Effects of Age, Environments and Sex on Plasma Metabolite Levels in Young Holstein Calves

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ABSTRACT : Thirty Holstein calves were used to determine effects of age, environment and sex on blood metabolite concentrations during 1 to 90 d of age. Calves were weaned at 75 d of age. Environmental effects are grouped by the difference in month at birth and site of feeding. Blood samples were obtained every 2 or 3 d. The mean metabolite concentration every 3 d was used for the statistical analysis. Dairy bodyweight gain was not affected by environmental group and sex effect. Concentrations of plasma glucose, nonesterified fatty acids (NEFA), triglyceride, total cholesterol and total ketone changed with growth. These developmental changes in metabolite levels would be caused by ruminal maturation with increment of grain intake. Levels of plasma urea nitrogen, glucose, NEFA, triglyceride and total cholesterol drastically changed during a few weeks after birth, indicating that the physiological state in calves greatly changed during that time. Effects of the environmental group and sex were significant in almost all metabolites. Temperature influenced plasma metabolite concentrations. The plasma metabolite concentrations were affected more intensely by heat stress in the infant period than in the neonatal period. ***(Asian-Aust. J. Anim. Sci. 2002. Vol 15, No. 5 : 637-642)***

Key Words : Dairy Cattle, Young Calf, Metabolite, Plasma

INTRODUCTION

Many previous reports have described changes in the plasma metabolite concentrations of urea nitrogen, glucose, nonesterified fatty acids (NEFA) and cholesterol with the growth of young calves (Abe et al., 1987; Doppenberg and Palmquist, 1991; Quigley et al., 1991; Quigley and Bernard, 1992; Okamoto et al., 1999). Quigley et al. (1991) reported early grain feeding and consequent volatile fatty acids (VFA) production possibly caused an increase in metabolic activity of ruminal epithelium, thereby increasing production of a larger amount of β-hydroxybutyrate (BHBA). They found that the concentrations of plasma BHBA, acetoacetate and glucose were correlated with grain intake. Quigley and Bernard (1992) reported the concentration of plasma urea nitrogen was also changed in relation to grain intake, indicating extensive ruminal degradation of dietary protein and carbohydrate, metabolism of absorbed amino acids, and, possibly, urea recycling. However, the seasonal effect and the effect of sex on the metabolite concentrations have not been reported.

The purpose of the present study was to determine the effects of birth month, site of feeding and sex on plasma metabolite concentrations in young calves.

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MATERIALS AND METHODS

Thirty Holstein calves (21 females, 9 males) were used until 90 d of age. These calves were born in the National Agricultural Research Center for Hokkaido Region from 1992 to 1994. They were assigned into five environmental groups by their birth month and site of feeding (table 1): born from January to February, fed in a cowshed (EG1); born from January to February, fed in a calf hatch (EG2); born from March to May, fed in a calf hatch (EG3); born from June to August, fed in a cowshed (EG4); and born from June to August, fed in a calf hatch (EG5). The calf hatch was placed outside the cowshed. The calves were fed twice a day at 09:00 and 16:00 h. Whole milk was given 5.0 kg/d during 1 to 60 d of age and 2.0 kg/d during 61 to 75 d of age. Calves were weaned at 75 d of age. Calf starter was fed 0.2 kg/d during 21 to 30 d of age and 0.6 kg/d during 31 to 60 d of age. Calves were fed 1.0 kg/d of calf starter and 0.4 kg/d of formula feed during 61 to 75 d of age, and 1.2 kg/d of calf starter and 1.2 kg/d of formula feed after 76 d of age. Hay and water were given calves *ad libitum* during the experimental period. Chemical composition of calf starter and formula feed are shown in table 2. Body weights were measured at 1 and 90 d of age. Daily bodyweight gain from 1 to 90 d of age was calculated.

Blood samples were obtained once before feeding at 09:00 h by jugular venipuncture into evacuated tubes with heparin at 2 d of age and thereafter every 2 d until 90 d of age (13 females, 5 males). In a same way, blood samples were also obtained at 3 d of age and thereafter every 3 d until 90 d of age from 8 females, 4 males. The analytical data every 3 d were used for the statistical analysis. Thus,

Table 1. The number of the animals in each environmental group (EG) and sex

	Female	Male
EG1[1]	4	1
EG2	5	1
EG3	6	1
EG4	4	4
EG5	2	2

[1] Born from January to February, fed in a cowshed (EG1); born from January to February, fed in a calf hatch (EG2); born from March to May, fed in calf hatch (EG3); born from June to August, fed in a cowshed (EG3); born from June to August, fed in a calf hatch (EG5).

Table 2. Chemical composition of calf starter and formula feed (±SE)

	Calf starter[1]	Formula feed[2]
Dry matter (%)	86.4±0.5	85.5±0.8
Crude protein (% DM)	22.2±1.6	20.6±1.9
Crude fat (% DM)	3.7±0.6	3.8±0.4
Crude fiber (% DM)	5.2±0.3	9.0±1.1
Crude ash (%DM)	7.3±0.4	7.8±0.6

[1] Mean value of 26 samples.
[2] Mean value of 10 samples.

the experimental period was divided into 30 phases of 3 d each. Each phase was from (3×n-2) d to (3×n) d after birth (1<=n<=30, an integer). The data every 2 d were also used. The data from every 2 d were converted to 3 d data by calculating the means of all the data within each of the 30 phases as in the case of the 3 d sampling. Plasma was stored under -20°C prior to analysis. Urea nitrogen (Kainos Laboratories Inc., Japan), glucose (Kainos Laboratories Inc., Japan), NEFA (Kainos Laboratories Inc., Japan), triglyceride (Kainos Laboratories Inc., Japan), total cholesterol (Kainos Laboratories Inc., Japan), and total ketone (Nittobo Medical Inc., Japan) were analyzed by 7250 automatic analyzer (Hitachi Ltd., Japan) using commercially supplied reagent kits.

The statistical analysis for estimating the effects of age, environmental group, sex and these interactions used the following model by GLM procedure of Statistical Analysis System (SAS, 1990). The model for plasma metabolites concentration was

$$YM_{ijkl}=\mu+D_i+T_j+S_k+e_{ijkl}$$

where:

YM_{ijkl}= an observation of plasma metabolite concentration on the i^{th} animal in the ijk^{th} subclass,
μ= overall mean,
D_i= effect of i^{th} age,
T_j= effect of j^{th} environmental group,
S_k= effect of k^{th} sex,
e_{ijkl}= residual.

The model for daily bodyweight gain was

$$YG_{jkl}=\mu+T_j+S_k+e_{jkl}$$

where:

YG_{jkl}= an observation of daily bodyweight gain on the i^{th} animal in the jk^{th} subclass,
μ= overall mean,
T_j= effect of j^{th} environmental group,
S_k= effect of k^{th} sex,
e_{jkl}= residual.

RESULTS

Least square means of daily bodyweight gain in EG1, EG2, EG3, EG4 and EG5 were 0.66±0.03, 0.64±0.03, 0.66±0.03, 0.67±0.02 and 0.69±0.04 kg/d (±SE), respectively. Least square means of daily bodyweight gain was 0.68±0.02 in female and 0.66±0.02 kg/d (±SE) in male. The daily bodyweight gain was not affected by the environmental group or sex.

The metabolite concentrations are shown in figure 1 in terms of age. Concentrations of plasma glucose, NEFA, triglyceride, total cholesterol and total ketone changed with age ($p<0.01$). There was a marked decrease in plasma glucose concentration from 3 to 9 d of age ($p<0.01$) and then a continuing decrease to 27 d ($p<0.01$). Plasma NEFA concentration was lower at 30 d than 3 d ($p<0.01$) and at 90 d than 75 d ($p<0.01$). There was a large decrease in plasma triglyceride from 3 to 9 d of age ($p<0.01$) and a further decrease from 15 to 24 d ($p<0.01$). Plasma total cholesterol increased steeply from 3 to 30 d ($p<0.01$) but decreased from 69 to 90 d ($p<0.01$). Plasma total ketone increased from 30 to 90 d ($p<0.01$). Plasma urea nitrogen rapidly increased from 6 to 21 d of age ($p<0.01$), decreased from 21 to 45 d ($p<0.01$), and increased after 69 d (NS). The effect of age on the plasma urea nitrogen concentration during the whole experimental period was not significant.

Least square means of plasma metabolite concentrations in each environmental group and sex are shown in table 3 and table 4. The environmental group had significant effects ($p<0.01$) on the concentrations of plasma urea nitrogen, glucose, NEFA, triglyceride, total cholesterol and total ketone. Sex had significant effects on the concentrations of plasma glucose ($p<0.01$), triglyceride ($p<0.01$), total ketone ($p<0.01$) and total cholesterol ($p<0.05$). Concentrations of plasma urea nitrogen, glucose, triglyceride, total cholesterol and total ketone were high in EG1 and EG2 ($p<0.01$). Levels of plasma NEFA, triglyceride and total ketone were

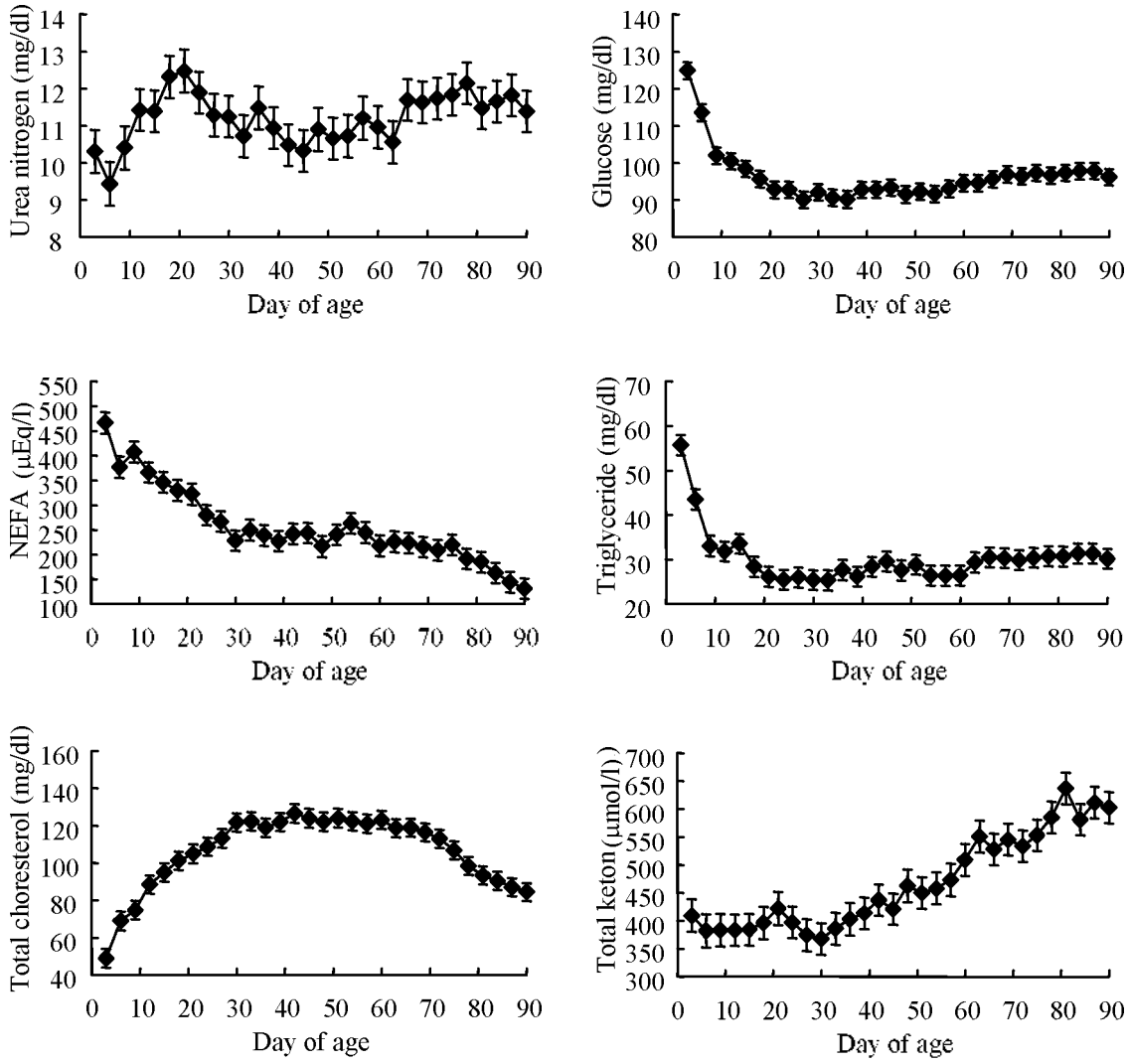

Figure 1. Least square means of concentrations of plasma urea nitrogen, glucose, NEFA, triglyceride, total cholesterol and total ketone from 3 to 90 d of age. Vertical bars denote the standard error at each sampling day.

higher in EG2 than the other groups ($p<0.01$). Levels of plasma urea nitrogen, triglyceride and total ketone were lower in EG3 than the other groups ($p<0.01$). Levels of glucose, triglyceride and total ketone were higher in female than in male ($p<0.01$). Levels of total cholesterol were higher in male than in female ($p<0.05$).

DISCUSSION

The developmental changes in plasma metabolite concentrations of calves from 3 to 90 d after birth were investigated in this study. The trends found to 30 d of age are similar to the results of previous reports. The levels of plasma urea nitrogen (Abe et al., 1987; Quigley and Bernard, 1992), glucose (Doppenberg and Palmquist, 1991; Quigley et al., 1991) and NEFA (Doppenberg and Palmquist, 1991; Quigley et al., 1991; Quigley and Bernard, 1992) decreased and of plasma cholesterol (Doppenberg and Palmquist, 1991) increased from birth to weaning. The metabolite transitions after 60 d of age tended to agree with many reports. After weaning, the plasma urea nitrogen (Quigley and Bernard, 1992), glucose (Quigley et al., 1991; Quigley and Bernard, 1992) and ketone (Quigley et al., 1991; Quigley and Bernard, 1992) levels increased, whereas the plasma NEFA (Quigley et al., 1991) and cholesterol (Doppenberg and Palmquist, 1991; Okamoto et al., 1999) levels decreased. Blood ketone is a function of ruminal butyrate production and absorption by ruminal epithelium (Quigley and Bernard, 1992). Murdock and Wallenius (1980) reported that giving calves solid feed enhanced ruminal butyrate and then increased the rate of ruminal maturation because butyrate influences the metabolic activity of ruminal epithelium. Early grain feeding and consequent VFA production possibily caused an increase

Table 3. Least square means of concentration of plasma urea nitrogen, glucose, nonesterified fatty acid (NEFA), triglyceride, total cholesterol and total ketone in each environmental group (±SE)

	Environmental group				
	1	2	3	4	5
Urea nitrogen (mg/dl)	13.2±0.3[a]	12.6±0.2[a]	8.8±0.2[b]	11.6±0.2[c]	9.9±0.3[d]
Glucose (mg/dl)	104.4±1.0[a]	103.5±1.0[a]	89.0±0.9[b]	89.3±0.8[b]	95.6±1.1[c]
NEFA (μEq/l)	244±10[a]	311±9[b]	245±9[a]	225±7[a]	255±11[a]
Triglyceride (mg/dl)	35.7±1.0[a]	41.8±1.0[b]	19.5±0.9[c]	28.2±0.8[d]	25.7±1.1[d]
Total cholesterol (mg/dl)	117±2[a]	116±2[a]	101±2[b]	102±2[b]	93±2[c]
Total ketone (μmol/l)	668.0±13.0[a]	727.5±12.1[b]	146.7±11.7[c]	429.6±10.1[d]	347.9±14.3[e]

Mean value within rows with different superscript letters were significantly different ($p<0.01$).

Table 4. Least square means of concentration of plasma urea nitrogen, glucose, nonesterified fatty acid (NEFA), triglyceride, total cholesterol and total ketone in each sex (±SE)

	Female	Male	p
Urea nitrogen (mg/dl)	11.4±0.1	11.0±0.2	NS
Glucose (mg/dl)	101.0±0.5	91.7±0.8	$p<0.01$
NEFA (μEq/l)	262±5	250±7	NS
Triglyceride (mg/dl)	31.7±0.5	28.7±0.8	$p<0.01$
Total cholesterol (mg/dl)	104±1	108±2	$p<0.05$
Total ketone (μmol/l)	494.5±6.4	441.4±10.0	$p<0.01$

in metabolic activity of ruminal epithelium, thereby increasing production of a larger amount of BHBA per unit feed intake (Quigley et al., 1991). Walker and Simmonds (1962) reported that the metabolism of butyrate by ruminal wall of young lambs was activated with growth, and presumably, by dry feed intake. In the present study, calf starter was fed at 20 d of age and the amount was increased from 30 d of age. This change promoted an acceleration of ruminal maturation and the inducement of a plasma total ketone increase after 30 d of age. The concentration of plasma urea nitrogen increased after 69 d of age. The plasma urea increment was related to grain intake, indicating extensive ruminal degradation of dietary protein and carbohydrate, metabolism of absorbed amino acids, and, possibly, urea recycling (Quigley and Bernard, 1992). Increases in grain intake and ruminal maturation also were thought to lead to higher plasma glucose and triglyceride levels from about 60 d of age. Quigley et al. (1991) compared two groups of calves separated by different weaning times, 28 or 56 d after birth. They found that post-weaning increases in plasma propionate and butyrate depressed NEFA in calves weaned early. This led them to consider that limited milk feeding and minimal dry feed intake relative to energy requirements in late-weaned calves may cause a relatively higher concentration of plasma NEFA. When the energy requirement increases together with the reduction in the plasma glucose level caused by fasting, the plasma NEFA level increases (Rule et al., 1985; Sejrsen et al., 1984). Plasma NEFA levels did not increase in this study, so the energy supply was thought to be sufficient for the calves weaned at 75 d after birth. Doppenberg and Palmquist (1991) and Okamoto et al. (1999) reported the developmental change in cholesterol concentration. They did not refer to the mechanism of the developmental change in cholesterol concentration and it would be difficult to deduce from the present result.

The change in plasma metabolite concentration during a few weeks after birth was not found in previous studies because most reports started at two weeks after birth and blood samples were collected weekly. The mechanism by which the plasma metabolite concentration changed within a few weeks from birth would be difficult to deduce only from the present results. However the plasma glucose, NEFA and triglyceride levels decreased and the plasma urea nitrogen and total cholesterol levels increased drastically during the few weeks after birth. These results suggested that the physiological state in calves drastically changed shortly after birth and this was thought to be an adaptation process for the changes in nutritional pathway from the umbilical cord to independent food intake through the mouth and digestive organs.

The daily bodyweight gain was not affected by the effects of the environmental group or sex. Therefore the difference of growth rate among environmental groups and sex would not influence the plasma metabolites concentration.

The temperature is considered to be a key factor affecting the plasma metabolite concentration. The mean temperatures during the 11 month from January to November of 1980 to 1990 at the National Agricultural Research Center for Hokkaido Region were -6.6, -5.8, -1.6, 4.9, 10.0, 14.4, 18.1, 20.5, 15.8, 9.4, and 2.6°C, respectively (Miyata, 1992). The maximum difference was 27.1°C between January and August. The coldest environmental group was EG2 followed by EG1. Concentrations of plasma glucose in EG1 and EG2 were larger than in EG3, EG4 and EG5. The plasma NEFA level was highest in EG2 among all environmental groups. Where the animal was exposed to the cold environment, the plasma glucose (Fujita et al.,

1976; Shaffer et al., 1981; Shijimaya et al., 1985) and NEFA levels (Fujita et al., 1976; Shijimaya et al., 1985) were reported to increase. Therefore, elevated plasma metabolite levels in EG1 and EG2 were considered as the result of cold stress. Fujita et al. (1976) found that when sheep were exposed to cold, their heat production increased in relation to the low temperature and, by the increased energy demand, the NEFA was mobilized from adipose tissue, and the plasma NEFA concentration rose. In the present study, plasma glucose increased simultaneously with an NEFA increment. Therefore, catecholamine, which is induced by cold stress, was thought to elevate the plasma NEFA concentration in EG2. The increase in the plasma ketone concentration was considered due to the metabolization of NEFA (Sato, 1987). The increase in the plasma urea nitrogen concentration in EG1 and EG2 was considered due to the amino acid mobilization to provide more energy under cold stress (Fujita et al., 1976).

Plasma urea nitrogen (Shaffer et al., 1981), glucose (Hassan and Roussel, 1975; Shaffer et al., 1981) and cholesterol (Shaffer et al., 1981) were low in a hot environment. Shaffer et al. (1981) reported that a depression in the metabolite concentration from increasing the environmental temperature may be due to a hemodilution effect. This effect may be explained by the supply of a large amount of water to the circulatory system for evaporative cooling, the significant decline in feed consumption, and by the marked increase in respiratory activity in responses to the heat stress. The calves in EG4 and EG5 had the hottest season in the neonatal period and those in EG3 had the hottest season in the infant period. The plasma metabolite levels were lower in EG3 than in EG4 and EG5. It was thus suggested that the concentrations of plasma metabolites were affected more intensely by heat stress in the infant period than in the neonatal period.

Min et al. (1993) reported that the plasma urea nitrogen concentration at 3.5 month of age and the plasma glucose and NEFA concentrations at 7 month of age differed significantly by sex. The effect of sex on the concentrations of plasma glucose, triglyceride, total cholesterol and total ketone was significant. Despite immaturity, the difference of sex already began to affect the concentration of plasma metabolites before 90 d of age.

CONCLUSION

The concentrations of plasma glucose, NEFA, triglyceride, total cholesterol and total ketone changed with the overall growth of calves, whereas the levels of plasma urea nitrogen, glucose, NEFA, triglyceride and total cholesterol changed drastically in the first few weeks after birth. Therefore, the physiological state of calves dramatically changed within a few weeks of birth. These developmental changes would be caused by increased grain intake. Effects of environmental group and sex were significant in almost all metabolites. Concentrations of plasma NEFA and total ketone were high in the cold state because catecholamine, which is induced by cold stress, raised the level of plasma NEFA. Concentrations of plasma metabolites were affected more intensely by heat stress in the infant period than in the neonatal period.

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