samples of two laying hens randomly chosen from each treatment (two layers per replicate) were obtained from the wing vein using heparin as anticoagulant. Blood samples were centrifuged at 3000 rpm for 10 min, and plasma was collected and stored at −20 °C until analysis of biochemical parameters. Plasma cholesterol (lot 0047), high-density lipoprotein (HDL) – cholesterol fraction (lot 0097) –, triglycerides (TG) (lot 0027), and glucose concentrations (lot 0066) were measured using enzymatic assay kits (Bioclin®) in an automated clinical chemistry analyzer (Mindray BS2000®, São Paulo, SP, Brazil). Plasma concentration of very low-density lipoprotein (VLDL) was calculated with Friedwald equation, dividing TG levels by 5 (Friedewald et al., 1972).

For the analysis of the deposition of Cr in egg yolk, a pool of three egg yolks per repetition on the last day of each cycle was used. The yolks were dried at 50 °C for 72 h and frozen at −20 °C for further analysis. The pool of three egg yolks was freeze dried (L108 – Liobras, São Carlos, SP, Brazil), milled (Geno, Grinder 2010 – Sprex Samples Prep, Metuchen, NJ, USA), and then subjected to ultrasonic extraction (UNIQUE model USC-DC ultrasonic cell disruptor, Campinas, SP, Brazil). The Cr content in the yolk was determined using the graphite furnace atomic absorption spectroscopy technique (SHIMADZU AA-6800 – Shimadzu Corporation, Kyoto, Japan) according to Silva et al. (2006) with modifications.

At the end of the experimental period (112 d), six laying hens per treatment were weighed and sacrificed by cervical dislocation. The liver was removed, weighed, and frozen at −5 °C for further analysis of fat and glycogen content. Abdominal fat was also removed and weighed. After extraction, liver fat was determined gravimetrically with the method of Folch et al. (1957). Briefly, the lipids were extracted with chloroform: methanol solution. After centrifugation, aliquots of the lower phase, which contains the tissue lipids, were transferred to previously weighed petri dishes and left to dry (65 °C). Hepatic glycogen was extracted with 30% KOH and precipitated with alcohol (Carroll et al., 1956), and the amount recovered was determined by the colorimetric anthrone method of Collowick and Kaplan (1957), using a spectrophotometer (Bel Photonics 2000UV, Monza, Milan, Italy).

2.5. Statistical analysis

Data were subjected to analysis of variance (ANOVA) using the General Linear Model procedure of SAS (Statistical Analysis System, version 9.0). When necessary, the treatment means were compared by Tukey's test ($P<0.05$). Regression analyses of the Cr factor were not performed when the model was not significant or when adequate biological results were not obtained ($R^2<0.70$), according to the PROC REG statement. The statistical model applied for performance, egg quality, plasma cholesterol, HDL cholesterol fraction, TG, glucose concentrations, Cr in egg yolk, hepatic fat, hepatic glycogen, and abdominal fat was:

$$Y_{ij} = \mu + AME_i + Cr_j\,(AME \times Cr)_{ij} + \varepsilon_{ij},$$

in which Y_{ij} = dependent variable, μ = overall mean, AME_i = apparent metabolizable energy, Cr_j = chromium levels, $AME \times Cr$ = interaction between apparent metabolizable energy and chromium levels, and ε_{ij} = random error.

3. Results

No effect of Cr yeast supplementation ($P>0.05$) on feed intake, egg weight, egg production, intact eggs, egg mass, and feed conversion ratio was observed in this study (Table 2). Egg quality parameters and Cr content in the yolk were not significantly ($P>0.05$) affected by the different levels of supplemental Cr (Table 3). The yolk percentage showed interaction ($P<0.05$) between Cr level and dietary energy (Table 3), and a linear effect of Cr supplementation on yolk percentage was detected in hens fed 2,780 kcal AME kg^{-1} (yolk percentage = 24.90033 + 0.168x; $R^2 = 0.98$).

Table 2 - Performance parameters of laying hens supplemented with chromium yeast and two energy levels

		Feed intake (g day^{-1})	Egg weight (g)	Egg production (%)	Intact eggs (%)	Egg mass (g day^{-1})	Feed conversion ratio (kg dz^{-1})
Cr yeast (ppm)							
0.0		116.00	65.04	92.14	99.05	59.94	1.95
0.2		113.33	65.30	88.47	98.71	57.73	2.01
0.4		114.92	64.85	92.47	98.84	59.93	1.93
0.8		116.50	64.33	92.42	98.66	59.44	1.98
AME (kcal kg^{-1})							
2,780		115.66	64.95	90.54	98.73	58.77	1.99
2,900		114.71	64.82	92.22	98.90	59.75	1.94
Cr	AME						
0.0	2,780	113.67	65.09	90.22	99.00	58.74	1.96
0.2	2,780	114.67	65.84	86.64	98.46	57.00	2.08
0.4	2,780	116.50	65.31	92.41	98.88	60.33	1.94
0.8	2,780	118.33	63.55	92.87	98.60	59.02	2.02
0.0	2,900	118.83	64.98	94.08	99.11	61.14	1.95
0.2	2,900	112.00	64.76	90.29	98.96	58.46	1.94
0.4	2,900	113.33	64.40	92.52	98.81	59.53	1.92
0.8	2,900	114.67	65.12	91.97	98.71	59.86	1.94
P-value							
Cr		0.515	0.690	0.465	0.866	0.638	0.630
AME		0.551	0.825	0.425	0.651	0.486	0.175
Cr × AME		0.138	0.265	0.796	0.952	0.871	0.705
SEM		0.809	0.284	1.013	0.167	0.664	0.021
CV		4.78	3.11	7.91	1.25	8.12	7.72

AME - apparent metabolizable energy; SEM - standard error of the mean; CV - coefficient of variation.

The interaction (P<0.05) between AME levels and levels of Cr yeast inclusion (Table 3) demonstrated that in the two treatments without Cr supplementation a lower yolk percentage was observed in laying hens fed a diet with 2,780 kcal AME kg^{-1}. Dietary Cr yeast addition at 0.8 ppm resulted in a lower yolk percentage in laying hens fed a diet with 2,900 AME kg^{-1}.

An interaction (P<0.05) between AME levels and supplementation of Cr levels on hepatic glycogen content was found (Table 4). In laying hens fed 2,780 kcal AME kg^{-1}, a quadratic effect was observed on hepatic glycogen content (hepatic glycogen = 0.77831 + 0.94695x – 1.50928x^2; R^2 = 0.83) by increasing Cr yeast supplementation. Laying hens supplemented with 0.2 ppm Cr yeast showed higher (P<0.05) hepatic glycogen content when fed a diet containing 2,900 kcal AME kg^{-1}. However, in birds fed diet supplemented with 0.4 ppm Cr yeast, lower glycogen content was observed in the treatment with 2,900 kcal AME kg^{-1}. Chromium supplementation did not affect the hepatic glycogen content in laying hens fed 2,780 kcal AME kg^{-1} (Table 4).

The Cr yeast and AME levels used in this study did not affect the plasma cholesterol, HDL, VLDL, or TG levels of the layers (Table 4). An increase (P<0.05) in abdominal fat was detected in the treatments with 2,900 kcal AME kg^{-1} (Table 4).

4. Discussion

In the present study, no effects of Cr yeast supplementation (0.2, 0.4, or 0.8 mg kg^{-1}) on production performance of laying hens were observed, which is consistent with other studies. Eseceli et al. (2010) reported that egg production and egg weight were unchanged in laying hens fed the diet supplemented with Cr yeast at 0.15 mg kg^{-1}. Similarly, no effect of CrMet (400 µg kg^{-1}) and CrPic (0.4-0.6 mg kg^{-1}) supplementation on egg production or egg weight was detected in laying hens (Karami et al., 2018; Zhang et al., 2018). In laying quails, egg production and egg weight were not affected by dietary

treatment with Cr chloride ($CrCl_3$; 1000 ppm) and CrPic (100 ppm; Yeşilbağ and Eren, 2009). Nonetheless, another study showed that CrPic (0.2-0.8 mg kg^{-1}) supplementation improved egg weight and egg production in late-phase laying hens (Ma et al., 2014). In addition, dietary supplemental CrPic (250, 500, 750, and 1000 ppb) increased performance parameters, particularly egg production in laying quails (Yildiz et al., 2004).

Several factors could explain the discrepancy between the results, such as age of birds, experimental period, stressful conditions, supplementation level, and bioavailability of Cr source. Chromium yeast was used in this study, yet the other organic and inorganic Cr sources have been used in several other studies. Previous research has confirmed that organic Cr has higher bioavailability than inorganic Cr, reaching values closer to 25% compared with the low digestibility of the inorganic source, which is around 1% (Piva et al., 2003; Valera et al., 2019).

In general, the beneficial effects of Cr to improve performance parameters can be more efficiently found in animals under stressful conditions, especially in birds reared under not only cold or heat stress (Lin and Lin, 1999; Sahin et al., 2001a; Sahin et al., 2002c; Khan et al., 2014; Jahanian and Rasouli, 2015), but also high stocking density (Mirfendereski and Jahanian, 2015). Similarly, investigations in ruminants have shown that dietary supplemental Cr in stressed feeder calves improved humoral immune function and performance (Kegley et al., 1996); in contrast, Cr supplementation did not reveal positive results in growth performance of non-stressed feeder calves (Chang et al., 1995). It is important to highlight that the results of the present study were obtained from laying hens subjected to neither environmental nor management stressful conditions. In dairy cows, CrMet supplementation during dry period did not indicate positive results on milk production, colostrum, or immunoglobulin

Table 3 - Egg quality parameters and chromium content in the egg yolk of laying hens supplemented with chromium yeast and two energy levels

		Specific gravity (g L^{-1})	Shell thickness (mm)	Egg shell (%)	Albumen index (%)	Haugh unit	Cr yolk (µg kg^{-1}*10)	Yolk (%)[1]
Cr yeast (ppm)								
0.0		1.088	0.39	9.07	65.48	80.69	45.97	25.45
0.2		1.088	0.38	9.11	65.57	79.86	46.23	25.33
0.4		1.088	0.38	9.13	65.22	81.06	45.91	25.65
0.8		1.089	0.39	9.22	64.84	81.45	46.25	25.88
AME (kcal kg^{-1})								
2,780		1.088	0.38	9.14	65.34	81.36	46.16	25.49
2,900		1.089	0.39	9.13	65.22	80.16	46.01	25.67
Cr	AME							
0.0	2,780	1.088	0.38	9.12	65.94	80.76	46.11	24.96B
0.2	2,780	1.088	0.38	9.08	65.56	80.44	46.55	25.21
0.4	2,780	1.089	0.38	9.17	65.35	81.71	45.91	25.47
0.8	2,780	1.088	0.39	9.20	64.50	82.54	46.06	26.30A
0.0	2,900	1.088	0.39	9.02	65.03	80.61	45.79	25.94A
0.2	2,900	1.088	0.39	9.14	65.58	79.27	45.90	25.44
0.4	2,900	1.088	0.38	9.10	65.08	80.41	45.91	25.82
0.8	2,900	1.090	0.38	9.25	65.18	80.34	46.25	25.46B
P-value								
Cr		0.709	0.464	0.836	0.596	0.214	0.5492	0.388
AME		0.661	0.819	0.474	0.121	0.681	0.5056	0.265
Cr × AME		0.766	0.756	0.792	0.119	0.899	0.3686	0.028
SEM		0.001	0.010	0.034	0.121	0.460	0.186	0.111
CV		0.18	3.55	2.66	1.21	4.09	1.58	2.78

AME - apparent metabolizable energy; SEM - standard error of the mean; CV - coefficient of variation.
[1] Linear effect in laying hens fed 2,780 kcal AME kg^{-1} (yolk percentage = 24.90033 + 0.168x; R^2 = 0.98).
In the interactions between factors, means followed by lowercase letters at the same AME level (2,780 or 2,900 kcal kg^{-1}) differ by Cr level, and those followed by uppercase letters at the same Cr level (0.0, 0.2, 0.4, and 0.8 ppm) differ by AME level by 5% Tukey's test.

(IgG) parameters, and the authors attributed it to the positive effects of Cr on the immune cells of cows after parturition (Gultepe et al., 2018). Thus, the referred studies in birds and dairy cows may help corroborate the hypothesis of the present study, which supports that the lack of expected effects of Cr supplementation on performance parameters is closely linked to good management practices and stress-free animals.

Supplemental dietary Cr yeast (0.2, 0.4, or 0.8 mg kg^{-1}) did not affect egg quality in this study, which is a response also obtained in previous studies. Eseceli et al. (2010) reported that eggshell thickness and Haugh unit were unchanged in eggs laid by hens fed diets supplemented with Cr yeast at 0.15 mg kg^{-1}. Similarly, CrPic addition did not affect eggshell thickness, eggshell strength, yolk color, or Haugh unit of brown-egg laying hens (Zhang et al., 2018). Lien et al. (1996) and Uyanik et al. (2002) demonstrated no effect in shell thickness of laying hens supplemented with CrPic (0.4 or 0.6 mg kg^{-1}) and $CrCl_3.6H_2O$ (20 ppm), respectively. However, Lien et al. (1996) found increased Haugh unit as well as reduced eggshell strength and eggshell thickness in White Leghorn laying hens fed diets supplemented with CrPic at 0.4 or 0.6 mg kg^{-1}. Ma et al. (2014) described that CrPro supplementation (0.4 or 0.6 mg kg^{-1}) improved eggshell thickness and reduced Haugh unit in hens during their late laying period. Haugh unit is a frequently used index for assessing egg internal quality, and studies have shown some improvement on Haugh unit after Cr supplementation. Sahin et al. (2018) found an improvement in Haugh unit of layers subjected to heat stress fed diet supplemented with Cr histamine. The above inconsistent results may have been due to the different lineages and ages of layers, Cr sources, and environmental conditions.

Table 4 - Blood and hepatic biochemical parameters and abdominal fat in laying hens supplemented with chromium yeast and two energy levels

		Cholesterol (mg dL^{-1})	TG (mg dL^{-1})	VLDL (mg dL^{-1})	HDL (mg dL^{-1})	Glucose (mg dL^{-1})	Hepatic fat (%)	Hepatic glycogen (%)[1]	Abdominal fat (%)
Cr yeast (ppm)									
0.0		111.69	1008.57	201.71	15.32	225.20	7.42	0.856	2.017
0.2		120.64	1148.20	229.64	14.38	226.77	9.32	1.000	2.099
0.4		113.73	1074.42	214.88	12.99	217.89	7.19	0.786	2.021
0.8		116.83	1036.32	207.26	13.00	224.29	7.28	0.647	1.854
AME (kcal kg^{-1})									
2,780		114.61	1047.28	209.46	14.31	224.11	8.04	0.779	1.866B
2,900		116.84	1086.48	217.30	13.53	222.96	7.56	0.834	2.130A
Cr	AME								
0.0	2,780	108.96	973.42	194.68	227.89	16.03	7.18	0.799	1.733
0.2	2,780	122.92	1180.52	236.10	226.08	16.75	11.14	0.787B	1.944
0.4	2,780	116.32	1068.13	213.62	219.24	12.82	7.00	0.936A	1.999
0.8	2,780	110.23	967.05	193.41	223.25	11.65	6.85	0.567	1.790
0.0	2,900	114.42	1043.72	208.74	222.50	14.60	7.66	0.927ab	2.302
0.2	2,900	118.36	1115.88	223.17	227.47	12.00	7.50	1.161Aa	2.255
0.4	2,900	111.14	1080.71	216.14	216.55	13.15	7.39	0.606Bb	2.044
0.8	2,900	123.43	1105.60	221.12	225.34	14.35	7.71	0.723ab	1.918
P-value									
Cr		0.4398	0.2310	0.2310	0.6006	0.0571	0.0823	0.0202	0.5576
AME		0.5863	0.4337	0.4337	0.5863	0.6291	0.4649	0.2362	0.0389
Cr × AME		0.3335	0.5245	0.5245	0.3233	0.6468	0.0647	0.0073	0.4556
SEM		2.024	4.879	4.976	0.707	1.220	0.356	0.045	0.188
CV		12.17	16.09	16.09	35.67	3.66	29.10	25.52	21.38

TG - triglycerides; VLDL - very low-density lipoproteins; HDL - high density lipoproteins; AME - apparent metabolizable energy; SEM - standard error of the mean; CV - coefficient of variation.
[1] Quadratic effect in laying hens fed 2,780 kcal AME kg^{-1} (hepatic glycogen = $0.77831 + 0.94695x - 1.50928x^2$; $R^2 = 0.83$).
A,B - Means in the same column followed by different uppercase letters are different by F test ($P<0.05$).
In the interactions between factors, means followed by lowercase letters at the same AME level (2,780 or 2,900 kcal kg^{-1}) differ by Cr level, and uppercase letters at the same Cr level (0.0, 0.2, 0.4, and 0.8 ppm) differ by AME level by 5% Tukey's test.

In the current study, Cr yeast supplementation did not affect the Cr level in the yolk, the same way that Cr supplementation as $CrCl_3$, Cr yeast, and Cr aminoniacinate did not affect the Cr level in the yolk of eggs of laying hens in the study by Piva et al. (2003). In contrast, supplemental NanoCrPic resulted in Cr accumulation in the egg yolk of old post-molt laying hens (Sirirat et al., 2013). The above-mentioned inconsistent results may be attributed to the different laying phases of layers, as well as different Cr sources and digestibility. Previous studies have demonstrated that the transport of NanoCr, compared with CrPic and $CrCl_3$, exhibited considerably higher absorption efficiency (Zha et al., 2008), as well as increased cellular uptake (Win and Feng, 2005).

A linear effect of Cr yeast supplementation on egg yolk percentage was detected in this study in laying hens fed 2,780 kcal AME kg^{-1}. These results confirm those obtained by Abdallah et al. (2013), who observed that the more the dietary CrPic levels increased, the more the egg yolk percentage raised in laying hens, and those by Sahin et al. (2001b), who found the same effect in Japanese quails. Hepatic lipogenesis from glucose was increased by about 60% due to Cr^{+3} supplementation in turkey poults (Rosebrough and Steele, 1981). Lipogenesis from [U^{-14C}] glucose by isolated hepatocytes was significantly enhanced by CrPic supplementation in broilers (Lien et al., 1999). In the present study, the higher yolk percentage observed in laying hens fed 2,780 kcal AME kg^{-1} and Cr addition may be due to their increased hepatic lipid metabolism. Lipids are major precursors of yolk formation in the liver (Yin et al., 2000), which are released into the bloodstream and then become available for ovarian follicle growth; thus, it may result in a larger-sized yolk.

Previous studies in broilers reported that Cr supplementation could lower abdominal fat (Lien et al., 1999; Sahin et al., 2002a; Toghyani et al., 2006). However, this effect preventing abdominal fat accumulation was not observed in this study; on the contrary, a higher abdominal fat level was found in laying hens fed diets containing 2,900 kcal AME kg^{-1}, yet with no hepatic fat accumulation. Hepatic lipogenesis is very responsive to dietary changes (Leveille et al., 1975; Kersten, 2001). The diet with the highest energy level used in this study (2,900 kcal AME kg^{-1}) may have stimulated hepatic lipogenesis and contributed to higher accumulation of abdominal fat in layers. In avian species, the *de novo* lipogenesis is restricted to the adipose tissue and does not occur in the ovary. Thus, TG storage in these compartments depends on the availability of a plasma lipid substrate originating from either the diet or hepatic lipogenesis, as the liver is the major site of fatty acid synthesis (Scanes and Braun, 2013). Additionally, Cr in association with the diet with higher energy level may have contributed to the accumulation of abdominal fat, since Cr has been reported to have an insulinomimetic action and to possibly increase apolipoprotein A1 (ApoA-1) transcript by either direct and indirect mechanisms via PPARα or insulin responsive elements on the ApoA-1 promoter (Mooradian et al., 2006; Siripurkpong and Na-Bangchang, 2009).

The effects of Cr supplementation on blood lipids are still controversial in birds. In the present study, Cr yeast supplementation did not affect the plasma lipid concentration in laying hens. These results are in accordance with the findings of Nakaue and Hu (1997), who identified no difference in blood TG induced by CrPic supplementation (200 and 800 ppb) in young or old laying hens. In addition, CrPro supplementation did not affect plasma cholesterol in late-phase laying hens (Ma et al., 2014). However, other studies have shown that organic Cr supplementation (Cr yeast and CrPic) resulted in lower plasma cholesterol, VLDL, and TG, as well as higher HDL in laying hens (Lien et al., 1996; Lien et al., 2004; Du et al., 2005).

There was significant interaction between AME levels and Cr supplementation levels in liver glycogen content. The hepatic glycogen content was higher only with 0.2 ppm Cr yeast in both levels of dietary energy. Moreover, laying hens fed the diet containing 2,900 kcal AME kg^{-1} presented the highest hepatic glycogen content when supplemented with 0.2 ppm Cr yeast, while those fed 2,780 kcal AME kg^{-1} had the highest content when supplemented with 0.4 ppm Cr yeast. Nevertheless, Cr yeast effects on the hepatic glycogen were not observed in the laying hens fed diets supplemented with a higher Cr yeast level (0.8 ppm). These results cannot be explained since the highest level of Cr was expected to cause the highest hepatic glycogen concentration. In a previous study, an increase in liver glycogen content was reported in turkeys fed $CrCl_3$ (20 ppm) due to increased glycogen synthetase-enzyme activity (Rosebrough and Steele, 1981).

5. Conclusions

The results observed in the present study support the hypothesis that the lack of positive effects of Cr yeast supplementation (0.2-0.8 ppm) on production performance and egg quality of laying hens may be related to good management practices, as the birds were not subjected to stressful conditions.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

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Acknowledgments

The authors are grateful to Alltech do Brasil, Curitiba, which supported this study through grants and supplied the Cr yeast (Co-Factor III). This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001.

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