

## Plasma Metabolites Concentrations in Calves until 90 Days of Age for Estimating Genetic Ability for Milk Production Traits

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**ABSTRACT :** The aim of this study was to identify useful secondary traits for estimating genetic ability of milk production traits. We investigated the value of using plasma metabolites concentrations. Two hundred and nineteen cattle out of 271 had only milk production traits records (G1), 33 had only metabolites records (G2), and 19 had both milk production traits and metabolites records (G3). Fifty two calves with metabolites records (G2 and G3) were born from 1992 to 1997. Forty three calves (29 females, 14 males) were used from 10 to 90 d of age and the others (3 females, 6 males) from 10 to 60 d of age. A total of 566 records of milk yield, fat yield and protein yield for 240 to 305 d on 238 heads (G1 and G2) were collected. The collected blood samples were divided into three age groups: AG1, 10 to 30 d; AG2, 40 to 60 d; and AG3, 70 to 90 d. Heritabilities of milk yield, fat yield and protein yield were  $0.45 \pm 0.04$ ,  $0.50 \pm 0.04$  and  $0.38 \pm 0.04$ , respectively. Heritability of plasma glucose concentration at AG1 was  $0.45 \pm 0.08$ . Genetic correlations between plasma glucose concentration and milk yield, fat yield and protein yield were  $-0.35 \pm 0.28$ ,  $0.64 \pm 0.24$  and  $0.36 \pm 0.35$ , respectively. When the plasma glucose concentration at AG1 was used to estimate genetic ability of these milk production traits, reliability of milk yield of animals without milk record increased 8.2%, fat yield increased 24.2% and protein yield increased 9.5%. Heritability of plasma total cholesterol concentration at AG3 was  $0.83 \pm 0.04$ . Genetic correlation between plasma total cholesterol concentration and milk yield, fat yield and protein yield were  $0.58 \pm 0.21$ ,  $0.42 \pm 0.20$  and  $0.45 \pm 0.22$ , respectively. When the plasma total cholesterol concentration at AG3 was using to estimate genetic ability of these milk production traits, reliability of milk yield of animals without milk record increased 19.0%, fat yield increased 9.6%, and protein yield increased 13.5%. The annual genetic gain is in proportion to the reliability of selection. These results show that the plasma metabolite concentrations would be useful for improvement of genetic ability for milk production traits in the genetic improvement in herd of cows, where half of the animals selected are from a herd without its own milk record. (*Asian-Aust. J. Anim. Sci.* 2002, Vol 15, No. 12 : 1813-1821)

**Key Words :** Dairy Cattle, Young Calf, Metabolite, REML, Reliability

### INTRODUCTION

In the selection of calves to maintain the number of animals in a herd, the prediction of milk ability is not accurate. The estimation of the genetic ability for milk production requires many records of the dam and her relatives. However, genetic progress in milk production is limited, since the milk production traits are expressed only in the mature females (Sinnott-Smith et al., 1987). If some traits, which are significantly correlated with genetic advantages for milk production, could be measured in calves, the generation interval could be shortened, and genetic improvements could be accelerated (Tilakaratne et al., 1980; Sejrsen et al., 1984). Mackenzie et al. (1988) reported that the calves with higher genetic merit for fat yield maintained significantly higher levels of circulating insulin and glucose, were less sensitive to the stimulation

of glycogenolysis or glyconeogenesis by the glucagon challenge, and showed greater sensitivity of peripheral tissues to insulin than those with lower genetic merit. The concentrations of plasma metabolites related to the energy metabolism, e.g. glucose, urea nitrogen, non esterified fatty acids (NEFA) and  $\beta$ -hydroxybutyrate, are mentioned as possible candidates for the estimation of genetic ability for milk production (Hart et al., 1978). Sejrsen et al. (1984) suggested that in early lactation the nutrient and energy supply to the mammary gland is strongly dependent on the cow's ability to mobilize body reserves and may be also the ability to reduce the requirements of peripheral tissues. It is reasonable to suggest that the physiological basis for differences between animals in their genetic capacity is found in differences in the endocrine system. The advantage of plasma metabolites is not limited by sex or age and these are simple to measure (Woolliams and Lovendahl, 1991). However, the genetic parameters for plasma metabolite concentration, e.g. heritability, genetic correlation with other plasma metabolite concentrations and genetic correlation with milk production traits, are rare or not reported.

The present study was conducted to estimate the

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heritability of the plasma metabolite concentrations in calves from 10 to 90 d of age and the genetic correlations with the milk production traits. We investigated the effect of using plasma metabolites concentrations to estimating genetic ability of milk production traits.

## MATERIALS AND METHODS

Two hundred and seventy one Holstein cattle of the National Agricultural Research Center for Hokkaido Region were used. Two hundred and nineteen of the 271 had only milk production traits records (G1), 33 had only metabolites records (G2), and 19 had both milk production traits and metabolites records (G3). Fifty two calves with metabolites record (G2 and G3) were born from 1992 to 1997. Forty three calves (29 females, 14 males) were used from 10 to 90 d of age and the others (3 females, 6 males) from 10 to 60 d of age. They were divided into environmental groups (EG): two sex, three groups of birth month (January to March, April to May, June to August), and two groups of feeding places (pen in cowshed, calf hatch out of cowshed). Since there was no calf in an EG, which was male, born in June to August, and feed in calf hatch, the calves were assigned into 11 EG. Calves were fed twice a day at 09:00 and 16:00 h. Whole milk was fed 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 provided *ad libitum* during the whole experimental period. Blood samples were obtained one or two days from 9 to 11 d of age, one time per day, before feeding at 09:00 h by jugular venipuncture into evacuated tubes with heparin. The means of plasma metabolite concentrations were used for statistical analysis as the data at 10 d of age. In the same way, the blood samples of every 10 d were collected until 90 d of age. Plasma was stored at -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.

A total of 566 records of milk yield, fat yield and protein yield for 240 to 305 d on 238 cattle (G1 and G2) were collected from 1 January, 1983 to 31 March, 1999. The lack of milk production data before 305 d was estimated by the method of Wood (1967). In the milk yield records, the method of Sasaki et al. (1999) was used to

correct for the effect of age at delivery.

Sasaki et al. (2002) reported that the postnatal transition in plasma metabolite concentration changed twice, at 30 and 60 d of age. Then, the collected plasma samples were divided into three groups by age: 10 to 30 d (AG1), 40 to 60 d (AG2), and 70 to 90 d (AG3). Genetic parameters were estimated in each age group. The report also showed that the month of birth and interaction between site of feeding and sex had a significant effect on the plasma metabolite concentration, so the statistical model for estimation of genetic parameters included the effect of EG. The statistical analysis for estimation of genetic parameters for plasma metabolite concentration used the following model:

$$Y1_{ijk} = MHS_i + D_j + u1_k + e_{ijk}$$

where:

$Y1_{ijk}$  = observation of plasma metabolite concentrations on the  $k$  th animal in the  $ij$  th subclass.

$MHS_i$  = fixed effect of  $i$  th EG,

$D_j$  = fixed effect of  $j$  th sampling day in each age group,

$u1_k$  = total merit of additive genetic and group effects on  $k$  th animal.

$e_{ijk}$  = residual effect.

The genetic parameters for milk yield, fat yield and protein yield were estimated by the model number 2 of Sasaki et al. (1999). The model was

$$Y2_{ijk} = YP_i + M_j + u2_k + e_{ijk}$$

where:

$Y2_{ijk}$  = observation of milk production trait on the  $k$  th animal in the  $ij$  th subclass.

$YP_i$  = fixed effect of  $i$  th year-parity group. The delivery years are grouped every three years. The year groups were divided into three parity subclasses (first, second and after second lactation). The records in 1998 were allocated to the groups from 1995 to 1997.

$M_j$  = fixed effect of  $j$  th calving month.

$u2_k$  = total merit of additive genetic and group effects on  $k$  th animal.

$e_{ijk}$  = residual effect.

Relationship data were traced back to three generations from the cows with milk record; the relationship data included the progenies of these cows. A total of 699 animals (543 female, 156 male) constructed the relationship data. All the animals with plasma metabolite measurements were included in the relationship data. Unknown sires were assigned into three genetic groups in terms of the birth year of their offspring (before 1960, 1960 to 1969, after 1969) as

in Sasaki et al. (1999). In the same way, unknown dams were assigned into eight genetic groups in terms of the birth year of their offspring (before 1960, 1960 to 1964, 1965 to 1969, 1970 to 1974, 1975 to 1979, 1980 to 1984, 1985 to 1989, and after 1989).

Restricted maximum likelihood for multiple traits animal model was used for estimation of heritability and genetic correlation. The VCE4.2 program (Groeneveld and Cortés, 1998) was used for this estimation. The statistical model with all metabolites and all milk production traits did not show convergence. Therefore, genetic and residual variances were estimated by the single trait model for each metabolite and milk production trait. Genetic and residual covariances were estimated using the two trait model.

The two-trait mixed model equation was,

$$\begin{bmatrix} X'R^{-1}X & 0 & X'R^{-1}Z \\ 0 & G_0 \otimes A_g' A^{-1} A_g & G_0 \otimes (-A_g' A^{-1}) \\ Z'R^{-1}X & G_0 \otimes (-A^{-1} A_g) & Z'R^{-1}Z + G_0 \otimes A^{-1} \end{bmatrix} \begin{bmatrix} \hat{b} \\ \hat{g} \\ \hat{u} \end{bmatrix} = \begin{bmatrix} X'R^{-1}y \\ 0 \\ Z'R^{-1}y \end{bmatrix}$$

with

$$E \begin{bmatrix} u \\ e \end{bmatrix} = 0, \quad V \begin{bmatrix} u \\ e \end{bmatrix} = \begin{bmatrix} G & 0 \\ 0 & R \end{bmatrix}$$

where:

- $y$  =  $N \times 1$  vector of observation,
- $\hat{b}$  =  $p \times 1$  vector of fixed effect of EG in metabolites, and year-parity groups and calving month in milk production traits,
- $\hat{g}$  =  $n \times 1$  vector of fixed effect of genetic group,
- $\hat{u}$  =  $(q \times 2) \times 1$  vector of the total merit of additive genetic and group effects ( $\hat{u} = a + A_g g$ ),
- $\hat{a}$  =  $(q \times 2) \times 1$  vector of additive genetic effect,
- $e$  =  $N \times 1$  vector of random residuals,
- $X$  =  $N \times p$  incidence matrix for fixed effects,
- $A$  =  $q \times q$  matrix of additive relationships,
- $A_g$  =  $q \times n$  matrix of additive relationships between animals and groups,
- $Z$  =  $N \times (q \times 2)$  incidence matrix for random effects,
- $G = G_0 \otimes A$  with  $\otimes$  the direct product operator (Searle, 1982),
- $G_0$  = genetic covariance matrix,
- $R = \begin{bmatrix} R_{11} & R_{12} \\ R_{12}' & R_{22} \end{bmatrix}$ ,
- $R_{11}$  =  $N_1 \times N_1$  residual covariance matrix of first trait,
- $R_{22}$  =  $N_2 \times N_2$  residual covariance matrix of second trait,
- $R_{12}$  =  $N_1 \times N_2$  residual covariance matrix between first and second traits,
- $N_1$  = number of records of first trait,
- $N_2$  = number of records of second trait,
- $N = N_1 + N_2$ ,
- $p$  = total number of fixed effects of first and second

traits.

$n$  = number of genetic group effects.

$q$  = number of animals.

The reliability of breeding value for milk production trait was estimated by the method of Da et al. (1989), and modified for multiple traits model. When the reliability was estimated, the first genetic group effect was restricted to zero.

$$\gamma_i = \sqrt{1 - (d_i / \sigma_a^2)}$$

where:

$\gamma_i$  = reliability of  $i$  th rank,

$d_i$  = diagonal element of  $i$  th rank of  $(M_x + M_a)^{-1}$ ,

$\sigma_a^2$  = additive genetic variance of milk production trait,

$$M_x = Z'R^{-1}Z - Z'R^{-1}X(X'R^{-1}X)^{-1}X'R^{-1}Z,$$

$$M_a = G^{-1} \otimes (A^{-1} - T_{gg}' T_{gg}^{-1} T_{gg}),$$

$$T_{gg} = -A_g' A^{-1},$$

$$T_{gg} = A_g' A^{-1} A_g.$$

The reliabilities of milk production traits were estimated 2 times. The first was estimated using milk records only (E1), and the other was estimated using both milk and metabolite records (E2). The reliabilities of milk production traits of the animals at G2 were chosen from the result of E1 and E2, respectively. The mean reliability of E1 (E1G2) was compared with that of E2 (E2G2). Similarly, the reliabilities of the animals at G3 were chosen from the result of E1 and E2, respectively. The mean reliability of E1 (E1G3) was compared with that of E2 (E2G3).

In the statistical analysis, the GLM procedure of Statistical Analysis System (SAS, 1990) was used for estimation of least square means of metabolites concentrations and reliability of milk production traits.

## RESULTS

Least square means of plasma metabolite concentrations from 10 to 90 d of age are shown in Table 1. Means of milk yield, fat yield and protein yield were  $8101 \pm 1.565$  kg,  $282.7 \pm 67.3$  kg and  $232.8 \pm 52.6$  kg, respectively. The mean milk yields indicated were after adjustment for the effect of delivery age.

Genetic and residual variances and heritabilities of plasma metabolite concentrations and milk production traits are shown in Table 2. Heritabilities of plasma urea nitrogen, glucose and total ketone concentrations were high in all age groups. Heritabilities of plasma total cholesterol and triglyceride were high at AG2 and AG3. Heritabilities of plasma metabolites concentrations were high at AG3 and low at AG1 except for the plasma NEFA concentration. Heritability of plasma NEFA concentration at AG1 did not converge. Therefore, the analysis after this was not

**Table 1.** Least square means of plasma metabolite concentrations every 10 days from 10 to 90 days of age and the standard errors

Age (d)	10	20	30	40	50	60	70	80	90
Urea nitrogen (mg/dl)	10.04±0.40	11.20±0.40	10.27±0.40	10.47±0.40	10.35±0.40	10.61±0.40	11.55±0.44	12.25±0.44	12.23±0.44
Glucose (mg/dl)	99.3±1.4	92.9±1.4	90.0±1.4	93.1±1.4	91.8±1.4	93.3±1.4	94.3±1.5	93.4±1.5	93.1±1.5
NEFA <sup>1)</sup> (μEq/l)	349±12	290±12	242±12	234±12	209±12	213±12	200±13	162±13	125±13
Triglyceride (mg/dl)	33.3±1.5	28.6±1.5	27.5±1.5	25.3±1.5	26.0±1.5	25.6±1.5	27.4±1.6	28.3±1.6	28.9±1.6
Total cholesterol (mg/dl)	78±3	102±3	119±3	115±4	114±3	121±3	110±4	92±4	81±4
Total ketone (μmol/l)	278±25	291±25	278±25	328±25	341±25	384±25	429±27	496±27	493±27

<sup>1)</sup> Non esterified fatty acid.

performed about NEFA concentration at AG1.

Genetic correlations between each plasma metabolite concentration are shown in Table 3. Genetic correlations among urea nitrogen, glucose, triglyceride and total ketone were high in all age groups, except that the genetic correlation between urea nitrogen and glucose was low in AG2. Plasma total cholesterol concentration had a high genetic correlation with four other plasma metabolites, urea nitrogen, glucose, triglyceride and total ketone levels at

AG2 and AG3. Plasma total cholesterol had a high genetic correlation with the plasma glucose concentration at AG1. Concentration of plasma NEFA had a high genetic correlation with total ketone at AG2 and AG3.

Genetic correlations between plasma metabolite concentration and milk production traits are shown in Table 4. Milk yield had a high and positive genetic correlation with plasma urea nitrogen and total cholesterol levels at AG2 and AG3. Milk yield had a high and negative genetic correlation with the plasma glucose concentration at AG1 and AG2. Milk yield had a high and negative genetic correlation with the plasma NEFA concentration at AG3. Fat yield had a high and positive genetic correlation with the plasma glucose level at AG1, the total cholesterol concentration at AG3, and the NEFA concentration at AG2. The genetic correlation between fat yield and plasma total ketone concentration at AG2 was high and negative. Protein yield had high and positive genetic correlation with the plasma glucose concentration at AG1, the total cholesterol concentration at AG3 and the NEFA concentration at AG3.

Least square means of reliability of milk, fat and protein yield in E1G2 and their standard errors were 0.49±0.01, 0.50±0.01 and 0.47±0.01, respectively. Least square means of reliability of milk, fat and protein yield in E1G3 and their standard errors were 0.78±0.01, 0.80±0.01 and 0.74±0.01, respectively. The reliability of milk, fat and protein yields in E2G2 and E2G3 are shown in Table 5. The reliability of milk yield in E2G2 was 8.2 to 19.0% higher ( $p<0.01$ ) than in E1G2 by using plasma metabolite concentration, urea nitrogen at AG2 and AG3, glucose at AG1 and AG2, and total cholesterol at AG3. The reliability of fat yield in E2G2 was 6.4 to 24.2% higher ( $p<0.01$ ) than in E1G2 by using plasma metabolite concentration, glucose at AG1, total cholesterol at AG3 and total ketone at AG2. The reliability of protein yield in E2G2 was 9.5 to 20.1% higher ( $p<0.01$ ) than in E1G2 by plasma concentration, glucose at AG1, total cholesterol at AG3 and NEFA at AG2. The reliability of milk yield, fat yield and protein yield in E2G3 was not higher than in E1G3 at any metabolite concentration.

**Table 2.** Genetic and residual variances, and heritability and its standard error of milk production traits and plasma metabolite concentrations in each age group<sup>1)</sup>

	Genetic variance	Residual variance	Heritability
AG1			
Urea nitrogen	3.048	4.942	0.38±0.08
Glucose	53.075	65.607	0.45±0.08
Total cholesterol	168.441	424.536	0.28±0.09
NEFA <sup>2)</sup>	NC <sup>3)</sup>	NC	NC
Triglyceride	33.323	103.323	0.24±0.09
Total ketone	23935.258	2971.713	0.89±0.02
AG2			
Urea nitrogen	5.003	3.472	0.59±0.06
Glucose	70.312	51.635	0.58±0.07
Total cholesterol	469.653	301.001	0.61±0.07
NEFA	1647.468	4633.476	0.26±0.08
Triglyceride	99.873	45.373	0.69±0.05
Total ketone	35644.083	3428.025	0.91±0.02
AG3			
Urea nitrogen	6.794	2.465	0.73±0.05
Glucose	65.057	33.905	0.66±0.07
Total cholesterol	489.510	105.875	0.83±0.04
NEFA	300.885	3650.710	0.08±0.08
Triglyceride	97.179	34.345	0.74±0.05
Total ketone	43556.494	4351.370	0.91±0.02
Milk production traits			
Milk yield	526706.939	632734.174	0.45±0.04
Fat yield	860.648	864.952	0.50±0.04
Protein yield	327.621	544.859	0.38±0.04

<sup>1)</sup> The blood samples were obtained from 10 to 30 d of age (AG1), 40 to 60 d of age (AG2), and 70 to 90 d of age (AG3).<sup>2)</sup> Non esterified fatty acid<sup>3)</sup> Not convergence

**Table 3.** Genetic correlation and its standard error between two plasma metabolite concentrations in each age group<sup>1)</sup>

	Plasma metabolites concentration				
	Glucose	Total cholesterol	NEFA <sup>2)</sup>	Triglyceride	Total ketone
	AG1				
Urea nitrogen	0.30±0.21	0.07±0.25	NE <sup>3)</sup>	0.60±0.26	0.53±0.14
Glucose		-0.53±0.22	NE	0.31±0.21	0.63±0.12
Total cholesterol			NE	-0.11±0.27	-0.11±0.19
NEFA				NE	NE
Triglyceride					0.56±0.16
	AG2				
Urea nitrogen	-0.01±0.10	0.41±0.15	0.18±0.20	0.42±0.13	0.58±0.11
Glucose		0.28±0.15	0.10±0.21	0.31±0.14	0.25±0.14
Total cholesterol			0.19±0.21	0.80±0.08	0.61±0.10
NEFA				0.26±0.18	0.46±0.18
Triglyceride					0.66±0.09
	AG3				
Urea nitrogen	0.40±0.15	0.44±0.14	0.07±0.39	0.70±0.10	0.28±0.14
Glucose		0.33±0.16	0.20±0.44	0.47±0.14	0.51±0.13
Total cholesterol			-0.01±0.35	0.63±0.10	0.47±0.12
NEFA				0.08±0.35	0.27±0.40
Triglyceride					0.72±0.09

<sup>1)</sup> The blood samples were obtained from 10 to 30 d of age (AG1), 40 to 60 d of age (AG2), and 70 to 90 d of age (AG3).<sup>2)</sup> Non esterified fatty acid<sup>3)</sup> Not estimated

## DISCUSSION

**Table 4.** Genetic correlation between plasma metabolite concentrations and milk production traits in each age group<sup>1)</sup>

	Milk yield	Fat yield	Protein yield
	AG1		
Urea nitrogen	0.08±0.27	-0.01±0.25	-0.04±0.14
Glucose	-0.35±0.28	0.64±0.24	0.36±0.35
Total cholesterol	0.10±0.33	-0.03±0.30	-0.23±0.34
NEFA <sup>2)</sup>	NE <sup>3)</sup>	NE	NE
Triglyceride	0.01±0.34	-0.13±0.29	-0.19±0.33
Total ketone	0.01±0.34	-0.10±0.21	0.11±0.24
	AG2		
Urea nitrogen	0.35±0.27	-0.07±0.23	0.00±0.27
Glucose	-0.42±0.27	0.14±0.27	0.05±0.29
Total cholesterol	0.26±0.23	0.06±0.23	0.08±0.25
NEFA	0.17±0.34	0.30±0.32	0.61±0.32
Triglyceride	-0.02±0.25	-0.06±0.23	-0.23±0.25
Total ketone	-0.15±0.25	-0.27±0.20	-0.01±0.24
	AG3		
Urea nitrogen	0.43±0.23	0.22±0.23	0.10±0.26
Glucose	-0.08±0.29	0.12±0.28	0.01±0.30
Total cholesterol	0.58±0.21	0.42±0.20	0.45±0.22
NEFA	-0.32±0.58	0.15±0.50	0.48±0.62
Triglyceride	0.05±0.22	-0.06±0.16	-0.17±0.23
Total ketone	-0.16±0.26	-0.18±0.23	0.02±0.26

<sup>1)</sup> The blood samples were obtained from 10 to 30 d of age (AG1), 40 to 60 d of age (AG2), and 70 to 90 d of age (AG3).<sup>2)</sup> Non esterified fatty acid.<sup>3)</sup> Not estimated.

Rowlands et al. (1983) estimated that the heritabilities of plasma glucose and urea nitrogen concentration were 0.41 and 0.29, respectively, in a herd of 428 British Friesian males, aged 3 to 15 mo. They also reported that the heritabilities of plasma globulin and potassium concentration were 0.65 and 0.82, respectively. These results suggested that many plasma metabolites concentrations would be heritable traits. Heritabilities of plasma urea nitrogen, glucose and total cholesterol concentration were high in all age groups, ranging from 0.38 to 0.91 in this study. Heritabilities of plasma triglyceride and total ketone concentrations were also high at AG2 and AG3 and ranged from 0.61 to 0.83. Heritabilities of plasma urea nitrogen, glucose, total cholesterol, triglyceride, and total ketone concentration increased with growth. These increments agree with the report of Xing et al. (1988) which showed that the variance of plasma glucose concentration after one night fast decreased with growth. The animals need to be exposed to some metabolic stress, when plasma metabolite measures are made for estimation of genetic ability for milk production trait. (Tilakaratne et al., 1980; Sejrsen et al., 1984; Sinnett-Smith et al., 1987; Woolliams and Lovendahl, 1991). The blood samples were collected 17 h after the last feeding so the animals were exposed to light fasting in this study. The plasma glucose concentration after one-night fast sometime between 11 to 26 d of age was high in calves with

**Table 5.** Least square mean of the reliabilities of milk production traits

	E2G2 <sup>1)</sup>			E2G3		
	Milk yield	Fat yield	Protein yield	Milk yield	Fat yield	Protein yield
	AG1 <sup>2)</sup>					
Urea nitrogen	0.49	0.50	0.47	0.78	0.80	0.74
Glucose	0.53**	0.62**	0.51**	0.79	0.83	0.75
Total cholesterol	0.49	0.50	0.48	0.78	0.80	0.74
NEFA <sup>3)</sup>	NE <sup>4)</sup>	NE	NE	NE	NE	NE
Triglyceride	0.49	0.50	0.47	0.78	0.80	0.74
Total ketone	0.49	0.51	0.47	0.78	0.80	0.74
	AG2					
Urea nitrogen	0.53**	0.50	0.47	0.79	0.80	0.74
Glucose	0.55**	0.51	0.47	0.79	0.80	0.74
Total cholesterol	0.51*	0.50	0.47	0.79	0.80	0.74
NEFA	0.50	0.52	0.56	0.78	0.81	0.77
Triglyceride	0.49	0.50	0.49	0.78	0.80	0.75
Total ketone	0.50	0.53**	0.47	0.78	0.81	0.74
	AG3					
Urea nitrogen	0.54**	0.51	0.47	0.80	0.81	0.74
Glucose	0.49	0.50	0.47	0.78	0.80	0.74
Total cholesterol	0.58**	0.55**	0.53**	0.81*	0.82	0.76
NEFA	0.49	0.50	0.48	0.78	0.80	0.74
Triglyceride	0.49	0.50	0.47	0.78	0.80	0.74
Total ketone	0.50	0.51	0.47	0.78	0.81	0.74

<sup>1)</sup> The mean reliability of E2G2 was compared with that of E1G2. The mean reliability of E2G3 was compared with that of E1G3 (\*  $p < 0.05$ ; \*\*  $p < 0.01$ ). The means of E1G2, E1G3, E2G2 and E2G3 were shown in the text.

<sup>2)</sup> The blood samples were obtained from 10 to 30 d of age (AG1), 40 to 60 d of age (AG2), and 70 to 90 d of age (AG3).

<sup>3)</sup> Non esterified fatty acid.

<sup>4)</sup> Not estimated.

high genetic potential for fat yield (Xing et al., 1988). Thus, the length of fasting in this study was sufficient. Heritability of plasma NEFA concentration could not estimate at AG1 and it was too small in AG3. The plasma NEFA concentration at AG1 and AG3 was thought to be affected by the environment e.g. feeding and nutritional state, so these would not be suitable for the estimation of genetic ability for milk production traits. The plasma NEFA concentration in a condition of negative energy balance has a positive correlation with milk yield (Hart et al., 1978; Tilakaratne et al., 1980), fat yield (Sejrsen et al., 1984), and fat plus protein yield (Robinson et al., 1992). The reason seems to be that a large amount of NEFA is mobilized from adipose tissues in high genetic merit calves (Broster et al., 1969). Sasaki et al. (1998) reported that the changes in plasma glucose, urea nitrogen and triglyceride concentrations of calves at 5 mo of age during 24 h fasting were correlated with the genetic ability for milk yield. Xing et al. (1988) reported that the plasma glucose concentration in calves from 11 to 26 d of age was higher in calves of high genetic merit for fat yield than in low ones. These reports suggested that the plasma metabolite concentrations in young calves can be a supporting trait for estimation of genetic ability for milk production.

Heritabilities of milk yield, fat yield and protein yield agree with the results of the multiple traits model from the

same herd (Sasaki et al., 1999) and another herd (Welper and Freeman, 1992; Suzuki et al., 1994).

The genetic correlation among plasma metabolite concentrations was not reported previously and not given much attention. These correlations were high and positive among plasma urea nitrogen, triglyceride, and total ketone in all age groups. The variation in plasma total ketone concentration in pre-weaning calves was thought to depend on an increment in the ketogenic rate along with maturation of rumen epithelium (Quigley et al., 1991). Most plasma  $\beta$ -hydroxybutyrate, a major component of total ketone, originated from alimentary ketogenesis (Quigley et al., 1992). Plasma urea nitrogen concentration was also affected by fermentation of food protein and carbohydrate in rumen (Quigley and Bernard, 1992). Thus, the concentrations of plasma metabolites were considered to be related to the development of the rumen. The plasma NEFA concentration had a high genetic correlation with plasma triglyceride and total ketone concentrations at AG2 and AG3, and the plasma total ketone concentration was increased by development of the rumen. Quigley et al. (1991) suggested that the source of peripheral circulating glucose changed from small intestine absorption in the pre-weaning stage to hepatic gluconeogenesis via ruminal fermentation of carbohydrate to propionate in post-weaning. So it is natural that the genetic correlations among plasma metabolites

concentrations at AG1, corresponding to the pre-ruminal development stage, were different from those at AG2 and AG3, in the ruminal developing stage. Ruminal maturation was thought to correlate closely with the amount of dry matter intake (Savage and McCay, 1942), and the plasma metabolite concentration was considered to be affected by feed (Quigley et al., 1991; Quigley et al., 1992). The plasma metabolite changes seemed to proceed in a mutual genetically stable relationship during the ruminal development stage, because genetic correlations among plasma metabolites concentrations were reproducible, and those at AG2 were similar to those at AG3.

Genetic correlations between plasma urea nitrogen and milk yield increased along with growth. These genetic correlations were positive and higher at AG2 and AG3 in the ruminal development stage than at AG1 in the pre-ruminal development stage. Plasma urea nitrogen, however, did not genetically correlate with the fat and protein yield. The trends in the genetic correlations reported between plasma urea nitrogen concentration and milk production traits have been different. Some investigators (Tilakaratne et al., 1980; Sinnett-Smith et al., 1987) found that the plasma urea nitrogen concentration correlated negatively with the genetic ability for milk yield, while others (Olbrich-Bludau et al., 1990) showed that the correlation was positive. In the same way, conflicting results were reported on the correlation between genetic ability for fat yield, and the plasma urea nitrogen concentration was negative (Tilakaratne et al., 1980; Sinnett-Smith et al., 1987) or absent (Mackenzie et al., 1988). The correlation between genetic ability for the sum of fat and protein yield and plasma urea nitrogen concentration was positive (Woolliams et al., 1992) or absent (Robinson et al., 1992). The experimental conditions, e.g. feed, breed, sex, age and so on, were different in each study. Plasma urea nitrogen was reportedly affected by the state of nutrition (Robinson et al., 1992) and age (Woolliams and Smith, 1988). These results indicated that the high and reproducible genetic correlation between the plasma urea nitrogen concentration and milk production traits would be found only in restricted conditions.

Plasma glucose concentration had high and negative genetic correlation with milk yield at AG1 and AG2. Its genetic correlation was low at AG3. Tilakaratne et al. (1980) reported that the plasma glucose concentration during fasting positively correlated with the genetic merit of milk yield. On the other hand, Sinnett-Smith et al. (1987) indicated that the plasma glucose concentration did not genetically correlate with the genetic merit of milk yield. Glucose metabolism in the peripheral tissue was high in the high genetic merit calf for milk production trait and low in the low genetic merit calf under ordinary conditions (Mackenzie et al., 1988). However, if the high genetic merit

calf was exposed to fasting, the glucose metabolism decreased in the peripheral tissue. These findings show that the genetic correlation between plasma glucose concentration and milk production trait would be sufficiently changed with the rate of nutrition. Therefore the genetic correlation between plasma glucose concentration and milk yield was negative under the light fast in this study. Many studies suggested that plasma glucose concentration during fasting had a positive correlation with genetic ability for fat yield (Sejrsen et al., 1984; Mackenzie et al., 1988; Xing et al., 1988; Min et al., 1993), in agreement with the present study.

The same correlation between plasma NEFA concentration and genetic ability for milk yield is not in the literature. It was suggested that this correlation was positive (Tilakaratne et al., 1980; Barnes et al., 1985), or that there was no correlation (Sinnett-Smith et al., 1987; Olbrich-Bludau et al., 1990). The reason why the genetic correlation between plasma NEFA concentration during fasting and milk production trait differed with each report was not clear. Tilakaratne et al. (1980) suggested that animals with high genetic capacity for milk production have a higher capacity to mobilize body reserves. Sejrsen et al. (1984) reported that the plasma NEFA was positively correlated with breeding value of fat yield during the first 2 days of fasting, but negatively correlated after 3 days of fasting. And they suggested that the peripheral tissues of animals with high breeding value have a reduced requirement, and consequently body stores are depleted more slowly during periods of limited nutrient supply. The genetic correlation between plasma NEFA and milk production traits would be affected by feeding because the heritability of the plasma NEFA concentration was low. Variation of plasma NEFA concentration in reaction to environmental stress makes it difficult to estimate the genetic potential for milk production traits (Sinnett-Smith et al., 1987).

Many reports indicated that the fat metabolism during fasting genetically and positively correlated with milk yield (Tilakaratne et al., 1980; Barnes et al., 1985) and fat yield (Sejrsen et al., 1984). Therefore, the plasma metabolite-related fat metabolism, e.g. NEFA, triglyceride and total ketone, would have a genetic correlation with milk production traits. The genetic correlation between the plasma total cholesterol concentration and milk yield increased with age, and at AG3 it was positive and the highest in this study. The plasma total cholesterol concentration at AG3 also showed a high and positive genetic correlation with fat yield and protein yield. The plasma triglyceride concentration did not correlate with milk production traits. The plasma total ketone concentration genetically and negatively correlated only with fat yield at AG2.

The efficiency of annual genetic gain each year depends on reliability, selection intensity and generation interval. Thus, the annual genetic gain is mainly in proportion to the reliability of selection, when the selection intensity and generation interval are fixed in a breeding plan. In animals without milk records, the use of the plasma metabolite concentrations increased the reliability of milk production traits (E2G2) compared to that in animals not measured for their plasma metabolite concentration (E1G2).

These results show that the record of plasma metabolite concentration was useful especially to assure greater accuracy in estimation of the genetic ability-related milk production trait in an individual animal without a milk record, e.g. immature male and female calves. Plasma metabolite concentrations would be expected to be useful for improvement of genetic ability for milk production traits in the genetic improvement in herd of cows, where half of the individuals selected are from a herd without its own milk record. Because the metabolite concentrations could be measured simply from the neonatal stage, the rate of genetic improvement could be accelerated. However, this would shorten the generation interval and limit the new criteria for selection.

## CONCLUSION

The plasma metabolite concentration before morning feeding in calves until 90 d of age was considered useful information for genetic improvement of milk production traits. The measurement of the plasma metabolite concentration in the present study did not require special management and was easy, so it was considered highly useful. However, since sufficient consideration has not been given to the measurement conditions, there is the possibility that, due to change in dietary conditions, the genetic correlation between plasma metabolite levels and milk production traits may vary.

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