Effects of Prepartum Supplementary Fat and Muscle Hypertrophy Genotype on Cold Tolerance in Newborn Calves[1]

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ABSTRACT: Effects of feeding pregnant dams supplemental dietary fat during the last 55 d of gestation on cold tolerance of newborn crossbred calves with (Piedmontese cross, P, n = 15) or without (Hereford cross, H, n = 16) the muscle hypertrophy allele was determined. Primiparous F1 dams gestating F2 calves of the respective breeds were assigned randomly within breed to receive gestation diets containing either 2.2 (Low Fat; LF) or 5.1% fat (High Fat; HF). Safflower (*Carthamus tinctorius* L.) seeds containing 37% oil with 79% linoleic acid were the supplemental fat source in diets formulated to be isocaloric-isonitrogenous. At parturition, calves were separated from their dams, fed 38°C pooled dairy cow colostrum (30 mL/kg BW), muzzled to prevent suckling, and returned to their dams in a heated (22°C) room for 3.5 h. At 4 h of age (birth = 0 h), a catheter was inserted into the jugular vein. At 5 h of age, calves were placed in a 0°C room for 140 min, and rectal temperatures and blood samples were obtained at 10- and 20-min intervals. Blood was assayed for cortisol and glucose. Rectal temperature was affected by diet ($P < .05$), time, diet × time, and breed × time ($P < .01$ for time and the interactions). Cortisol and glucose concentrations were not affected by diet, breed, or the diet × breed interaction, but they were affected by time, breed × time (both $P < .01$), and diet × time ($P = .06$). Calves from HF dams had higher rectal temperatures than calves from LF dams, and the HF calves maintained higher rectal temperatures throughout cold exposure. Cortisol concentrations were lower ($P = .06$) in calves from HF dams, and these calves had more ($P = .06$) glucose available for metabolic heat production than calves from LF dams. Piedmontese-cross calves maintained higher ($P < .01$) rectal temperatures and had higher cortisol and glucose (both $P < .01$) concentrations than did H-cross calves. We conclude that feeding dams supplemental fat during late gestation increased heat production in newborn calves and potentially could increase calf survival; calves with muscle hypertrophy may have a different ratio of shivering vs nonshivering thermogenesis due to differences in body composition or relationships among uncoupling proteins.

Key Words: Dietary Fat, Newborn Animals, Cold Tolerance, Muscular Hypertrophy, Heat Production

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Introduction

Calf mortality due to cold stress varies among years and geographic location, but thermogenesis plays a major role in determining survival of newborn calves. Approximately 50% of the heat production in newborn calves is dependent on shivering and 50% on nonshivering thermogenesis (Alexander and Williams, 1968). Nutrition of the dam in late gestation could affect thermogenesis in the neonate (Alexander, 1962, 1978; Carstens et al., 1987). Studies with rodents demonstrated that fat supplementation of the dam during gestation increased nonshivering thermogenesis in newborn pups (Nedergaard et al., 1983). Lammoglia et al. (1999) fed safflower seeds containing 37% fat to primiparous beef heifers during late

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gestation and found increased glucose concentrations in calves from the fat-supplemented dams. In addition, calves from fat-supplemented dams that were placed in a cold room maintained increased body temperature longer than did calves from non-supplemented dams. They hypothesized that calves from dams receiving the supplemental fat were potentially more cold-tolerant.

Mortality due to cold stress in neonatal calves is genetically influenced (Josey et al., 1987), suggesting that some breeds may be more cold-tolerant than others. Buckley et al. (1990) reported that Hereford calves had greater fat content than Simmentals at 2 d of age. Karima and Berg (1985) reported that muscular-hypertrophy cattle had less subcutaneous, intermuscular, and carcass cavity fat than Hereford cattle. We hypothesize that fat content of the gestation diet and quantity of neonatal body fat could potentially influence thermogenesis in newborns. The objective of this experiment was to evaluate the previously reported (Lammoglia et al., 1999) effects of prepartum dietary fat on newborn calves exposed to a cold environment and determine whether these responses differed in muscular-hypertrophy calves.

Materials and Methods

Crossbred, primiparous heifers (n = 16 F1, Hereford sire × three-way composite [¼ Charolais, ¼ Tarentaise, and ½ Red Angus]; and n = 15 F1, Piedmontese sire × composite [¼ Charolais, ¼ Tarentaise, and ½ Red Angus] backcrossed to similar F1 Hereford or Piedmontese sires, respectively) gestating F2 calves were assigned randomly to the experiment within genotype and breeding date (Table 1). Calves were genotyped using the method of Smith and Fahrenkrug (M.D. Grosz, personal communication), and all Piedmontese-cross calves contained at least one copy of the Piedmontese (C313X; Grobet et al., 1998) myostatin allele.

The experiment consisted of feeding late-gestation diets containing either 2.2 (low fat, **LF**) or 5.1% (high fat, **HF**) dietary crude fat (Table 2). Safflower (*Carthamus tinctorius* L.; variety, Centennial) seeds, containing 37% oil, with a composition of 79.1% linoleic, 6.2% palmitic, 2.1% stearic, 10.3% oleic, and 2.3% other fatty acids, were used as the supplemental fat source. Seeds were processed through a roller mill with enough pressure to crack approximately 90% of the seed hulls, but not to extract the oil. In an *in situ* digestion study, this process increased 48-h DM disappearance of the seed from 12.0% when unprocessed to 56.5% when processed. Fatty acid composition of the diets expressed as a percentage of total fat were determined using GLC, and values are shown in Table 2. Diets were formulated to be approximately isocaloric and isonitrogenous and for the heifers to gain .5 kg/d. At d 230 ± 1.9 of gestation and again at 10 d before predicted calving, heifers were weighed and body condition was scored (1 = thinnest to 10 = fattest). Heifers were fed for the last 55 d (± 4 d) of gestation and calved early in the spring.

Heifers were observed for signs of parturition every 2 h, and, once parturition started, they were observed continuously. All calves subsequently placed in the cold room were born without obstetrical assistance. Within 30 min after calving and before nursing, newborn calves were weighed and fed 30 mL/kg of birth weight, 38°C pooled colostrum (obtained from a local dairy) via esophageal tube, and placed back with their dams in a heated barn (22°C) for 3.5 h to facilitate maternal-offspring bonding. No breed difference in maternal behavior was apparent. During this time, nursing was prevented by placing muzzles on the calves. At 4 h of age (birth = 0 h), an indwelling Teflon catheter (.11 cm i.d.) was inserted into the jugular vein using local anesthesia and aseptic procedures. A 1-m extension was connected to the catheter to allow blood collection without disturbing the calves. At catheterization, a blood sample was taken, and rectal temperature was measured (sample –60). After catheterization, calves were maintained separately from the dam and continued in the 22°C environment for 1 h.

At 5 h of age, calves were placed in a 0°C-controlled-temperature room for 140 min. We estimated that at least 95% of the placental fluid had

Table 1. Experimental design and subgroup numbers

Gestation diet	Crude fat, %	Sire		Total number
		Hereford	Piedmontese	
Low fat	2.2	8	7	15
High fat	5.1	8	8	16

Table 2. Composition (DM basis) of experimental diets

Item	Diet	
	Control (2.2% fat)	High fat (5.1% fat)
Ingredient, % of total diet		
Corn silage	60.7	73.4
Crested wheatgrass hay (ground)	17.8	13.8
Safflower seeds	—	9.7
Barley	18.0	—
Soybean meal	3.5	3.1
Analyses		
Crude protein, %	10.2	10.1
Dry matter, %	35.4	34.7
TDN, %	64.8	61.8
Fatty acids[a]		
Linoleic (18:2)	42.4	67.0
Oleic (18:1)	21.0	14.3
Palmitic (16:0)	15.5	8.2
Stearic (18:0)	2.7	2.5

[a]Expressed as percentage of total diet fat.

been removed by the dam or had evaporated from the calf at the time they were placed in the cold room. Rectal temperatures (digital thermometer; Becton Dickinson and Company, Franklin Lakes, NJ) and blood samples were taken at 0, 10, 20, 30, 40, 50, 60, 80, 100, 120, and 140 min, and blood volume (8 mL/sample) was replaced with 38°C sterile physiological saline solution (.9% NaCl). After collection, blood was placed into 16 × 125 mm test tubes. Blood was processed to yield serum, which was stored at −20°C until concentrations of cortisol and glucose were determined. Cortisol concentrations were determined using RIA kits (kit 031; Pantex, Santa Monica, CA) and the intra- and interassay CV were 5.4 and 11.9% respectively. Spectrophotometric techniques were used to determine glucose concentrations (kit 1520; DMA, Inc., Arlington, TX).

Statistical Analyses

Data were analyzed with methods for repeated measures (Gill and Hafs, 1971). Rectal temperatures, cortisol, and glucose values were analyzed as a split-plot in time with effects of diet, sire, and the interaction in the whole plot. Whole plot error was the pooled variation among calves treated alike (Error A). Time and interactions with time were in the subplot and tested with the residual mean squares (Error B). Birth weights were analyzed using standard GLM procedures. Calf birth weight was used as a covariate in all repeated measures analyses, and data were pooled over calf sex due to zero calves in one subgroup and because of nonsignificant sex main effects in a previous study (Lammoglia et al., 1999). Main effects and two-way interactions were tested for significance in the final analyses. Higher order interactions were not significant in preliminary analyses and were absorbed into the error term for final analyses. All procedures were performed using GLM procedures for analyses of variance, and mean separation was accomplished using the PDIFF option (SAS, 1994).

Results and Discussion

Statistical analyses and main-effect means are summarized in Tables 3 and 4, respectively. Initial and precalving body weights of the dam were not affected by diet, breed, or the diet × breed interaction. Dam body condition scores did not differ with the exception of the breed effect on initial condition score, which approached significance ($P = .08$). This effect was due to Hereford-cross dams (**F1HX**) having a condition score .5 points higher than Piedmontese-cross dams (**F1PX**). However, this breed difference was not significant precalving.

Calf birth weights were not affected by main or interaction effects. This lack of effect is in contrast to results reported by Lammoglia et al. (1999) who reported higher birth weights in calves from dams that received a high fat diet during the last 53 d of gestation and suggests that effects on birth weight are not consistent.

Calf rectal temperatures were affected by diet ($P < .05$), time ($P < .01$), and the interactions of diet × time ($P < .01$) and breed × time ($P < .01$). Rectal temperatures of calves from dams receiving the HF diet averaged .3°C higher than those of calves from LF dams. This is in contrast with the work of Lammoglia et al. (1999) who reported a nonsignificant main effect of diet on average rectal temperature of the calf. The effect of time was caused by an increase in rectal temperature with time in the cold room that was maximum after 20 to 50 min of cold exposure and then decreasing to approximately the preexposure temperature at 140 min. Increased rectal temperature in response to cold exposure has been previously reported by Jessen (1990) and Lammoglia et al. (1999). The mechanism involves thermoreceptors present in the skin, spinal cord, and hypothalamus that perceive cold sensation, resulting in stimulation of thermogenesis (Jessen, 1990). Differences in rectal temperature across dietary treatments were apparently caused by differences in fat content and composition of the diets fed to the dams during the last 55 d of gestation. However, this main effect must be interpreted in light of the diet × time and breed × time interactions. These interactions are summarized in Figures 1 and 2, respectively.

Higher rectal temperatures were evident in calves from HF dams throughout the cold room exposure, and the increase in rectal temperature when calves were placed in the cold room averaged .50°C in calves from HF dams compared to .36°C in those from LF dams. In addition, the decrease in average temperatures during cold exposure was .19 and .34°C in the HF and LF calves, respectively (Figure 1). Thus, initial rectal temperature response was greater in the HF calves, and the HF calves maintained the higher temperature longer than did LF calves.

Linoleic acid is the major fuel for heat production in brown adipose tissue (**BAT**), and pregnant rats consuming high-fat diets rich in linoleic acid had pups with increased BAT activity (Cresteil, 1977). In addition, feeding rats high-fat gestation diets resulted in increased sympathetic nervous system activity (Schwartz et al., 1983) and BAT thermogenesis (Nedergaard et al., 1983) in the newborn pups. In cattle, linoleic acid concentration in perirenal fat was markedly increased when steers were fed high-fat diets rich in linoleic acid for 8 wk (Cook et al., 1972).

Lammoglia et al. (1999) concluded that feeding high-fat diets to pregnant heifers could potentially improve cold tolerance in their newborn calves. Results of the present study support that conclusion. However, results of the two studies do not establish whether the effect of dietary fat is dependent on quantity of fat or on fatty acid composition (specifically linoleic) of the supplemental fat in the gestation diet of the dam. This awaits further investigation.

The breed × time interaction is summarized in Figure 2 and shows little breed difference in rectal

Table 3. Analyses of variance mean squares and significance

Source	df	Dam body wt, kg		Dam condition score		Birth wt, kg	Rectal temp, °C	Cortisol, ng/mL	Glucose, mg/dL
		Initial	Precalving	Initial	Precalving				
Diet	1	310.93	11.14	.01	.23	56.08	5.94*	4,733.38	7,234.22
Breed	1	71.01	456.14	1.70†	.20	.01	.84	5,019.20	4,339.01
Diet × breed	1	109.66	17.73	.01	1.70	11.26	1.80	160.72	3,952.31
Error A	27	1,093.15	1,705.45	.54	.64	22.37	1.11	4,508.26	3,949.47
Time	11	—	—	—	—	—	.65**	1,900.67**	306.08**
Diet × time	11	—	—	—	—	—	.07**	379.69†	186.36†
Breed × time	11	—	—	—	—	—	.12**	496.67**	329.94**
Error B	297	—	—	—	—	—	.02	216.90	108.48

*$P < .05$.
**$P < .01$.
†$P < .10$.

temperatures until 60 min of cold exposure. From that time until removal from the cold room, F2 Piedmontese-cross calves (**F2PX**) maintained higher temperatures than did the F2 Hereford-cross calves (**F2HX**) with the difference in temperatures being .28°C at 140 min. This breed difference merits discussion.

Brown adipose tissue is specialized for heat production in neonates and accounts for approximately 2% of the body weight of newborn calves (Alexander et al., 1975), with the major deposit located in the perirenal region. This tissue is capable of generating heat due to the presence of an uncoupling protein (**UCP**) located in the inner mitochondrial membrane (Himms-Hagen, 1990). Fleury et al. (1997) reported a second UCP (**UCP2**), similar to the one present in BAT, that is widely distributed throughout the body and suggested that UCP2 may play a major role in energy balance, immunity, and thermoregulatory response to infection. Boss et al. (1997) and Vidal-Puig et al. (1997) recently reported a third UCP (**UCP3**) found principally in skeletal muscle as well as BAT. Uncoupling protein 3 has 71% identity to UCP2 and 57% identity to UCP1 and has been suggested to be an important energy regulator and mediator of adaptive thermogenesis (Vidal-Puig et al., 1997) and to be involved in respiratory control in skeletal muscle (Boss et al., 1997). Muscle hypertrophy fetuses develop more muscle fibers in utero than normal-muscled fetuses (Swatland and Kieffer, 1974; Gerrard et al., 1995). Karima and Berg (1985) reported that mature muscle-hypertrophy cattle had greater muscle mass and less total fat than normal muscled cattle. Approximately 50% of the thermogenesis in newborn calves is dependent on shivering and 50% on nonshivering thermogenesis (Alexander and Williams, 1968). Because muscle-hypertrophy calves and mature cattle have been shown to differ in body composition, we hypothesize that differences in muscle mass, fat depot (including BAT), or relationships among the three uncoupling proteins may change the ratio of shivering and nonshivering thermogenesis. Shivering in the

Table 4. Least-squares mean values for main effects[a]

Main effect	Item	No.	Dam body wt, kg		Dam condition score		Birth wt, kg	Rectal temp, °C	Cortisol, ng/mL	Glucose, mg/dL
			Initial	Precalving	Initial	Precalving				
Diet	Low fat	15	417	469	4.8	5.4	31.5	39.1	67.2	78.9
	High fat	16	410	468	4.7	5.2	34.2	39.4	60.0	88.0
Breed	Hereford cross	16	412	465	5.0	5.4	32.8	39.2	59.9	79.9
	Piedmontese cross	15	415	472	4.5	5.2	32.8	39.3	67.3	86.9
Time, min	–60	31	—	—	—	—	—	39.0	58.4	90.5
	0	31	—	—	—	—	—	39.0	54.2	80.2
	10	31	—	—	—	—	—	39.2	71.3	81.9
	20	31	—	—	—	—	—	39.4	76.8	83.4
	30	31	—	—	—	—	—	39.4	72.8	86.0
	40	31	—	—	—	—	—	39.4	69.6	85.7
	50	31	—	—	—	—	—	39.4	70.5	86.3
	60	31	—	—	—	—	—	39.3	62.0	84.0
	80	31	—	—	—	—	—	39.3	59.9	82.9
	100	31	—	—	—	—	—	39.2	56.6	80.1
	120	31	—	—	—	—	—	39.2	57.5	80.2
	140	31	—	—	—	—	—	39.1	53.8	80.1

[a]See Table 3 for significance.

F2PX calves may have generated more heat, with a decrease in that generated by nonshivering thermogenesis. This hypothesis awaits further investigation.

Serum cortisol concentrations were not affected by diet, breed, or the diet × breed interaction. However, concentrations were affected by time ($P < .01$), diet × time ($P = .06$), and breed × time ($P < .01$). Cortisol concentrations rose rapidly when the calf was placed in the cold room, reaching the peak value at 20 min and generally decreasing thereafter to the approximate concentration found at the 0-time sample. The interaction effects of diet × time and breed × time are shown in Figures 3 and 4, respectively. Cortisol concentrations in calves from dams that received the LF diet exceeded those found in calves from HF dams at all samplings obtained in the cold room. This effect may or may not be a reflection of stress elicited by cold exposure, but it is apparent that the cortisol response in calves from HF dams was less than that elicited in calves from LF dams. Cortisol concentrations in F2PX calves were greater in response to cold exposure than in F2HX calves. This breed difference in cortisol concentrations may be related to the breed difference in rectal temperatures discussed above and be a reflection of breed differences in the ratio of thermogenic pathways.

Elevated concentrations of cortisol in cold environments have been reported to be involved in shivering (Bell and Thompson, 1979) and nonshivering (Bassett and Alexander, 1971) thermogenesis. Furthermore, greater concentrations of cortisol in blood have been reported to stimulate production of substrates from lipid and glycogen stores (Bell and Thompson, 1979) and BAT (Bassett and Alexander, 1971) for the shivering muscle, while having a negative effect on nonshivering thermogenesis (Scarpace et al., 1988). Findings from the present and cited studies support our statement that the thermogenic mechanisms ratio (shivering and nonshivering) supporting cold tolerance may differ in muscle-hypertrophy calves and normal-muscled calves.

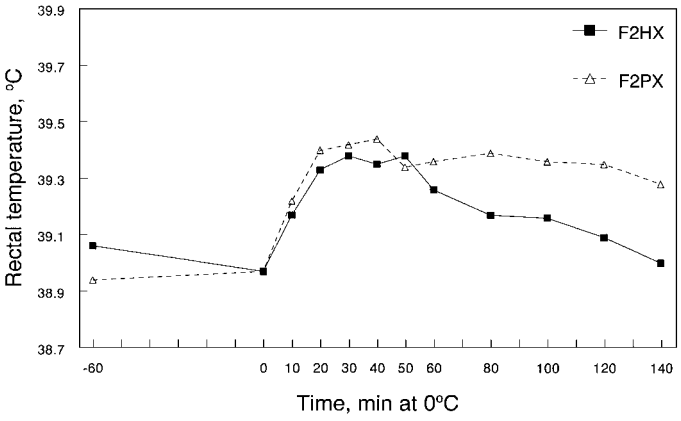

Figure 2. Least squares mean plot (Pooled SEM = .04) of rectal temperatures of newborn calves exposed to 0°C for 140 min as affected by the breed × time interaction ($P < .01$); F2HX = Hereford cross calves and F2PX = Piedmontese cross calves; n = 31.

Plasma glucose concentrations were not affected by diet, breed, or the diet × breed interaction, but they were affected by time ($P < .01$), diet × time ($P = .06$), and breed × time ($P < .01$). The time effect was due to an increase in glucose concentrations when the calves were placed in the cold room with a peak concentration reached at 50 min of cold exposure. Concentrations then decreased to approximately the level noted at the start of cold exposure. The diet × time and breed × time interactions are summarized in Figures 5 and 6. Greater glucose concentrations occurred in calves from dams that received the HF gestation diet throughout the sampling period, with the exception of the sample at 140 min. This diet effect is in agreement with previous findings from our laboratory (Lam-

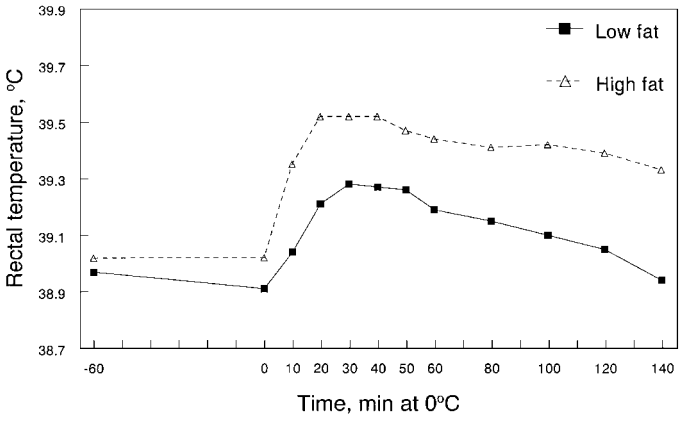

Figure 1. Least squares mean plot (Pooled SEM = .04) of rectal temperatures of newborn calves exposed to 0°C for 140 min from dams receiving 2.2 (LF) or 5.1% (HF) fat in the gestation diet as affected by diet × time interaction ($P < .01$), n = 31.

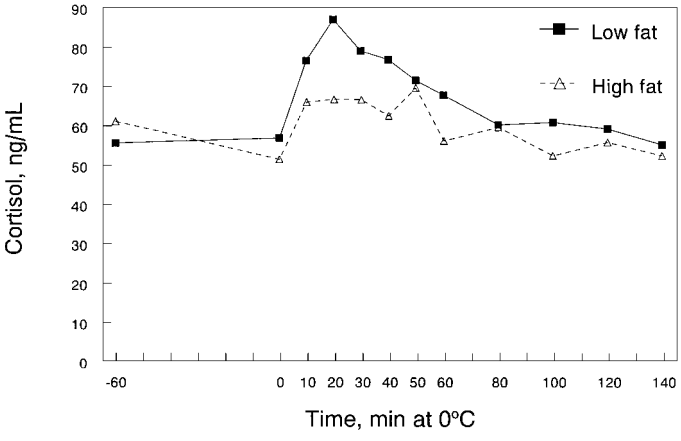

Figure 3. Least squares mean plot (Pooled SEM = 3.75) of serum cortisol concentrations in newborn calves from dams that received a 2.2 (LF) or 5.1% (HF) fat in the gestation diet as affected by the diet × time interaction ($P = .06$), n = 31.

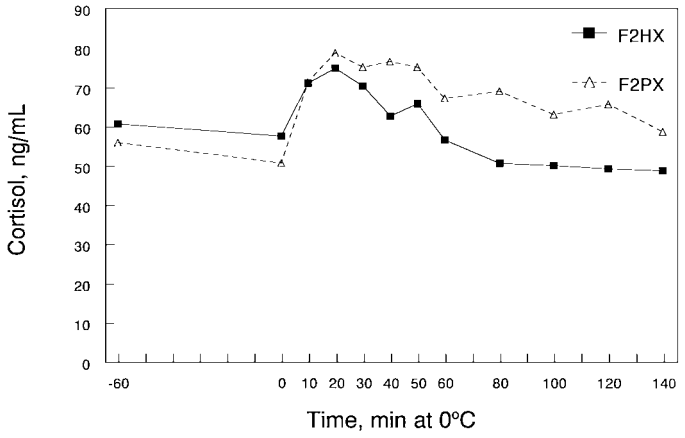

Figure 4. Least squares mean plot (Pooled SEM = 3.83) of serum cortisol concentrations in F2HX (Hereford cross) and F2PX (Piedmontese cross) newborn calves as affected by the breed × time interaction ($P < .01$), n = 31.

moglia et al., 1999), and we interpret the results from the two studies as indicating that more glucose may be available in these calves for metabolism and heat production. What would happen under conditions of lower temperatures and(or) longer cold exposure needs study.

The breed × time interaction resulted from greater glucose concentrations in F2PX calves than in F2HX at all samplings until the 120 min sample. At that sample, the breed difference reversed with greater glucose concentrations in F2HX calves. We believe this difference is also related to possible differences in the ratio of thermogenic and possibly metabolic pathways between the two breeds and is probably associated with the breed difference in cortisol concentrations shown in Figure 4.

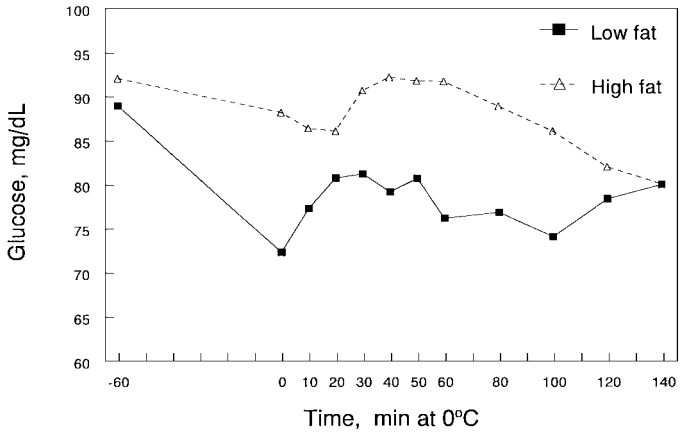

Figure 5. Least squares mean plot (Pooled SEM = 2.78) of serum glucose concentrations in newborn calves from dams that received 2.2 (LF) or 5.1% (HF) fat in the gestation diet as affected by the diet × time interaction ($P = .06$), n = 31.

Concentrations of glucose in blood increased in calves (Olson et al., 1981; Okamoto et al., 1986; Godfrey et al., 1991) and lambs (Alexander and Williams, 1968) that were exposed to cold environments. From 30 d prior to parturition to approximately 2 d after lambing, fetal ovine perirenal adipose tissue had a high rate of lipogenesis. During the lipogenic process, acetate, glucose, and lactate contributed to the formation of fatty acid synthesis by 50, 17, and 33%, respectively (Robertson et al., 1981). Furthermore, newborn calves suffering hypothermia due to excessive heat loss during cold exposure had increased serum glucose and NEFA concentrations, possibly reflecting a final attempt to increase body temperature (Okamoto et al., 1986; Godfrey et al., 1991). If liver glycogen content was greater in calves from HF dams and in F2PX calves, this could have resulted in greater concentrations of glucose in these calves when exposed to cold temperatures.

Massip (1980) reported increased concentrations of blood glucose in calves resulting from glycogen mobilization induced by adrenaline and cortisol secretions. Godfrey et al. (1991) reported glucose-concentration differences between *Bos indicus* and *Bos indicus* × *Bos taurus* crossbred calves. However, Carstens et al. (1997) showed no differences in concentrations of glucose in blood of newborn Brahman-, Tuli-, or Angus-sired calves when maintained in a thermoneutral environment and challenged with norepinephrine. This disagreement with our study could be caused by response differences between cold exposure vs norepinephrine challenge and(or) breed of dam differences between experiments.

We interpret results from the cited studies and ours that blood concentrations of glucose are associated with thermogenesis and potential cold tolerance in newborn calves. Furthermore, the results of this work support that of Lammoglia et al. (1999). We conclude that blood glucose concentrations and thermogenesis

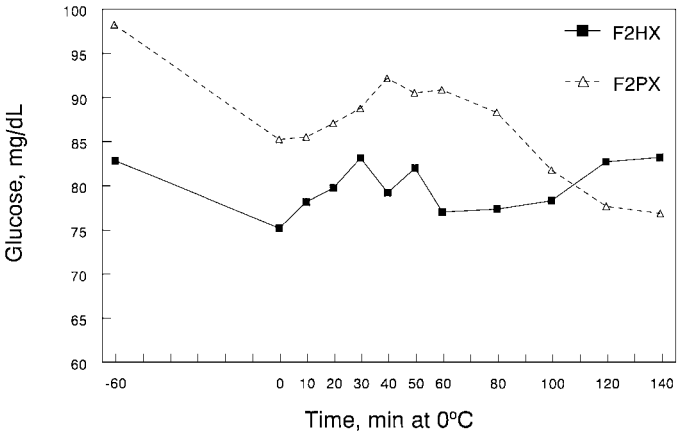

Figure 6. Least squares mean plot (Pooled SEM = 2.74) of serum glucose concentrations in F2HX (Hereford cross) and F2PX (Piedmontese cross) newborn calves as affected by the breed × time interaction ($P < .01$), n = 31.

in newborn calves can be affected by prepartum dietary fat of their dams, and these effects may differ in muscle-hypertrophy calves.

Implications

Feeding pregnant dams supplemental fat during late gestation could potentially improve survival rate of newborn calves by improving cold tolerance. Positive response has been obtained by feeding safflower seeds, high in linoleic acid, but further research is needed to determine whether different fat sources and fatty acid compositions could have similar thermogenic impact on newborn calves. Breed differences in rectal temperatures and blood concentrations of glucose and cortisol suggest possible differences in thermogenic mechanisms between calves with muscular hypertrophy and normal-muscle calves.

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