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Effects of a supplement containing multiple types of gluconeogenic precursors on production and metabolism in Holstein bull calves during heat stress

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Article Info

Highlights

- •We evaluated the effects of a commercial glucose precursors during heat stress in bull calves.
- •We assessed the effects of this product on productive performance of bull calves during heat stress.
- •We examined changes in the level of blood parameters during heat stress and in combination with a dietary treatment.

- •This product changed insulin level but not productive performance and body temperature indices.

Abstract

Glucose appears to be a preferred systemic fuel during heat stress (HS) in a variety of species. Increasing the dietary grain content can enhance the post-absorptive carbohydrate status, but providing excessive fermentable starch can cause rumen disorders and this is especially true during HS. Current study objectives were to evaluate the effects of a glycerol based supplemental product on growth and metabolic variables in Holstein bull calves during controlled HS. Before the start of the experiment, bull calves (n=14; 163.6±30.1 kg body weight) were subjected to thermal neutral conditions [26.5±3.4 °C and a temperature–humidity index (THI) of 70.4±2.8] for 7 d (period 1; P1). During this period, productive parameters as well as blood metabolites were measured and used as covariates for the subsequent HS period. Following P1, a cyclical HS pattern was implemented for 21 d (P2) where daily ambient temperatures ranged from 29.1 to 39.7 °C and the THI was >74 for 24 h/d and >83 for at least 14 h/d. During P2, half of the HS calves (n=7) received a control diet (CON) and the other half received the control diet supplemented with a product (300 g/d) containing gluconeogenic precursors (GLU). Throughout each period respiration rate, rectal temperature and skin temperature at the shoulder and rump were recorded at 0600, 1100 and 1500 h daily. Blood samples were obtained prior to and 4 h post the a.m. feeding during both periods. Although HS markedly reduced DMI (18%) and growth as expected, supplemental GLU did not affect body weight gain. Supplemental GLU decreased the shoulder temperature at 0600 and 1500 h (P<0.01), and decreased respiratory rate at 1500 h (P<0.02). Feeding GLU did not affect blood urea nitrogen (BUN), glucose or nonesterified fatty acids (NEFA) concentrations, but increased circulating insulin prior to the a.m. feeding (P<0.03) and this demonstrates that GLU was effective at enhancing the post-absorptive carbohydrate status. Our results suggest that feeding supplemental GLU improves some body temperature indices but did not enhance growth performance in Holstein bull calves during HS.

Keywords:

Bull calf, Glucose, Heat stress, Insulin

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ABSTRACT

Glucose appears to be a preferred systemic fuel during heat stress (HS) in a variety of species. Increasing the dietary grain content can enhance the post-absorptive carbohydrate status, but providing excessive fermentable starch can cause rumen disorders and this is especially true during HS. Current study objectives were to evaluate the effects of a glycerol based supplemental product on growth and metabolic variables in Holstein bull calves during controlled HS. Before the start of the experiment, bull calves ($n=14$; 163.6 ± 30.1 kg body weight) were subjected to thermal neutral conditions [26.5 ± 3.4 °C and a temperature–humidity index (THI) of 70.4 ± 2.8] for 7 d (period 1; P1). During this period, productive parameters as well as blood metabolites were measured and used as covariates for the subsequent HS period. Following P1, a cyclical HS pattern was implemented for 21 d (P2) where daily ambient temperatures ranged from 29.1 to 39.7 °C and the THI was >74 for 24 h/d and >83 for at least 14 h/d. During P2, half of the HS calves ($n=7$) received a control diet (CON) and the other half received the control diet supplemented with a product (300 g/d) containing gluconeogenic precursors (GLU). Throughout each period respiration rate, rectal temperature and skin temperature at the shoulder and rump were recorded at 0600, 1100 and 1500 h daily. Blood samples were obtained prior to and 4 h post the a.m. feeding during both periods. Although HS markedly reduced DMI (18%) and growth as expected, supplemental GLU did not affect body weight gain. Supplemental GLU decreased the shoulder temperature at 0600 and 1500 h ($P<0.01$), and decreased respiratory rate at 1500 h ($P<0.02$). Feeding GLU did not affect blood urea nitrogen (BUN), glucose or nonesterified fatty acids (NEFA) concentrations, but increased circulating insulin prior to the a.m. feeding ($P<0.03$) and this demonstrates that GLU was effective at enhancing the post-absorptive carbohydrate status. Our results suggest that feeding supplemental GLU improves some body temperature indices but did not enhance growth performance in Holstein bull calves during HS.

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1. Introduction

Heat stress (HS) compromises efficient animal production and although difficult to accurately quantify, the economic impact on the global livestock industries is likely greater than $100 billion annually (Baumgard and Rhoads, 2013). Economic losses due to HS can be attributed to decreased milk production, increased incidence of metabolic disorders and health problems (e.g., rumen acidosis), slowed and inconsistent growth, compromised milk quality, reduced reproductive performance, and mortality (West, 1999).

A variety of amelioration strategies for HS are available, and can be implemented alone or in a coordinated manner. These include: (1) physically modifying the environment (shades, fans, evaporative cooling, etc.), (2) management adaptations (timing of milking, feeding, etc.), (3) genetic selection and (4) dietary modifications. To date, genetic selection for HS tolerance is frequently associated with production drag during thermal neutral conditions (Baumgard and Rhoads, 2013). Investing in adequate HS abatement facilities maybe financially challenging for many producers; this is especially true in developing countries and for small stakeholders. Identifying nutritional strategies that can be easily incorporated into the diet to alleviate the negative effect of HS is beneficial for optimizing production of high quality protein during the stressful summer months.

There are several nutritional strategies to consider during HS and they typically concentrate on increasing the energy density of the diet and minimizing the thermic effect of feeding (see reviews by West, 1999; Kadzere et al., 2002). Recently, some studies have demonstrated that heat-stressed farm animals including growing and lactating ruminants preferentially utilize glucose for processes other than milk and muscle synthesis (see reviews by Baumgard and Rhoads, 2012, 2013). Consequently, heat-stressed animals appear to be in a negative post-absorptive carbohydrate status and thus altering insulin action and increasing glucose availability may be a viable approach during HS (Rhoads et al., 2013). One method of enhancing hepatic glucose output is to feed additional starch, but feeding excessive grain needs to be carefully considered given that heat-stressed ruminants are susceptible to ruminal acidosis (Kadzere et al., 2002). Consequently, safely providing rumen substrates that are gluconeogenic themselves (glycerol; if absorbed intact by the intestine or rumen wall) or are metabolized into glucose precursors (propionate) may increase productivity during the warm summer months (Baumgard and Rhoads, 2012). Dietary ionophores safely increase propionate production and can increase hepatic glucose output (Baumgard et al., 2011). Another dietary option to increase propionate production is via supplementing glucose precursors, such as glycerol and propylene glycol, which have been used for prophylactic and metaphylactic treatment for ketosis (Johnson, 1954; Fisher et al., 1973).

We hypothesized that supplementing a product containing a variety of gluconeogenic molecules could enhance the post-absorptive carbohydrate status during HS and improve animal performance. Therefore, our study objectives were to evaluate the effects of a supplement containing multiple types of glucose precursors (mainly consisting of glycerol and propylene glycol), on productive performance and circulating bioenergetic markers in Holstein bull calves during HS.

2. Material and methods

2.1. Animals and experimental design

Holstein bull calves were cared for according to the guidelines of the Iranian Council on Animal Care (1995). In order to acclimate to the diet and stalls growing Holstein bull calves ($n=14$; 163.6 ± 30.1 kg BW) were selected and randomly assigned to individual tie stalls (4×2 m^2; with individual feeders and waters) two weeks prior to experiment initiation. Animals were maintained in tie-stall stanchions at the University of Zanjan's research farm. The sunlight was able to enter the facility via 2 windows, but the condition was the same for 2 groups. All calves were randomly assigned to treatments and individually fed a TMR twice a day at 0700 and 1400 h to achieve 5–10% orts. Hay (alfalfa) to concentrate (primary barley grain) ratio was 80.2:19.8 which was formulated to meet or exceed NRC (1996) requirements for energy, protein, minerals, and vitamins (Table 1). This experiment consisted of two periods (P). During P1, production variables were recorded daily for 7 d. Blood samples were collected on d 2 and 7 of P1. During P1 all calves were kept in thermal neutral conditions [26.5 ± 3.4 °C and a temperature–humidity index (THI; Buffington et al., 1981) of 70.4 ± 2.8; 14 h/10 h light/dark cycle, Fig. 1]. During P2,

Table 1
Ingredients and chemical composition of diet (DM basis)[a].

Item	
Ingredient composition (g/kg DM)	
Alfalfa hay	198.0
Ground barely grain	543.0
Ground corn grain	31.0
Fish meal	23.0
Cottonseed whole	32.0
Cottonseed meal	95.0
Beet pulp	47.0
Sodium bicarbonate	12.0
Salt	5.0
Mineral-vitamin premix[b]	14.0
Chemical composition	
Diet DM (g/kg)	927.6
ME[c] (MJ/kg DM)	11.17
Crude protein (g/kg DM)	134.0
Crude fat (g/kg DM)	27.0
TDN[c] (g/kg DM)	740.0
NDF (g/kg DM)	288.5
ADF (g/kg DM)	161.0

[a] Composition of the basal diet to which 300 g of Glukosa was added as a top-dress. Glukosa contained 330 g/kg of glycerol, 94.5 g/kg of mono propylene glycol, 70.5 g/kg of Ca propionate, 470 mg/kg of niacin and 185 mg/kg of cobalt sulfate. Effective material was 49.5% and the rest of material was colloidal silica as a carrier.

[b] Provided (per kg of DM): 700000 IU of Vitamin A; 600,000 of IU Vitamin D; 1000 of mg Vitamin E; 250 g of Ca; 200 g of Mg; 8 mg of Co; 800 mg of Cu; 40 mg of I; 3200 mg of Mn, 10 mg of Se; 3000 mg of Zn.

[c] Estimated using the NRC (1996) individual dietary ingredients.

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all calves were subjected to cyclical HS conditions (29.1 to 39.7 °C and a THI greater than 74 for more than 24 h/d and a THI of more than 83 at least for 14 h/d; Fig. 1) for 21 d. The HS room was warmed using a central heating system which was controlled manually. Fans were utilized to evenly distribute the heat load within the room and fans were angled to prevent hot air blowing directly on the animals. During P2, half of the calves were offered the same TMR as during P1 (CON) and the other half were offered the CON diet that was top-dressed with 300 g of a multiple glucose precursor/d (GLU; Glukosa, Novation Co. Madrid, Spain) during the a.m. feeding. Glukosa contained glycerol (330 g/kg), mono propylene glycol (94.5 g/kg), Ca propionate (70.5 g/kg), niacin (470 mg/kg) and cobalt sulfate (185 mg/kg) and colloidal silica as the carrier. Calves were fed ad libitum during both P1 and P2.

Samples of all TMR and diet ingredients were analyzed for CP (AOAC, 2000; 984.13), ether extract (AOAC, 2000; ID 920.39), ash (AOAC, 2000; ID 942.05), NDF and ADF (Van Soest et al., 1991), and lignin(sa) (AOAC, 2000; ID 973.18). An alpha amylase and sodium sulfite were not used in the NDF assay (Udén et al., 2005).

During each period, DMI and water intake were recorded daily, where intake was calculated as the offered amount minus the orts the following day. Body temperature indices (respiration rate, skin and rectal temperatures) were obtained three times a day at 0600, 1100, and 1500 h. Respiration rates (RR) were determined by counting flank movements for a 60-s period. Skin temperatures at the shoulder and rump were measured on a shaved area ($\sim 5 \times 5$ cm^2) with an infrared temperature gun (model MiniTemp MT6, Raytek Corp. Santa Cruz, CA). Rectal temperatures were measured using a standard digital thermometer (RT; PIC Vedodigit II, Digital Thermometer; Pic Solution Co., Como, Italy; with 0.1 °C accuracy of measurement). Body weight (BW) was recorded at the beginning and the end of P1, and weekly during the P2. For each calf, ADG was calculated as the difference between two consecutive BW measurements divided by 7 during P2. Initial and final BW of calves during P2 were 175.9 ± 35.9 and 191.3 ± 41.4 kg, respectively.

In both periods, ambient temperature, but not humidity, was tightly controlled. The temperature and humidity data were continuously monitored by a data logger (MASTECH, model MS-6505, Precision Mastech Enterprises Co. Taipei, Taiwan) which recorded environmental data in 15 min intervals. The data logger was suspended at a height of approximately 1.5 m from the floor for the duration of the experiment.

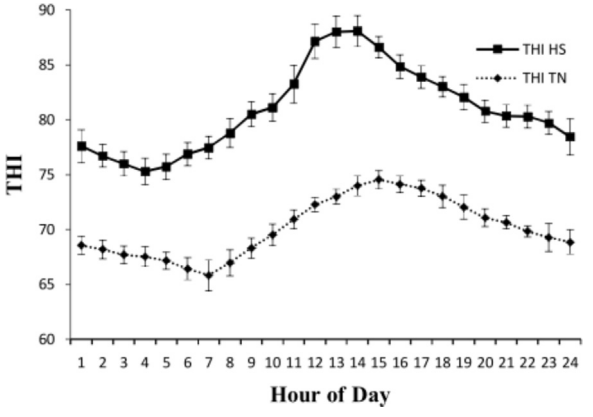

Fig. 1. Average diurnal patterns (mean ± SD) of temperature–humidity index (THI) during thermal neutral (TN) or heat stress (HS) conditions.

2.2. Blood sampling

Blood samples were collected during both periods from the jugular vein (5 mL in collection tubes containing 200 United States Pharmacopeia units of sodium heparin) before and 4 h after morning feeding on d 2 and 7 in P1, and d 2, 8, 15 and 21 in P2. Blood was centrifuged at $2000 \times g$ for 15 min to obtain plasma, and then stored at −20 °C for later analysis.

2.3. Rumen sampling

Rumen fluid samples ($n=12$, 6 per treatment) were collected once on d 20 of P2 using the stomach tube technique 4 h after the morning feeding (Shen et al., 2012). Briefly, a plastic hose (25 mm in diameter) with a probe head was passed through the mouth into the rumen; after reaching to the rumen, an electrical power pomp attached to out-side part of hose created a negative pressure whereby rumen fluid moved out of rumen. The pH was determined immediately (pH meter, pH 340i/set, 2A30-1112, WTW, Berlin, Germany). The samples were then squeezed through a four-layer cheesecloth and one milliliter of rumen fluid was collected and acidified with 20 μL of $0.5H_2SO_4$ and frozen at −20 °C for later analysis of volatile fatty acids (VFA). After the trial, ruminal fluid samples were centrifuged at $15{,}000 \times g$ for 15 min at 4 °C, and supernatants were collected. The centrifuged supernatant was analyzed for VFA concentration by gas chromatography according to the method described by Khorasani et al. (1996).

2.4. Plasma analyses

Blood metabolites were analyzed as previously described (Mahjoubi et al., 2014). Plasma glucose (GOD-PAR, Pars Azmun Laboratory, Tehran, Iran), blood urea nitrogen (BUN; BUN assay kit, Pars Azmun Laboratory, Tehran, Iran), and NEFA (NEFA-HR(2) assay kit, Wako Chemicals GmbH, Neuss, Germany) concentrations were determined enzymatically. The plasma samples were analyzed by a BT 1500 automatic biochemistry analyzer (Biotechnica Instruments S.p.A, Rome, Italy) which simultaneously measures plasma samples for all the aforementioned diagnostic tests. To evaluate insulin concentration in duplicate, an ELISA kit (Mercodia Bovine Insulin ELISA, Mercodia AB, Uppsala, Sweden) was used. The assay was conducted using Infinite® M200 microplate reader (TECAN Group Ltd., Männedorf, Switzerland) in 96-well microplates and read at 450 nm. Inter- and intra-assay coefficients for the plasma insulin assay were 4.7 and 5.4%, respectively. To gain a better appreciation for how feed intakes influences circulating insulin, the pre-feeding insulin concentrations were divided by each calves DMI in the relevant sampling day and multiplied by 100. To indirectly evaluate insulin sensitivity, an insulin sensitivity check index (RQUICKI)

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was calculated according the equation suggested by Holtenius and Holtenius (2007): RQUICKI = 1/[log (glucose in mg/dL) + log (insulin in μU/mL) + log (NEFA in mmol/L)].

2.5. Statistical analysis

All recorded data in P1 for body and blood parameters were condensed into a single average which is represented as day −1 on the figures. Statistical analyses were carried out using the MIXED procedure of SAS (version 9.1; SAS Inst. Inc. Cary NC) to evaluate the fixed effects of treatment, day (of P2), and their interactions in a completely randomized design with the respective covariate and calf as a random effect. All P1 measurements (when available) were utilized as a covariate during analysis of P2 data. Repeated measurements over time (DMI, water intake, body temperature indices, and blood metabolites) for each calf were assessed with an auto-regressive covariance structure and day as the repeated effect. The following model was used:

$$Y_{iik} = \mu + T_i + bull(T_i) + Day_j + Cov_k + (T \times Day)_{ij} + e_{ijk}$$

where, Y_{ijk} = dependent variables, T_i = fixed effect of treatment, bull (T_i) = random effect of bull calves nested in treatment, Day_j = fixed effect of time, Cov_k = fixed effect of covariate and e_{ijk} = random error term. Data were reported as least square means ± standard deviation (SD) and were considered significant if $P < 0.05$ and a tendency if $P < 0.1$.

3. Results

3.1. Performance and body temperature indices

All body thermal indices were elevated ($P < 0.01$) in P2 (Table 2) and influenced by day ($P < 0.01$). Respiration rate at 1500 h (7 breaths per minutes) and shoulder temperature at 0600 and 1500 h (0.58 and 0.19 °C, respectively) were decreased in the GLU-fed compared to CON bulls ($P < 0.01$; Table 2).

During P1, there was no difference in DMI between groups (Table 2). During P2, there was an immediate reduction in DMI (18%; $P < 0.01$; Fig. 2) that was similar in both treatments. Although diet had no effect on DMI (Table 2), DMI tended ($P < 0.07$) to increase with time and this is especially apparent from d1 to d2 (Fig. 2). Water intake did not differ between treatments (Table 2), but increased (36%; $P < 0.01$) during P2 compared to P1. There were no treatment differences detected for ADG and gain: feed ratio, but there was an effect of time whereby ADG increased as P2 progressed ($P < 0.05$; Fig. 3).

3.2. Blood metabolites

Overall, neither pre- nor post-feeding plasma concentrations of glucose, NEFA or BUN were affected by treatment (Table 3). There were minor but inconsistent effects of day on the pre- and post-feeding glucose concentration (Fig. 4A and B). Overall pre-feeding circulating insulin concentrations were

Table 2
Effects of feeding a glucose precursor on body thermal indices and production variables in growing Holstein bull calves during heat stress.

Item	P1[c]	Treatment (Trt)[a]		*P*-value[b]		
		CON	GLU	Trt	day	Trt × day[d]
N	14	7	7	–	–	–
Rectal temperature (°C)						
0600 h	38.57 ± 0.23	39.45 ± 0.62	39.64 ± 0.42	0.14	0.01	0.65
1100 h	38.85 ± 0.30	39.81 ± 0.52	39.84 ± 0.39	0.76	0.01	0.21
1500 h	39.05 ± 0.26	40.56 ± 0.49	40.59 ± 0.42	0.78	0.01	0.65
RR[e] (breaths/min)						
0600 h	50.6 ± 14.34	79.22 ± 19.21	77.28 ± 17.10	0.41	0.01	0.26
1100 h	55.0 ± 12.98	105.32 ± 21.16	101.75 ± 19.71	0.15	0.01	0.78
1500 h	61.5 ± 17.65	135.97 ± 19.92	128.93 ± 19.85	0.01	0.01	0.71
Surface temperature (°C)						
Shoulder						
0600 h	32.30 ± 0.97	35.93 ± 0.69	35.35 ± 1.78	0.01	< 0.01	0.43
1100 h	34.48 ± 1.01	38.42 ± 0.63	38.34 ± 0.57	0.36	0.01	0.10
1500 h	35.76 ± 0.57	39.77 ± 0.62	39.58 ± 0.73	0.01	0.01	0.11
Rump						
0600 h	32.00 ± 1.13	35.20 ± 0.90	35.23 ± 0.76	0.77	0.01	0.58
1100 h	34.40 ± 1.02	38.23 ± 0.75	38.26 ± 0.68	0.77	0.01	0.75
1500 h	35.78 ± 0.51	39.77 ± 0.75	39.70 ± 0.83	0.51	0.01	0.54
DMI (kg/d)	5.08 ± 1.23	4.20 ± 1.02	4.40 ± 0.83	0.31	0.07	0.99
Water intake (L/d)	24.9 ± 6.95	34.80 ± 10.24	33.00 ± 13.06	0.28	0.01	0.52
W:DMI	5.09 ± 1.61	8.41 ± 2.53	7.77 ± 3.27	0.23	< 0.01	0.38
Total ADG (kg/d)	1.40 ± 0.41	0.77 ± 0.36	0.95 ± 0.29	0.34	–	–
Total G:F[f]	0.28 ± 0.07	0.18 ± 0.08	0.22 ± 0.05	0.41	–	–

[a] Treatments were control diet without Glukosa (CON) and control diet + Glukosa as a top-dress (GLU); least square means ± SD.
[b] *P*-values are only related to the treatment effect during period 2 (CON and GLU) not period 1.
[c] Average values for each item during period 1 ± SD.
[d] Treatment × day interaction.
[e] Respiration rate.
[f] Gain to feed ratio.

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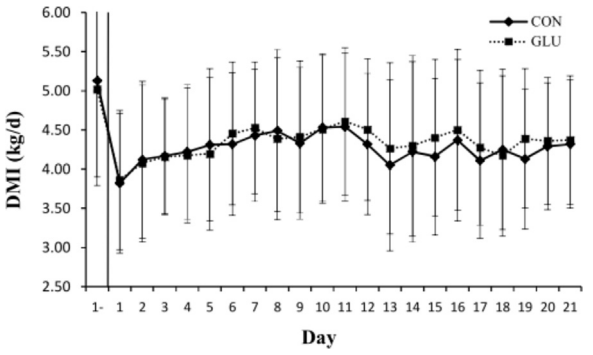

Fig. 2. Effects of feeding a control diet (CON; $n=7$) or control diet + glucose precursor (GLU; $n=7$) on DMI during heat stress. Vertical line separates P1 from P2 and day −1 represents the average DMI in thermal neutral conditions. Each point represents least square means ± SD.

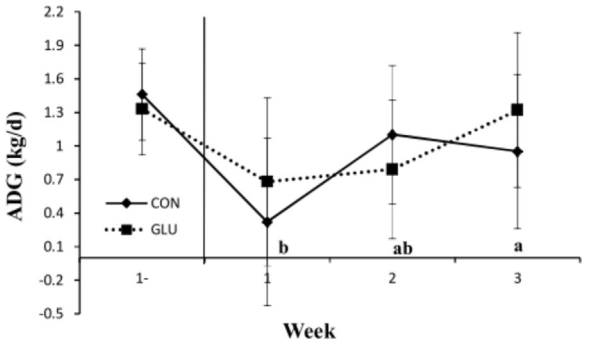

Fig. 3. Effects of feeding a control diet (CON; $n=7$) or control diet + glucose precursor (GLU; $n=7$) on ADG during heat stress. Vertical line separates P1 from P2 and day −1 represents ADG in thermal neutral conditions. There was a week effect and data points with different letters (a, b) on X axis represent differences among weeks of P2 ($P<0.05$). Each point represents least square means ± SD.

increased (Table 3; $P<0.01$) in the GLU-fed calves, and there was a day by treatment interaction ($P<0.01$) as pre-insulin levels were specifically increased on d 2 and 21 (Fig. 5A) in the GLU-fed calves. Pre-feeding NEFA concentrations decreased early in P2 but increased with time ($P<0.05$) and returned to P1 levels by d 8 (Fig. 6A). Post-feeding NEFA decreased immediately during P2 and increased with time ($P<0.01$), but remained decreased compared to P1 (Fig. 6B). BUN increased during P2 and there was a temporal effect on BUN as it peaked on d 2 and then decreased with time for both the pre- and post-feeding (Fig. 7A and B). Despite increased circulating insulin in GLU-fed calves, RQUICKI was not influenced by dietary treatments (Table 3). The insulin:DMI ratio was increased (~52%) in GLU-compared to CON-fed calves ($P<0.05$, Table 3) and this proxy of the pancreas's sensitivity to dietary nutrients increased (~ 33%) from P1 to P2 in both treatments.

3.3. Rumen metabolites

Supplementing GLU did not affect rumen pH or the content of acetate, propionate, or butyrate (Table 4). Rumen isovalerate content tended ($P<0.09$) to increase in the GLU-fed calves compared to the CON-fed bulls (Table 4).

4. Discussion

Heat stress adversely affects many (if not all) aspects of animal agriculture production. Despite recent advances in heat abatement technologies, animal performance still decreases during the summer. Dietary supplementation is an easily managed strategy that may ameliorate economic losses due to HS. In both monogastric and ruminant farm animals it appears that the preferred fuel of peripheral tissues during HS is glucose (Baumgard and Rhoads, 2013). However, modifying the post-absorptive glucose status in ruminants is challenging because ingested carbohydrates are primarily fermented to short-chain fatty acids in the rumen. Therefore, ruminants rely almost exclusively on gluconeogenesis to meet their glucose requirements (Aschenbach et al., 2010). Propionate is the major substrate for gluconeogenesis during positive energy balance and it mostly originates from starch fermentation (Huntington, 1990). Consequently, increasing the dietary grain content is typically utilized to enhance hepatic glucose output, but heat-stressed cows are predisposed to ruminal acidosis for a variety of biological and behavioral reasons so feeding additional concentrates needs to be carefully considered (Kadzere et al., 2002). Therefore, identifying alternative feed sources that can safely enhance hepatic glucose output maybe an effective strategy to maximize production during HS.

4.1. Performance and body temperature indices

As expected, HS immediately reduced DMI and the magnitude of decrease (~15%) is similar to that of another HS calf experiment (O'Brien et al., 2010). Feeding GLU did not influence DMI but this is not surprising as there are inconsistencies within the literature on how glycerol affects feed intake in ruminants (DeFrain et al., 2004; Bodarski et al., 2005; Boyd et al., 2013).

Heat stress markedly reduced the rate of BW gain and this was most pronounced during the first week (Fig. 3). Overall, ADG decreased by 39% in P2 compared to P1 and did not differ between dietary treatments. It is of interest to see how ADG would have responded to treatment if the experiment had lasted longer. However, interpreting production data needs to be conducted with care as the experiment likely did not have enough animals to detect treatment differences, especially because the variation of growth increased during HS (standard deviation of ADG was 0.41, 0.75, 0.62 and 0.68 kg/d for wk −1, 1, 2 and 3 respectfully). Regardless, the improvement in rate of gain (Fig. 3) as HS progressed implies that calves were acclimating to their environment and this agrees with our previous results in growing lambs (Mahjoubi et al., 2014), calves (O'Brien et al., 2010) and pigs (Pearce et al., 2013; Sanz-Fernandez et al., 2013). Interestingly, although ADG increased as HS progressed, feed intake did not (i.e. DMI did not appreciably differ between d 2 to 21) and this suggests that the efficiency of converting dietary nutrients

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Table 3
Effects of feeding a glucose precursor on blood metabolites in growing Holstein bull calves during heat stress.

Item	P1[c]	Treatment (Trt)[a]		*P*-value[b]		
		CON	GLU	Trt	Day	Trt × day[d]
N	14	7	7	–	–	–
Glucose (mg/dL)						
Pre-feeding	95.4 ± 15.42	82.9 ± 10.69	82.6 ± 5.55	0.94	0.06	0.57
Post-feeding	97.1 ± 14.96	83.7 ± 5.64	87.3 ± 4.86	0.15	0.08	0.25
BUN (mg/dL)						
Pre-feeding	8.5 ± 2.05	10.9 ± 3.94	10.3 ± 2.73	0.68	< 0.01	0.35
Post-feeding	9.6 ± 2.03	10.2 ± 3.76	8.4 ± 2.32	0.25	0.01	0.66
Insulin (ng/mL)						
Pre-feeding	0.36 ± 0.26	0.36 ± 0.21	0.57 ± 0.26	0.03	0.84	0.05
Post-feeding	0.90 ± 0.53	1.22 ± 0.84	1.55 ± 0.78	0.35	0.06	0.85
NEFA (mmol/L)						
Pre-feeding	0.13 ± 0.04	0.13 ± 0.05	0.11 ± 0.05	0.36	0.03	0.85
Post-feeding	0.11 ± 0.04	0.08 ± 0.04	0.07 ± 0.02	0.29	0.01	0.15
RQUICKI[e]						
Pre-feeding	0.53 ± 0.15	0.53 ± 0.10	0.49 ± 0.06	0.34	0.08	0.01
Post-feeding	0.44 ± 0.10	0.50 ± 0.19	0.42 ± 0.04	0.05	0.24	0.31
Insulin:DMI ratio	8.07 ± 7.24	8.56 ± 4.69	13.01 ± 7.08	0.05	0.90	0.05

[a] Treatments were control diet without Glukosa (CON) and control diet+Glukosa as a top-dress (GLU); least square means ± SD.
[b] *P*-values are only related to the treatment effect during period 2 (CON and GLU) not period 1.
[c] Average values for each item during period 1 ± SD.
[d] Treatment × day interaction.
[e] RQUICKI: Revised quantitative insulin sensitivity check index.

into tissue accretion was enhanced as P2 advanced. To better understand how HS alters growth energetics we used established equations to predict ADG from actual DMI (Lofgreen and Garrett, 1968). In this scenario, animals were assumed to be in thermal neutral conditions and based on their measured DMI, we calculated how much feed was used for maintenance and how much was allotted to growth. Surprisingly, based on the inputted information (actual DMI, metabolic BW, metabolizable energy content of the diet, feed required for zero energy balance, and the net energy content of the rest of feed for gain) the predicted ADGs for weeks −1, 1, 2 and 3 were 1.1, 0.71, 0.73 and 0.66 kg/d, respectively, compared to the observed ADG of 1.4, 0.50, 0.94 and 1.13 kg/d. The increase in actual compared to predicted growth could not likely have occurred if maintenance cost had increased as is frequently reported (Kleiber, 1961; Morrison, 1983). The presumed decrease in maintenance costs and ostensible increase in tissue accretion efficiency as HS progressed agrees with our recent experiments in growing pigs (Johnson et al., 2014). Remarkably, animals in the current study did not lose body mass but had a slower growth rate and this indicates that they were not severely heat-stressed. This is important because a quadratic relationship between environment and bioenergetics has been proposed, where maintenance costs and total body energy expenditure decline with mild HS but rapidly increase during severe HS (Yunianto et al., 1997; Baumgard and Rhoads, 2013). Thus, the magnitude of the heat load and the amount of time the HS is applied appears to temporally but markedly affect whole-animal bioenergetics.

All thermal indices were increased during P2 and this demonstrates that HS conditions were successfully created. There were no treatment differences detected in rectal temperature, but GLU-feeding decreased surface temperature at the shoulder at 0600 and 1500 h. Reasons why shoulder temperatures did not differ at 1100 h or why rump surface temperature was not influenced by treatment are not-clear. Supplementing GLU also reduced RR during the hottest part of the day (1500 h) and the small improvements in both measurements implies that GLU had some effect on either heat production or heat dissipation. Whether the minor effects on body temperature indices have practical implications are unknown but are of obvious interest. It should be noted that the GLU product also contained some niacin and although results vary, niacin has been shown to reduce body temperature in lactating heat-stressed cows (Zimbelman et al., 2010; Rungruang et al., 2014). However, niacin in the GLU product is not in the appropriate rumen protected form (Campbell et al., 1994) and the dose used in the current experiment is more than 70 fold less than that required to influence body temperature in the previous experiments (Zimbelman et al., 2010). Consequently, the likelihood that niacin is having a biological effect on the aforementioned body temperature indices is unlikely.

4.2. Blood metabolites

In contrast with our expectations (and despite numerically increased circulating glucose concentrations in the post-feeding measurement; Fig. 4B) neither pre-feeding nor post-feeding glucose concentrations were statistically affected by dietary treatment. However, glucose precursors (such as glycerol, the main component of Glukosa) that are orally drenched appear to be more effective at increasing blood glucose levels than glycerol mixed homogenously in the diet (Linke et al., 2004). Nonetheless, utilizing glucose "concentration" as a measure of hepatic glucose flux is complicated because the "content" of a metabolite or

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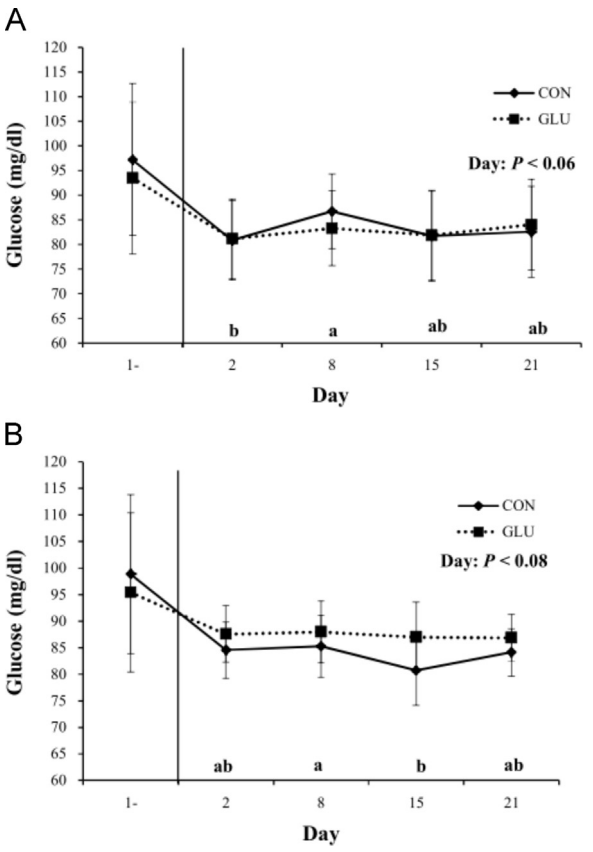

Fig. 4. Effects of feeding a control diet (CON; $n=7$) or control diet + glucose precursor (GLU; $n=7$) on pre-feeding (A) and post-feeding (B) glucose concentrations during heat stress. Vertical line separates P1 from P2 and day −1 represents the average glucose concentration in thermal neutral conditions. Data points with different letters (a, b) on X axis represent differences among days of P2 ($P<0.05$). Each point represents least square means ± SD.

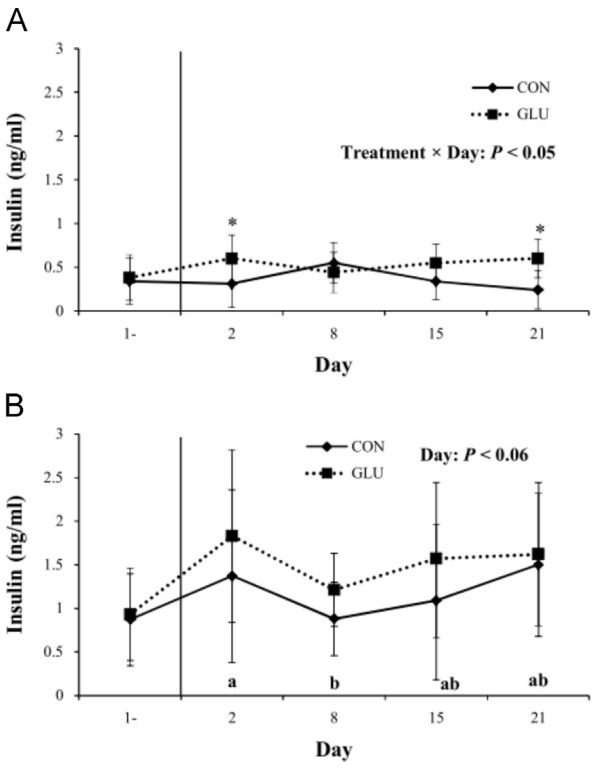

Fig. 5. Effects of feeding a control diet (CON; $n=7$) or control diet + glucose precursor (GLU; $n=7$) on pre-feeding (A) and post-feeding (B) insulin concentrations during heat stress. Vertical line separates P1 from P2 and day −1 represents the average insulin concentration in thermal neutral conditions. The signs * show a treatment by day interaction on the corresponding days ($P<0.05$). Data points with different letters (a, b) on *X* axis represent differences among days of P2 ($P<0.05$). Each point represents least square means ± SD.

hormone is the balance between pool entry and pool removal. This is especially true for strict homeostatically regulated molecules like glucose. Thus, determining whether or not feeding GLU increased the post-absorptive carbohydrate status requires interpreting changes in all of the bioenergetics variables within context and within relation to each other (see below paragraph on insulin parameters). Regardless of dietary treatments, basal glucose concentrations markedly decreased (~14%) during HS and this agrees with a variety of long-term HS experiments (Baumgard and Rhoads, 2012). Reasons for HS-induced hypoglycemia are not clearly delineated, but likely include the contribution of decreased feed intake coupled with increased glucose utilization by skeletal muscle and/or the immune system (Baumgard and Rhoads, 2013).

Although supplemental GLU did not statistically influence plasma glucose content, it did increase pre-feeding insulin concentrations and numerically increased post-feeding insulin levels compared to CON-fed calves. As already mentioned, because glucose is homeostatically regulated, evaluating GLU's effectiveness at altering post-absorptive carbohydrate status maybe better appreciated by evaluating changes in blood insulin (insulin secretion is sensitive to glucose flux). Consequently it appears that GLU was very likely increasing hepatic glucose output as expected. In contrast to previous ruminant experiments (Baumgard and Rhoads, 2013), basal insulin (i.e. pre-feeding) did not increase during HS. However, it is surprising that basal insulin parameters did not decrease considering that DMI was reduced (15%) during HS, as insulin secretion typically mirrors feed intake. In fact, the insulin:DMI ratio suggests that dietary nutrient's ability to stimulate insulin secretion is actually increased (~33%) when comparing P1 vs. P2. This increase in insulin during HS is especially apparent in the post-feeding insulin concentration which increased (~54%) during HS compared to P1. The increased post-feeding insulin during HS is akin to the increased insulin response to a glucose tolerance test in cattle (O'Brien et al., 2010; Wheelock et al., 2010). Reasons for the increased insulin despite the decreased feed intake during HS is not known, but may include insulin's role in the cellular heat shock response (Baumgard and Rhoads, 2013).

Despite the fact that DMI decreased during heat stress (~15%), circulating NEFA concentrations did not increase, which agrees with previous studies in bull calves (O'Brien et al., 2010), dairy cows (Wheelock et al., 2010) and pigs (Pearce et al., 2013; Sanz-Fernandez et al., 2013). In fact, NEFA concentrations actually decreased 23 and 50% on d 2 of heat

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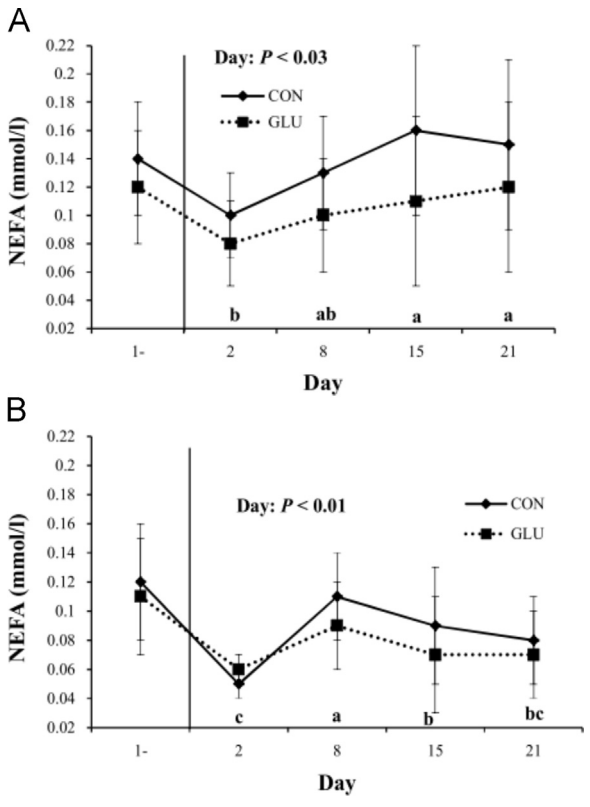

Fig. 6. Effects of feeding a control diet (CON; $n=7$) or control diet+glucose precursor (GLU; $n=7$) on pre-feeding (A) and post-feeding (B) NEFA concentrations during heat stress. Vertical line separates P1 from P2 and day −1 represents the average NEFA concentration in thermal neutral conditions. Data points with different letters (a, b, c) on X axis represent differences among days of P2 ($P<0.05$). Each point represents least square means ± SD.

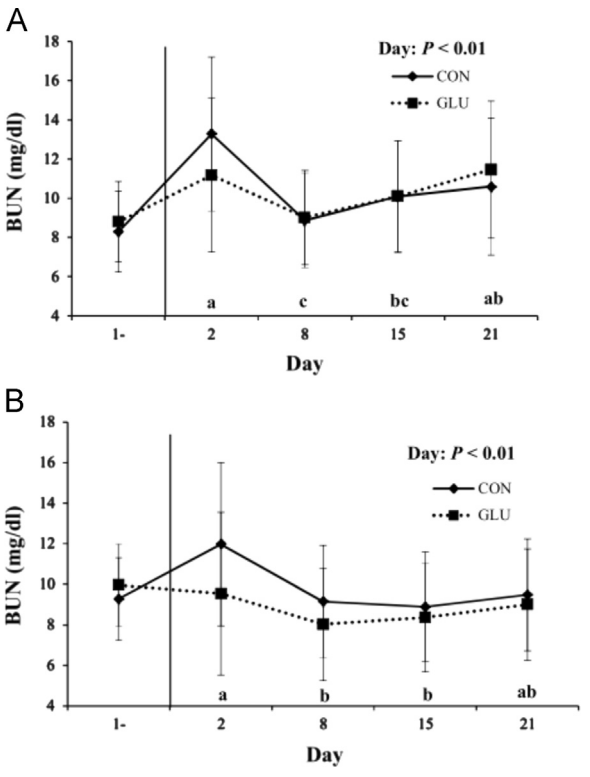

Fig. 7. Effects of feeding a control diet (CON; $n=7$) or control diet+glucose precursor (GLU; $n=7$) on pre-feeding (A) and post-feeding (B) BUN concentrations during heat stress. Vertical line separates P1 from P2 and day −1 represents the average BUN concentration in thermal neutral conditions. Data points with different letters (a, b, c) on *X* axis represent differences among days of P2 ($P<0.05$). Each point represents least square means ± SD.

stress in the pre-feeding and post-feeding samples, respectively. In growing animals reared under thermal neutral conditions, inadequate nutrient consumption initiates metabolic adaptations to support the growth of high priority tissues like skeletal muscle, resulting in decreased adipose tissue insulin sensitivity and subsequent NEFA mobilization (Bauman and Currie, 1980). However, in heat-stressed animal models, NEFA contribution to whole-animal energetics is blunted and the mechanism by which this occurs is not exactly known, but likely includes increased insulin action on the adipocyte as insulin is a potent anti-lipolytic molecule (Vernon, 1992). The evolutionary rationale for why this occurs is not well-understood but could be due to differences in the efficiency of capturing ATP from glucose compared to fatty acid oxidation (Baldwin et al., 1980), resulting in increased basal heat production when lipids are a primary fuel (see review by Baumgard and Rhoads, 2013). Heat stress also tends to increase adipose tissue lipoprotein lipase (Christon, 1988), indicating that adipose tissue from hyperthermic animals may in fact have an increased capacity to extract and redirect fatty acids from circulating triglycerides into storage. The lack of an increase in NEFA and actual acute NEFA decrease agrees with our postulation that maintenance requirements actually decrease (see above) and thus whole-body energy balance maybe increased.

Table 4
Effects of feeding a glucose precursor on rumen metabolites in growing Holstein bull calves during heat stress.

Item	Treatment[a]		*P*-value
	CON	GLU	
N	6	6	–
Ruminal VFA, m*M*	104.78 ± 23.69	101.26 ± 13.77	0.76
VFA (molar %)			
Acetate	55.20 ± 9.77	52.58 ± 9.61	0.65
Propionate	36.88 ± 10.90	37.10 ± 7.34	0.97
Butyrate	11.20 ± 3.49	10.42 ± 2.07	0.62
Valerate	1.00 ± 0.43	0.66 ± 0.16	0.11
Isovalerate	0.30 ± 0.06	0.50 ± 0.25	0.09
Acetate:Propionate	1.55 ± 0.25	1.47 ± 0.35	0.65
Ruminal pH	5.88 ± 0.31	5.99 ± 0.31	0.53

[a] Treatments were control diet without Glukosa (CON) and control diet+Glukosa as a top-dress (GLU); least square means ± SD.

In agreement with previous studies in calves (O'Brien et al., 2010) and lactating cows (Wheelock et al., 2010), BUN concentrations were increased (~25%) during HS (Fig. 7). The period effect is primarily explained by the rapid rise in BUN within the first 2 d of HS, but then BUN

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concentrations gradually decreased as HS progressed (Fig. 7A). The temporal BUN pattern is very similar to that observed in pigs (Pearce et al., 2013; Sanz-Fernandez et al., 2013), calves (O'Brien et al., 2010), lambs (Mahjoubi et al., 2014) and lactating dairy cattle (Wheelock et al., 2010). Although BUN is not an ideal marker of skeletal muscle breakdown, many other reports in a variety of species indicate an increase in proteolysis during HS (see review by Baumgard and Rhoads, 2013). Interestingly, the rapid increase in post-feeding BUN was only observed in CON-fed calves and the lack of a post-feeding BUN response in the GLU-supplemented animals temporally (d 2; Fig. 7B) coincides with the numerical increase in post-feeding insulin (Fig. 5B), and this is not surprising because insulin is a potent anti-proteolytic hormone (Allen, 1988). However, BUN should be interpreted with caution as increased circulating levels could also result from inefficient rumen nitrogen metabolism (Baumgard and Rhoads, 2013). Regardless, the different acute temporal BUN pattern between dietary treatments provides further support that the GLU was altering post-absorptive carbohydrate metabolism as we expected.

4.3. Rumen metabolites

Volatile short-chain fatty acids are the principal byproducts of rumen fiber fermentation and they (acetate in particular) are the primary energy source in ruminants. Total rumen VFA content and pH did not differ between dietary treatments and this agrees with the previous literature (Ramos and Kerley, 2012). In contrast to our expectations, supplemental GLU did not alter the primary VFA patterns and specifically did not increase propionate. Although only a minor VFA component, supplemental GLU did increase the rumen isovalerate content and this suggests that GLU was slightly altering fermentation as has been previously reported (Boyd et al., 2013). Reasons why we did not detect changes in the main VFA (acetate, propionate and butyrate) pattern are not clear, but one possible explanation might be the timing of sample collection, as 4 h post feeding may have been missed the opportunity to observe altered VFA concentrations if the product fermented more rapidly than we expected. Despite the fact that glycerol is thought to be extensively fermented to propionate (Rémond et al., 1993; Bergner et al., 1995) previous reports on the VFA pattern in response to glycerol feeding (the main component of Glukosa) are inconsistent (DeFrain et al., 2004; Donkin and Doane, 2007). When glycerol is ingested, microbial fermentation to VFA is considered to be the main route of digestion (Rémond et al., 1993), but the level of feeding and method of delivery may affect the amount of glycerol escaping fermentation (DeFrain et al., 2004). In fact, it is possible that some glycerol is absorbed intact via the rumen wall and glycerol absorbed intact via the rumen or small intestine is probably a more efficient glucose precursor because it would enter into the gluconeogenic pathway closer to glucose than propionate.

5. Conclusion

This study evaluated the effect of dietary gluconeogenic precursors on body temperature indices, bioenergetics parameters and productive performance in Holstein bull calves during HS. As expected, HS decreased DMI and thus ADG compared to thermal neutral conditions. Our results suggest that maintenance costs may have decreased and thus the efficiency of converting dietary nutrients into tissue accretion was enhanced as HS progressed. Heat stress increased circulating insulin parameters, blunted adipose tissue mobilization and appeared to acutely stimulate skeletal muscle proteolysis. Although the dietary gluconeogenic precursors improved some body temperature indices, and enhanced the post-absorptive carbohydrate status, it did not benefit gross measures of productivity during HS. Knowing whether or not the metabolic changes and improvements in thermal indices with feeding additional gluconeogenic precursors described in this small experiment translate into meaningful production gains in larger commercial trials is important to the global animal agriculture industries.

Conflict of interest statement

There is no conflict of interest in the manuscript.

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