# Dietary nano-chromium tri-picolinate increases feed intake and decreases plasma cortisol in finisher gilts during summer

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**Abstract**

Chromium (Cr) is an essential mineral element and has been used in pig diets to improve growth performance, insulin sensitivity, immune response, carcase traits and to reduce heat or other stress responses. The aims of this study were to determine the impact of nano-sized chromium tripicolinate (nCrPic) on growth performance, feed efficiency and carcase characteristics of finisher gilts during the summer period. A total of sixty finisher Large White x Landrace gilts were stratified on initial weight and then within strata randomly allocated into two treatment groups in three replicates during mid-summer for 28 days. All pigs were housed in individual pens and had *ad libitum* access to feed and water. Pigs were fed either a control finisher diet (wheat based diet containing 13.8 MJ DE/kg and 0.56 g available lysine/MJ DE) or a control diet containing 400 ppb Cr as nCrPic. Dietary nCrPic supplementation increased feed intake by 6% over the entire study (P=0.05). In particular, dietary nCrPic increased ADFI by 8% (P=0.02) during the final 2 weeks of the study. Moreover, dietary nCrPic tended to improve ADF over the entire study (P=0.09). However, there were no significant effects of nCrPic on FCR, final weight, hot standard carcase weight (HCWT), P2 depth or dressing percentage. Plasma cortisol was decreased by 25% (P=0.06) by dietary nCrPic supplementation. However, there were no effects of nCrPic on plasma glucose, insulin and nonesterified fatty acids (NEFA), might because of the higher feed intake. In conclusion, this study demonstrates that dietary nCrPic supplementation at 400 ppb can increase feed intake in finisher gilts during

mid-summer suggesting that nCrPic can ameliorate some of the negative effects of heat stress in pigs, possibly via decreased of circulating cortisol.

## Introduction

A hot environment has negative impacts on production, reproduction, metabolism, health status and immune response. Despite advances in the construction and design of animal housing facilities and cooling technologies (Armstrong. 1994), animal production can still be severely affected by heat stress (St-Pierre et al. 2003). The heat-induced economic burden is due to a combination of increased mortality and decreased growth performance, nutrient utilization, sow performance, and carcase quality (St-Pierre et al. 2003). Consequently, climate change threatens the global protein supply chain and may decrease the competitiveness of the pig industry (Godfray et al. 2010). Heat-stressed animals decrease their feed intake, presumably in an effort to reduce heat production and the resultant reduced nutrient intake is responsible, at least in part, for the reduction in performance (Quiniou et al. 2000). Finishing pigs are particularly susceptible to high temperatures due to their decreased evaporative critical temperature and the high stocking density often found during this phase of production (Kouba et al. 2001; Spencer et al. 2005).

Cr is an essential mineral and has been included in pig diets to improve growth performance, insulin sensitivity, immune response, carcase traits and to reduce the

65 impact of heat or other stressors (Hung et al. 2010). The interest in Cr
66 supplementation during the finisher phase of pig production is primarily for its
67 potential impact to improve body composition, and to a lesser extent, for any
68 associated improvement in performance. However, the effect of Cr on growth
69 performance is inconsistent. For example, Cr supplementation studies conducted with
70 pigs have been shown to either result in improve (Page et al. 1993; Lindemann et al.
71 1995; Mooney and Cromwell, 1995, 1997), or have no effect (Evock-Clover et al.
72 1993; Xi et al. 2001; Matthews et al. 2003) on growth performance. A meta-analysis
73 conducted by Sales and Jančík (2011) reported that dietary Cr can increase average
74 daily gain (ADG) and feed efficiency (FCR), whereas there is no effect on average
75 daily feed intake (ADFI). At least some of the variation in response to Cr may be
76 related to the low and variable digestion, absorption and availability of Cr and there is
77 potential to improve this by producing nano- or micro-sized Cr. Lien et al. (2009)
78 reported that nano-sized Cr had 1.66 fold greater digestibility than normal size.
79 Gonzales-Eguia et al. (2009) also indicated that availability of copper is higher in
80 nano sized particle copper. Moreover, the study reported by Hung et al. (2014) also
81 found benefits of Cr inclusion in finisher diets, particularly when CrPic was ground to
82 a small particle size (nCrPic). Carcase P2 was decreased and longissimus muscle area
83 increased when gilts were fed a high-fat finisher diet supplemented with 400 ppb
84 nCrPic. A meta-analysis conducted by Hung et al. (2010) reported that dietary nCrPic
85 can increase loin muscle area and decreased fat depth to a greater extent than normal

(ca. 0.5 mm) sized CrPic. Previous studies have demonstrated the potential of Cr to reduce the negative effects of heat stress in broiler and layer poultry (Sahin et al. 2002a; Sahin et al. 2002b; Al-Saiady et al. 2004; 2005). In a rodent model, dietary nCr improved growth rate and feed efficiency in heat-stressed rats (Zha et al. 2009). The risk of high rigour temperature of beef carcasses increases with increasing insulin resistance (Warner et al. 2014) and it has been proposed that the ability of dietary Cr to improve insulin sensitivity may provide a means of ameliorating heat shortening in beef cattle (DiGiacomo et al. 2014) and heat stress in dairy cattle (Dunshea et al. 2013). However, there are no studies investigating the effect of Cr on heat stress or during hot condition in finishing pigs. Therefore, this study was conducted the impact of nCrPic on growth performance, feed efficiency and carcase characteristics of female finisher pigs during summer.

## Methods and Materials

*Animals and treatments*

All animal procedures were approved by the Rivalea Pty Ltd. Corowa, NSW, Australia (Rivalea Australia) Animal Ethics Committee. Sixty Large White × Landrace cross breed finishing gilts (PrimeGro$^{TM}$ Genetics) from the Research and Development facility at Rivalea Australia were selected at approximately 17 weeks of age (initial live weight 67.7± 0.46 kg, mean± standard error (SE), kg). Pigs were stratified on weight and then within strata randomly allocated into two treatments in

three replicates during the mid-summer (January-February, 2011). The average maximum temperature during the experiment was 29.7 $^{o}$C, with a total of 24 days where the daily maximum temperature was above 28 $^{o}$C. All pigs were housed in individual pens in a shed with no additional cooling and had *ad libitum* access to feed and water. The experiment included one-week acclimatization and 28 days experimental period. During the acclimatization period, pigs were fed with commercial standard grower diet (Rivalea Australia). During experimental period, pigs were fed a control finisher diet (wheat based diet containing 13.9 MJ digestible energy (DE)/kg and 0. 52 g available lysine/MJ DE) or control diet plus 400 ppb Cr as nCrPic (Table1). The nCrPic was prepared according to our previous study (Hung et al. 2014). Briefly, the raw CrPic material was ground and then passed through appropriate sized end-plates sieves to collect nCrPic.

*Husbandry and management*

Pigs were housed in individual pens in the Rivalea Research and Development boar test facility. Individual live weights were obtained at day 0, 14 and 28 of the study. Feed intake was recorded weekly throughout the experimental period as estimated by feed disappearance. In addition back fat thickness was measured via real time ultrasound (Pork Scan Pty. Ltd. Australia) at the P2 site (65 mm from the midline over the final rib) and leg (60 mm to the right of the midline base of the tail) on day 0 and 28. At the end of experiment, pigs were slaughtered at a commercial abattoir to

128 determine hot carcase weight (HCWT) and dressing percentage. HCWT was
129 standardized as head on (including tongue); kidneys removed (kidney fat remaining),
130 fore and hind trotters on.
131
132 *Blood sampling*
133 Pigs were fasted for 16 hours prior to blood collection. Fifteen animals were selected
134 randomly from each treatment (five pigs/ replicate) for blood collection on day 27.
135 After collection of blood, the samples were placed on ice for 1 h, and then centrifuged
136 for 15 min at 1,500×g. Plasma was collected and frozen (-20 $^{o}$C) until subsequent
137 analysis for glucose, insulin, and NEFA concentrations. Plasma insulin (Millipore
138 Corporation, USA) and cortisol (Diagnostica, Finland) levels were used commercial
139 kit and determined by radioimmunoassay. Plasma glucose (Thermo Fisher Scientific
140 Inc. USA), and NEFA (NEFA-C kit, Wako Chemical Industries Ltd, Osake, Japan)
141 concentrations were determined by enzymatic colorimetric procedures.
142
143 *Statistical analyses*
144 Data were analysed by analysis of variance using GENESTAT release 11.1 (VSN
145 International Ltd. UK). Initial live weight was used as a covariate for ADG, final live
146 weight and HCWT and initial P2 were used as covariate for final P2. The quantitative
147 insulin sensitivity check index (QUICKI baseline) was calculated using the formula,
148 QUICKI=1/ (log fasting insulin, μU/ mL + log fasting glucose, mg/dL) (Katz et al.,

2000). Normal QUICKI values are around 0.45 with values closer to 0.30 indicating insulin resistance, an increase in QUICKI is associated with increased insulin sensitivity (Katz et al., 2000). The homeostatic model assessment (HOMA) was calculated using the formula, HOMA= (fasting insulin × fasting glucose)/ 22.5 (Katz et al., 2000). A decrease in HOMA is associated with reduced insulin resistance.

## Results

*Growth performance*

While there were no significant effect of nCrPic on ADFI during the first 14 days (2.31 vs 2.37 kg/day for control and nCrPic diet, respectively, P=0.43), as the study progressed dietary nCrPic increased ADFI such that during the final 2 weeks of the study ADFI was 9% higher in pigs supplemented with dietary nCrPic (2.53 vs 2.75 kg/day, respectively, P=0.02) (Table 2). Consequently, ADFI over the entire study was increased by 6% by dietary nCrPic (2.42 vs 2.56 kg/day, respectively, P=0.05). There were no significant effects of nCrPic on ADG during the first 14 days (0.88 vs 0.95 kg/day, respectively, P=0.12) or over the period from 14 to 28 days (1.00 vs 1.02 kg/day, respectively, P=0.59). However, ADG tended to be increased by 5% dietary nCrPic when measured over the entire study (0.94 vs 0.99 kg/day, P=0.09). Dietary nCrPic had no effect on FCR in the current study (Table 2).

*Carcase characteristics*

In line with effects on ADFI and ADG, dietary nCrPic tended to increase live weight at the completion of the study (94.0 vs 95.4 kg respectively, P=0.09) (Table 3). However, dietary nCrPic did not significantly alter HSCW (70.2 vs 71.1 kg, P=0.14) or dressing percentage (74.4 vs 74.4 %, P=0.71). The depth of back fat at the P2 site (P=0.94) or over the leg (P=0.45) at the end of the study did not differ with treatment (Table 3).

*Plasma metabolites*

Dietary nCrPic tended to decrease cortisol level (17.8 vs 15.2 nM/L, for control and nCrPic diet, respectively, P=0.06) (Table 4). However, there was no effect of dietary nCrPic on plasma glucose (P=0.48), insulin (P=0.96), NEFA (P=0.69) concentration or the HOMA (P=0.91) and QUICKI (P=0.98) measures on day 27 of the study (Table 4).

## Discussion

Thermal stress reduces animal productivity and decreases ADFI in farm animals and therefore it is desirable to develop nutritional strategies, likely based on physiological and/or metabolic adaptations to help maintain pig performance during summer. The major finding from the present study was that dietary nCrPic supplementation during a hot summer period can increase ADFI with resultant improvements in ADG and slaughter weigh. High ambient temperatures can reduce the efficiency and

profitability in pig production (Nardone et al. 2010) with pigs being particularly susceptible during the late finishing phase due to their lower evaporative critical temperature. Evaporative heat loss from the respiratory tract is the major mechanism by which pigs dissipate heat (Giles et al. 1988; Marai et al. 2007). When pigs reach the point where evaporative heat loss from the respiratory tract and skin is at a maximum, the upper critical temperature is also reached (Giles et al. 1988). Beyond this point, pigs will voluntarily reduce ADFI in order to reduce their heat production. For example, Le Bellego et al. (2002) reported that pigs reduced ADFI by 55 g per $^{o}$C above 22 $^{o}$C and ceased eating when their body temperature reached about 41.3 $^{o}$C. The reduction in ADFI during high ambient temperatures was more pronounced in pigs during the finishing phase than during the growing phase (78 vs 35 g /day per $^{o}$C above 22 $^{o}$C for finishing phase and growing phase pig, respectively) (Le Bellego et al. 2002). This is because the upper critical temperature decreases with the increasing of live weight (Giles et al. 1988; Quiniou et al. 2000) which may explain why the improvement in ADFI became more pronounced as the present study progressed. An improvement in growth performance, after CrPic supplementation during heat stress, also been observed in poultry. For example, dietary supplementation of CrPic ameliorated the detrimental effects of heat stress on growth and egg production (Sahin et al. 2002a; Sahin et al. 2005). Also, supplementation of CrPic at levels from 200 to 1200 μg/kg linearly increased feed intake, live weight gain, and egg production rate of laying quail reared under heat stress condition (Sahin et al. 2002a). Finally, dietary Cr

212 Yeast (4 g/head per day) increased feed intake in Holstein cows under heat stress
213 (Al-Saiady et al. 2004). These data suggested that dietary Cr may be able to use in
214 farm animals to alleviate the negative effects of high environment temperature.
215
216 The results from our previous study (Hung et al. 2014), in conjunction with other
217 literatures (Wang and Xu, 2004; Wang et al. 2007; Wang et al. 2009a) suggested that
218 dietary nano Cr could reduce the body fat content in pigs. However, in the present
219 study, there was no effect of dietary nCrPic on P2 backfat and leg fat depth of pigs.
220 Verstegen et al. (1973) indicated that pigs reared in hot environment typically yield
221 carcasses with a higher percent lean. The reduction in fat content of pigs under heat
222 stress was due to a reduction in total energy intake in the hot environment. Dietary
223 nCrPic increased feed intake during the day 14 to 28 in this study suggested that pigs
224 feed with nCrPic increased total energy intake in the late finishing period compared
225 with the control group. Increased energy intake without changed the P2 back fat and
226 leg fat depth implied that pigs fed with nCrPic had better efficiency to metabolized fat.
227 It should be noted that, by commercial standards, the pigs in the current study were
228 already very lean and had a low P2 backfat.
229
230 Heat stress likely affects many aspects of metabolism, including alterations in
231 substrate uptake and utilization, much of which is not yet fully understood. Metabolic
232 adaptations during high ambient temperatures likely occur in order to increase

survival probability. Denbow et al. (1986) reported insulin concentration are lower in summer than in winter and spring in Holstein cattle, possibly as a result of reduced feed intake. In lactating sows, plasma insulin was lower during high ambient temperature compared to sows housed under thermo-neutral conditions despite similar glucose concentrations (de Bragança and Prunier, 1999). Conversely, Achmadi et al. (1993) reported that heat-exposer had no effect on fasting insulin level. Circulating glucose concentrations were increased in pigs (Prunier et al. 1997) and cattle (Denbow et al. 1986), but decreased in sheep (Achmadi et al. 1993) and cattle (Shwartz et al. 2009; Wheelock et al. 2010), and unchanged in pigs in another study (de Bragança and Prunier, 1999) under hot conditions. Heat exposure increased plasma NEFA in sheep (Sevi et al. 2002), but decreased NEFA in lactating cattle (Itoh et al. 1998; Shwartz et al. 2009; Wheelock et al. 2010). These differences between basal hormone and metabolite concentrations may relate to differences in experimental conditions such as ambient temperature and feeding manner, physiological status, and animal species. Importantly, it worth to understand the degree to which feed intake is impacted by high ambient temperatures as reduced feed intake generally results in increased NEFA and decreased glucose and insulin. Also, insulin resistance increase with fatness and the rate of fat deposition in pigs (Dunshea and Cox et al. 2008). There in now increasing evidence that insulin resistant and diabetic individuals suffer from thermal intolerance, exhibiting an inability to control body temperature (Ohtsuke et al. 1995). In part this is because skin blood flow and

skin thickness are reduced in diabetic individuals (Forst et al. 2006), thereby reducing the ability of thermoregulation. If dietary nCrPic can improve insulin sensitivity then it may explain why feed intake is improved with dietary nCrPic during summer and that the effect was greater during latter part of the study when the pigs would be fatter.

Chromium is a trace mineral that is widely distributed throughout the body, being necessary for maintenance of insulin function and glucose uptake by insulin-sensitive tissues (Anderson et al. 1985). However, the effects of dietary Cr on fasting glucose concentrations in pigs are inconsistent. Some studies reported that dietary Cr significantly reduces fasting glucose (Lien et al. 2001; Wang et al. 2001; Wang et al. 2009b), whereas others reported no effect (Amoikon et al. 1995; Matthews et al. 2001). Amoikon et al. (1995) reported that CrPic increased insulin sensitivity as assessed by increased glucose clearance rate and decreased glucose half-life during a glucose tolerance test and insulin challenge test in pigs. It is reported that CrPic can increase the rate of insulin internalizations and uptake of glucose into skeletal muscle cells (Evans and Bowman, 1992). Under heat stress condition, dietary CrPic decreased glucose level in quails (Quiniou et al. 2000) and broiler chickens (Samanta et al. 2008) indicating improved glucose clearance and insulin sensitivity. Zha et al. (2009) indicated that heat-stressed male Sprague-Dawley rats fed with nano-Cr had lower insulin. However, supplementation of CrPic linearly increased insulin concentration at levels from 200 to 1200 μg/kg (Sahin et al. 2002a). Yari et al. (2010)

suggested that an improvement in peripheral insulin sensitivity as evidence by an intravenous GTT revealed linear reductions in AUC of insulin during 0-90 and 0-120 minute after glucose infusion in calf fed with Cr-L-methionine in summer. As reported by Hung et al. (2014), dietary increased insulin sensitivity as indicated by a decrease in both fasting insulin and HOMA, and an increase in QUICKI. In the present study, however, dietary nCrPic had no significant effect on glucose, insulin, NEFA, HOMA, and QUICKI in finisher gilts during summer. The lack of effect on these measures of insulin sensitivity and intermediary metabolism may be due to the increase in feed intake over the latter phase of the study which would have the effect of increasing plasma glucose and insulin, and lowering plasma NEFA (ie. opposite effects of improving insulin sensitivity in the face of no change in feed intake).

Circulating cortisol concentration was tended to decrease by dietary nCrPic supplementation. A reduction in plasma cortisol is a classic metabolic consequence of Cr in farm animals suggesting there was a reduction in stress, especially when animals are challenged with stressors such as a thermal-stress. For example, in heat-stressed broiler chickens, dietary CrPic decreased serum cortisol concentration (Samanta et al. 2008). While, Sahin et al. (2002a) reported that dietary CrPic linearly decreased serum corticosterone concentration across the range of 200-1200 μg/kg in heat-stressed laying quail. A stress-induced increase in circulating cortisol may contribute to increase hepatic gluconeogenesis and heat production, thereby escalating

296 the heat load of the animal (McDonald et al. 2011). Farm animals tend to reduce feed
297 intake to adapt to high environment temperature (Nardone et al. 2010). Zha et al.
298 (2009) suggested that nCrPic can balance circulating cortisol and insulin level to
299 maintaining homeostasis. The anorexigenic actions of cortisol can mediated via
300 reducing circulating ghrelin levels as well as GHSR1a-LR (biologically active ghrelin
301 receptor) expression and/or suppressing neuropeptide Y expression (Janzen et al.,
302 2013). These data suggest that the improvement in feed intake in dietary nCrPic
303 supplemented pigs may be via the decreased cortisol secretion. Alternatively, the
304 reduction in cortisol in pigs supplemented with dietary nCrPic may mean they are
305 under less stress.
306
307 **Conclusion**
308 These data clearly show that dietary nCrPic supplementation at 400 ppb can increase
309 feed intake and tended to increase ADG in finisher gilts during mid-summer
310 suggesting that nCrPic can ameliorate some of the negative effects of heat stress in
311 pigs possibly via decreased circulating cortisol. However, this study failed to detect
312 any effects of dietary nCrPic on the levels of plasma glucose, insulin, NEFA, HOMA
313 and QUICKI under these conditions, might because of the increased feed intake.
314 Further studies are required to examine the growth performance and physiological
315 response in animal under heat stress condition.
316

**The authors declare that they have no conflict of interest**

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Table 1. Ingredient and nutrient composition of experimental diets

| Ingredient | |
|---|---|
| Wheat | 68.46 |
| Millmix | 16.5 |
| Canola meal 36% | 6.0 |
| Meat meal | 2.6 |
| Water | 1 |
| Porzyme 9310 | 0.02 |
| Natuphos 5000 | 0.01 |
| Tallow-mixer | 3.07 |
| Salt | 0.2 |
| Limstone | 1.5 |
| Lysine-HCl | 0.35 |
| DL-methionine | 0.01 |
| Threonine | 0.11 |
| Copper proteinate Micro | 0.1 |
| Growth Premix [a,b] | 0.07 |
| Total | 100 |
| **Calculated nutrient composition** | |
| DE, MJ/kg | 13.9 |
| Fat, % | 4.9 |
| Crude protein, % | 16.0 |
| Calcium, % | 0.89 |
| Available Phosphorus, % | 0.36 |
| Available lysine/MJ DE | 0.52 |

[a] Provided the following trace mineral per kilogram of diet: Se, 0.2 mg; Fe, 60; Mn, 25 mg; Zn, 50 mg; I, 0.2 mg; Cu, 10 mg.

[b] Provided the following vitamins per kilogram of diet: Vitamin A, 2.5 mg; Vitamin D3, 1mg; Vitamin E, 30 mg; Niacin, 10 mg; Ca-D-Pantothenate, 5 mg; Riboflavin, 2 mg; Vitamin B12 (Cyanocobalamin), 5 mg.

Table 2 Effect of nCrPic on growth performance, feed intake and feed efficiency in finisher gilts during summer

| | Control | nCrPic | sed[a] | P- value |
|---|---|---|---|---|
| **Day 0 to 14** | | | | |
| ADFI[b], kg/d | 2.31 | 2.37 | 0.076 | 0.43 |
| ADG[b], kg/d | 0.88 | 0.95 | 0.047 | 0.12 |
| FCR (Feed: gain) | 2.72 | 2.55 | 0.127 | 0.20 |
| **Day 14 to 28** | | | | |
| ADFI[b], kg/d | 2.53 | 2.75 | 0.085 | 0.02 |
| ADG[b], kg/d | 0.99 | 1.02 | 0.048 | 0.59 |
| FCR (Feed: gain) | 2.63 | 2.77 | 0.136 | 0.32 |
| **Day 0 to 28** | | | | |
| ADFI[b], kg/d | 2.42 | 2.56 | 0.070 | 0.05 |
| ADG[b], kg/d | 0.94 | 0.99 | 0.030 | 0.09 |
| FCR (Feed: gain) | 2.61 | 2.62 | 0.080 | 0.96 |

[a] Standard error of the difference

[b]– initial weight used as covariate

Table 3 Effect of nCrPic on carcass characteristics in finisher gilts during summer

| | Control | nCrPic | sed[a] | P- value |
|---|---|---|---|---|
| Final weight [b], kg | 94.0 | 95.4 | 0.83 | 0.09 |
| HCWT [b], kg | 70.2 | 71.1 | 0.69 | 0.14 |
| Dressing % | 74.4 | 74.4 | 0.44 | 0.71 |
| P2 fat depth [c], mm | 8.0 | 8.0 | 0.19 | 0.94 |
| Leg fat depth [c], mm | 9.0 | 9.1 | 0.20 | 0.45 |

[a] Standard error of the difference; [b] Initial weight used as covariate;

[c] Initial P2 and HCWT used as covariate

Table 4 Effect of nCrPic on plasma glucose, insulin, NEFA, HOMA and QUICKI in finisher gilts during summer period

| | Control | nCrPic | sed[a] | P- value |
|---|---|---|---|---|
| Cortisol nmol/L[b] | 1.25 (17.8) | 1.12 (13.2) | 0.067 | 0.06 |
| Glucose, mmol/L | 3.91 | 3.98 | 0.09 | 0.48 |
| Insulin, mU/L | 11.0 | 11.0 | 0.74 | 0.96 |
| NEFA, μM | 307 | 309 | 6.45 | 0.69 |
| HOMA | 1.91 | 1.93 | 0.174 | 0.91 |
| QUICKI | 0.62 | 0.62 | 0.017 | 0.98 |

[a] Standard error of the difference

[b] Data were log-transformed before analyses due to heterogeneity of variances. Values in parentheses are the geometric mean.

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