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1                   **Potential use of chromium to combat thermal stress in animals: a review**

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**44 Abstract**

45 Heat stress (HS) has adverse effects on the body: it decreases body weight, feed efficiency, feed  
46 intake, carcass quality, and nutrient digestibility. Chromium (Cr) can prevent lipid peroxidation  
47 induced by HS through its strong antioxidant activities, especially when it is added to the poultry  
48 diet. It improves the action of insulin and nutrient metabolism (of lipids, proteins, nucleic acid,  
49 and carbohydrates) through activation of enzymes associated with such pathways. The results of  
50 the studies on Cr added to diets with concentrations of 0.05 mg Cr/kg of Cr-methionine led to  
51 improved feed efficiency and DM intake by cows and Holstein dairy calves exposed to high  
52 environmental temperatures. Moreover, calves that received Cr at levels of 0.05 mg/kg of body  
53 weight tended to have higher serum concentrations of glucose and higher ratios of insulin to  
54 glucose. In heat-stressed pigs, Cr addition (200 ppb) increased blood neutrophils by about 37%.  
55 Several studies have asserted that Cr can inhibit inflammation in lactating cows by promoting the  
56 release of Hsp72, assisting production of IL-10 and inhibiting degradation of I $\kappa$ B $\alpha$  in HS  
57 conditions. In addition, Cr supplementation was observed to possibly have positive impacts on  
58 both cell-mediated and humeral immunity in heat-stressed buffalo calves. Studies over the last  
59 two decades have shown with certainty that chromium supplementation has an impact on many  
60 variables in chickens. Moreover, Cr is believed to increase insulin action in insulin-sensitive  
61 tissues (i.e., adipose and muscles), resulting in increased farm animal productivity through the  
62 improvement of feed intake, growth rate, carcass quality, reproductive parameters and immune  
63 functions.

64 **Keywords:** Heat stress; chromium; growth; thermoregulation; heat shock proteins.

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## 67 **1. Introduction**

68 Heat stress (HS) occurs when the heat produced by animals surpasses their capacity to  
69 dissipate the extra heat into their surrounding environment (Alagawany et al., 2017; El-Kholy et  
70 al., 2018; Farghly et al., 2018). There are two main categories of HS: chronic and acute. Because  
71 chronic HS comes from high temperatures over a long period, acclimatization to the environment  
72 is possible. Acute HS, on the other hand, comes from rapid increase in the environmental  
73 temperature over a short period (Alagawany et al., 2017). HS elicits many physiological  
74 consequences. Its impacts include increased body and rectal temperatures, reduced feed  
75 consumption, impaired immunity, alteration of blood electrolyte balance and pH, poor feed  
76 utilization, depressed cellular energy bioavailability, impaired reproductive functions and  
77 impaired endocrine functions (Loyau et al., 2015).

78 HS alters lipid, protein and carbohydrate metabolism. It also alters homeorhetic  
79 adaptations, which are largely caused by increased circulating insulin (Baumgard and Rhoads,  
80 2013). Hence, identification of nutritional approaches to ameliorate the effects of HS on health  
81 and production has become of interest recently (Khafaga et al., 2019). Alternation in utilization  
82 and substrate uptake is also a result of HS, but this phenomenon is not yet fully understood.  
83 When temperatures are high, metabolic adaptations occur to increase chances of survival. For  
84 example, feed intake in Holstein cattle during summer was found to result in lower insulin  
85 concentrations in summer than in winter and spring (Abdelnour et al., 2019). Likewise, plasma  
86 insulin was lower in heat-stressed lactating sows compared to a control group, although glucose  
87 concentration was the same (de Braganca and Prunier, 1999). In addition, Sevi et al. (2002)  
88 reported that plasma non-esterified fatty acids (NEFA) increased in sheep but declined in  
89 lactating cows due to heat exposure (Itoh et al., 1998; Shwartz et al., 2009; Wheelock et al.,

90 2010). Under stress conditions, Cr requirements are higher due to increased urinary excretion  
91 (Chang and Mowat, 1992; Pechova and Pavlata, 2007; Yari et al., 2010).

92 Chromium (a low-molecular-weight binding substance) activates insulin receptors and  
93 mobilizes the glucose transporter type 4 (GLUT 4) from the inside of the cell to the surface,  
94 allowing glucose to enter the cell (Vincent, 2015). Dietary Cr supplementation (0.5 to 1.5 mg/kg  
95 of dry matter (DM)) to heat-stressed buffalo calves improved heat tolerance, immune function,  
96 and insulin effectiveness. However, it had no effect on feed intake, growth rate, tri-iodothyronine  
97 (T3) or thyroxine (T4) concentrations (Kumar et al. 2015a).

98 The effects of Cr supplementation on production were previously reported for the cases of  
99 heat-stressed cows (Soltan 2010) and normal cows (Sadri et al. 2009). However, the information  
100 concerning the influences of Cr on growth rate in heat-stressed calves is not consistent (Kumar et  
101 al. 2015a; Sadri et al. 2009; Yari et al. 2010). These inconsistencies could be attributed to the  
102 chemical form of Cr used (organic vs inorganic, yeast vs chelated Cr) (Kegley et al. 1997), the  
103 environmental conditions (normal vs heat-load conditions), Cr dosage, and method of Cr  
104 delivery (starter vs liquid).

105 Several research papers described the influences of Cr supplementation to heat-stressed  
106 dairy calves on feed intake, growth rate, general health status, and blood metabolites. However,  
107 the way in which Cr affects the feed intake is not clear. To clarify this mechanism, investigation  
108 of ingestion behavior in calves supplemented with Cr is needed.

109 There are no review articles summarizing the beneficial impacts of Cr on heat-stressed  
110 poultry. Therefore, the aim of the present paper is to highlight the consequences of Cr on  
111 thermoregulatory responses, growth rate, metabolites of blood, health status, oxidative  
112 biomarkers, and immune indices in different heat-stressed ruminants.

## 113 **2. Heat stress in animals**

114 Heat stress has several direct and indirect effects on dairy animal health: it can disrupt  
115 normal metabolism as well as affect the physiological, hormonal, and immune status of animals  
116 (Abd El-Hack et al. 2019; Saeed et al. 2019). Incremental increases in environmental  
117 temperature exert direct adverse impact on the hypothalamus appetite center, with subsequent  
118 reduction in feed intake (Baile and Forbes, 1974). In lactating cows, feed intake starts to decrease  
119 at climatic temperatures of 25-26°C, and it may decline more rapidly at 30°C and drop by  
120 approximately 40% at 40°C (Rhoads et al., 2013). The purpose of this reduction in feed intake is  
121 to decrease production of heat in hot environments (Kadzere et al., 2002). As a result, animals  
122 may develop negative energy balance (NEB), with subsequent negative effect on body weight  
123 and body condition scores (Lacetera et al., 1996). Additionally, HS may affect the production  
124 and composition of milk in dairy animals (Bouraoui et al., 2002; West, 2003; Spiers et al., 2004;  
125 Upadhyay et al., 2009; Wheelock et al., 2010). Berman (2005) concluded that the stress-response  
126 systems in dairy cattle could be affected negatively in climatic temperatures above 35°C. Heat-  
127 stressed dairy cows developed reduced feed intake and NEB, which is subsequently responsible  
128 for milk synthesis decline (Wheelock et al., 2010).

129 Moreover, HS often alters the physiological mechanisms of the rumen, with resultant  
130 increases in health problems and metabolic disorders (Nardone et al., 2010; Soriani et al., 2013).  
131 Nonaka et al. (2008) concluded that heat-stressed animal suffer from diminished acetate  
132 production and from increased propionate and butyrate production due to alterations in rumen  
133 function. As a result, stressed animals consume less roughage and undergo alteration of rumen  
134 pH and microbial population (Hall, 2009), diminished rumination and rumen motility (Nardone  
135 et al., 2010; Soriani et al., 2013), decreased salivary production, and less dry-matter intake

136 (DMI) (Nardone et al., 2010; Soriani et al., 2013). Also, HS resulted in decreased animal growth,  
137 carcass quality and fertility, and in increased mortality rate and care costs (Baumgard and  
138 Rhoads, 2013; Mayorga et al., 2019).

139         It is therefore necessary to develop protocols to overcome the negative impacts of HS.  
140 Nutritional intervention is considered to be an economical method for increasing production in  
141 summer (Mayorga et al., 2019). Indeed, dietary chromium enhances cellular glucose uptake  
142 (Chen et al., 2006) by increasing the binding of insulin to its extracellular receptors (NRC, 1994;  
143 Vincent, 2015). In addition, studies showed that average daily feed intake (ADFI), reproduction,  
144 immune response and growth performance are improved by Cr supplementation (Burton et al.,  
145 1993b; Hayirli et al., 2001; Hung et al., 2014; Lindemann et al., 1995; Sadri et al., 2009; Sales  
146 and Jancik, 2011; Yasui et al., 2014). The adverse effect of HS in ruminants and their body  
147 reactions against it are summarized in Figure 1. Similarly, the adverse effect of HS on animal  
148 performance, production and reproduction is summarized in Figure 2.

### 149 **3. General properties of chromium and sources**

150         Trivalent Cr ( $\text{Cr}^{3+}$ ) has an effect on rodents (rats and mice), raising their sensitivity to  
151 insulin (Vincent, 2014). Despite the fact that, according to a report published by the European  
152 Food Safety Authority, there is no evidence that Cr is an essential trace element for either  
153 animals or humans (Vincent, 2014), Cr has for decades been thought to be an essential nutrient,  
154 and current studies show that high pharmacological and nutritional doses should be maintained  
155 as a beneficial element (Hua et al., 2012; Vincent, 2001; Vincent, 2017; Ibrahim et al., 2017).  
156 Farm animals, humans and laboratory animals have been the subject of studies of Cr as a dietary  
157 supplement. These studies showed that stress resulted in Cr deficiency (when Cr is considered  
158 essential) (e.g. see Lukaski et al., 1996) and in increased loss of Cr through urinary excretion.



159 Cr supplementation also overcomes the effects of stress stemming from animal shipping  
160 and meat quality improvement. The reviews made by the (NRC, 1994) in the mid-1990s and by  
161 Lindemann et al. (1995) are the most comprehensive reviews about the use of Cr  
162 supplementation for farm animals. No conclusions about the need for Cr supplementation in the  
163 diets of sheep, fish, rats, horses, and rabbits have been drawn or stated by the Animal Research  
164 Committee. Nor have any recommendations been made about Cr supplementation for poultry,  
165 cattle and swine, even though Cr does have beneficial impacts on heat-stressed cattle and pig  
166 leanness and on the effectiveness of reproduction (NRC, 1994).

#### 167 **4. Mode of action of chromium in animal nutrition**

168 Chromium chloride, chromium polynicotinate, and chromium picolinate (CrP, Figure 3)  
169 are considered to be nutritional supplements and micronutrients. Several investigations  
170 concluded that chromodulin is a naturally occurring biomolecule that binds to trivalent Cr and  
171 could explain the action of Cr in metabolism of lipids and carbohydrates (Davis and Vincent,  
172 1997). Chromodulin is able to stimulate insulin-dependent glucose metabolism in adipocytes  
173 (Yamamoto et al., 1988). Moreover, it could activate the membrane phosphotyrosine  
174 phosphatase in adipocyte membranes (Davis et al., 1996).

175 Supplemented levels of Cr are expected to induce little alteration on body weight and body  
176 composition. However, the administration of high levels of Cr could potentially lead to altered  
177 Cr status, with subsequent alteration in metabolisms (John, 2004).

178 In addition, CrP has the potential to control levels of blood sugar in diabetes, control blood  
179 pressure levels, and reduce cholesterol. Cr acts as a cofactor for insulin; therefore, it activates  
180 insulin receptor kinase and increases the number of insulin receptors. CrP attenuates weight gain,

181 but the mechanism for this is unknown (Anton et al., 2008). It may have an action through its  
182 influence on the balance of energy to either decrease feed intake or increase energy expenditure.

183 Cr affects reproduction by enlarging the seminal vesicles, increasing prostate weight and  
184 reducing testis weight (Marouani et al., 2012). It also affects weight loss by decreasing fat  
185 percentage and increasing lean body mass (Anderson, 1998).

186 Stress stimulates the hypothalamus to produce corticotropin, which stimulates the pituitary  
187 to produce the adrenocorticotrophic hormone, which in turn stimulates the adrenal cortex to  
188 release corticosterone. Corticoids depress the immune system and reduce the concentration of  
189 serum protein. They also raise blood glucose levels and decrease the utilization of glucose, thus  
190 functioning as antagonists to Cr, which influence the release of corticosteroids. The role of Cr<sup>3+</sup>  
191 against cellular glucose uptake is summarized in Figure 4.

## 192 **5. Impacts of chromium in heat-stressed animals**

193 The potential beneficial impact for dietary supplementation of Cr during HS could be  
194 obtained through several pathways. The main findings related to Cr supplementation in heat-  
195 stressed animals are summarized in Table 1 and discussed in the following sections.

### 196 **5.1 Thermoregulatory responses**

197 Thermoregulatory response is considered to be the first indicator of HS. HS is associated  
198 with increased respiration, pulse rate and body temperature in homothermic animals. A dietary  
199 supplement of 0.05 mg of Cr-methionine/kg of body weight significantly reduced the respiration  
200 rate (RR) in heat-stressed dairy calves (Mousavi et al. 2019b). Cr (0.05 mg/kg of body weight)  
201 had the same effect but had no effect on RT (Kargar et al., 2018b). In heat-stressed calves, Cr-  
202 methionine at 0.05 mg/kg of body weight reduced diarrhea to -0.9 d, pneumonia to -0.7 d and  
203 medication to -1.5 d before weaning (Kargar et al., 2018b; Mousavi et al., 2019a).

204 Most studies indicated that Cr decreases RT to overcome the effects of HS (Kargar et al.,  
205 2018b). Mousavi et al. (2019a) found no effect of Cr-methionine on diarrhea, pneumonia,  
206 medication days or RT in heat-stressed calves. Also, Kumar et al. (2015a) found no effect of 0.5,  
207 1 or 1.5 mg of inorganic Cr/kg on pulse rate (PR), RR or RT in heat-stressed buffalo calves. An-  
208 Qiang et al. (2009) measured the values of RR and RT for cows undergoing Cr supplementation  
209 (3.6, 7.2 or 10.8-milligram chromium/head per day) and cows without any supplementation and  
210 found no differences. According to the previous results, Cr has no impact on somatic cell count  
211 and or on RT, PR or RR in lactating cows (Qi et al. 2018b). A separate study indicated that Cr  
212 propionate (200 ppb) has no effect on RR, skin temperature (ST) or body temperature (BT) in  
213 heat-stressed pigs (Mayorga et al., 2019).

## 214 **5.2 Growth performance**

215 Thermal stress has been reported to decrease feed intake, weight gain and effectiveness of  
216 feed intake (Abdelnour et al., 2019). Separate studies corroborated that heat-load results in  
217 decreased feed intake and growth performance and that indicated that, in turn, it results in  
218 decreased energy expenditure for tissue synthesis (Johnson et al., 2015; Kerr et al., 2003; Le  
219 Bellego et al., 2002; Mayorga et al., 2019). To counter this, Cr supplementation can result in  
220 increased feed intake in heat-stressed animals and hence growth performance, as shown by  
221 several studies (Hayirli et al., 2001; Hung et al., 2014; Mayorga et al., 2019; Sahin et al., 2002).  
222 Likewise, Cr yeast increased feed intake in heat-stressed lactating dairy cows compared with a  
223 control group (21.24 and 19.56 kg/day, respectively) (Alsaiady et al., 2004; Hayirli et al., 2001).

224 Feed efficiency and average daily gain increased in the case of calves in their growth stage  
225 fed Cr at a level of 4 mg/head (Chang and Mowat, 1992). This result is due to the increase in  
226 total levels of immunoglobulins in serum (Burton, 1995; Burton et al., 1996; Chang and Mowat,

227 1992). Moreover, the supplementation of 0.05 mg Cr/kg of body weight of Cr-methionine in  
228 cows' diets increased feed efficiency and DMI (Arthington et al., 1997). Supplementation of 3.6,  
229 7.2 and 10.8 mg chromium/head/day to heat-stressed dairy cows resulted in a significant increase  
230 in DMI ( $p < 0.05$ ) according to An-Qiang et al. (2009). Mousavi et al. (2019a) reported that  
231 supplementation of Cr to dairy calves under HS resulted in increases in DMI ( $P = 0.002$ ) and in  
232 average daily gain (ADG) ( $p = 0.02$ ) but had no effect on effectiveness of feed ( $P = 0.93$ ) or on  
233 body weight ( $p = 0.14$ ). On the other hand, Kargar et al. (2018b) found that dietary  
234 supplementation of chromium improved the intake of feed and growth rate in dairy Holstein  
235 calves exposed to high environmental temperature (83.3 units). Also, the addition of Cr at a level  
236 of 0.05 mg/kg of body weight to a milk substitute or to diets for cattle during the pre- and post-  
237 weaning periods led to increases, at the end of the experiment, in feed intake, DM intake and  
238 average body weight gain, by 16.4%, 11.85% and 7.07% respectively. Cr supplementation  
239 improves growth rate and body weight by enhancing the absorption and digestion of nutrients  
240 (Ghorbani et al., 2012; Kargar et al., 2018a). Moreover, in finisher gilts exposed to high  
241 temperatures, the application of nano-chromium tripicolinate (400 ppb) resulted in a reduction in  
242 cortisol level by 25% and an increase in feed intake by 6% (Hung et al., 2014). Mayorga et al.  
243 (2019) discovered that final body weight increased in heat-stressed finishing pigs when fed with  
244 200 ppb of chromium propionate.

245 Ruminant activity was reported to decrease as a result of environmental heat load  
246 (Soriani et al., 2013). Decreased rumen motility as a result of a slowing in the fractional digesta  
247 passage rate has led to a decrease in feed intake (Nikkhah, 2012). Although the mechanism by  
248 which Cr affects rumination patterns is unclear, the insulin-potentiating pathway may be  
249 involved. In contrast with previous results, Cr supplementation given over 120 days to Murrah

250 buffalo calves had no significant effect on feed conversion ratio (FCR), ADG or intake of DM  
251 (Kumar et al., 2015a). Similar results recorded by Qi et al. (2018a), Subiyatno et al. (1996)  
252 showed that Cr has no influence on DM intake. Also, the results of tests performed under normal  
253 conditions by Bunting et al. (2000) and Ghorbani et al. (2012) showed that Cr has no influence  
254 on feed intake or total DMI. The timing of supplementation of Cr to the diet (pre-partum or  
255 immediately post-partum) had an impact on the response.

256 Overall, the addition of Cr yeast to the diet has been observed to attenuate the negative  
257 impacts of high environmental temperatures and result in a reduction in feed intake and  
258 efficiency. Cr requirements for calves increase under a variety of stress conditions (Chang and  
259 Mowat, 1992; Pechova and Pavlata, 2007). Such variations are due to changes in the  
260 concentration of supplementation and the method of adding Cr to milk only or colostrum plus  
261 milk. Cr requirements as a whole rise during thermal heat load; this is a result of increased Cr  
262 urinary excretion, which in turn results in diminished feed intake (Kargar et al., 2018b; Pantelic  
263 et al., 2018; Yari et al., 2010). In short, to attenuate the impacts of HS, dietary Cr should be used  
264 to improve both the performance of animals and their welfare (Kargar et al., 2018b).

### 265 ***5.3 Milk yield and composition***

266 Adding (4 g/head/day) of dietary chelated chromium to the diet of heat-stressed dairy cows  
267 has no effect on milk fats, protein, lactose, or the non-fat percentage of solids (Alsaady et al.,  
268 2004). A significant rise ( $p < 0.05$ ) in milk protein, fat and lactose levels by 1.043%, 1.066% and  
269 1.020% respectively was recorded by Nikkhah (2012) when heat-stressed dairy cows were  
270 supplied with 0.05 mg Cr/kg. Moderate levels of Cr resulted in an increase in the ingestion of  
271 feed, DM effectiveness and a decline in insulin levels, leading to a significant augmentation in  
272 milk secretion as a result of increased mammary nutrient flow (Winkelman and Overton, 2012).

273 Insulin growth factor (IGF) activity, which has functional and structural homology with  
274 insulin receptors, is supported through the improvement of milk production, which also mimics  
275 the action of bovine somatotropin (bST) (Alsaady et al., 2004). Likewise, milk production  
276 increased, resulting in a decline in lipolysis. In a separate study, increase in glucose uptake,  
277 which leads to a rise in DMI (NRC, 1994), resulted from receiving 10 mg/day of Cr from 21  
278 days pre-partum to 35 postpartum (McNamara and Valdez, 2005). Additionally, milk yield was  
279 found to increase ( $p=0.013$ ) after the supplementation of chromium picolinate to the diet of heat-  
280 stressed cows (An-Qiang et al., 2009). On the other hand, An-Qiang et al. (2009) reported that  
281 dietary Cr had no significant effect on milk components (protein, fat and lactose) in dairy cows  
282 under high temperature (79.61). The beneficial effect of chromium on glucose and NEFA  
283 metabolism in dairy cattle is illustrated in Figure 5.

#### 284 ***5.4 Blood variables***

285 Cr yeast (4 mg/day) fed to lactating cows failed to show any effect on the blood serum  
286 levels of hemagglutination (HA), cholesterol, triglycerides, urea, IGF-1, BHBA, albumen, and  
287 glucose (Mirzaei et al., 2011; Soltan, 2010) but did show a drop in total blood and protein  
288 concentration (Soltan, 2010). Serum, insulin and NEFA reduced significantly when 0.05 mg  
289 Cr/kg of body weight of Cr yeast was supplemented (Yari et al., 2010). Recently, in dairy farm  
290 calves suffering from HS, dietary supplementation with 0.05 mg of Cr-methionine/kg of body  
291 weight had no significant influence on glucose, albumin, A/G ratio, triglyceride cholesterol,  $\beta$ -  
292 Hydroxybutyrate ( $\beta$ HBA) or blood urea nitrogen (BUN). Nevertheless, the serum concentration  
293 of globulin tended to be higher in calves supplemented with  $\text{Cr}^{+3}$  during the experimentation  
294 period (Mousavi et al., 2019a).

295 Calves supplied with chromium at a level of 0.05 mg/kg body weight tended to have higher  
296 serum concentrations of glucose and higher ratios of insulin to glucose measured at the end of  
297 the experiment (Kargar et al. 2018b). The same results were recorded by Yari et al. (2010) and  
298 Ghorbani et al. (2012). Similarly, Jin et al. (2017) suggested that dietary supplementation of  
299 chromium picolinate (3.5 mg of Cr/cow daily) to lactating cows under HS conditions did not  
300 exhibit any changes in glucose, BUN, cholesterol or creatinine.

301 Mayorga et al. (2019) reported similar results as he demonstrated that heat-stressed  
302 finishing pigs supplied with 200 ppb of chromium propionate did not show any shift in most of  
303 the blood parameters except for neutrophils. Heat-stressed pigs treated with chromium  
304 propionate supplementation (200 ppb) showed an increase in blood neutrophils by 37%  
305 compared to the control group (Mayorga et al., 2019). Adding chromium ( $p < 0.01$ ) was found to  
306 raise albumin/globulin ratio as well as cholesterol level (Soltan, 2010). Enhanced insulin action  
307 allows glucose to enter the cell and can repress lipolysis. Reduced mobilization of fats was  
308 reported to decrease dependence on body storage and lessen NEFA (Yang et al., 1996). Reduced  
309 NEFA raises DMI through the reversed lipostatic mechanism: as the levels of NEFA increase,  
310 the intake of food will decrease (Forbes, 2007; McNamara and Valdez, 2005). Milk production  
311 was found to increase when insulin action is improved as a result of the reduction in NEFA and  
312 insulin levels despite fixed glucose levels (Hayirli et al., 2001). Yari et al. (2010) and Ghorbani  
313 et al. (2012) reported similar results. Similarity, Jin et al. (2017) suggested that dietary  
314 supplementation of 3.5 mg of chromium picolinate daily to lactating cows under heat-stress  
315 conditions did not exhibit any changes in cholesterol, glucose, BUN or creatinine levels. In  
316 finisher gilts reared under summer conditions, plasma glucose, insulin and NEFA levels in serum

317 did not change in chromium tripicolinate nanoparticles-treated pigs (400 ppb) compared to those  
318 in the control group (Hung et al., 2014).

319 Bareille and Faverdin (1996) found evidence indicating that insulin can affect rumination  
320 by increasing the motility of reticulum and rumen through enhancing the entry of glucose into  
321 the cells. They also reported an increase in rumination time when glucose was infused  
322 intravenously (2.75 mmol/kg) to dairy cows. However, supplemental Cr did not affect blood  
323 insulin concentration at weaning or rumination time during the pre-weaning period in their study,  
324 despite the fact that rumination patterns changed (decreased rumination frequency and increased  
325 rumination duration and interval). Therefore, the relationship between blood insulin  
326 concentration and rumination patterns needs to be investigated further.

327 Chromium supplementation had little impact on insulin and blood glucose levels for both  
328 control and heat-stressed calves (Ghorbani et al., 2012; Yari et al., 2010). Kumar et al. (2015a)  
329 reported a reduction in blood insulin concentration in buffalo calves under HS when 1.0 and 1.5  
330 mg Cr/kg of DMI was supplemented, but not in the case of 0.5 mg Cr/kg. Consistent with these  
331 results, Bunting et al. (2000) found that blood glucose decreased and insulin levels increased  
332 when 0.5 mg of Cr/kg of DMI was supplemented to dairy calves. Chromium supplementation  
333 increased both insulin concentrations and the ratio of insulin to glucose, suggesting that high  
334 levels of insulin were needed in order to make blood free from glucose (weak insulin sensitivity)  
335 and that resistance to insulin increased over that time period (Bunting et al., 2000).

### 336 **5.5 Hormones**

337 After 0.05 mg of Cr-methionine/kg of body weight was given in supplementation to dairy  
338 calves exposed to HS, Mousavi et al. (2019a) observed no notable effect on thyroxin (T3, T4,  
339 T3/T4 ratio), and cortisol hormone activities. However, they also reported that calves fed with Cr



340 recorded high insulin levels and insulin-to-glucose ratios compared to those in the control group.  
341 Similarly, Kargar et al. (2018b) reported that chromium supplementation (0.05 mg/kg of body  
342 weight) had no influence on serum levels of T3, T4 or cortisol when measured at pre- and post-  
343 weaning. Calves fed .04 mg Cr/kg of body weight showed no difference in T3 or T4 blood  
344 concentration.

345 The same results were observed in buffalo calves that received 0.5, 1.0, and 1.5 mg of  
346 Cr/kg of DMI (Kumar et al., 2015a). However, Cr supplementation of 0.03 mg of Cr/kg of body  
347 weight increased blood T4 concentration and reduced T3-to-T4 ratio in dairy calves (Ghorbani et  
348 al., 2012). On the other hand, it was reported that raising Cr doses (from 0 to 0.02 and 0.04 mg  
349 Cr/kg of body weight) resulted in a quadratic decline in serum T4, whereas blood T3 decreased  
350 only with the higher Cr dose supplementation in dairy calves (Yari et al., 2010).

### 351 **5.6 Antioxidants**

352 Cr supplementations do not influence malondialdehyde (MDA) or glutathione peroxidase  
353 (GPx) serum levels, but the concentration of superoxide dismutase (SOD) tended to increase in  
354 calves receiving Cr-methionine at levels of 0.05 mg Cr/kg of body weight at weaning.  
355 Furthermore, serum activities of catalase in calves fed Cr-methionine was higher after weaning  
356 ( $p < 0.001$ ) (Jin et al., 2017). Moreover, Mousavi et al. (2019a) revealed a significant reduction  
357 of creatine kinase as a response to yeast-chromium (Cr yeast, 8 mg/d) dietary supplementation to  
358 lactating heat-stressed cows; this result may be linked to application of different types of  
359 animals, suitable doses of chromium, and its chemical forms.

360 On the other hand, (Qi et al., 2018a) reported that supplementation of chromium picolinate  
361 (3.5 mg of Cr/cow daily) under hot conditions did not have an effect on antioxidant activities  
362 such as serum total antioxidant capacity (T-AOC), SOD activity, or MDA levels of lactating

363 cows (Qi et al. 2018a). Qi et al. (2018a) moreover suggested that Cr can inhibit inflammation in  
364 lactating cows by promoting the release of Hsp72, facilitating the production of IL-10, and  
365 inhibiting the degradation of I $\kappa$ B $\alpha$  under HS conditions.

### 366 **5.7 Immunity**

367 Kumar et al. (2015a) observed that 1mg Cr/kg DM had positive impacts on both cell-  
368 mediated and humeral immunity in heat-stressed buffalo calves. Kumar et al. (2015a)  
369 investigated different levels: 0.5, 1.0 and 1.5 mg of inorganic Cr/kg DM of the diet of buffalo  
370 calves under high environmental temperature conditions: buffalo calves receiving 1.5 mg/kg DM  
371 of Cr ( $p < 0.01$ ) showed the highest immune status values and plasma Cr concentration during  
372 the stages of the experiment. It was found that buffalo calves receiving 1.5 mg/kg of DM of Cr  
373 exhibited a boost in the proliferation of B or T lymphocyte values (+16.50%), the phagocytic  
374 activity of neutrophils (13.72%), fluorescence recovery after use of the photobleaching (FRAP)  
375 technique (27.88%) and immunoglobulin of plasma (13.14%) compared with the control buffalo  
376 calves (Kumar et al., 2015a). The negative influence of HS on cows can be reduced through  
377 improved immunological activities under the effect of chromium supplementation. A significant  
378 enhancement in lymphocytes proliferation and blastogenesis in inorganic Cr given as  
379 supplementation to periparturient buffaloes was reported by several researchers (Burton et al.,  
380 1993a; Chang and Mowat, 1992; Kumar et al., 2015b).

381 It was reported that beef calves given supplementation of 15 mg/kg DM of Cr) had a slight  
382 improvement in cell-mediated immunity. Supplementation of Cr may have improved immune  
383 status by increasing immunoglobulin (IgM) and decreasing blood cortisol levels in buffalo calves  
384 compared to those in the control group, although an increase in cortisol level after exposure to  
385 high temperatures was also observed (Abdelnour et al., 2019; Kumar et al., 2015b). This led to

386 depression in immunity by preventing proliferation of lymphocytes, the activation of  
387 lymphocytes factors, and the production of T-cell growth factors (Munck et al., 1984; Roth and  
388 Kaeberle, 1982). Wang et al. (2007) recorded that supplementation of 200 ppb Nano- Cr/kg in  
389 pigs increased the serum levels of IgG and IgM by 20.6% and 13.2% respectively. Likewise,  
390 serum total levels of Ig and IgM increased after supplementation of Cr yeast to the diets of  
391 stressed calves (Burton et al., 1993a). On the other hand, Kegley and Spears (1995) found that Cr  
392 nicotinate and Cr yeast supplementation for stressed calves had no impact on total levels of IgM.  
393 To develop immunoglobulin concentration and the proliferation of lymphocytes, advanced  
394 antioxidants are needed for the production of immunoglobulin (Bach et al., 2000).

#### 395 **5.8 Heat shock proteins (HSPs)**

396 Heat-stressed lactating Holstein cows with a chromium diet (Cr yeast, 8 mg/d) were found  
397 to have a significant elevation ( $p < 0.05$ ) of blood HSP mRNA expression on d 44 of exposure,  
398 and Kumar et al. (2015b) found that the addition of 1.5 mg of Cr/kg to the diet of heat-stressed  
399 buffalo calves decreased Hsp70 significantly. Similarly, Qi et al. (2018a) confirmed that the  
400 supplementation of 3.5 mg Cr/kg daily to the diets of heat-stressed lactating cows increased  
401 Hsp72 significantly (11.68 ppb) compared to those that did not receive Cr (6.21 ppb).

402

#### 403 **6. Conclusion and future perspectives**

404 The rapidly changing climate, global warming, and accompanying rapid growth in  
405 population size are sounding the alarm for the development of critical strategies to manage  
406 livestock productivity and reproducibility. As shown in this current review, HS has an effect on  
407 the nutritional metabolic and antioxidant status of livestock. When exposed to HS conditions,  
408 animals maintain thermoregulation and homeostasis via metabolic and physiological adjustments

409 that can have a negative influence on growth performance. Feed intake is lowered, and growth  
410 rates consequently decrease. Alterations in nutrient partitioning and utilization as well as in  
411 immune and metabolic functions have also been reported. Cr restores productivity, performance,  
412 nutrient digestibility, antioxidant profile and immune status while decreasing lipid peroxidation,  
413 cholesterol and fat content in animals after exposure to HS. Therefore, dietary supplementation  
414 with Cr may be recommended as a nutritional strategy to modulate the negative effects of HS in  
415 broilers.

416 Proper nutritional management of livestock so that animals maintain energy balance could  
417 have a positive effect on reproductive status and resulting embryos, future progeny and  
418 production. More in-depth studies using genomic and metabolomic technologies are needed to  
419 clearly investigate the genomic and epigenetic mechanism of action of HS on livestock  
420 development. Most of the literature discussing the effect of HS and/or NEB on livestock health is  
421 based on in vivo chromium effects. Because of its marked role in poultry performance and  
422 production, Cr could be considered as one of the very essential components in poultry feed  
423 during times of HS. Furthermore, the effect of Cr diet interaction with livestock health status  
424 during HS is a new area that should be considered for future research. Another future  
425 consideration is proper attention on breeding programs, for selection of HS-tolerant breeds,  
426 instead of only selection for high-production breeds.

427

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433

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701 **Figure legends**

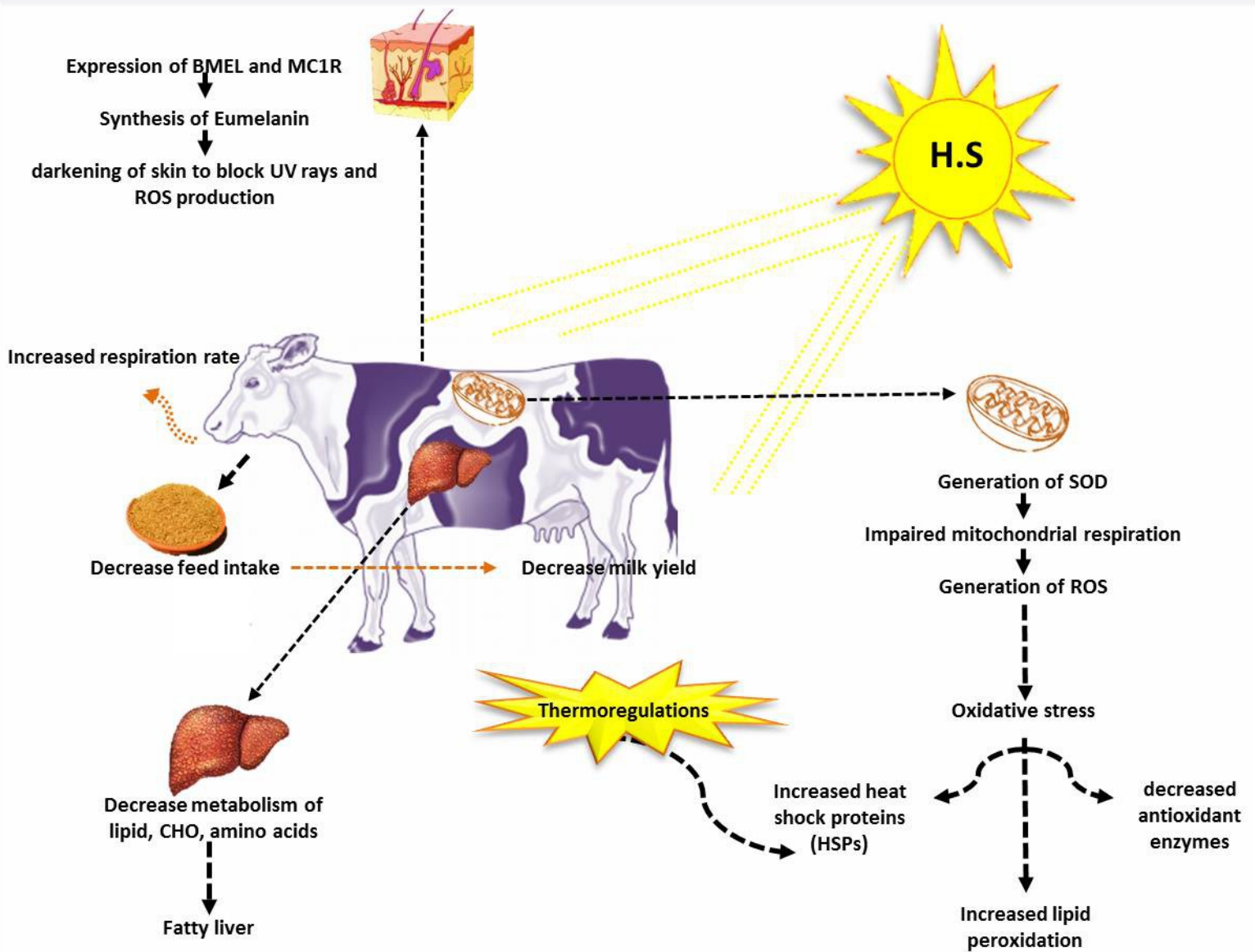
702 **Figure 1.** Summary of heat stress adverse impacts and body reactions in ruminants.

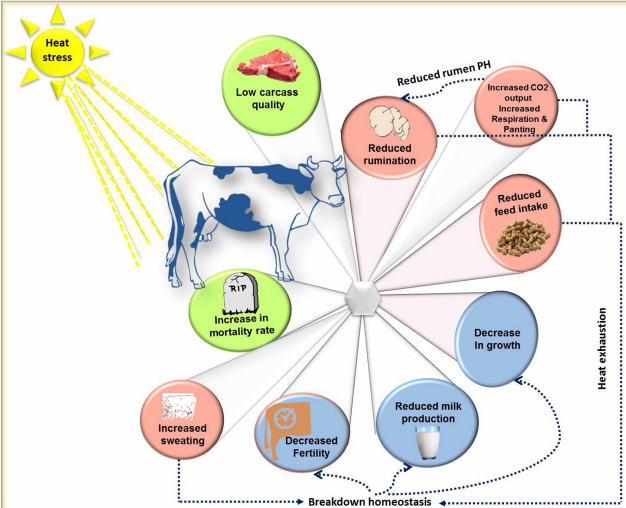
703 **Figure 2.** Effect of heat stress on animal performance, production and reproduction.

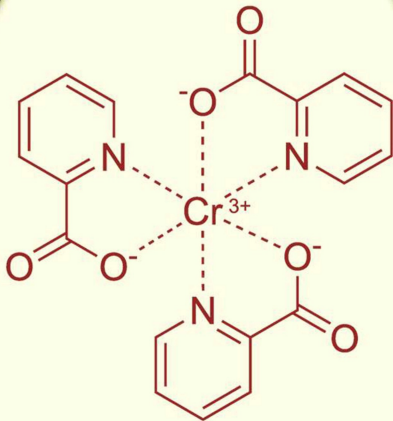
704 **Figure 3.** Chemical formula of Cr III picolinate.

705 **Figure 4.** The role of Chromium (III) against cellular glucose uptake (insulin binds to its  
706 receptor, which in turn initiates several protein-signaling cascades, including a signal that  
707 causes the translocation of the GLUT4 into the plasma membrane (PM). Cr (III) induces  
708 a loss of PM cholesterol. Accumulation of GLUT4 at the PM has been shown to be  
709 dependent on the Cr (III)-induced cholesterol loss and leads to an increase in cellular  
710 glucose uptake.

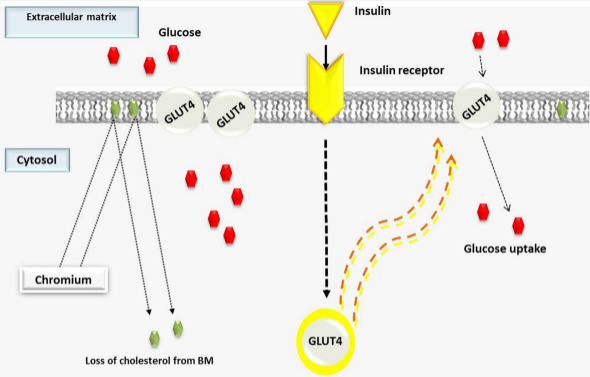
711 **Figure 5.** The beneficial effect of Chromium on glucose and non-esterified fatty acids (NEFA)  
712 metabolism in dairy cattle.







**Chromium(III) Picolinate**



Extracellular matrix

Glucose

Insulin

Insulin receptor

GLUT4

GLUT4

GLUT4

Cytosol

Chromium

GLUT4

Glucose uptake

Loss of cholesterol from BM



Increased glucose  
output from liver

Increased dry matter  
intake

Decreased NEFA

Increased availability  
of nutrient to  
mammary gland

Increased milk  
synthesis and  
production

Cows reach positive  
energy balance sooner

Improved reproduction

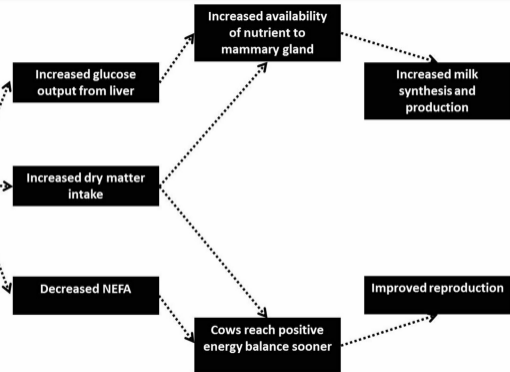


Table 1. The main findings related to Cr supplementation in heat stressed-animals

Parameter	Species	Form & dosage	Findings	References
1-Thermoregulatory responses	Dairy calves.	<ul style="list-style-type: none"> <li>• Cr-methionine (0.05 mg /kg BW0.75).</li> <li>• Cr (0.05 mg/kg BW0.75).</li> <li>• Cr-methionine at 0.05 mg/kg BW0.75</li> <li>-</li> </ul>	<ul style="list-style-type: none"> <li>• Significantly reduced the Respiration rate (RR).</li> <li>• Reduced the RR but has no effect on rectal temperature (RT).</li> <li>• Reduced the days with diarrhea (-0.9 d), pneumonia (-0.7 d) and total medication days (-1.5 d) before weaning in heat stressed calves.</li> <li>• No effect of Cr methionine on diarrhea, pneumonia, medication days and RT in heat stressed calves.</li> </ul>	<ul style="list-style-type: none"> <li>• (Mousavi et al., 2019b)</li> <li>• (Kargar et al., 2018b)</li> <li>• (Mousavi et al., 2019a)</li> <li>• (Mousavi et al., 2019a)</li> </ul>
	Buffalo calves.	<ul style="list-style-type: none"> <li>• Cr (0.5, 1 or 1.5 mg inorganic Cr/kg</li> </ul>	<ul style="list-style-type: none"> <li>• No effect on PR, RR and RT in heated buffalo calves.</li> </ul>	<ul style="list-style-type: none"> <li>• (Kumar et al., 2015)</li> </ul>
	Cows.	<ul style="list-style-type: none"> <li>• (3.6, 7.2 or 10.8 milligram chromium/head per day)</li> </ul>	<ul style="list-style-type: none"> <li>• Values of RR and RT for cows take Cr supplement and cows without any supplement were with no differences</li> <li>• According to the previous results Cr has no impact on somatic cell count and (RT, PR, RR) in lactating cows.</li> </ul>	<ul style="list-style-type: none"> <li>• (An-Qiang et al., 2009)</li> <li>• (Qi et al., 2018)</li> </ul>
	Pigs.	<ul style="list-style-type: none"> <li>• Cr propionate (200ppb).</li> </ul>	<ul style="list-style-type: none"> <li>• No effect on RR, ST and BT in heat stressed pigs.</li> </ul>	<ul style="list-style-type: none"> <li>• (Mayorga et al., 2019)</li> </ul>



2- Growth performance

Cows.	<ul style="list-style-type: none"> <li>• Cr yeast.</li> <li>• Cr–methionine at 0.05 mg Cr/kg.</li> <li>• Cr (3.6, 7.2 and 10.8 mg chromium/head/day)</li> </ul>	<ul style="list-style-type: none"> <li>• Increased feed intake in heat stressed lactating dairy cows compared with control group (21.24 and 19.56kg/day, respectively 21.24 and 19.56kg/day, respectively.</li> <li>• Increases feed efficiency and dry matter intake.</li> <li>• Significant increase (<math>p &lt; 0.05</math>) as reported in the DMI.</li> </ul>	<ul style="list-style-type: none"> <li>• (Alsaiady et al., 2004)</li> <li>• (Arthington et al., 1997)</li> <li>• (An-Qiang et al., 2009)</li> </ul>
Calves.	<p>-</p> <ul style="list-style-type: none"> <li>• 0.05 mg/kg BW0.75 Cr.</li> </ul> <p>-</p> <ul style="list-style-type: none"> <li>• 4 mg/head.</li> </ul> <p>-</p>	<ul style="list-style-type: none"> <li>• The supplement of Cr to heat stressed dairy calves resulted in increasing total DMI (<math>P = 0.002</math>), ADG (<math>P = 0.02</math>) but has no effect on feed efficiency (<math>P = 0.93</math>) or final body weight (<math>P = 0.14</math>).</li> <li>• Dietary supplementation of Chromium improved feed intake and growth rate in Holstein dairy calves exposed to high environmental temperature (<math>THI=83.3</math> units), Also, the addition of Cr to a milk substitute or diets during pre and post-weaning cattle increases the feed intake, dry matter intake and average body weight gain at the end of the experiment of 16.4 %, 11.85 and 7.07 % respectively.</li> <li>• Cr supplement improves growth rate and body weight through enhancing the absorption and digestion of nutrients. Under a variety of stress conditions, Cr requirement of calves is increased.</li> <li>• Feed efficiency and average daily gain increased in case of growing calves fed Cr.</li> <li>• This result is due to increase the total levels of</li> </ul>	<ul style="list-style-type: none"> <li>• (Mousavi et al., 2019a)</li> <li>• (Kargar et al., 2018a)</li> <li>• (Ghorbani et al., 2012)</li> <li>• (Chang and Mowat, 1992)</li> <li>• (Burton et al., 1996)</li> </ul>

		-	<p>immunoglobulins in serum.</p> <ul style="list-style-type: none"> <li>• Cr supplementation to Murrah buffalo calves showed no significant effect on feed conversion ratio (FCR) over a period of 120 days, average daily gain (ADG) and dry matter intake.</li> <li>• Under normal conditions, Cr has no influence on feed intake or total DMI.</li> </ul>	<ul style="list-style-type: none"> <li>• (Kumar et al., 2015)</li> <li>• (Bunting et al., 2000)</li> </ul>
	Pigs	<ul style="list-style-type: none"> <li>• 200ppb of Chromium propionate.</li> </ul>	<ul style="list-style-type: none"> <li>• Final body weight has increased in heat stressed finishing pigs.</li> </ul>	<ul style="list-style-type: none"> <li>• (Mayorga et al., 2019)</li> </ul>

<b>3-Milk yield and composition</b>	Dairy cows.	<ul style="list-style-type: none"> <li>• (4g/head/day) of Dietary chelated chromium.</li> <li>• Cr at 0.05 mg Cr/kg).</li> <li>• Moderate levels of Cr.</li> </ul> <ul style="list-style-type: none"> <li>• 10 mg/day Cr from 21 days pre-partum to 35 postpartum.</li> <li>• Chromium picolinate.</li> </ul>	<ul style="list-style-type: none"> <li>• Adding to the diet of heat loaded dairy cows has no effect on milk fats, protein, lactose, and solids non-fat percentage.</li> <li>• A significant rise (<math>p &lt; 0.05</math>) in milk protein, fat and lactose levels by 1.043,1.066,1.02 %, respectively is recorded when heat stressed dairy cows are supplied with Cr.</li> <li>• Increase in feed intake, dry matter efficiency and a decline in the insulin levels leading to a significant increase in milk secretion as a result of increasing the mammary nutrient flow.</li> <li>• The activity of Insulin growth factor (IGF) that has functional and structural homology to insulin receptors are supported through the improvement of milk production which also mimic the action of bovine somatotropin (bST).</li> <li>• An increase in milk production which was associated with decrease lipolysis and increase glucose uptake that will result in DMI rise.</li> <li>• The milk yield has increased (<math>p=0.013</math>), dietary Cr has no significant on milk components (protein, fat and lactose) in dairy cows under high temperature (THI=79.61).</li> </ul>	<ul style="list-style-type: none"> <li>• (Alsaiady et al., 2004)</li> <li>• (Nikkhah, 2012)</li> <li>• (Winkelman and Overton, 2012)</li> <li>• (Alsaiady et al., 2004)</li> <li>• (McNamara and Valdez, 2005)</li> <li>• (An-Qiang et al., 2009)</li> </ul>
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4-Blood variables	Cows.	<ul style="list-style-type: none"> <li>• Chromium yeast (6mg/head /day).</li> <li>• 0.05 mg of supplemental Cr/kg of BW 0.75</li> <li>• Chromium picolinate (3.5 mg of Cr/cow daily).</li> <li>• 3.5m g/kg of Cr/cow.</li> </ul>	<ul style="list-style-type: none"> <li>• Failed to show any effect on blood serum level of hemagglutination test (HA), cholesterol, triglycerides, urea, IGF-1, BHBA, albumen, and glucose while there is a drop in total blood and protein concentration.</li> <li>• Serum, insulin and NEFA reduced significantly.</li> <li>• No changes in glucose, blood urea nitrogen (BUN), creatinine, and cholesterol of lactating cows under heat stress conditions.</li> <li>• Significantly increased Hsp72 (11.68 µg/L) compared to those did not receive Cr (6.21 µg/L), Cr may play an anti-inflammatory role in lactating cows by promoting the release of Hsp72, increasing the production of IL-10, and inhibiting the degradation of IκBα under summer hot situation.</li> </ul>	<ul style="list-style-type: none"> <li>• (Soltan, 2010).</li> <li>• (Yari et al., 2010)</li> <li>• (Jin et al., 2017)</li> <li>• (Qi et al., 2018)</li> </ul>
	Calves.	<ul style="list-style-type: none"> <li>• Cr-methionine (0.05 mg /kg BW 0.75).</li> <li>• Chromium at level 0.05 mg/kg BW 0.75</li> </ul>	<ul style="list-style-type: none"> <li>• Dairy farm calves suffering from heat stress had no significant influence on glucose, albumin, and A/G ratio, triglyceridecholesterol, βHBA and BUN. Nevertheless, the serum concentration of globulin tended to be higher in Cr+ calves during the experimental period.</li> <li>• Higher serum concentrations of glucose and a higher ratio of insulin to glucose measured at the end of the experiment.</li> <li>• Chromium supplementation had small effects on blood glucose and insulin in calves under normal or heat-stress conditions.</li> </ul>	<ul style="list-style-type: none"> <li>• (Mousavi et al., 2019a)</li> <li>• (Kargar et al., 2018b; Yari et al., 2010)</li> <li>• (Ghorbani et al., 2012)</li> </ul>

		<ul style="list-style-type: none"> <li>• 1 and 1.5 mg/kg of DMI.</li> <li>• 0.5 mg of Cr/kg of DMI.</li> <li>• Cr-methionine (0.05 mg /kg BW0.75).</li> <li>• 0.04 mg Cr/kg of BW0.75.</li> <li>• 0.5, 1, and 1.5 mg of Cr/kg</li> <li>• (0.03 mg of Cr/kg of BW0.75).</li> <li>• Raising Cr doses (from 0 to 0.02 and 0.04 mg Cr/kg of BW0.75).</li> </ul>	<ul style="list-style-type: none"> <li>• Blood insulin concentration reduced in heat-stressed buffalo calves but not at 0.5 mg/kg of DMI.</li> <li>• Blood concentrations of glucose and insulin decreased and increased, respectively, when dairy calves were supplemented with cr.</li> <li>- Hormones: <ul style="list-style-type: none"> <li>• No notable effect on the T3, T4, T3/T4 ratio, and cortisol hormones activities However, they also reported that calves fed with Cr, recorded higher blood insulin level and insulin to glucose ratio as compared to those in the control group.</li> </ul> </li> <li>• No influences on serum levels of T3, T4 and cortisol measured at pre and post weaning.</li> <li>• Similar findings were observed in buffalo calves that received 0.5, 1, and 1.5 mg of Cr/kg of DMI.</li> <li>• Increased blood T4 concentration and reduced T3 to T4 ratio in dairy calves.</li> <li>• Quadratic decline in serum T4, whereas blood T3 decreased only with the higher Cr dose supplementation in dairy calves.</li> </ul>	<ul style="list-style-type: none"> <li>• (Kumar et al., 2015)</li> <li>• (Bunting et al., 2000)</li> <li>• (Mousavi et al., 2019a)</li> <li>• (Kargar et al., 2018b)</li> <li>• (Kumar et al., 2015)</li> <li>• (Ghorbani et al., 2012)</li> <li>• (Yari et al., 2010)</li> </ul>
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	Cows	<ul style="list-style-type: none"> <li>• (Cr-Yeast, 8mg/d).</li> </ul>	<p>- Antioxidants:</p> <ul style="list-style-type: none"> <li>• Significant reduction of creatine kinase as a response to yeast-chromium dietary supplementation to lactating heat-stressed cows, which may be attributed to the use of different animal models, proper dose gradients of Cr, and chemical forms of Cr.</li> </ul>	<ul style="list-style-type: none"> <li>• (Mousavi et al., 2019a)</li> </ul>
	Calves.	<ul style="list-style-type: none"> <li>• 1.0 mg Cr/kg DM and above.</li> <li>• High Cr-yeast.</li> <li>• 4 mg of supplemental Cr/kg of DM and Cr nicotinate.</li> <li>• 1.5 mg of Cr/kg.</li> </ul>	<ul style="list-style-type: none"> <li>• Positive impacts in improving both humeral and cell-mediated immunity in heat-stressed buffalo calves.</li> <li>-Increased serum total Ig and IgM levels in high Cr-yeast supplemented calves following transport stress.</li> <li>• No change in total IgM in both high-Cr yeast and Cr nicotinate supplemented transport stressed calves.</li> <li>• To heat stressed buffalo calves, decreased significantly Hsp70.</li> </ul>	<ul style="list-style-type: none"> <li>• (Kumar et al., 2015)</li> <li>• (Burton et al., 1993)</li> <li>• (Kegley and Spears, 1995).</li> <li>• (Kumar et al., 2015), (Qi et al., 2018)</li> </ul>
	Pigs.	<ul style="list-style-type: none"> <li>• Chromium propionate supplementation (200 ppb).</li> <li>• Chromium tripicolinate nanoparticles (400 ppb).</li> <li>• 200 µg/kg Cr as Cr-Nano.</li> </ul>	<ul style="list-style-type: none"> <li>• Increase in blood neutrophils by 37% corresponded to the control group.</li> <li>• Plasma glucose, insulin and NEFA levels in serum were not changed in chromium tripicolinate nanoparticles-treated pigs (400 ppb) compared to those in the control group.</li> <li>• 13.2 and 20.6 % higher serum concentration of IgM and IgG.</li> </ul>	<ul style="list-style-type: none"> <li>• (Mayorga et al., 2019)</li> <li>• (Hung et al., 2014)</li> <li>• (Wang et al., 2007)</li> </ul>