



Original Research Article

Chromium propionate improves performance and carcass traits in broilers



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ABSTRACT

There is evidence to suggest that poultry may have a dietary requirement for metabolically available chromium (Cr) that exceeds the amount provided through wheat soybean meal diets. The objective of the present study was to investigate the effects of dietary supplemental organic Cr from Cr propionate at different dose levels (control = 0 µg/kg, T1 = 200 µg/kg, T2 = 400 µg/kg) on the growth performance, carcass traits, and meat quality of broilers. Weight gain and feed intake of each treatment were recorded at the start and after 14, 28 and 35 d, and feed conversion ratios (FCR) were calculated accordingly. At 35 d of age, birds were randomly selected and euthanized for carcass evaluation. Results of the first trial indicate that both Cr propionate treatments increased final body weight ($P < 0.05$), feed efficiency ($P < 0.05$) and body weight gain ($P < 0.0001$). Furthermore, Cr propionate supplementation improved ($P < 0.0001$) all carcass characteristics. Interestingly, with increased Cr dosage, carcass yield, dressing percentage and breast meat yield increased linearly ($P < 0.0001$). The second study reveals that the feed intake in the control group was significantly higher compared to both Cr propionate supplemented groups (T1 & T2). Furthermore, the Cr propionate supplemented T2 group displayed a significantly lower FCR than the control and T1 group ($P = 0.027$). Finally, Cr propionate supplementation increased the dressing percentage compared to control birds ($P < 0.0001$). In the third experiment, Cr propionate supplementation (T1 & T2) increased final body weight and decreased FCR compared with the control treatment. These effects were highly significant ($P < 0.0001$) throughout all feeding phases of the trial. Cr propionate supplementation also increased ($P < 0.0001$) carcass yield, dressing percentage, breast meat yield, leg and thigh weights compared with the control treatment. In conclusion, growth performance, feed conversion, carcass yield, breast and leg meats of broiler birds can be significantly improved by dietary inclusion of Cr propionate. Cr propionate can be supplemented to broiler birds from 1 d old of age at a level that provides 200 or 400 µg/kg organic Cr and can increase the efficiency of broiler production.

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1. Introduction

The intensification of broiler production (FAO, 2004) results in the search for nutrient compounds that can maximize broilers' zootechnical performance and carcass parameters. Nutrition is the key to capitalize on improved genetic potential on the farm. Nutrients must be supplied in the correct amounts and balanced to support rapid and efficient body weight gains. Micronutrients as trace minerals play a vital role in various metabolic, enzymatic and biochemical reactions ultimately leading to a better growth rate, egg production and feed efficiency (Van der Klis and Kemme, 2002). Awareness on the role of micronutrients in poultry farming is of interest for farmers, veterinarians and other

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investigators. For many years, chromium (Cr) has been considered by many nutritionists as an essential nutrient for animals and humans (NRC, 1997). Furthermore, there is evidence to suggest that pigs, ruminants and poultry may have a dietary requirement for Cr that exceeds that found in corn soya meal diets (Amata, 2013).

Cr has appeared as key mineral that plays a pivotal role in carbohydrate and lipid metabolism, along with protein synthesis, growth and longevity (Mertz, 1993). More specifically, Cr is thought to facilitate activation of insulin through the glucose tolerance factor (Schwarz and Mertz, 1959). For instance, Cr is essential for the synthesis of the specific low molecular weight Cr-binding substance that, upon conversion to chromodulin, activates the insulin signaling cascade. This results in greater cell permeability to insulin, with as subsequent stimulating effect on the metabolism of carbohydrates, lipids and proteins (Lukaski, 1999).

Based on literature data, the carbohydrate metabolism of chickens (and birds in general) is greatly different from that of mammals, which is reflected by higher physiological plasma glucose levels (Mátis et al., 2014). Further, elevated blood glucose concentration is associated with decreased plasma insulin levels and decreased insulin sensitivity in most extrahepatic tissues. Thereby, improving the insulin responsiveness by adding Cr might be of vital importance in fast growing broilers. Since insulin is an anabolic hormone and it is highly involved in the regulation of growth, studying Cr supplementation in chicken may be of special interest not only from comparative physiological approach, but from food production point of view, as well (Mátis et al., 2014).

Studies have shown that dietary Cr supplementation beneficially affects physiological functions such as cell preservation and immune response that are of utmost importance to animal homeostasis and thermoregulatory capacity under heat stress conditions (Dalolio et al., 2018). Furthermore, Cr has antioxidant properties which help to attenuate the negative effects of oxidative stress. A dietary supplementation of Cr has been suggested as a new approach to increase meat quality of animals (Ohh and Lee, 2005) as Cr supplementation is well-known to decrease the level of blood total cholesterol, low-density lipoprotein cholesterol, and triglyceride but increase the level of high-density lipoprotein cholesterol (Anderson, 1995).

Cr supplementation has been of interest to a variety of livestock production systems. In pigs, findings on the positive effects of Cr supplementation on carcass quality and on reproductive parameters have been impressive (Page et al., 1992, 1993; Lindemann et al., 1995; Mooney et al., 1995). In dairy cows, it has been shown (Burton et al., 1994; Mallard et al., 1994; Chang et al., 1996) that supplementation with Cr reduced blood cortisol concentrations and improved immunological activities during transition. Then, in turkeys, Rosebrough and Steele (1981) reported that Cr supplementation as CrCl₃ increased growth and feed efficiency. In a similar study with broilers, Kim et al. (1995) reported that FCR ratio was improved and that carcass fat was decreased by a diet that included 200 µg/kg Cr. Lien et al. (1999) also reported an increase in growth and a decrease in carcass fat in broilers fed diets supplemented with Cr picolinate. Lee et al. (2003) reported that Cr as Cr picolinate had no effect on growth performance but did decrease FCR and up-regulated immune responses in broilers. Sahin et al. (2001, 2002) reported that the use of Cr in broiler chickens under heat stress increases the concentration of vitamins C and E in serum.

Cr supplements can be either organic or inorganic. Organic sources of microminerals, such as proteinates and chelates with amino acids, have been found to have higher relative bioavailability than inorganic mineral sources such as oxides and sulfates (Pierce et al., 2009). Therefore, organic Cr has received much attention as it can be readily absorbed into the gut (Ohh and Lee, 2005). In dairy cattle, these organic sources of Cr, such as Cr propionate or

Cr-methionine have demonstrated firm positive effects on glucose use, feed intake, and milk production compared to other Cr mixtures (NRC, 1997; Hayirli et al., 2001). However, the vast majority of the research with Cr in poultry has only documented the effects of CrCl₃ or Cr picolinate. This implies that effect of organic sources of Cr, other than Cr picolinate, on broiler performance and carcass traits still needs to be explored.

Therefore, the purpose of this research was to evaluate the effect of Cr propionate on growth and carcass traits in poultry. Hereto, 3 experiments with broilers were conducted.

2. Materials and methods

2.1. Ethic statement of animal experiments

The procedures related to animal care used in these experiments were approved by the Animal Care and Use Committee of the Center Institutional and The University of Jordan (Exp. 1 and 3) and the University of Warmia and Mazury, Poland (Exp. 2). More specifically, the University of Jordan, i.e. the research Centre where Exp. 1 and 3 were carried out, is authorized by the Government of Jordan to employ animals for experimental or other scientific purposes. The latter experiments were approved by the Scientific Research Council of the University of Jordan. Animal welfare was monitored by the designated veterinarian; DVM Beshar Abdraboh. On the other hand, the animals employed in Exp. 2 were reared and treated in compliance with the Directive 2010/63/EU covering the protection of the animals used for experimental or other scientific purposes, adopted by the Polish legislation in 2010 (L 276/77, 20.10.2010). The University of Warmia and Mazury (REGON 510884205), i.e. the research Centre (veterinary number: 286271-147) where the study was carried out, is authorized by the Polish government to employ animals for experimental or other scientific purposes.

2.2. Experimental design

Three similar experiments were conducted at different time points to determine the effects of dietary Cr propionate on growth performance and carcass traits in commercial Ross 308 broilers from 0 to 35 d of age.

The trials were conducted to investigate the effect of dietary supplementation of Cr propionate from KemTRACE Cr propionate 0.4% dry (Kem Industries Inc., Des Moines, IA), which provides 0.4% Cr (lot numbers: Exp. 1, 1802106723; Exp. 2, 1803118642; Exp. 3, 1806101858) at different Cr dose levels (control = 0 µg/kg, T1 = 200 µg/kg, T2 = 400 µg/kg) on the performance and carcass characteristics of broiler birds. The design of the studies is presented in Table 1. In Exp. 3, along with Cr propionate, a microtracer (MTSE GmbH, Langerwehe, Germany) was included in the same proportions as KemTRACE (200 and 400 µg/kg) to test for the mixing homogeneity of Cr propionate in the feed mill.

2.3. Procedures for test product (KemTRACE Cr propionate 0.4% dry) mixing with the compound feed

In order to reach 200 µg/kg organic Cr, 50 mg KemTRACE propionate 0.4% dry was mixed per kilogram compound feed. In order to reach 400 µg/kg organic Cr, 100 mg KemTRACE propionate 0.4% dry was mixed per kilogram compound feed. The required amounts of KemTRACE Cr propionate 0.4% dry were weighed on a digital balance (METTLER AE240). Before mixing in the whole feed, the KemTRACE Cr propionate 0.4% dry was diluted with 1 kg of the vitamins and minerals premix, and mixed thoroughly in a horizontal mixer for 10 min (Mixture 1), then Mixture 1 was diluted

Table 1
Trial design and dosages of the efficacy trials performed.

Trial	Location	Duration, d	Total no. of animals (animals per replicate) Replicates per treatment	Breed sex	Composition feed (form)	Groups (organic Cr, µg/kg)
1	Jordan	35	900 (25) 12	Ross 308	Corn, soybean meal (Mash)	0
				Male		200
2	Poland	35	882 (14) 21	Ross 308	Corn, wheat, soybean meal (Pellet)	0
				Male		200
3	Jordan	35	1,080 (30) 12	Ross 308	Corn, soybean meal (Mash)	0
				Male		200
						400

again with 5 kg of basal feed and mixed in the same mixer for 15 min (Mixture 2). Then, Mixture 2 was added to the feed and uploaded to the bagging silo in the feed mill. Before bagging, the total amount of feed was mixed thoroughly in the silo for 2 min. The same method of dilution was applied for all treatments and in all phases within the treatment. The feed mill system was flushed between the production of 2 different phases, to avoid carry over from the highest concentration of the previous phase to the basal diet of the next phase. The following production and flushing protocol was followed in the feed mill: the starter basal diet (0 µg/kg Cr propionate 0.4% dry) was produced first. After the production of starter Control the system was flushed. Then the production of the basal diet starter + 200 µg/kg organic Cr from the Cr propionate. Finally, the basal diet starter + 400 µg/kg organic Cr from the Cr propionate was produced and then the system was flushed once more. The same protocol was repeated for the grower and finisher diets production. The final concentrations of Cr propionate added in all feed types were confirmed by analyzing representative samples from all diet types and from each phase.

2.4. Feeding

The diets were fed for a total period of 35 d and were provided in 3 phases as starter, grower and finisher mash (Exp. 1 & 3) and pellet (Exp. 2). The starter diets were fed from 0 to 14 d, the grower diets from 15 to 28 d and the finisher diets from 29 to 35 d. All birds were fed ad libitum. All groups received the same basal compound feed, which was mainly based on corn and soybean meal as in the control treatment. Table 2 (Exp. 1 & 3) and Table 3 (Exp. 2) show the proportion of ingredients (% wt/wt) used in the studies and the calculated nutrient content during the 3 phases of the study. The basal diets were all the same in composition apart from the test product content. The basal feeds were produced with no Cr propionate, no antibiotics, no anti-coccidial drug or coccidiostats. Except for the test product, the animals did not receive any other similar product for the whole study period. Two feeders per pen were available. To avoid Cr contamination from stainless steel, plastic feeders were available.

2.5. Husbandry conditions

Temperature and ventilation conditions were controlled automatically throughout the trials and were appropriate to the age as recommended by the breeder. Standard management and husbandry practices were used throughout the experiment. During the complete study duration, birds (Ross 308, Males) were provided with the following light schedule: 0 to 7 d, 23 h light: 1 h dark; 7 to 35 d, 18 h light: 6 h dark. Bird health was examined daily in the pens and any variation in appearance and/or behavior was recorded. Mortality was monitored on a daily basis and the animals that died or were culled during the study were weighed and necropsied by a

veterinary pathologist within 12 h after death or culling. Along with the additive, a microtracer (MTSE GmbH, Langerwehe, Germany) was included to test for the mixing homogeneity of the active substance Cr propionate in the feed mill. The results showed unequivocally that the active substance Cr propionate had been added correctly to the diets according to specifications.

2.6. Measurements and records

Body weight: total pen weight was measured on 0, 7, 14, 21, 28, and 35 d of the trial. The weight of the pen was based upon the weight of all birds within the pen. The birds were fasted 2 h prior to weighing process. Scales were of digital types and were calibrated on a regular basis, at the beginning of every weighing. Average weight of the birds per pen was calculated.

Table 2
Percentage composition of diets for 35-d growth trial in Exp. 1 and 3.

Item	Starter	Grower	Finisher
Ingredients			
Corn, yellow	59.24	61.14	63.50
Soy bean meal solvent extracted 47	34.81	31.33	29.27
Soybean oil	1.95	3.41	3.75
Dicalcium phosphate	1.55	1.73	1.48
Limestone	0.75	0.71	0.62
Dl-methionine	0.33	0.32	0.24
L-lysine hydrochloric acid	0.29	0.27	0.21
Sodium bicarbonate	0.23	0.22	0.20
Sodium chloride	0.20	0.20	0.20
Minerals and vitamins broiler ¹	0.10	0.10	0.10
L-threonine	0.12	0.13	0.10
Toxin dry	0.10	0.10	0.10
Endox dry	0.10	0.10	0.10
Choline chloride 50%	0.10	0.10	0.06
L-valine	0.04	0.04	0.00
Calculated composition			
Crude protein	21.48	19.98	18.97
Crude fat	4.26	6.10	6.48
Crude fiber	2.70	2.61	2.58
Ash content	6.00	5.59	5.17
Calcium	0.88	0.8	0.7
Total phosphorus	0.72	0.66	0.61
Available phosphorus	0.43	0.36	0.35
Sodium	0.15	0.15	0.15
Chloride	0.23	0.23	0.22
Lysine digestible poultry	1.20	1.10	0.97
Methionine + Cysteine digestible poultry	0.89	0.84	0.75
Tryptophan digestible poultry	0.21	0.19	0.18
Threonine digestible poultry	0.78	0.74	0.65
Valine digestible poultry	0.90	0.84	0.76

¹ Premix (Cargill, Efficient Broiler) provided the following per kilogram of premix: vitamin A 12,500,000 IU; vitamin D₃ 5,000,000 IU; vitamin E 70,000 mg; vitamin K₃ 3,800 mg; vitamin B₁ 2,500 mg; vitamin B₂ 7,500 mg; vitamin B₆ 4,300 mg; vitamin B₁₂ 25,000 µg; pantothenic acid 13,000 mg; niacin (B₃) 50,000 mg; folic acid 1,000 mg; biotin 200,000 mg; Mn (oxide) 62,000 µg; Fe (sulfate) 44,000 mg; Zn (oxide) 50,000 mg; Cu (sulfate) 10,000 mg; I (K-iodide) 1,300 mg; Se (Na-selenite) 230 mg.

Table 3
Percentage composition of diets for 35-d growth trial in Exp. 2.

Item	Starter	Grower	Finisher
Ingredients			
Wheat	25.13	35.16	37.68
Corn	34.31	29.63	30.15
Rapeseed meal	4.02	3.02	3.02
Soybean meal	30.99	25.85	23.04
Soybean oil	1.33	2.27	2.01
Poultry lard	1.01	1.01	1.66
Chalk	1.32	1.18	0.99
Monocalcium phosphate	0.72	0.57	0.42
Sodium bicarbonate	0.20	0.25	0.22
Sodium chloride	0.22	0.21	0.23
Vitamin and mineral premix ¹	0.10	0.10	0.10
Methionine (DL-methionine 99%)	0.29	0.28	0.21
Valine	0.02	0.06	0.01
L-lysine hydrochloric acid	0.28	0.31	0.23
L-threonine	0.11	0.15	0.09
M2342 glucose-xylose	0.05	0.05	0.05
Calculated composition			
Crude protein	22.10	20.19	19.00
Crude fiber	3.07	5.28	2.84
Crude fat	4.50	2.88	5.67
Ash content	5.60	5.08	4.63
Starch	36.05	38.98	40.75
Calcium	0.90	0.80	0.70
Total phosphorus	0.43	0.39	0.35
Sodium	0.15	0.16	0.16
Chlorine	0.23	0.23	0.23
Methionine + Cysteine	0.89	0.84	0.75
Threonine	0.78	0.74	0.65

¹ Premix (Cargill, Efficient Broiler) provided the following per kilogram of premix: vitamin A 12,500,000 IU; vitamin D₃ 5,000,000 IU; vitamin E 70,000 mg; vitamin K₃ 3,800 mg; vitamin B₁ 2,500 mg; vitamin B₂ 7,500 mg; vitamin B₆ 4,300 mg; vitamin B₁₂ 25,000 µg; pantothenic acid 13,000 mg; niacin (B₃) 50,000 mg; folic acid 1,000 mg; biotin 200,000 mg; Mn (Oxide) 62,000 µg; Fe (sulfate) 44,000 mg; Zn (oxide) 50,000 mg; Cu (sulfate) 1,000 mg; I (K-iodide) 1,300 mg; Se (Na-selenite) 230 mg.

Feed intake: average daily feed intake (ADFI) per pen, during the treatment periods 0 to 14 d, 15 to 28 d, 29 to 35 d, 0 to 35 d were recorded. Feed intake per pen was used to calculate the average feed conversion ratio (FCR) during the periods 0 to 14 d, 15 to 28 d, 29 to 35 d, 0 to 35 d.

Feed conversion ratio (FCR): the FCR was calculated considering the total feed intake per pen for each replicate (pen). Losses due to mortality or culling were considered for the calculation of the FCR. Feed conversion ratio was calculated based on kilograms of feed consumed per kilogram of body weight gain during the specified period of the trial.

Carcass parameters: at the end of the trial (35 d), one bird was randomly selected from each pen (replication). Birds were selected, euthanized and directly taken to perform the following measurements: carcass yield, breast meat yield, weight of the abdominal fat pad, weight of the liver, weight of the heart, weight of the gizzard, weight of the kidney, weight of the bursa, weight of the thymus, and weight of the spleen. The relative weight of internal organs was calculated as a ratio by dividing the organs weight by the birds live body weight (gram per kilogram of body weight). Weight gain and feed intake for each treatment were recorded at the start and after 14, 28 and 35 d of fattening, and FCR were calculated accordingly (and corrected for mortality).

2.7. Calculations and statistical analyses

The Statistical Analysis System (SAS Institute, 2010, Version 9.1.3) was used to conduct all statistical analyses. Mean, standard deviation and pooled standard errors (SEM) were calculated for each variable. Data were tested for normality using proc univariate

procedure of SAS. All variables showed a normal distribution (Kolmogorov–Smirnov, $P < 0.05$). Data were analyzed with ANOVA implemented in the GLM procedure of SAS, that included the effects of treatment (Cr level = 0, 200 or 400 µg/kg) and replicates. Data were collected for all pens and all time points (no missing data). No data points were excluded from the analysis. Each pen or replicate was considered as the experimental unit for the performance outcome parameters. For the carcass trait evaluation, the animal was considered as experimental unit. Differences were accepted as representing statistically significant differences when $P < 0.05$. Post hoc Tukey's test was used to separate the means.

3. Results

3.1. Growth performances in broilers fed with Cr propionate

In Table 4, all details on growth performance can be studied across treatments and experiments.

Experiment 1: during the growing phase, T2 showed the maximum ($P < 0.0001$) average gain compared to the other treatments, whereas the control treatment showed the lowest average gain ($P < 0.0001$). T1 showed a significant increase in average daily gain compared to the control. During the finisher phase of the trial, T2 showed the maximum ($P < 0.001$) average gain while the control showed the lowest body gain, with no significant difference when comparing the control to the T1 treatment. Overall, the T2 treatment had the maximum ($P < 0.0001$) average daily gain throughout the trial compared with the T1, while the control treatment had the lowest average daily gain. With increased Cr propionate concentrations, the daily gain increased linearly ($P < 0.0001$). During the starter phase, the control treatment showed a high ($P < 0.0001$) rate of feed intake compared with the T1 and T2 treatments. During the growing phase, T2 exhibited a higher feed intake than the other treatments, while no significant differences in feed intake were observed between the T1 and the control group during the same phase. In contrast, during the finisher phase, all birds showed the same rate of average daily feed intake. When the feed intake was calculated and expressed over the whole study period (d 0 to 35 of age), there was a numerical trend throughout the trial that the Cr propionate increased feed intake in both T1 and T2 when compared with the control group, but this increase was not significant. The results showed that there was a highly significant effect ($P < 0.0001$) of dietary Cr supplementation on FCR. The maximum feed efficiency was achieved by birds from the T2 group compared with the other treatments, and this trend was observed throughout the study period. During the starter phase, both organic Cr levels (T1 and T2) showed a lower FCR than the control treatment. During the growing phase, the best FCR was obtained by birds from the T2 group, followed by T1, while the highest FCR was obtained by birds receiving the control treatment. Over the whole study period (0 to 35 d of age), T2 showed the lowest ($P < 0.0001$) FCR (1.31), followed by the T1 treatment (1.36), and the highest rate of FCR was obtained by the control treatment (1.42). These differences were highly significant ($P < 0.0001$). The effect of organic Cr on FCR was dose-dependent, and this effect was linear ($P < 0.0001$).

Experiment 2: at the start of experiment there were no significant differences in body weight across treatments. There were no significant differences in body weight and gain during experimental periods: 15 to 28 d, 0 to 28 d and 29 to 35 d. The birds from all treatment groups reached the final body weight lower than the Ross 308 standards, the latter being 2.283 kg. There were significant differences in feed intake in all feeding periods across treatment groups, and the birds from control group consumed more feed than one or both Cr propionate supplemented groups (T1 & T2). The feed intake calculated for the entire experiment was significantly higher in

Table 4
Daily feed intake, body weight and feed conversion ratio during starter, grower, finisher phase and the total period.

Item	Treatment ¹	Feed intake, g/d				Body weight, g			Feed conversion ratio, g/g			
		Starter (0 to 14 d)	Grower (15 to 28 d)	Finisher (29 to 35 d)	Total (0 to 35 d)	Starter (14 d)	Grower (28 d)	Finisher (35 d)	Starter (0 to 14 d)	Grower (15 to 28 d)	Finisher (29 to 35 d)	Total (0 to 35 d)
Exp. 2 ¹	0	504.0 ^a	1,629.7 ^b	1,137.2	3,270.9	455.8 ^b	1,593.8 ^c	2,339.7 ^c	1.22 ^a	1.43 ^a	1.53 ^a	1.42 ^a
	200	495.1 ^b	1,649.9 ^{ab}	1,183.8	3,328.7	464.8 ^{ab}	1,702.5 ^b	2,490.3 ^b	1.17 ^b	1.33 ^b	1.50 ^a	1.36 ^b
	400	493.1 ^b	1,661.9 ^a	1,176.3	3,331.3	470.2 ^a	1,749.6 ^a	2,580.2 ^a	1.16 ^b	1.30 ^c	1.42 ^b	1.31 ^c
	Pooled SEM	1.37	10.93	18.60	22.25	3.74	10.10	20.13	0.01	0.01	0.02	0.01
	P-value	< 0.0001	0.0453	NS	NS	0.010	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Exp. 3 ²	0	473.2	1,512.0 ^a	1,032.5 ^a	3,045.0 ^a	446	1,490	2,147	1.15 ^{ab}	1.45	1.58	1.43 ^b
	200	467.6	1,478.4 ^{ab}	1,000.1 ^{ab}	3,020.5 ^b	433	1,470	2,111	1.17 ^b	1.43	1.61	1.43 ^b
	400	459.2	1,461.6 ^b	994.0 ^b	2,936.5 ^c	438	1,476	2,108	1.14 ^a	1.43	1.58	1.42 ^a
	Pooled SEM	0.77	7.32	6.15	15.93	0.002	0.005	0.008	0.005	0.005	0.008	0.003
	P-value	NS	0.015	0.023	0.013	NS	NS	NS	0.029	NS	NS	0.027
Exp. 2 ³	0	548.4	1,605.6 ^a	1,178.8	3,332.7 ^a	488.2	1,575.4 ^c	2,281.5 ^c	1.23	1.48 ^a	1.67 ^a	1.49 ^a
	200	538.8	1,555.1 ^b	1,169.0	3,262.9 ^b	490.6	1,618.0 ^b	2,345.7 ^b	1.20	1.38 ^b	1.61 ^a	1.42 ^b
	400	538.5	1,528.9 ^b	1,160.4	3,227.8 ^b	491.7	1,675.4 ^a	2,443.1 ^a	1.20	1.29 ^c	1.51 ^b	1.34 ^c
	Pooled SEM	4.79	11.67	8.45	13.16	4.84	7.98	13.32	0.02	0.02	0.02	0.01
	P-value	NS	< 0.0001	NS	< 0.05	NS	< 0.0001	< 0.0001	NS	< 0.05	< 0.05	< 0.0001

NS = not significant.

^{a-c} Means within a column with a different superscript differ significantly ($P \leq 0.05$).

¹ Treatments included 0, 200, and 400 µg of organic Cr /kg diet.

² Reported values are means of 12 replicates.

³ Reported values are means of 21 replicates.

control group compared to Cr propionate supplemented groups (T1 & T2), and the feed intake in T1 was significantly higher than in T2. Differences in FCR between treatments have been calculated for the first period. More specifically, between 0 and 14 d the birds from Cr propionate supplemented T2 group and from the control group better converted feed than those from T1 group. Between 15 and 28 d the birds from both T1 & T2 were characterized by a near-significantly better FCR than the control birds. The same tendency was observed for the 0 to 28 d period. However, only birds from Cr propionate supplemented group T2 were characterized by a near-significantly better FCR than the control birds. The FCR calculated for the entire experiment (35 d) was found in T2 significantly lower than in control and T1 (lower dose of Cr propionate in feed) groups ($P < 0.05$). The liveability in all treatments ranged from 97.6% of T2 to 99.0% of T1 and no significances in this parameter between treatment groups were observed. The main reasons of death in the flock were sudden death syndrome and cachexia and the reason of culling were weak legs and cachexia.

Experiment 3: during the growing phase, T2 showed the maximum ($P < 0.0001$) average gain compared to the other treatments, followed by T1. The control treatment showed lower body gain than T1 and T2. In the same manner, T2 showed the highest body gain during the finisher phase compared to other treatments, and there was also a significant difference between T1 and the control. Overall, broiler birds supplemented with 400 µg/kg of Cr as Cr propionate 0.4% dry (T2) had the maximum ($P < 0.0001$) average daily gain throughout the trial compared with the control. During the first week of the trial, no significant differences in feed intake were observed between T1, T2 and the control. In line with this, when the feed intake was calculated over the whole starter phase, it showed no significant differences among treatments. During the grower phase, the T1 and T2 birds consumed ($P < 0.0001$) the lowest amount of feed compared with the control treatment. During the finisher phase no significant differences among treatments in total feed intake were retrieved. The total amount of feed consumed during the whole trial period was highest in the control treatment compared with other treatments. The results showed that there was a highly significant effect ($P < 0.0001$) of dietary Cr supplemented as Cr propionate 0.4% dry on FCR. The maximum feed efficiency was achieved by birds receiving the 400 µg/kg of organic Cr level compared with the

other treatments, and this trend was observed throughout the study period. During the starter phase, no significant differences in feed efficiency were noted between the treatments. During the grower phase, T1 and T2 showed a lower FCR than the control treatment. Over the whole study period (0 to 35 d of age), T2 showed the lowest ($P < 0.0001$) FCR (1.34; kg feed: kg gain), followed by T1 (1.42), and finally the control (1.49).

3.2. Carcass traits in broilers fed with Cr propionate

In Table 5, details on carcass characteristics are presented across treatments and across experiments.

Experiment 1: the highest ($P < 0.0001$) carcass yield was obtained from the birds receiving T2 (1,892.9 g), followed by T1 (1,767.4 g) and the lowest carcass yield was obtained by the control treatment (1,633.0 g). These differences were highly significant ($P < 0.0001$) and the organic Cr level-carcass weight response was linear ($P < 0.0001$). The dressing percentages (ratios of hot carcass weight to the live birds' weight) were consistent with these results. T2 exhibited the highest ($P < 0.0001$) percentage of dressing (73.85%), compared with 72.34% obtained by T1, and the lowest ($P < 0.0001$) rate of dressing was obtained by the control group (70.12%). The breast meat yield was consistent with the above carcass results being the highest ($P < 0.0001$) in the T2 treatment and the lowest ($P < 0.0001$) in the control treatment (no Cr). In the same manner, the leg (thigh and drumstick) also showed the highest cuts in the T2 treatment and the lowest cuts in the control treatment. The effect of the level of Cr propionate on all carcass traits was linear ($P < 0.0001$). The largest ($P < 0.0001$) abdominal fat pads were retrieved from the control treatment (14.55 g/kg of live body weight), while the smallest ($P < 0.0001$) fat pads were collected from the T2 treatment (10.01 g/kg of live body weight). These responses were linearly affected by the organic Cr level coming from Cr propionate 0.4% dry. This is also consistent with the carcass results where T2 obtained highest dressing percentage. The relative weights of liver and heart were not affected in T1 compared with the control level, while they were slightly decreased ($P < 0.05$) in T2. The gizzards' relative weight was the lowest ($P < 0.0001$) in T2 and the highest ($P < 0.0001$) in the control treatment. Kidneys' weights were slightly affected ($P < 0.05$) by the Cr level being lower in the 200 and 400 µg/kg Cr group compared to the control group.

Table 5
Carcass traits during the whole period.

Item	Treatment ¹	Carcass weight, g	Dressing percentage, %	Breast meat yield, g	Abdominal fat, %	Liver, %	Heart, %	Gizzard, %	Kidneys, %	Bursa, %	Spleen, %
Exp. 2 ¹	0	1,633.0 ^c	70.12 ^c	511.43 ^c	1.46 ^a	2.10 ^a	0.52 ^a	1.73 ^a	0.46 ^a	0.030 ^a	0.070 ^a
	200	1,767.4 ^b	72.34 ^b	584.91 ^b	1.21 ^b	2.02 ^{ab}	0.50 ^{ab}	1.66 ^b	0.42 ^b	0.029 ^{ab}	0.067 ^{ab}
	400	1,892.9 ^a	73.85 ^a	665.03 ^a	1.00 ^c	1.93 ^b	0.48 ^b	1.56 ^c	0.42 ^b	0.029 ^b	0.064 ^b
	Pooled SEM	13.65	0.19	4.32	0.025	0.049	0.009	0.023	0.009	0.0004	0.001
P-value	<0.0001	<0.0001	<0.0001	<0.0001	0.0205	0.0023	<0.0001	0.0018	0.0299	0.0094	
Exp. 3 ²	0	1514	70.17 ^c	220.9	–	2.29	0.42	0.75	0.57	0.180	0.109
	200	1505	71.24 ^b	214.9	–	2.17	0.42	0.73	0.57	0.196	0.099
	400	1523	72.11 ^a	222.0	–	2.16	0.42	0.73	0.55	0.178	0.108
	Pooled SEM	0.008	0.198	1894	–	0.029	0.155	0.017	0.007	0.005	0.004
P-value	NS	<0.001	NS	–	NS	NS	NS	NS	NS	NS	
Exp. 2 ³	0	1,517 ^b	70.28 ^b	413.47 ^b	0.97 ^a	2.15 ^a	0.571 ^a	2.221 ^a	0.554 ^a	0.167	0.169 ^a
	200	1,658 ^a	71.37 ^b	498.04 ^a	0.42 ^b	1.76 ^b	0.392 ^b	1.602 ^b	0.425 ^b	0.146	0.164 ^a
	400	1,757 ^a	72.04 ^a	540.87 ^a	0.36 ^b	1.63 ^b	0.335 ^b	1.545 ^b	0.399 ^b	0.138	0.111 ^b
	Pooled SEM	37.22	0.19	15.16	0.054	0.105	0.026	0.115	0.033	0.01	0.012
P-value	<0.0001	<0.0001	<0.0001	<0.05	<0.05	<0.0001	<0.0001	<0.05	NS	<0.05	

NS = not significant.

^{a-c} means within column with a different superscript differ significantly ($P \leq 0.05$).

¹ Treatments included 0, 200, and 400 µg of organic Cr /kg diet.

² Reported values are means of 12 replicates.

³ Reported values are means of 21 replicates.

The relative weights of bursa, thymus and spleen were slightly higher in the control treatment than in T2, with no significant difference when compared with T1.

Experiment 2: the dressing percentage calculated for both Cr propionate supplemented groups (T1 & T2) was significantly higher compared to control birds and for T2 group significantly higher than for T1 group ($P < 0.0001$). There were differences in liver weight between treatments, and the weight of this organ was significantly (the relative weight – near significantly) lower in both Cr propionate supplemented groups (T1 & T2) than in control birds. The thymus weight was the highest in T1 (lower dose of Cr propionate in feed), significantly higher than in T2 (higher dose of Cr propionate in feed) and near-significantly than in control birds. The relative weight of thymus was significantly higher in T1 than in T2 and control.

Experiment 3: the highest ($P < 0.0001$) carcass yield (1,757.2 g/bird) was obtained from the birds in T2, followed by the T1 (1,658.1 g/bird). These differences were highly significant ($P < 0.0001$). The dressing percentages (ratios of hot carcass weight to the live birds' weight) were consistent with these results. The T2 treatment exhibited the highest ($P < 0.0001$) dressing percentage (72.0%), compared with 71.4% obtained by T1. The breast meat yield as well as the thigh and leg were in line with the above carcass results being the highest ($P < 0.0001$) in T1 and T2 and the lowest ($P < 0.0001$) in control treatments. The largest ($P < 0.0001$) abdominal fat pads were retrieved from the control treatment (9.68 g/kg of live body weight), while the T1 and T2 had significantly smaller fat pads than the control. Internal organs relative weight: the smallest livers were retrieved from T1 and T2, compared with the control groups. The smallest ($P < 0.0001$) hearts were obtained from T1 and T2 while the largest ones were obtained from the control. The largest ($P < 0.0001$) gizzards were obtained from the control, and the lowest ones were obtained from T1 and T2. Kidneys weights were also the least in T1 and T2 and the highest in control birds. Bursa and thymus showed no significant differences between the control and the T1 and T2 treatments. Spleens' weights were slightly affected ($P < 0.05$) by the Cr propionate level being lower in T2 compared to T1 and the controls.

4. Discussion

Interest in Cr as an essential nutrient for livestock in manipulating growth performance and improving carcass composition

has been reported as early as 1960s (Amata, 2013). The present study confirmed that Cr propionate can be utilized to enhance broiler performance. The significant improvement of broiler performance with 400 µg/kg dietary Cr supplementation was evident on increased body weight, higher average daily gain and decreased FCR. Cr propionate revealed a linear effect on body weight after 14 d of treatment, and this effect continued toward the end of the trials. This can indicate that Cr propionate has a dose-dependent effect on body weight. Our results are in consistence with previous studies on Cr supplementation in broilers (Sands and Smith, 1999; Sahin et al., 2003; Toghiani et al., 2006).

No single mortality case was recorded in two of the three trials, and this indicates that Cr propionate has no negative impact on birds' health even at high concentrations (400 µg/kg). In the same manner, Cr supplementation was previously reported not to influence livability of healthy birds (Kim et al., 1996; Anandhi et al., 2006).

Regarding the carcass parameters, all experiments revealed significant positive effects of Cr supplemented as Cr propionate on carcass traits. The carcass yield, dressing percentage, breast meat and the leg cuts were generally higher in T1 and T2 treatments compared to the control. These results are in line with the previous results reported by many authors who confirmed the improvements in carcass quality with Cr supplementation (Anandhi et al., 2006; Sahin et al., 2003). The amount of meat a farmer will get from an animal depends on the dressing percentage and the carcass cutting yields (Whiteheart, 2012). Therefore, associated factors such as carcass yields, amount of meat and the proportion of meat in relation to live weight are of great importance to all the parties involved – the producers, processors and consumers.

Interestingly, experiment 2 did not reveal drastic effects on performance but did reveal impacts of the Cr on carcass traits. This is in line with previous studies which documented that the effect of Cr on carcass traits is more consistent than the effect of Cr on growth performance (NRC, 1997; Matthews et al., 2005; Uyanik et al., 2005). It is important to consider that the effect of Cr propionate seems obvious on abdominal fat. The Cr treated groups significantly showed lower fat pad sizes compared to the control group. This may be due to the active involvement of Cr in the glucose metabolism (Vincent, 2000). Cr apparently potentiates the action of insulin in glucose utilization (Vincent, 2000). This might indicate that, in our studies, Cr supplied as Cr propionate enhanced the utilization of glucose in the body cells and less glucose was

converted to fat. Hence, this could explain the leaner carcass of Cr treated groups compared with the control treatment. The latter was reflected in smaller fat pads in the Cr treated groups.

Reports on the effect of Cr on growth performance in broilers have been variable (NRC, 1997), and our results agree, at least to a certain extent, with this variability. In Exp. 1 and 3, Cr supplementation improved most aspects of growth performance (resulting in a decreased FCR) in the grower and finisher phase, whereas this was not observed in Exp. 2. Our results and the review by the National Research Council (NRC, 1997) regarding the variability of Cr supplementation are consistent with recent publications. Also, Lee et al. (2003) reported that Cr picolinate had no effect on growth, but feed efficiency was increased. One factor causing the variability between the presented studies might be the difference in feed formulations; in Exp. 1 and 3 chicken were with the same diet formulation (corn, soybean meal), whereas in Exp. 2, broilers were fed with a different diet formulation (corn, wheat and soybean meal). Another factor causing the variability between the studies might be stress. Sahin et al. (2002) reported that Cr as Cr picolinate improved growth performance of broilers during heat stress, but this response may be due to the stress. Indeed, Cr supplementation has been recognized to reduce the negative effects of environmental stress (Mowat, 1994; Lien et al., 1999; Sahin et al., 2001). This effect of Cr in relieving stress has been well documented. Various reports have confirmed decreased sensitivity to stress in animals fed with Cr supplements due to reduced concentrations of cortisol in the blood (Chang and Mowat, 1992; Moonsie-Shager and Mowat, 1993; Pechova et al., 2002). Nevertheless, there is still no clear-cut evidence for the association between Cr and cortisol as an indicator of stress in animals (Pechova and Pavlata, 2007).

It is important to consider that organic sources of Cr have greater biological availability than Cr from inorganic sources (Anderson et al., 1985, 1993; Kim et al., 1996). This does not imply that the organic form is more dangerous. On the contrary, the inorganic form is shown to have a much higher retention in the body. More specifically, a recent work (Kottwitz et al., 2009) demonstrated that in rats the final whole-body retention of Cr from Cr-chloride was more than twice that from Cr picolinate despite the considerable higher absorption of Cr from Cr picolinate compared to Cr-chloride. Kottwitz et al. (2009) found that due to the absorption of Cr picolinate in the form of intact molecule, its major portion was directly excreted by kidney before the degradation in the liver can occur. Furthermore, it has been shown that the mean bioavailability in terms of tissue deposition for Cr propionate, Cr-methionine and Cr-yeast in pigs, expressed in relative values to Cr picolinate, is 13.1%, 50.5% and 22.8%, respectively (Lindemann et al., 2008).

Trivalent Cr (Cr³⁺) is the most stable oxidation state in which Cr is found in living organisms and is considered to be a highly safe form of Cr (Lindemann et al., 1996). Collected data also confirm that Cr³⁺ is not metabolized in the body (ECHA, 2008). The Cr is excreted in the form of Cr³⁺ complexes with glutathione (ECB RAR, 2005). Furthermore, Cr oxide (Cr₂O₃) has been used for decades as a digestibility marker in cattle, sheep and pigs at levels up to 3,000 mg Cr/kg feed without signs of acute toxicity (Titgemeyer, 1997).

In Europe, the feed additive based on Cr has not yet been authorized. In the USA, the organic Cr propionate is more accepted than any other form of Cr. In this context, 2 organic forms of Cr (Cr propionate and Cr picolinate) are currently permitted for addition to swine diets in the USA at levels not exceeding 0.2 mg/kg (200 µg/kg) of supplemental Cr. Cr propionate is a source of readily absorbed organically-bound Cr. Other Cr products on the market include non-bound Cr salts, organically-bound species with documented health risks of the carrier anion (e.g. Cr picolinate), and ill-defined admixtures of such salts. Traditional quality control methods for the latter are typically unable to distinguish and quantify organically-

bound from non-bound Cr in these products. However, Cr³⁺ propionate is a novel and structurally well-defined compound that lends itself to an accurate quality control evaluation.

In conclusion, growth performance, feed conversion, carcass yield, breast and leg meats of broiler birds can be significantly improved by dietary inclusion of Cr propionate. Cr propionate can be supplemented to broiler birds from 1 d old at a level that provides 400 µg/kg Cr. The effects reported on carcass traits are very important as the carcass yield of a broiler chicken is of primary concern to the producer, processor and the dressing percentage is the key trait of economic importance (Omojola et al., 2004).

Conflict of interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

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