



## Original Research Article

# Dietary nano chromium picolinate can ameliorate some of the impacts of heat stress in cross-bred sheep



Alex T. Hung<sup>a</sup>, Brian J. Leury<sup>a</sup>, Matthew A. Sabin<sup>b</sup>, Fahri Fahri<sup>a</sup>, Kristy DiGiacomo<sup>a</sup>, Tu-Fa Lien<sup>c</sup>, Frank R. Dunshea<sup>a, d, \*</sup>

<sup>a</sup> Faculty of Veterinary and Agricultural Sciences, The University of Melbourne, Parkville, VIC, 3010, Australia

<sup>b</sup> Murdoch Children's Research Institute, The University of Melbourne, Parkville, VIC, 3010, Australia

<sup>c</sup> Department of Animal Science, National Chiayi University, China

<sup>d</sup> Faculty of Biological Sciences, The University of Leeds, Leeds LS2 9JT, United Kingdom

## ARTICLE INFO

## Article history:

Received 14 October 2019

Received in revised form

21 June 2020

Accepted 29 July 2020

Available online 21 December 2020

## Keywords:

Chromium picolinate

Sheep

Heat stress

Nanotechnology

Physiology

## ABSTRACT

Two studies were conducted to evaluate the effect of nano chromium picolinate (nCrPic) during heat stress (HS) in sheep. In the initial study, 36 Merino × Poll cross-bred sheep were individually penned and allocated to 3 dietary treatments (0, 400 and 800 µg/kg nCrPic) for 8 wk. Body composition was determined at the beginning and end of the experiment using dual energy X-ray absorptiometry. The sheep remained in their dietary groups but were then placed in metabolic cages and randomly allocated within the dietary group to differing ambient temperature regimes, i.e., thermo-neutral (TN) ( $n = 18$ ) and HS ( $n = 18$ ), for 3 wk. Dietary nCrPic had no effect on growth performance and body composition during the initial study conducted under TN conditions. Heat stress decreased average daily feed intake (ADFI) ( $P = 0.002$ ) whereas sheep under HS had reduced average daily gain (ADG) and indeed lost weight ( $P < 0.001$ ). Dietary nCrPic increased both ADFI ( $P = 0.041$ ) and ADG ( $P = 0.049$ ) under both TH and HS conditions such that the performance of sheep receiving supplemental nCrPic and exposed to HS was similar to that of control sheep maintained under TN conditions. Heat stress increased rectal temperature ( $P < 0.001$ ) and respiration rate ( $P < 0.001$ ), particularly during the hottest parts of the day as indicated by interactions ( $P < 0.001$ ) between time of day and thermal treatment. Rectal temperature was lower in sheep fed nCrPic ( $P = 0.050$ ), particularly under peak HS conditions during the afternoon as indicated by the interactions between dietary nCrPic and time of day ( $P < 0.001$ ) and dietary nCrPic, thermal treatment and time of day ( $P = 0.010$ ). Similarly, respiration rate was lower in sheep fed nCrPic under peak HS conditions during the afternoon as indicated by the interactions between dietary nCrPic and thermal treatment ( $P < 0.001$ ) and dietary nCrPic and time of day ( $P = 0.030$ ). In conclusion, dietary nCrPic can partially ameliorate the negative effects of HS as indicated by the maintenance of ADFI and decreased physiological responses, such as elevations in rectal temperature and respiration rate.

© 2021, Chinese Association of Animal Science and Veterinary Medicine. Production and hosting by Elsevier B.V. on behalf of KeAi Communications Co., Ltd. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

\* Corresponding author.

E-mail address: [fdunshea@unimelb.edu.au](mailto:fdunshea@unimelb.edu.au) (F.R. Dunshea).

Peer review under responsibility of Chinese Association of Animal Science and Veterinary Medicine.



Production and Hosting by Elsevier on behalf of KeAi

<https://doi.org/10.1016/j.aninu.2020.07.004>

2405-6545/© 2021, Chinese Association of Animal Science and Veterinary Medicine. Production and hosting by Elsevier B.V. on behalf of KeAi Communications Co., Ltd. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

## 1. Introduction

Animal productivity is negatively impacted by environmental factors, including elevated ambient temperature causing heat stress (HS). Acclimation to the external environment is a phenotypic response developed by the animal as its environment changes. The acclimation of the animals to meet high ambient temperatures results in a reduction of feed intake, increased respiration rate, decreased activity, and increased water consumption to facilitate the dissipation of excess heat (Nardone et al., 2010; Sejian et al., 2018). In sheep, evaporation is the most important means of heat

dissipation, because sweating is much less important than respiratory evaporation due to the presence of a wool cover. Moreover, voluntary feed intake decreases during HS in order to reduce metabolic heat production (McGuire et al., 1989; Nardone et al., 2010; Gaughan et al., 2019). The reduction of feed intake can result in an essential nutrient imbalance and, therefore, decrease tissue anabolism and increase tissue catabolism.

In many countries, sheep are raised in arid and semiarid regions characterized by extreme summer temperatures (Alhidary et al., 2012; Dunshea et al., 2017). Positive physiological and production in responses to dietary chromium (Cr) supplementation have been observed in sheep exposed to external stressors, such as transportation stress (Al-Mufarrej et al., 2008; Kraidees et al., 2009), isolation stress (Sano et al., 1999) and HS (Dunshea et al., 2013; Sahin et al., 2002a, 2002b, 2005; Samantha et al., 2008). Dietary Cr picolinate (CrPic) has been shown to increase feed intake in quail (Sahin et al., 2002a, 2010) and Holstein cows (Al-Saiady et al., 2004) under HS. However, the performance responses of farm animal to dietary Cr supplementation are inconsistent. For example, Estrada-Angulo et al. (2013) found that dietary yeast bound Cr increased growth rate and feed efficiency with no change in feed intake in hairy sheep whereas Moreno-Camarena et al. (2015) found no effect on growth performance in sheep. Sales and Jančík conducted a meta-analysis that indicated that the effects of Cr supplementation on performance varied considerably among swine studies (Sales and Jančík, 2011). At least some of the variation in response to dietary Cr may be related to low and variable absorption and particle size reduction of Cr may offer a means of ensuring a more consistent response to dietary CrPic (Hung et al., 2015).

In the rodent model, nano-sized Cr particles (nCr) improved Cr digestibility approximately three-fold higher (Lien et al., 2009) and improved growth rate and immune response in rats during HS (Zha et al., 2009). Hung and colleagues also found that dietary nano Cr picolinate (nCrPic) can improve feed intake in finisher pigs during the mid-summer (Hung et al., 2014). The positive feed intake response under high ambient temperature indicated that dietary Cr may be able to ameliorate the negative physiological response of animal under HS. Chromium improves insulin sensitivity and may enhance skin micro-circulation (Forst et al., 2006) and vasodilation (Abebe et al., 2010) to facilitate heat dissipation during HS. Also, fat deposition tends to increase with increasing temperature in sheep (Degen and Young, 2002) which may be related to decreased lipid mobilization as a result of hyperinsulinemia as reported in HS cattle (O'Brien et al., 2010). Dietary Cr can decrease plasma insulin concentrations (Zhou et al., 2013) so may reduce the effects of HS on hyperinsulinemia during maintaining normal insulin action. However, there are no studies investigating the effect of dietary nCrPic on the ability of sheep to counter HS. Therefore, the aims of this study were to examine the effects of dietary nCrPic supplementation on growth, body composition and physiological responses of sheep under both thermo-neutral and HS conditions. The hypothesis to be tested in the study is that if sheep exposed to the HS regime display clinical signs of HS such as increased respiration, rectal and skin temperature, then dietary nCrPic will reduce responses to HS by reducing the heat load on the animal, which will result in increased feed intake and weight gain.

## 2. Methods and materials

All procedures undertaken in this study were conducted in accordance with the guidelines set out in the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes and were approved by the University of Melbourne, Science, Optometry & Vision Sciences and Land & Environment animal ethics committee.

### 2.1. Animals, diet and experimental design for feeding studies

There were two experiments conducted within a larger study which was conducted over three replications. Animals were housed in individual pens under cover in a shed for an 8-wk feeding study conducted at the Dookie campus of The University of Melbourne. Following the 8-wk dietary treatment, animals were placed in metabolic cages for a secondary 4-wk HS study (including 1-wk acclimation to new surroundings). Nutrient requirements for growing sheep were determined using guidelines established by the NRC (2007). During both studies, the lambs were fed either a basal control sheep grower pelleted diet (wheat- and barley-based diet containing 11.3 MJ ME/kg and 15.5% CP) or the basal diet supplemented with either 400 or 800 µg/kg Cr as nCrPic (Table 1). Water and pelleted feed were provided ad libitum. All sheep also offered barley straw 100 g/d per animal in conjunction with the pelleted feed.

### 2.2. Nano chromium picolinate preparation and particle size determination

The nCrPic particles were prepared according to our previous study (Hung et al., 2015). Nano-sized Cr powder were prepared at

**Table 1**  
Ingredients and nutrient composition of experimental diets (% dry matter basis).

Item	Content
Ingredients	
Wheat	40.0
Millmix	24.9
Barley	20.0
Canola meal (36%)	2.93
Lupin kernels (33%)	4.53
Water	1.09
Molasses	1.59
Salt	1.30
Limestone	2.13
Calcium sulphate	0.33
Ammonium chloride	0.54
Rumen buffer <sup>1</sup>	0.54
Lamb premix <sup>2,3</sup>	0.11
Lasalocid <sup>4</sup>	0.011
Total	100
Calculated nutrient composition <sup>5</sup>	
ME, MJ/kg	12.3
Fat	2.12
Crude protein	16.8
Calcium	1.08
Total phosphorus	0.52
Analyzed nutrient composition <sup>6</sup>	
Cr, µg/kg	780

<sup>1</sup> Acid Buf, (Feedworks Australia, Romsey, VIC, Australia).

<sup>2</sup> Alltech Lienert Australia, Roseworthy, SA, Australia. 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, 25 mg.

<sup>3</sup> Provided the following vitamins per kilogram of diet: vitamin A, 2.5 mg; vitamin D<sub>3</sub>, 1 mg; vitamin E, 30 mg; niacin, 10 mg; Ca-D-pantothenate, 5 mg; riboflavin, 2 mg; vitamin B<sub>12</sub> (Cyanocobalamin), 5 mg.

<sup>4</sup> Bovatec, 20%, (Zoetis, Silverwater, NSW, Australia).

<sup>5</sup> Calculated from formulation package (Saltbush Feedmania, Armidale, NSW, Australia).

<sup>6</sup> Chromium content of the high chromium diet formulated to be 800 µg/kg.

Hsin-fang Nanotech Co. Ltd. by grinding in a dry cryo-nanonization grinding system through sieve end plate with appropriate size in order to collect nanonized CrPic. No solvent was used and the temperature was controlled under 40 °C during the grinding process.

The particles size distribution was determined using the method reported by Gonzales-Eguia and colleagues with some modifications (Gonzales-Eguia et al., 2009). Briefly, the ground CrPic powders (average particle size  $310 \pm 129 \mu\text{m}$ ) were vibrated in an alcohol solution, depositing the ultrafine fragments on a copper grid covered by a thin carbon film (CF200–Cu, Electron Microscopy Science) in the solution. The bright and dark field images of samples were obtained by using a JEM-2100F field emission transmission electron microscope (FE-TEM; JEOL Ltd, Tokyo, Japan) operating at 200 kV. The images were then manipulated by the software “Digisscan Image Acquisition System” to achieve the area data of each recognized particle. These data were then converted into particle diameter data. Total of 40 random selected recognized particles data were used for particle size distribution. The average particle size of nCrPic, was  $49.7 \pm 12.37 \text{ nm}$  (mean  $\pm$  SD). Concentration of dietary Cr was determined by a modification of the method of Williams et al. (1962) using atomic absorption spectrophotometry (SpectrAA-200 System, Varian Australia Pty. Ltd., Mulgrave, VIC, Australia).

### 2.3. Initial feeding study (experiment 1)

Thirty-six 9-month-old Merino  $\times$  Poll cross-bred ewes and wethers (initial live weight  $33.8 \pm 1.04 \text{ kg}$ , mean  $\pm$  standard error kg) in three replicates of 12 sheep were selected from The University of Melbourne grazing flock and housed in indoor group pens for a 1-wk acclimation period before the study commenced. During this period, sheep were fed ad libitum the basal diet containing no supplemental Cr. After the acclimation period, initial body composition was determined using dual-energy X-ray absorptiometry (DXA) (Hologic QDR4500 Fan Beam X-Ray Bone Densitometer; Hologic Inc., Waltham, MA, USA) (Hunter et al., 2011). Sheep were then stratified within sex group according to body weight and body composition and allocated to three dietary treatments containing either 0, 400 or 800  $\mu\text{g/kg}$  supplemental nCrPic and housed in individual pens. All animals had *ad libitum* access to feed and water for 8 wk with individual live weight and feed intake being recorded weekly. Body composition was again determined by DXA at end of the initial 8 wk feeding study.

### 2.4. Heat stress study (experiment 2)

After the initial feeding study, the sheep were transported to the climate control rooms. The sheep were acclimatized to their new surroundings for 1 wk, followed by a 3-wk experimental thermal treatment period. All sheep were shorn before the HS study. During the thermal study, the sheep were housed in individual metabolism crates housed in one of two climate control rooms which were maintained at either thermo-neutral (TN) ( $n = 18$ ) or HS ( $n = 18$ ) conditions. The TN room was maintained at average 22 °C ambient temperature and 70% to 80% relative humidity (RH) for 24 h throughout the experimental period. The sheep in the HS room experienced cyclical daily temperatures beginning at 09:00 when the temperature was increased (15 °C/h) to a maximum of 40 °C where it remained until 17:00 before being decreased to 22 °C where it was maintained until 09:00 the next morning. The RH was approximately 50% during the day and 70% overnight. Both rooms were

maintained under a 12-h light and 12-h dark cycle. Access to feed and water was provided by troughs and buckets attached to the side of the cage. Feed and water was available *ad libitum*. Feed and water disappearance were recorded daily throughout the experimental period. After the final physiological measures at 17:00 on d 20, sheep were fitted with indwelling jugular catheters to conduct intravenous insulin and glucose challenges and these data will be reported elsewhere.

Temperature-humidity index (THI) was calculated and recorded during both experimental periods. The THI is commonly used as an indicator of the degree of stress on animals caused by weather conditions. The THI was calculated by combining temperature and RH with the following equation (LPHSI, 1990):

$$\text{THI} = T - (0.31 - 0.31 \text{ RH}) \times (T - 14.4),$$

where  $T$  is the temperature (°C) and RH is the relative humidity. The THI averaged  $21.8 \pm 0.83$  and  $36.7 \pm 2.13$  for the TN and HS conditions indicating an absence of HS and severe HS, respectively (Marai et al., 2007).

### 2.5. Physiological measurements

During the thermal experimental period, rectal, skin temperatures, and respiration rate were measured at 09:00 (before room temperature increased), 13:00 and 17:00 daily. Rectal temperature was measured using a digital thermometer (Omron Australia MC34110, Port Melbourne, Victoria, Australia), inserted approximately 3 cm into the rectum and held in place approximately 30 s until a stable temperature was obtained. Skin temperature was measured using a handheld thermometer by placing the reader end of the measuring device in between the wool folds and pressed gently against the skin of the animal. Respiration rate was recorded by counting the number of breaths (flank movements) per minute.

### 2.6. Statistical analysis

The growth performance and tissue deposition data from the initial feeding experiment were analyzed by ANOVA with the main contrasts being sex (male vs. female), dietary Cr (0 vs. 400 and 800  $\mu\text{g/kg}$  Cr as nCrPic) and within dietary Cr (400 vs. 800  $\mu\text{g/kg}$  Cr as nCrPic) and their interactions with replicate as a blocking factor. For the thermal component of the study, the growth performance data were analyzed by ANOVA with the main contrasts being sex (male vs. female), temperature (TN vs. HS), dietary Cr (0 vs. 400 and 800  $\mu\text{g/kg}$  Cr as nCrPic) and within dietary Cr (400 vs. 800  $\mu\text{g/kg}$  Cr as nCrPic) and their interactions with replicate as a blocking factor. The physiological data were analyzed by restricted maximum likelihood (REML) suitable for repeated measures with the main contrasts being sex (male vs. female), temperature (TN vs. HS), day (d 1 vs. 2 ... vs. 20), time of day (09:00 vs. 13:00 vs. 17:00), dietary Cr (0 vs. 400 and 800  $\mu\text{g/kg}$  Cr as nCrPic) and within dietary Cr (400 vs. 800  $\mu\text{g/kg}$  Cr as nCrPic) and their interactions with individual sheep and replicate as random factors. Since there were no main or interactive effects of sex in the thermal study the data have been pooled across sexes for presentation. Also, there were no main or interactive effects of within Cr dose in both studies data so the Cr effects will be discussed as contrasts between sheep fed 0  $\mu\text{g/kg}$  Cr and the pooled data from sheep fed 400 and 800  $\mu\text{g/kg}$  Cr. All data were analysed using the GenStat statistical package (GenStat release 18; VSN International Ltd., Hemel Hempstead, UK) with appropriate covariates and data transformation where indicated in the text or table legends.

### 3. Results

#### 3.1. Growth performance and tissue deposition in initial feeding study

There was no effect of sex on average daily feed intake (ADFI) (1,188 vs. 1,322 g/d for females and males respectively,  $P = 0.14$ ), males had greater average daily gain (ADG) (128 vs. 182 g/d,  $P = 0.028$ ), feed conversion efficiency (FCE) (0.101 vs. 0.132,  $P = 0.040$ ) and lean tissue deposition (78 vs. 121 g/d,  $P = 0.010$ ) than females (Table 2). There were no significant effects of dietary Cr on growth performance or tissue deposition rate in the initial feeding study (Table 2).

#### 3.2. Growth performance during thermal study

There were no main or interactive effects of sex on growth performance in the thermal study so data have been pooled for presentation. Heat stress decreased ADFI (1,343 vs. 1,163 g/d,  $P = 0.002$ ) whereas sheep under HS had a reduced ADG and indeed lost weight (114 vs. -55 g/d,  $P < 0.001$ ) (Table 3). Dietary nCrPic increased both ADFI (1,171 vs. 1,294 g/d,  $P = 0.04$ ) and ADG (-24 vs. 56 g/d,  $P = 0.05$ ) under both TH and HS conditions. In this context, the ADFI of sheep maintained under TN conditions and not supplemented with nCrPic was not different to that of sheep receiving supplemental nCrPic and exposed to HS (1,299 vs. 1,223 g/d). Feed conversion efficiency was decreased by HS (0.088 vs. -0.039,  $P < 0.001$ ) but not significantly altered by dietary nCrPic (-0.010 vs. 0.041,  $P = 0.13$ ). Water disappearance was increased by HS (3.63 vs. 4.29 L/d,  $P = 0.04$ ) and tended to be increased by dietary nCrPic (3.54 vs. 4.17 L/d,  $P = 0.08$ ). The ratio of water disappearance to feed consumed was increased by HS (2.59

vs. 3.73 L/kg,  $P < 0.001$ ). Heat stress decreased ADFI on the first day of treatment, particularly in those sheep consuming the control diet whereas ADFI declined more slowly in those sheep fed nCrPic (Fig. 1). The ADFI of the sheep consuming the control diet was lower than for sheep consuming the nCrPic diet from d 1 of HS and remained lower for most of the study except for a few days towards the end of the HS period where they converged and then separated again (Fig. 1). However, there were no significant interactions ( $P > 0.33$  for all) between thermal treatment, day of treatment or dietary nCrPic (Fig. 1).

#### 3.3. Body temperature response during thermal study

Rectal temperature increased in response to HS (39.27 vs. 39.92 °C for TN and HS conditions, respectively,  $P < 0.001$ ) and increased over the day (39.21, 39.75, and 39.85 °C at 09:00, 13:00 and 17:00 respectively,  $P < 0.001$ ). However, there was an interaction ( $P < 0.001$ ) between thermal treatment and time such that the rectal temperature increased to a greater extent over the day in the sheep exposed to HS compared to those housed under TN conditions (Fig. 2). In addition, there were interactions (both  $P < 0.001$ ) between dietary nCrPic and thermal treatments and dietary nCrPic and time such that average rectal temperature was lower in sheep consuming nCrPic during HS (40.01 vs. 39.89 °C) but not under TN conditions (39.25 vs. 39.28 °C). Rectal temperature was highest on the first day of HS before declining to reach a nadir between d 4 and 6 before increasing again and reaching a relatively stable plateau (Fig. 3). Rectal temperature was higher for the sheep consuming the control diet for the entire HS period (Fig. 3) and there was no diet x thermal treatment interaction.

Skin temperature increased in response to HS (37.74 vs. 39.23 °C for TN and HS conditions, respectively,  $P < 0.001$ ) and increased

**Table 2**  
Effect of sex and dietary nano chromium picolinate on growth performance and tissue deposition in sheep<sup>1</sup>.

Sex	Female, µg/kg			Male, µg/kg			SED <sup>2</sup>	P-value			
	Chromium (Cr), µg/kg	0	400	800	0	400		800	Sex <sup>3</sup>	Cr <sup>4</sup>	within Cr <sup>5</sup>
ADFI, g/d		1200	1238	1125	1357	1305	1303	108.7	0.14	0.71	0.60
ADG, g/d		116	139	129	171	176	199	28.8	0.028	0.49	0.82
Feed conversion efficiency		0.0856	0.107	0.112	0.119	0.132	0.146	0.0180	0.043	0.15	0.60
Lean deposition, g/d		66.3	84.3	83.0	111	117	134	19.27	0.010	0.35	0.68
Fat deposition, g/d		42.9	44.1	35.8	46.4	44.6	46.5	8.89	0.50	0.80	0.72
Ash deposition, g/d		1.49	2.27	1.80	1.93	1.70	3.03	0.651	0.49	0.38	0.52

<sup>1</sup> There were no interactions between sex and chromium so main effects are presented.

<sup>2</sup> Standard error of the difference for effect of Sex × Cr.

<sup>3</sup> Female vs. male ( $n = 18$  vs. 18).

<sup>4</sup> Control vs. Cr ( $n = 12$  vs. 24).

<sup>5</sup> 400 vs. 800 µg/kg Cr ( $n = 12$  vs. 12).

**Table 3**  
Effect of heat stress and dietary nano chromium picolinate on growth performance and tissue deposition in sheep<sup>1</sup>.

Temperature (T)	Thermo-neutral			Heat stress			s.e.d. <sup>2</sup>	P-value			
	Chromium (Cr), µg/kg	0	400	800	0	400		800	T <sup>3</sup>	Cr <sup>4</sup>	within Cr <sup>5</sup>
ADFI <sup>6</sup> , g/d		1299	1336	1395	1043	1212	1233	68.3	0.002	0.044	0.52
ADC <sup>7</sup> , g/d		2.648 (94.6)	2.667 (115)	2.683 (132)	2.316 (-143)	2.501 (-33.0)	2.556 (9.7)	0.0673	<0.001	0.049	0.61
Feed conversion efficiency		0.079	0.084	0.100	-0.098	-0.027	0.008	0.0361	<0.001	0.13	0.51
Water disappearance, L/d		3.22	3.56	4.10	3.86	4.38	4.62	0.392	0.041	0.076	0.36
Water disappearance:feed intake, L/kg		2.33	2.67	2.76	3.85	3.54	3.80	0.355	<0.001	0.73	0.63

<sup>1</sup> There were no main or interactive effects of sex so data are pooled across sexes. There were no interactions between temperature and Cr so main effects are presented.

<sup>2</sup> Standard error of the difference for effect of temperature × Cr.

<sup>3</sup> Thermo-neutral vs. heat stress ( $n = 18$  vs. 18).

<sup>4</sup> Control vs. Cr ( $n = 12$  vs. 24).

<sup>5</sup> 400 vs. 800 µg/kg Cr ( $n = 12$  vs. 12).

<sup>6</sup> Initial body weight was used as a covariate.

<sup>7</sup> Transformed values  $[\log(x+350)]$  with back-transformed values are in parentheses below. Data were transformed due to heterogeneity in variances.

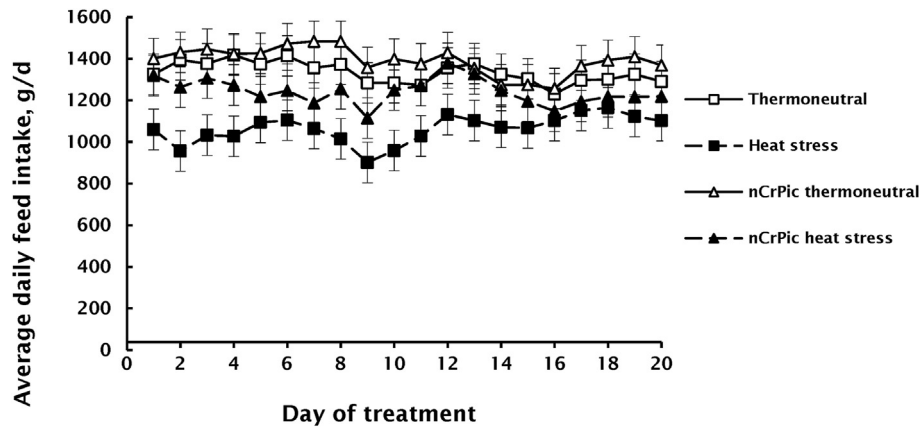


Fig. 1. Average daily feed intake in response to cyclic thermal treatment (T), day of treatment (D) and dietary nCrPic (Cr) in sheep. Data are mean ± standard error of the difference for D × T × Cr. The P-values for the effect of T, D and Cr were 0.004, <0.001 and 0.07, respectively. There were no significant interactions ( $P > 0.33$  for all).

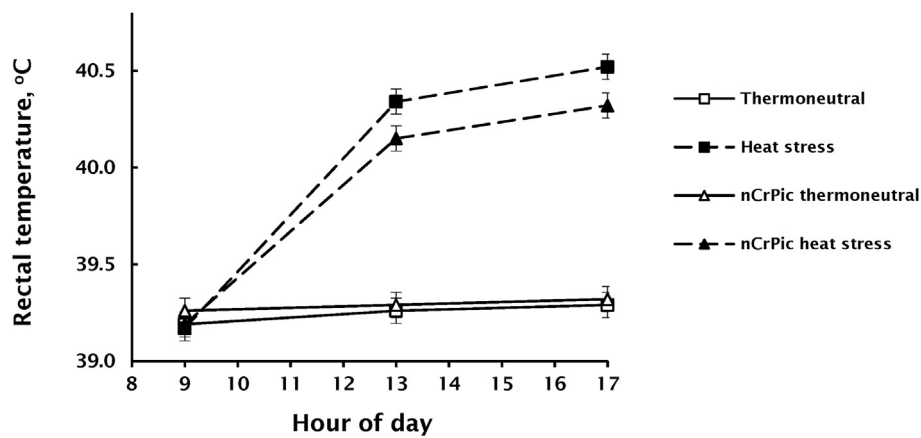


Fig. 2. Rectal temperature responses to cyclic thermal treatment (T), hour of day (H) dietary nCrPic (Cr) in sheep. Data are mean ± standard error of the difference for T × H × Cr. The P-values for the effect of T, H, Cr, T × H, T × Cr, H × Cr and T × H × Cr were <0.001, <0.001, 0.050, <0.001, <0.001, <0.001 and 0.010, respectively.

over the day (37.81, 38.78, and 38.86 °C at 09:00, 13:00 and 17:00 respectively,  $P < 0.001$ ) (data not shown). However, there was an interaction ( $P < 0.001$ ) between thermal treatment and time such that the skin temperature increased to a greater extent over the day in the sheep exposed to HS compared to those housed under TN conditions. There were no main or interactive effects of dietary nCrPic on skin temperature.

Respiration rate increased in response to HS (76 vs. 170 breaths per min for TN and HS conditions, respectively,  $P < 0.001$ ) and increased over the day (77, 142 and 149 breaths per min, at 09:00, 13:00 and 17:00, respectively,  $P < 0.001$ ) (Fig. 4). However, there was an interaction ( $P < 0.001$ ) between thermal treatment and time such that the respiration rate increased to a greater extent over the day in the sheep exposed to heat compared to those

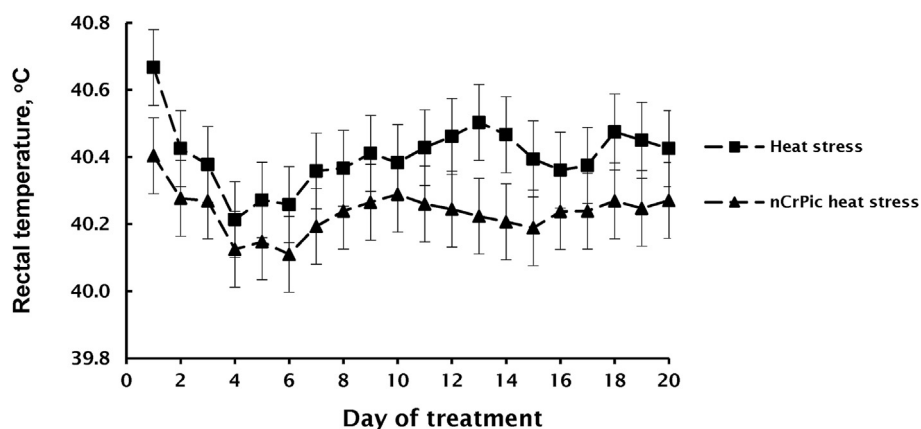


Fig. 3. Average rectal temperature during peak HS (average of 13:00 and 17:00 on each day) in sheep fed either a basal diet or a diet containing nCrPic (Cr) on each day (D) of treatment. Data are means ± standard error of the difference for D × Cr. The P-values for the effects of D, Cr and D × Cr were <0.001, 0.04 and 0.78, respectively.

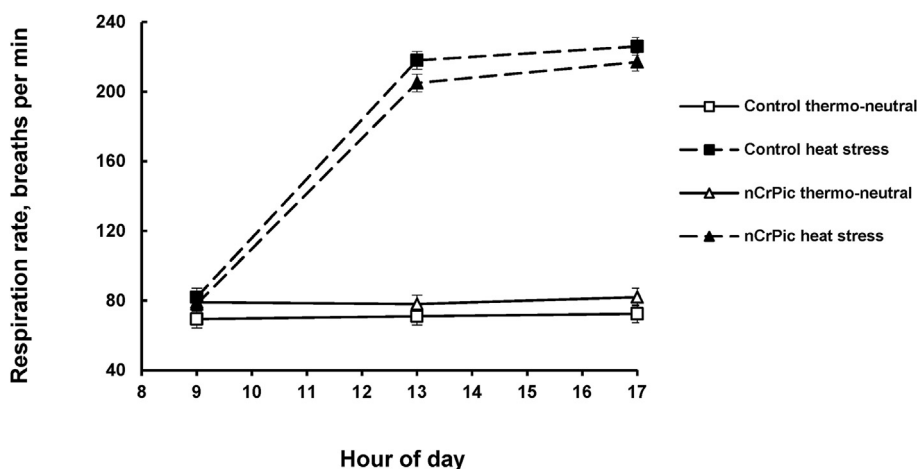


Fig. 4. Respiration rate responses to cyclic thermal treatment (T), hour of day (H) dietary nCrPic (Cr) in sheep. Data are mean ± standard error of the difference for T × H × Cr. The P-values for the effect of T, H, Cr, T × H, T × Cr, H × Cr and T × H × Cr were <0.001, <0.001, 0.90, <0.001, 0.030, <0.001 and 0.39, respectively.

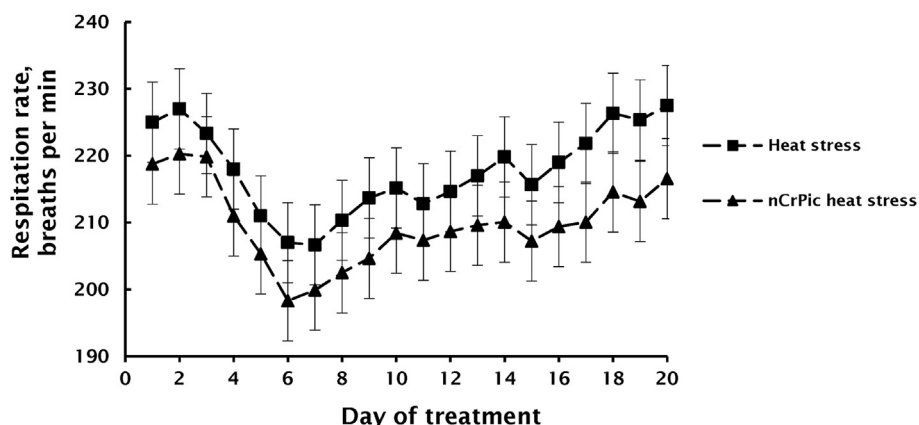


Fig. 5. Average respiration rate during peak HS (average of 13:00 and 17:00 h on each day) in sheep fed either a basal diet or a diet containing nCrPic (Cr) on each day (D) of treatment. Data are mean ± standard error of the difference for D × Cr. The P-values for the effects of D, Cr and D × Cr were <0.001, 0.09 and 0.99, respectively.

housed under TN conditions (Fig. 4). There was no main effect ( $P = 0.90$ ) of nCrPic on respiration rate, there was an interaction between dietary nCrPic and thermal treatment ( $P < 0.001$ ) and dietary nCrPic and time ( $P = 0.030$ ) such that respiration rate was lower in sheep consuming nCrPic during peak HS (222 vs. 211 breaths per min) but not under TN conditions (71 vs. 80 breaths per min) (Fig. 5).

#### 4. Discussion

The aims of this study were to examine the effects of dietary nCrPic supplementation on growth, body composition and physiological responses of sheep under HS. nCrPic has much smaller particle size (50 nm) compared with normal particle size CrPic (310 μm). The major findings of this study were that dietary nCrPic reduced the negative impact of HS on rectal temperature, respiration rate and ADFI allowing the sheep to continue to gain weight, or at least prevent weight loss, during HS. Since feed intake is a primary indicator of HS, the ability to reverse some of the effects on feed intake with dietary Cr is an important finding. Greater ADFI has been reported in heat-stressed quails (Sahin et al., 2002a, 2005), broilers (Smantha et al., 2008; Sahin et al., 2003), pigs (Hung et al., 2014) and cows (Al-Saiady et al., 2004) consuming diets supplemented

with Cr. Dietary Cr supplementation also increased ADFI in pregnant ewes that face a different type of physiological stress (Mousaie et al., 2017) and likely to be insulin resistant (Pettersson et al. 1993, 1994). Moreover, the net energy balance for lactation was improved by dietary Cr supplementation of dairy cows (Al-Saiady et al., 2004). The reduction in ADFI is a metabolic adaptation that occurs with HS (Baumgard and Rhoads, 2011; Sejian et al., 2018; Gaughan et al., 2019). When animals are consuming inadequate feed, they typically entered into negative energy balance and consequently, enhance the catabolic processes, as indicated by increased adipose tissue’s lipoprotein lipase (indicator of lipolytic response) and increased plasma urea nitrogen (indicator of muscle catabolism) (Wheelock et al., 2010; Baumgard and Rhoads et al., 2011; Rhoads et al., 2011). However, increased catabolism also leads to increased heat production (McDonald et al., 2011). The present data suggest that dietary nCrPic could prevent negative the impacts of HS since ADFI was increased in sheep supplemented with nCrPic. Moreover, dietary nCrPic also appears to decrease metabolic heat production or increase the ability of the animal to dissipate heat during HS as respiration rate and rectal temperature were lower in nCrPic fed sheep, despite the higher ADFI. Respiration rate and rectal temperature both increase with increasing level of ADFI in sheep exposed to HS (Gonzalez-Rivas et al., 2016).

Economic losses are incurred in livestock industries when farm animals are raised in locations and seasons where effective temperature conditions are outside their zone of thermal comfort for some or all of the time (St-Pierre et al., 2003; Sejian et al., 2018). High environmental temperature and humidity are detrimental to the productivity of farm animals (Fuquay, 1981; Sejian et al., 2018; Gaughan et al., 2019). Exposure of sheep to elevated temperature results in a decrease in ADG and ADFI (Marai et al., 2007). The upper critical temperature for sheep lies between 25 and 30 °C and HS occurs when sheep are exposed to temperatures higher than 30 °C (Fuquay, 1981). Marai and colleagues indicated that sheep are under extreme severe HS when the THI value is over 25.6 (Marai et al., 2007). The mean THI value at the height of heat treatment during the afternoon was  $36.7 \pm 2.13$  in the present study which is equivalent to extreme severe HS. The findings of Srikandakumar and colleagues indicated that respiration rate and rectal temperature were elevated by HS in both Omani and Merino sheep (Srikandakumar et al., 2003). In the current study, rectal, skin temperature and respiration rate increased as THI increased with the increase of these physiological responses sufficient to suggest that these animals were under HS.

During HS, evaporation loss through respiration is the most important avenue for heat dissipation, since sweating in sheep is much less important than other species due to the presence of wool (Fuquay, 1981). Under chronic high ambient temperatures, animals develop certain mechanisms to reduce body heat production such as decreased ADFI and metabolic heat production (Fuquay, 1981). Increased rectal temperature is an indicator of elevated core temperature that is often observed in sheep experiencing HS. At the height of HS in the afternoon, rectal temperature was 1.16 °C higher in sheep fed the basal diet than their counterparts under TN conditions, whereas the sheep consuming the diet containing nCrPic had a more moderate increase in rectal temperature of 0.93 °C. As previously mentioned, respiration is the most important avenue for heat evaporation and can account for up to 60% of heat loss in sheep (Marai et al., 2007). Macfarlane and colleagues suggested that the normal core temperature of sheep is approximately 39.6 °C, and any increase above this temperature indicates that the sheep's thermo-regulatory functions are insufficient (Macfarlane et al., 1958). In addition to reducing rectal temperature dietary nCrPic decreased respiration rate indicating sheep were able to dissipate heat better or produce less heat during increased heat load. Dietary CrPic also decreased rectal temperature and respiration rate in pigs exposed to a similar temperature regime as in the present study (Liu et al., 2017). Skin temperature increased in response to HS although there were no main or interactive effects of nCrPic on this response. In part, this may be because the sheep were not exposed to solar radiation in the heat chamber model and so the HS may not be as severe as when sheep are exposed to solar radiation. In this context, Sevi et al. (2001) found that lactating sheep exposed to solar radiation had higher rectal temperature than sheep not exposed to solar radiation even though the average ambient temperature were similar. It is possible that effects of nCrPic on skin temperature may become apparent if sheep were exposed to solar radiation.

In ruminants, the mechanisms of heat generation include the cost of eating and ruminating and heat produced by hepatic and the gut tissue as the heat increment (Webster et al., 1975). The heat increment is usually defined as the increase in heat production associated with the acute ingestion of food and the associated post-absorptive heat production during chronic hyperphagia (Ge et al., 2008). The processes of ingestion and digestion account for approximately 25% to 30% of the total heat increment (Webster et al., 1975). Interestingly, in the present study, feed intake was higher in sheep supplemented with dietary nCrPic diet during HS,

but the rectal temperature and respiration rate were in fact less. A possible explanation is the dietary Cr can improve energy utilization. Previous studies indicated that dietary nutrient deficiencies, including vitamin A, copper, iron, potassium, sodium, and calcium, result in increased thermogenesis as a result of decreased efficiency of energy utilization (Ge et al., 2008; Sun and Zemel, 2004). It is well established that dietary Cr can improve energy utilization (Samantha et al., 2008; Anderson, 2008; Yan et al., 2010). For example, Hung and colleagues found that dietary Cr decreased backfat in finisher pigs fed a high fat diet suggesting that nCrPic can increase the efficiency to utilize fat as energy, and consequently, reduce metabolic heat production (Hung et al., 2015).

In the present study, peak rectal temperature and respiration rate were highest on the first afternoon of HS, most likely because of the carryover effects of normal ADFI from the previous day and insufficient time for chronic adaptive mechanisms to occur. However, the increase in rectal temperature and respiration rate were much less dramatic in the sheep supplemented with nCrPic than those fed the basal diet. In the sheep fed the basal diet there was a substantial decrease in ADFI (−20%) on the first day of HS whereas the decrease in the sheep supplemented with nCrPic there was a more modest decrease in ADFI (−6%). Rectal temperature and respiration rate then declined, the latter albeit more slowly, and having reached their nadir then increasing gradually again with the differences between sheep for the basal and nCrPic supplemented diets being maintained. The continued increase or apparent plateau in both rectal temperature and respiration rate suggest that there was no long-term adaptation to HS.

During the initial feeding study conducted under ambient temperatures similar to TN conditions there were no significant effects on dietary nCrPic on growth performance and tissue deposition. However, the ADG and FCE responses were qualitatively and quantitatively similar to those of the sheep housed under TN conditions in the thermal experiment. Growth and metabolic effects of Cr in ruminants are variable and this may be in part related to the level of underlying stress that the animal is exposed to (Dallago et al., 2011). Interestingly, it has been hypothesized that effects of Cr on glucose metabolism may only occur when there is a stressor (Dallago et al., 2015) and this may explain why the responses became most apparent during HS in the present study.

## 5. Conclusion

This experiment clearly demonstrated that dietary nCrPic can partially ameliorate the negative effects of HS as indicated by maintained ADFI and decreased rectal temperature and respiration rate when sheep were exposed to high ambient temperatures. The effects of cyclic HS are apparent for up to 3 wk as is the protection conferred by dietary nCrPic. However, it is unclear whether the reductions in physiological responses to HS are due to the metabolic effects of nCrPic or improved energy utilization.

## Conflict of interest

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

## Acknowledgments

This research was primarily funded by the Commonwealth Department of Agriculture and Water Resources. The financial support of the Australian Pork Cooperative Research Centre for a

post-graduate stipend for Alex Hung is gratefully acknowledged. The authors have declared no conflict of interest.

## References

- Abebe W, Liu JY, Wimborne H, Mozaffari MS. Effects of chromium picolinate on vascular reactivity and cardiac ischemia reperfusion injury in spontaneously hypertensive rats. *Pharmacol Rep* 2010;62:674–82.
- Alhidary IA, Shini S, Al Jassim RAM, Gaughan JB. Physiological responses of Australian Merino wethers exposed to high heat load. *J Anim Sci* 2012;90:212–20.
- Al-Mufarrej SI, Al-Haidary IA, Al-Kraidees MS, Hussein MF, Metwally HM. Effect of chromium dietary supplementation on the immune response and some blood biochemical parameters of transport-stressed lambs. *Asian Austral J Anim* 2008;21:671–6.
- Al-Saiady MY, Al-Shaikh MA, Al-Mufarrej SI, Al-Showeimi TA, Mogawer HH, Dirrar A. Effect of chelated chromium supplementation on lactation performance and blood parameters of Holstein cows under heat stress. *Anim Feed Sci Technol* 2004;117:223–33.
- Anderson RA. Chromium and polyphenols from cinnamon improve insulin sensitivity. *Proc Nutr Soc* 2008;67:48–53.
- Baumgard LH, Rhoads RP. Ruminant production and metabolic responses to heat stress. *J Anim Sci* 2011;90:1855–65.
- Dallago BS, McManus CM, Caldeira DF, Lopes AC, Paim TP, Franco E, Borges BO, Teles PH, Correa PS, Louvandini H. Performance and ruminal protozoa in lambs with chromium supplementation. *Res Vet Sci* 2011;90:253–6.
- Dallago BS, Braz SV, Marcola TG, McManus C, Caldeira DF, Campeche A, Gomes EF, Paim TP, Borges BO, Louvandi H. Blood parameters and toxicity of chromium picolinate oral supplementation in lambs. *Biol Trace Elem Res* 2015;168:91–102.
- Degen AA, Young BA. Effect of air temperature and energy intake on body mass, body composition and energy requirements in sheep. *J Agric Sci* 2002;138:221–6.
- Dunshea FR, Gonzalez-Rivas PA, Hung AT, DiGiacomo K, Chauhan SS, Leury BJ, et al. Nutritional strategies to alleviate heat stress in sheep. In: Sejian V, Bhatta R, Gaughan J, Malik PK, Naqvi SMK, Lal R, editors. *Sheep Production adapting to climate change*. Singapore: Springer Nature; 2017. p. 371–88 [Chapter 18].
- Dunshea FR, Leury BJ, Fahri F, DiGiacomo K, Hung A, Chauhan S, Clarke J, Collier R, Little S, Baumgard L, Gaughan JB. Amelioration of thermal stress impacts in dairy cows. *Amin Prod Sci* 2013;53:965–75.
- Estrada-Angulo A, Valdés YS, Carrillo-Muro O, Castro-Perez BI, Barreras A, López-Soto MA, Plascencia A, Dávila-Ramos H, Rios FG, Zinn RA. Effects of feeding different levels of chromium-enriched live yeast in hairy lambs fed a corn-based diet: effects on growth performance, dietary energetics, carcass traits and visceral organ mass. *Anim Prod Sci* 2013;53:308–15.
- Forst T, Caduff A, Talary M, Weder M, Braendle M, Brändle M, Kann P, Flacke F, Friedrich C, Pfützner A. Impact of environmental temperature on skin thickness and micro-vascular blood flow in subjects with and without diabetes. *Diabetes Technol Therapeut* 2006;8:94–101.
- Fuquay JW. Heat stress as it affects animal production. *J Anim Sci* 1981;52:164–74.
- Ge CR, Gao SZ, Jia JJ, Jois M. Diet-induced thermogenesis. *Agric Sci China* 2008;7:1133–9.
- Gaughan JB, Sejian V, Mader TL, Dunshea FR. Adaptation strategies: ruminant. *Anim Frontiers* 2019;9:47–53.
- Gonzales-Eguia A, Fu C, Lu F, Lien T. Effects of nanocopper on copper availability and nutrients digestibility, growth performance and serum traits of piglets. *Livest Sci* 2009;126:122–9.
- Gonzalez-Rivas PA, DiGiacomo K, Russo VM, Leury BJ, Cottrell JJ, Dunshea FR. Feeding slowly fermentable grains has the potential to ameliorate heat stress in grain-fed wethers. *J Anim Sci* 2016;94:2981–91.
- Hung AT, Leury BJ, Sabin MA, Collins CL, Dunshea FR. Dietary nano-chromium tripicolinate increases feed intake and decreases plasma cortisol in finisher gilts during summer. *Trop Anim Health Prod* 2014;46:1483–9.
- Hung AT, Leury BJ, Sabin MA, Lien TF, Dunshea FR. Dietary chromium picolinate of varying particle size improves carcass characteristics and insulin sensitivity in finishing pigs fed low- and high-fat diets. *Anim Prod Sci* 2015;55:454–60.
- Hunter TE, Suster D, Dunshea FR, Wark JD, Cummins LJ, Egan AR, Leury BJ. Dual energy X-ray absorptiometry (DXA) can be used to predict live animal and whole carcass composition of sheep. *Small Rumin Res* 2011;100:143–52.
- Kraidees MS, Al-Haidary IA, Mufarrej SI, Al-Saiady MY, Metwally HM, Hussein MF. Effect of supplemental chromium levels on performance, digestibility and carcass characteristics of transport-stressed lambs. *Asian Austral J Anim* 2009;22:1124–32.
- Liu F, Cottrell JJ, Wijesiriwardana U, Kelly FW, Chauhan SS, Pustovit RV, Gonzalez-Rivas PA, DiGiacomo K, Leury BJ, Celi P, Dunshea FR. Effects of chromium supplementation on physiology, feed intake and insulin related metabolism in growing pigs subjected to heat stress. *Trans Anim Sci* 2017;1:116–25.
- Lien TF, Yeh HS, Lu FY, Fu CM. Nanoparticles of chromium picolinate enhance chromium digestibility and absorption. *J Sci Food Agric* 2009;89:1164–7.
- LPHSI. *Livestock and poultry heat stress indices agriculture engineering technology guide*. SC, USA: Clemson University; 1990.
- Macfarlane WV, Morris RJH, Howard B. Heat and water in tropical Merino sheep. *Aust J Agric Res* 1958;9:217–28.
- Marai IFM, El-Darawany AA, Fadiel A, Abdel-Hafez MAM. Physiological traits as affected by heat stress in sheep—A review. *Small Rumin Res* 2007;71:1–12.
- McDonald P, Edwards RA, Greenhalgh JFD, Morgan CA. *Animal nutrition*. 7th ed. Harlow, England: Pearson; 2011. 2011.
- McGuire MA, Beede DK, DeLorenzo MA, Wilcox CJ, Huntington GB, Reynolds CK, Collier RJ. Effects of thermal stress and level of feed intake on portal plasma flow and net fluxes of metabolites in lactating Holstein cows. *J Anim Sci* 1989;67:1050–60.
- Moreno-Camarena L, Domínguez-Vara I, Bórquez-Gastelum J, Sánchez-Torres J, Pinos-Rodríguez J, Mariezcurrera-Berasain A, Morales-Almaráz E, Salem AZM. Effects of organic chromium supplementation to finishing lambs diet on growth performance, carcass characteristics and meat quality. *J Integr Agric* 2015;14:567–74.
- Mousaie A, Valizadeh R, Chamsaz M. Selenium methionine and chromium-methionine supplementation of sheep around parturition: impacts on dam and offspring performance. *Arch Anim Nutr* 2017;71:134–49.
- NRC. *Nutrient requirements of small ruminants sheep, goats, cervids and new world camelids*. Washington, DC: Natl. Acad. Press; 2007.
- Nardone A, Ronchi B, Lacetera N, Ranieri MS, Bernabucci U. Effects of climate changes on animal production and sustainability of livestock systems. *Livest Sci* 2010;130:57–69.
- O'Brien MD, Rhoads RP, Sanders SR, Duff GC, Baumgard LH. Metabolic adaptations to heat stress in growing cattle. *Domest Anim Endocrinol* 2010;38:86–94.
- Petterson JA, Dunshea FR, Ehrhardt RA, Bell AW. Pregnancy and undernutrition alter glucose metabolic responses to insulin in sheep. *J Nutr* 1993;123:1286–95.
- Petterson JA, Slepetic R, Ehrhardt RA, Dunshea FR, Bell AW. Pregnancy but not moderate undernutrition attenuates insulin suppression of fat mobilization in sheep. *J Nutr* 1994;124:2431–6.
- Rhoads RP, La Noce AJ, Wheelock JB, Baumgard LH. Alterations in expression of gluconeogenic genes during heat stress and exogenous bovine somatotropin administration. *J Dairy Sci* 2011;94:1917–21.
- Sahin N, Akdemir F, Tuzcu M, Hayirli A, Smith MO, Sahin K. Effects of supplemental chromium sources and levels on performance, lipid peroxidation and proinflammatory markers in heat-stressed quails. *Anim Feed Sci Technol* 2010;159:143–9.
- Sahin K, Ozbey O, Onderci M, Cikim G, Aysondu MH. Chromium supplementation can alleviate negative effects of heat stress on egg production, egg quality and some serum metabolites of laying Japanese Quail. *J Nutr* 2002a;132:1265–8.
- Sahin K, Sahin N, Onderci M, Gursu F, Cikim G. Optimal dietary concentration of chromium for alleviating the effect of heat stress on growth, carcass qualities, and some serum metabolites of broiler chickens. *Biol Trace Elem Res* 2002b;89:53–64.
- Sahin K, Sahin N, Kucuk O. Effects of chromium, and ascorbic acid supplementation on growth, carcass traits, serum metabolites, and antioxidant status of broiler chickens reared at a high ambient temperature. *Nutr Res* 2003;23:225–38.
- Sahin N, Sahin K, Onderci M, Gursu MF, Cikim G, Vijaya J, Kucuk O. Chromium picolinate, rather than biotin, alleviates performance and metabolic parameters in heat-stressed quail. *Br Poultry Sci* 2005;46:457–63.
- Sales J, Jančík F. Effect of dietary chromium supplementation on performance, carcass characteristics, and meat quality of growing-finishing swine: a meta-analysis. *J Anim Sci* 2011;89:4054–67.
- Sano H, Kato Y, Takebayashi A, Shiga A. Effects of supplemental chromium and isolation stress on tissue responsiveness and sensitivity to insulin in sheep. *Small Rumin Res* 1999;33:239–46.
- Sejian V, Bhatta R, Gaughan JB, Dunshea FR, Lacetera N. Review: adaptation of animals to heat stress. *Animal* 2018;12:s431–44.
- Sevi A, Annicchiarico G, Albenzio M, Taibi L, Muscio A, Dell'Aquila S. Effects of solar radiation and feeding time on behavior, immune response and production of lactating ewes under high ambient temperature. *J Dairy Sci* 2001;84:629–40.
- Smantha S, Haldar S, Bahadur V, Ghosh TK. Chromium picolinate can ameliorate the negative effects of heat stress and enhance performance, carcass and meat traits in broiler chickens by reducing the circulatory cortisol level. *J Sci Food Agric* 2008;88:787–96.
- Srikandakumar A, Johnson EH, Mahgoub O. Effect of heat stress on respiratory rate, rectal temperature and blood chemistry in Omani and Australian Merino sheep. *Small Rumin Res* 2003;49:193–8.
- St-Pierre NR, Cobanov B, Schnitkey G. Economic losses from heat stress by US livestock industries. *J Dairy Sci* 2003;86:E52–77.
- Sun X, Zemel MB. Calcium and dairy products inhibit weight and fat regain during ad libitum consumption following energy restriction in Ap2-agouti transgenic mice. *J Nutr* 2004;134:3054–60.
- Webster AJF, Osuji PO, White F, Ingram JF. The influence of food intake on portal blood flow and heat production in the digestive tract of sheep. *Br J Nutr* 1975;34:125–9.
- Wheelock JB, Rhoads RP, VanBaale MJ, Sanders SR, Baumgard LH. Effects of heat stress on energetic metabolism in lactating Holstein cows. *J Dairy Sci* 2010;93:644–55.
- Williams CH, David DJ, Iismaa O. The determination of chromic oxide in faeces samples by atomic absorption spectrophotometry. *J Agric Sci* 1962;59:381–5.
- Yan XG, Zhang FY, Li D, Zhu XP, Jia ZH. Effects of chromium on energy metabolism in lambs fed with different dietary protein levels. *Asian Austral J Anim* 2010;23:205–12.
- Zha L, Zeng J, Sun S, Deng H, Luo H, Li W. Chromium(III) nanoparticles affect hormone and immune responses in heat-stressed rats. *Biol Trace Elem Res* 2009;129:157–69.
- Zhou B, Wang H, Luo G, Niu R, Wang J. Effect of dietary yeast chromium and L-carnitine on lipid metabolism of sheep. *Biol Trace Elem Res* 2013;155:221–7.