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Dietary chromium supplementation for heat-stressed broilers

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Studies have shown that dietary chromium (Cr) supplementation beneficially affects physiological functions such as cell preservation, antioxidant activity and immune response that are of utmost importance to animal homeostasis and thermoregulatory capacity under heat stress conditions. For instance, Cr is essential for the synthesis of the specific low molecular weight Cr-binding-substance (LMWCr) that upon conversion to chromodulin, activates the insulin signalling cascade. This results in greater cell permeability to insulin, with a subsequent positive effect on the metabolism of carbohydrates, lipids and proteins. Furthermore, Cr has antioxidant properties which help to attenuate the negative effects of oxidative stress. With regards to meat quality, Cr decreases lipid peroxidation. It has been suggested that Cr supplementation increases antibody responses and lymphocyte counts in broiler chickens exposed to high environmental temperatures. In addition, trivalent Cr supplementation increases growth performance and decreases the circulating levels of undesirable metabolites and hormones such as cholesterol and corticosterone in broiler chickens exposed to heat stress. The aim of this review was to report the effects of Cr supplementation as a nutritional strategy to increase growth performance, immune response, carcass characteristics and meat quality of broilers produced under hot conditions. This knowledge may contribute to improve the productivity and sustainability of broiler production in a context of global warming and development of livestock production in hot climate areas.

Keywords: heat stress; chromium; immune system; performance; poultry

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Introduction

When exposed to heat stress, broilers maintain their body temperature via thermoregulatory responses that negatively impact growth performance and metabolism, resulting then in important economic losses (Renaudeau *et al.*, 2012). In addition, it has been strongly suggested that the negative effects of heat stress will increase in the coming decades due to temperature rises associated with global warming. Broiler chickens have been genetically selected to improve growth and muscle deposition and are considered to be more susceptible to high temperatures, in relation to older or native breeds, because of their greater metabolic heat production (Brossi *et al.*, 2009).

Among the strategies used to attenuate the negative effects of heat stress in broiler production systems, different approaches including the use of air conditioning, ventilation and evaporative cooling systems, management of drinking water, vitamin and mineral supplementation and variation of the dietary electrolyte balance have been employed (Borges *et al.*, 2003; Gamba *et al.*, 2015). The use of low heat increment diets through the reduction of dietary crude protein content and inclusion of fat is one of the nutritional strategies most studied and utilised under commercial/practical conditions (Silva *et al.*, 2014). During heat stress, a reduction in the circulating levels of vitamins and minerals is observed, which may be associated with low feed intake and high water consumption by the broilers. Furthermore, the antioxidant capacity in heat-stressed broilers is reduced in association with the decreases in glutathione peroxidase, superoxide dismutase and catalase synthesis (Altan *et al.*, 2003). In contrast, positive effects of dietary mineral and vitamin supplementation under heat stress conditions have been reported (Borges *et al.*, 2003; Khan *et al.*, 2012) and are able to reduce cellular oxidation reactions (Silva *et al.*, 2015).

Research has supported the use of Cr supplementation as a nutritional strategy to improve growth performance (Ghazi *et al.*, 2012), nutrient metabolism (Sahin *et al.*, 2003; Jahanian and Rasouli, 2015), immune responses (Oba *et al.*, 2012), antioxidant function and stress response (Ghazi *et al.*, 2012; Khan *et al.*, 2014) in broiler chickens exposed to heat stress. The objective of this review is therefore to present chromium as a strategy for mitigating the negative effects of heat stress in livestock production systems.

Biological functions of chromium

Chromium is a trace mineral present in small amounts in raw ingredients commonly used in broiler diets, such as grains and oilseeds (Anderson *et al*., 1992). It is a metal element found in the oxidation states (2^-, 0, 2^+, 3^+, 6^+), but trivalent (3^+) is the most stable form. In this state, it is recognised as an essential trace element required for the metabolism of carbohydrates, proteins and lipids (Vincent, 2000a).

Different factors affect Cr absorption (Sirirat *et al.*, 2012), however it is well accepted that organic sources are more available and better absorbed than inorganic sources (Lukaski 1999; Zha *et al.*, 2009). Inorganic sources of Cr show lower availability (1 to 3%) (Hossain *et al.*, 1998) than organic sources (15 to 30%) (Oba *et al.*, 2012). The higher availability of organic Cr sources can be explained by its chelation within live yeasts, amino acids or other compounds. Organic Cr is usually found as Cr-L-methionine, Cr- histidinate, Cr-picolinate, Cr-nicotinic acid complex, and Cr-yeast (Sahin *et al.*, 2010; 2017). Although Cr absorption mechanisms are not yet well understood, it is hypothesised that Cr absorption occurs in the first portion of the jejunum and in

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combination with the chelated elements (Khan *et al.*, 2014). Chen *et al.* (1973) reported a small amount of Cr absorbed in the ileum and duodenum of rats.

The absorption and excretion of Cr is inversely proportional to the level of supplementation in the diet (Anderson *et al.*, 1992), and its excretion occurs almost exclusively through the urinary system (Pechova and Pavlata, 2007). According to Hunt and Stoecker (1996), Cr absorption is increased in the presence of amino acids, ascorbic acid and high carbohydrate diets; while phytate, due to binding, decreases Cr concentrations in the blood and tissues. Chen *et al.* (1973) reported that chromium oxalate showed high Cr absorption in rats. However, its absorption decreased in the presence of phytic acid. Furthermore, Cr can compete with other minerals (*e.g.*, zinc and iron) for the same absorption sites, which influences its absorption in the gut. In the bloodstream, Cr primarily circulates bound to transferrin (Vincent, 2010; Eze *et al.*, 2014); however, some studies have reported that Cr can also be transported by albumin (Borguet *et al.*, 1990).

The function and recycling of transferrin is dependent on insulin that stimulates the translocation of transferrin receptors from the vesicles towards the membrane of insulin responsive cells (Kandror, 1999). An increase in the number of membrane receptors, saturated with transferrin-Cr, stimulates endocytosis and subsequent release of the metal ion into the acidic pH of the newly formed vesicles (Vincent, 2000b). Vincent (2000a; 2010) described the mechanism of increased insulin sensitivity caused by the low-molecular weight Cr-binding-substance (LMWCr). The LMWCr is composed of glycine, cysteine, glutamate and aspartate with the carboxylates comprising more than half of the total amino acid residues (Yamamoto *et al.*, 1987; Davis and Vincent 1997a). Davis and Vincent (1997b) evaluated the effects of various minerals (V, Cr, Mn, Fe, Co, Ni, Cu, Zn and Mo) on the activation of rat adipocyte membrane protein kinase activity using fragments of gastrin as substrate associated with apoLMWCr in the presence of insulin. They identified that only Cr was able to stimulate the insulin dependent protein kinase and concluded that this stimulus is directly dependent on the concentration of Cr.

Chromodulin is a saturated oligopeptide that enhances the effects of insulin by stimulating protein tyrosine kinase dependent on insulin (Vincent, 2000a). This occurs without changing the insulin concentration, suggesting that chromodulin plays an intrinsic role in insulin function and regulation (Vincent, 2000b). According to Davis *et al.* (1996) insulin action on adipocytes occurs by activation of the phosphotyrosine phosphatase in cell membranes, which diversifies the action of Cr in animal metabolism. Moreover, this cascade of intracellular signals increases glucose uptake, and up regulates mRNA levels of the insulin receptor glucose transporter type 4 (GLUT4), glycogen synthase and uncoupling protein-3 in skeletal muscle cells (Davis *et al.*, 1996). Consequently, an increase in insulin sensitivity with consequent increased uptake of glucose and amino acids is observed. It is believed that Cr acts jointly with the LMWCr stimulating insulin sensitivity (Vincent 2000a; 2000b), however this is only consensus in studies involving rats and humans; because glucose metabolism in broilers is different from mammals, with glucose concentration becoming high and insulin level low (Brooks *et al.*, 2016). White *et al.* (1991) and Wang *et al.* (1994) state that birds only have GLUT-1, 2, 3 and 8. However, Sahin *et al.* (2017) identified GLUT-4 expression in the liver of broilers raised on heat stress with dietary Cr supplementation in the diet. Thus, the need for trials to clearly elucidate the biological mechanisms of action of Cr in poultry.

Metabolism of carbohydrates in animals is increased under heat stress conditions (Rhoads *et al.*, 2013) it there may be greater circulating insulin in the postprandial period as a consequence of a greater contribution of circulating blood glucose. Based on this reasoning, Toghyani *et al.* (2006) and Sahin *et al.* (2010) stated that this is the

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cause for the improvement of performance of broiler chickens raised under heat stress conditions. In agreement, Cupo and Donaldson (1987) reported a 16% increase in glucose utilisation in broilers receiving 20 ppm Cr-chloride, which resulted in increased cell sensitivity to insulin and consequently greater glucose uptake in the muscle and adipose tissues. The increase in anabolism, associated with greater glucose uptake, increases serum concentrations of growth hormone and associated factors such as insulin growth factor-1, increasing the synthesis of proteins (Mohammed *et al.*, 2014). Hazelwood (1986) argued that the importance of insulin in the metabolism of broiler chickens is questionable and does not work in the same way as in mammals, and therefore evidence of its direct effect on lipid metabolism should be viewed with caution.

According to Jackson *et al.* (2008) supplementation with 0.80 mg/kg of Cr-propionate in broiler diets increased feed efficiency from 15-42 days of age due to the increased glucose use by the liver and the skeletal musculature. Increased insulin sensitivity and greater glucose metabolism in response to Cr supplementation decreased gluconeogenesis in the liver, conserving amino acids for growth and protein deposition (Souza *et al.*, 2010). Cr supplementation in the form of Cr-proprionate (0.43-0.45 mg/kg) to broiler diets increased insulin activity and optimised the use of glucose, resulting in lower serum concentrations of non-esterified fatty acids by reducing lipolysis (Brooks *et al.*, 2016). According to Gursoy (2000), the supplementation of organic Cr in broiler diets improved feed efficiency and carcass quality while reducing the fat content. This fact was further observed by Dębski *et al.* (2004), who reported an improvement in weight of breast meat when 0.20 mg/kg Cr-yeast was added to the broiler diet.

Dietary chromium supplementation as a strategy to mitigate the effects of heat stress in broilers

Heat stress provokes negative effects on growth performance, immune system activity and nutrient absorption of broiler chickens (Rimold *et al.*, 2015). Khan *et al.* (2014) reported that the use of Cr causes physiological changes such as the relative decrease in corticosterone concentrations, greater immune function and attenuation of oxidative stress in broilers reared under heat stress.

According to Bahrami *et al.* (2012), supplementation with 1.20 mg/kg of Cr as Cr-methionine in the diets of heat-stressed broilers (33°C) decreased serum concentrations of corticosterone. They reported that supplementation with 1.20 mg/kg Cr-methionine increased the antibody response when broilers were challenged with Newcastle disease. Corticosterone has a suppressive effect on broiler chickens and decreases insulin sensitivity, directly affecting nutrient metabolism (Zhao *et al.*, 2009). In this sense, the reduction in concentration of corticosteroids caused by Cr supplementation is the main cause of the greater immune response in broilers under heat stress (Siegel, 1995). According to Mirfendereski and Jahanian (2015) cortisol impairs the function of lymphocytes and decreases the proliferation of leukocytes. Increasing amounts of pro-inflammatory cytokines decreases antibody titres and lymphoid organs, and enhances the synthesis of corticosterone in heat-stressed broilers (Ogle *et al.*, 1997).

According to Calefi *et al.* (2017) there is a direct connection (neuroimmunomodulation) between the central nervous system, endocrine and immune system that, through stressors such as heat, share efferent responses through various substances such as hormones, neurotransmitters, neuropeptides, cytokines and growth. Based on this, Sahin *et al.* (2017) found that supplementation with 0.78 mg/kg of Cr as Cr-histidine for broilers reared under heat stress influenced the expression of growth factors considered as biomarkers of oxidative stress, with increase in hepatic Nrf2

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expression and reduction on NF-κB expression in the breast muscle. Nrf2 is involved in the transcription of several genes that condition oxidative enzymes. NF-κB is linked to the transcription of genes involved in the coding of stress-related cellular responses (cytokines and free radicals). This indicates that the physiological mechanisms of the reaction of broilers to heat stress are multifactorial and have direct consequences on immunity and later performance. *Table 1* summarises the effects of Cr supplementation on physiological parameters and immune function in heat-stressed broilers.

Table 1 Effects of Cr supplementation on physiological parameters and immune function in heat-stressed broilers.

Cr source	ED (mg/kg)	Results	References
Cr-picolinate	0.20-1.20	Linear effect with decrease** in corticosterone and increase** in insulin, T3 and T4	Sahin *et al.* (2002)
Cr-picolinate	1.00	Increase* in haemoglobin and mean corpuscular haemoglobin concentration	Toghyani *et al.* (2006)
Cr-picolinate	1.50	Increase* in immunoglobulin G concentration and increase* in antibody titres against influenza virus and Newcastle	Toghyani *et al.* (2007)
Cr-picolinate	0.50	Decrease* in serum concentrations of glucose, protein, cortisol and cholesterol	Samanta *et al.* (2008)
Cr-nicotinic	1.50	Decrease* in serum concentrations of glucose and TG	Toghyani *et al.* (2012)
Cr-methionine	0.80	Increase* in serum concentration of HDL and decrease* in LDL, cholesterol and TG	Noori *et al.* (2012)
Cr-methionine	0.80	Increase* in serum concentration of antibody titres for Newcastle and infectious bronchitis, increase* in antibody production, and decrease* in H/L	Ebrahimzadeh *et al.* (2012)
Cr-methionine	1.20	Increase*in the immune response in broilers challenged with influenza and Newcastle virus. Increase* in immunoglobulin G and decrease* in corticosterone	Bahrami *et al.* (2012)
Cr-methionine	1.20	Increase** in the antibody response and decrease* in H/L. Increase in RWT** and RWS*	Ghazi *et al.* (2012)
Cr-chloride	2.00	Increase** in the immune response to Newcastle disease virus vaccine	Norain *et al.* (2013)
Cr-methionine	1.20	Decrease* in cholesterol concentration	Habibian *et al.* (2013)
Cr-chloride	1.20	Increase* in insulin concentration	Perai *et al.* (2014)
Cr-propionate	1.00 - 3.20	Quadratic increase** in antibody response for Newcastle vaccination and decrease* in H/L ratio	Rajalekshmi *et al.* (2014)

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Table 1 Continued

Cr source	ED (mg/kg)	Results	References
Cr-yeast	0.50	Decrease* in content of glucose and cholesterol	Mohammed *et al.* (2014)
Cr-methionine	0.50	Increase* in response to the Newcastle virus and decrease** in corticosterone	Jahanian and Rasouli (2015)
Cr-yeast	2.00	Increase* in SOD activity and decrease* in lipid peroxidation	Rao *et al.* (2016)
Cr-propionate	0.80-1.60	Linear decrease** in serum TG and LDL	Xiao *et al.* (2016)
Cr- histidinate	0.78	Increase** expression of GLUT-4 and Nrf2 in the muscle and GLUT-2 in the liver. Decrease** NF-κB. Increase in serum concentrations of glucose, cholesterol, creatine, and enzymes (AST, ALT, GGT, LDH and CK)	Sahin *et al.* (2017)

Abbreviations used: ED, effective doses; T3, triiodothyroxine; T4, thyroxine; H/L, heterophil/lymphocyte ratio; RWT, relative weight of thymus; RWS, relative weight of spleen; SOD, superoxide dismutase; GLUT-2, glucose transponder 2; GLUT-4, glucose transponder 4; Nrf2, nuclear factor erythroid 2-related factor 2; NF-κB, nuclear factor kappa B; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, γ-glutamyl transferase; LDH, lactate dehydrogenase; CK, creatine kinase. *, (P<0.05); **, (P<0.01)

Sahin *et al.* (2003) reported an increase in T3 and T4 concentrations, a decrease in lipid peroxidation and improved carcass characteristics of heat-stressed broilers (32°C) fed 0.40 mg/kg of Cr as Cr-picolinate with or without inclusion of 250 mg/kg of vitamin C to the diet. Vitamin C has been reported to decrease production and increase degradation of plasma glucocorticoids in broilers reared under cyclic heat stress (30°C) (Mahmoud *et al.*, 2004). Due to its antioxidant action, supplementation with 0.40 mg/kg Cr-propionate increased humoral immunity in broilers reared in high ambient temperatures (Eze *et al.*, 2014). In addition, Cao *et al.* (2004) reported that supplementation with 0.20 to 0.40 mg/kg Cr-chloride in broiler diets stimulated production of antibodies against Newcastle disease (Cao *et al.*, 2004). This mechanism of action is still poorly understood; however, mRNA expression was increased through interferon-gamma synthesis in broilers supplemented with Cr (Bhagat *et al.*, 2008). Ghazi *et al.* (2012) observed that supplementation with 1.20 mg/kg Cr-methionine for broilers under cyclic heat stress (37°C) induced a two-fold reduction in the heterophil/lymphocyte ratio.

Heat stress induces oxidative stress and cellular damage (unfolding, aggregation, and apoptosis) (Rhoads *et al.*, 2013). According to Sahin *et al.* (2009), the production of heat shock proteins (HSPs) and reactive oxygen species (ROS) indicates a physiological response of the organism to prevent heat-induced damage. Supplementation with 0.80 mg/kg Cr-histidinate increased cellular function and decreased oxidative stress by 27.2 and 55.6% respectively in the expression of HSPs in Japanese quails reared under cyclic heat stress (34°C) compared the control group (22°C) (Akdemir *et al.*, 2015). The decrease in expression of HSPs is associated with lower production of ROS, which attenuates the deleterious effects caused by heat stress. From currently available literature, there are no studies with broilers evaluating HSP expression due to Cr supplementation in broilers diets.

Past studies reported that LMWCr and cromodulin act as Cr carriers (Vincent 2000b;

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Vincent, 2010), which mobilise this mineral towards deficient proteins and enhancing cell membrane fluidity (Evans and Bowman, 1992). Therefore, insulin metabolism influences the peroxidation of lipids, and Cr, as an insulin cofactor, acts as a secondary antioxidant (Króliczewska *et al.*, 2004). Consequently, broilers exposed to high temperatures may present greater immune response and lower meat deterioration by reducing the formation of peroxides.

Supplementation with 0.50 mg/kg Cr-yeast in the diet of broilers decreased serum levels of total cholesterol, LDL, triglycerides and glucose, and increased HDL levels in association with greater body weights, weight gain and feed efficiency (Króliczewska *et al.*, 2004). Xiao *et al.* (2016) evaluated different levels Cr-propionate (0.20, 0.40, 0.80, 1.60 mg/kg) in the diets for broiler chickens raised under heat stress (35°C) from 21 to 42 days of age and reported a linear decrease in serum levels of triglyceride and LDL. Souza *et al.* (2010) suggested this probably occurred because Cr acts to inhibit the enzyme hydroxymethyl-CoA reductase responsible for the synthesis cholesterol. In agreement, Souza *et al.* (2010) reported a 6.53% reduction in total cholesterol in breast meat from broilers supplemented with Cr-picolinate.

Growth performance

Heat stress decreases feed intake and increases water consumption. Oliveira *et al.* (2006) observed a 31% lower feed intake in heat-stressed broilers (32°C) compared to those housed in thermoneutral conditions (25°C). Bruno *et al.* (2011) observed a 28% increase in water intake in broilers reared in a hot environment (34°C) compared to those in thermoneutral (25°C) conditions. According to Sands and Smith (1999), supplementation with 0.40 mg/kg Cr-picolinate increased weight gain and feed conversion ratio in broilers independent of their environment. Rajalekshmi *et al.* (2014) reported positive effects of Cr-propionate supplementation on growth performance in heat-stressed broilers. According to Jahanian and Rasouli (2015), heat stress increases Cr urinary excretion, resulting in marginal deficiency. Therefore, Cr supplementation is highly recommended when growing broilers are exposed to heat stress.

According to Oba *et al.* (2012), supplementation with 0.40 to 1.20 mg/kg Cr-yeast increased the feed conversion ratio and immune response by increasing haemaglutination values in broilers reared under heat stress (32°C). Toghyani *et al.* (2012) reported 4% greater feed efficiency in broilers exposed to high ambient temperatures (33°C) fed diets supplemented with 1.50 mg/kg Cr-nicotinic compared to those fed diets without Cr supplementation. Heat stress induced an increase in corticosterone, impaired growth performance, increased water intake and induced disturbances in the osmolarity and pH of the digestive tract (Puvadolpirod and Thaxton, 2000). Samanta *et al.* (2008) evaluated supplementation with 0.50 and 1.00 mg/kg of Cr as Cr-picolinate in broilers exposed to high temperatures (35°C) and reported greater feed efficiency with 0.50 mg/kg of Cr as Cr supplementation. *Table 2* summarises the effects of Cr supplementation on growth performance in heat-stressed broilers.

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Table 2 Effects of Cr supplementation on growth performance in heat-stressed broilers.

Cr source	ED (mg/kg)	Days of age	N	Results	References
Cr-picolinate	1.20	21-42	150	Increase* in FI and WG	Sahin *et al.* (2002)
Cr-picolinate	1.00	21-42	240	Increase* in FI and WG	Toghyani *et al.* (2006)
Cr-picolinate	0.50	1-40	225	Increase* in WG and decrease* in FCR	Samanta *et al.* (2008)
Cr-picolinate	0.50	21-42	200	Increase* in WG and decrease* in FCR	Zha *et al.* (2009)
Cr-nanocomposite	0.50	21-42	200	Increase* in ADG and decrease* in FCR	Zha *et al.* (2009)
Cr-nicotinic	0.60	21-42	432	Increase* in FI and WG	Naghieh *et al.* (2010)
Cr-chloride	2.50	1-42	150	Increase* in WG	Olufemi and Bosede (2011)
Cr-methionine	0.80	21-42	288	Increase* in FI and WG	Noori *et al.* (2012)
Cr-yeast	0.40	1-47	960	Decrease* in FCR and mortality	Oba *et al.* (2012)
Cr-methionine	1.20	1-49	150	Not significant	Ghazi *et al.* (2012)
Cr-chloride	2.00	1-35	80	Increase** in WG and FI	Norain *et al.* (2013)
Cr-methionine	0.80	1-42	288	Decrease* in mortality	Ebrahimzadeh *et al.*(2013)
Cr-picolinate	1.00	1-42	288	Decrease* in mortality	Pan *et al.* (2013)
Cr-methionine	1.20	1-49	150	Not significant	Habibian *et al.* (2013)
Cr-methionine	0.40	1-30	672	Increase* in WG and decrease* in FCR	Zhang and Kim (2014)
Cr-picolinate	0.50	1-42	128	Increase* in WG	Tawfeek *et al.* (2014)
Cr-methionine	0.70	1-41	400	Decrease* in FCR	Silva *et al.* (2014)
Cr-propionate	3.20	1-42	700	Not Significant	Rajalekshmi *et al.* (2014)
Cr-yeast	0.50	1-42	225	Increase* in WG	Mohammed *et al.* (2014)

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Table 2 Continued

Cr source	ED (mg/kg)	Days of age	N	Results	References
Cr-methionine	0.50	1-42	450	Increase** in FI, WG and FE	Jahanian and Rasouli (2015)
Cr-yeast	2.00	1-21	200	Increase* in FI, WG and FE	Rao *et al.* (2016)
Cr-propionate	2.00	15-42	250	Increase* in ADG	Huang *et al.* (2016)
Cr-histidinate	0.78	22-42	1200	Increase* in FI	Sahin *et al.* (2017)

Abbreviations used: ED, effective doses; n, number of birds; FI, feed intake; WG, weight gain; FCR, feed conversion ratio; ADG, average daily gain; FE, feed efficiency; *, (P<0.05); **, (P<0.01)

Carcass characteristics

Heat stress negatively affects carcass characteristics since it increases the production of free radicals, lipid peroxidation, protein and vitamin denaturation, exudate production and muscle glycogen consumption, thereby compromising meat quality (Song and King, 2015). Zhang *et al.* (2012) observed that broilers reared under cyclic heat stress had increased activity of pyruvate kinase with higher lactic acid content, reducing meat pH which accelerates meat glycolysis, and can result in decreased meat quality. Altan *et al.* (2003) reported changes in antioxidant capacity in broilers exposed to acute heat stress (38°C) in association with changes in catalase and glutathione peroxidase concentrations.

Habibian *et al.* (2013) evaluated different levels (0.60 and 1.20 mg of Cr per kg) and sources (Cr-chloride and Cr-methionine) of dietary Cr in broilers exposed to cyclic heat stress. They observed that those consuming diets supplemented with 1.20 mg/kg of Cr as Cr-methionine had higher breast yields. Zha *et al.* (2009) using three sources of Cr (Cr-chloride, Cr-picolinate, Cr-nanocomposite) at 0.50 mg of Cr per kg of feed reported an increase in carcass yield irrespective of the Cr source in broilers reared under heat stress conditions (35°C). In addition, protein concentration in the breast of broiler chickens fed diets containing Cr-nanocomposite was higher than those of the control group.

There is evidence that Cr supplementation can reduce skeletal muscle weight loss in stress situations and enhance mRNA expression of myosin and actin (Pan *et al.*, 2013; Rao *et al.*, 2012). Additionally, Cr supplementation inhibits the production of tumour necrosis factor (TNF-alpha) (Jain and Kannan, 2001) which can inhibit lipogenesis in adipocytes due to decreases in levels of lipoprotein lipase (LPL), glucose transporter type 4 (GLUT-4) and acetyl-coenzyme A synthetase (Sethi and Hotamisligil, 1999). Consequently, there is a reduction in fat content and an increase in protein deposition in the carcass of broilers. Huang *et al.* (2016) observed reduced abdominal fat content in broilers fed diets with Cr supplementation and submitted to a temperature of 33°C. Cr supplementation can also decrease cholesterol levels in the meat (Navidshad *et al.*, 2010; Javed *et al.*, 2010; Lin *et al.*, 2014). Moeini *et al.* (2011) reported high protein concentration and low cholesterol content in the skeletal muscle of broilers reared at 33°C and supplemented with 1.20 mg/kg of Cr as Cr-methionine in the feed. *Table 3* summarises the effects of Cr supplementation on carcass characteristics in heat-stressed broilers.

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Table 3 Effects of Cr supplementation on carcass characteristics in heat-stressed broilers.

Cr source	ED (mg/kg)	Results	References
Cr-picolinate	1.20	Increase* in carcass yield and decrease* in abdominal fat	Sahin *et al.* (2002)
Cr-picolinate	1.00	Increase* in carcass yield and decrease* in abdominal fat	Toghyani *et al.* (2006)
Cr-yeast	0.40	Increase* in L of the meat	Oba *et al.* (2007)
Cr-picolinate	0.50 -1.00	Linear effect in EE and quadratic effect in CP in carcass	Samanta *et al.* (2008)
Cr-nanocomposite	0.50	Increase* in carcass yield, breast yield, leg quarter yield, and decrease* in abdominal fat. Increase* in protein content of breast and tight, and decrease* in cholesterol content of breast and tight	Zha *et al.* (2009)
Cr-picolinate	0.50	Decrease* in abdominal fat and cholesterol content of tight. Increase* in protein content of tight	Zha *et al.* (2009)
Cr-yeast	1.20	Decrease* in lipid content and decrease** in lipid oxidation	Toghyani *et al.* (2010)
Cr-chloride	0.25	Decrease* in cooking weight loss with no changes on meat yield and quality	Olufemi and Bosede (2011)
Cr-nicotinic	1.50	Increase* in carcass yield and decrease** in abdominal fat	Toghyani *et al.* (2012)
Cr-methionine	0.80	Increase* in carcass yield and decrease* in abdominal fat	Ebrahimzadeh *et al.* (2013)
Cr-chloride	2.00	Increase** in carcass yield	Norain *et al.* (2013)
Cr-propionate	1.00 - 3.20	Increase* in breast yield	Rajalekshmi *et al.* (2014)
Cr-methionine	0.40	Increase* in L value of the meat	Zhang and Kim (2014)
Cr-propionate	2.00	Decrease* in b* value of the meat, and cooking loss of breast	Huang *et al.* (2016)

Abbreviations used: ED, effective doses; L, lightness; EE, ether extract; CP, crude protein; b*, yellow intensity; *, (P<0.05); **, (P<0.01)

Despite the numerous advantages of Cr supplementation in heat-stressed broiler chickens, some authors have reported no significant effects (Anandhi *et al.*, 2006; Vaz *et al.*, 2009; Ghanbari *et al.*, 2012). According to Huang *et al.* (2016) the discrepancy among studies may be explained by differences in type of sources, Cr concentration, different basal diets and experimental treatments. Standardised methodologies in future work would be useful in making data more directly comparable.

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One factor that must be taken into account when using Cr as a supplement in the diets of broiler chickens is the concentration of this metal in the meat of the animals. The determination of metals in foods is of utmost importance for the safety of consumers as it provides toxicological information necessary for the safety of the products. Ribeiro *et al.* (2011) conducted an experiment to detect the presence of Cr in chicken meat samples by spectrophotometry and did not detect traces of Cr (III), Cr (IV) and Cr (VI). Islam *et al.* (2016) evaluated the concentration of Cr in chicken meat samples from different farms in Bangladesh and found a high concentration in meat. Debski *et al.* (2004) found concentrations of 40 to 52 µg Cr/kg of moist tissue in meat from animals fed diets supplemented with 0.2 mg Cr/kg. According to EFSA (European Food Safety Authority) Panel on Dietetic Products, Nutrition and Allergies (2014), these values are within recommended daily intake range for adult men and women of 30-100 µg/day. However, they are in excess for infants less than one year of age (20-40 µg/day). In this sense, it is necessary to develop studies that evaluate the concentration of Cr and other heavy metals in foods of animal origin destined for human consumption, since toxicity presents extreme health risks.

Conclusions

When exposed to heat stress conditions, broilers maintain homeostasis and thermoregulation through physiological and metabolic adjustments that can have a negative effect on growth performance. Feed intake is reduced and consequently growth rates decline. Changes in nutrient utilisation and partitioning, as well as metabolic and immune function have also been reported. There is a positive relationship between dietary Cr supplementation and growth performance, physiological and immune functions when broilers are exposed to heat stress conditions. Chromium supplementation has been associated with increased growth rate, immune function and antioxidant capacity while decreasing lipid peroxidation, cholesterol and fat content in heat-stressed broilers. Therefore, dietary supplementation with Cr may be recommended as a nutritional strategy to modulate the negative effects of heat stress in broilers.

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