FULL PAPER *Clinical Pathology*

Relationships of Metabolic Profiles to Milk Production and Feeding in Dairy Cows

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ABSTRACT. The aim of this study was to assess the practicality of the metabolic profile test (MPT) for feeding evaluation in dairy cattle. Stepwise regression analysis was used to evaluate the relationships of MPT to feeding and milk production of 4,679 cows in 343 commercial dairy herds. Significant explanatory variables were determined by forward set-up selection, among the deviated values from the reference values of 10 blood metabolites and body condition score, to predict dependent variables, i.e., milk production and the rate of feeding to nutrient requirements, in each or all lactation stages and the dry period. The milk production model of the all-lactation stage showed the greatest goodness-of-fit (adjusted R^2=0.214, p<0.0001) with high positive regression coefficients for serum cholesterol, magnesium, urea nitrogen and albumin, and negative for glucose and calcium. In the feeding models, goodness-of-fit of crude protein was relatively high (R^2=0.072, p<0.0001) with a positive relationship to blood urea nitrogen. Although the other feeding models were low in goodness-of-fit, several significant explanatory variables to feeding were found. All feeding models in the late lactation stage and the dry period, in which the feeding was stable, had greater goodness-of-fit than those in the early lactation stage in which milk production varied. It was concluded that the values which deviated from the reference values for the MPT components could assess milk production and feeding, and the MPT is a practical tool for auxiliary feeding evaluation.

KEY WORDS: dairy cow, feeding and nutrition, metabolic profile test.

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Since Payne *et al.* [32] established a metabolic profile test (MPT) for dairy cattle, many researchers have applied the MPT for feeding evaluation. Blowey *et al.* [5] reported that changes in the blood metabolite concentration could be associated with changes in diet, by repeatedly conducting the MPT in 12 dairy herds. Parker and Blowey [31] investigated the relationships of blood components to nutrition and fertility of the dairy cow in 15 commercial dairy farms. They found that the most consistent correlations were the regressions of starch intake to nonesterified fatty acids, the ratio of starch and protein intake to plasma urea, protein intake to plasma urea and phosphorus intake to plasma phosphorus, but factors other than nutrition accounted for a large part of the variation.

Adams *et al.* [1] reported that a number of factors limited the usefulness of MPT. These include sampling problems, low correlations with nutrient intake, and inconsistent disease patterns. Lee *et al.* [26] also reported that concentrations of metabolites were of almost no practical use for individual cows because of extreme variations in diet required to generate abnormal concentrations of blood metabolites.

On the other hand, Kronfeld *et al.* [25] used multiple regression analysis to study the relationships between blood metabolite concentrations and feeding of 391 cows in 21 herds, and reported that good serum predictors of ration variables were glutamic-oxaloacetic, glutamic pyruvic transaminases, cholesterol, phosphorus, triglyceride and globulin. The blood metabolite concentrations were used as parameters of the nutritive background in many studies on the causes of reproductive disorder and periparturient disease [3, 4, 6, 7, 13, 16, 18, 20, 21, 27, 35, 36, 42].

Nowadays anyone can compose dairy cattle diets based on excellent feeding standards, such as the NRC nutrient requirements of dairy cattle [28, 29], the Japanese feeding standard [9, 10], or a computer program i.e., Spartan [39]. But in most dairy farms there is not a little difference in feed quantity between theoretical composition by computer and actual feeding by farmers [41]. The difference in quantity between theoretical and actual feeding sometimes causes problems in health or milk depression in dairy cows [22].

MPT in conjunction with assessment of animal condition, housing, body condition, and feeding, would be a precise tool for feeding evaluation [3, 41].

Kida [24] established 10-day criteria of blood metabolites to interpret MPT, which reflects physiological changes in the dairy cow such as milk production, feeding and management, every 10 days after calving or dry off. These criteria were interpreted by the deviations from the reference mean values of metabolites rather than the actual values. The aim of this study was to assess the practicality of MPT by using 10-day criteria for feeding evaluation in dairy cattle. Stepwise regression analysis was used to determine the MPT components that are able to evaluate feeding and milk production in numerous commercial dairy herds.

MATERIALS AND METHODS

Cow selection: The MPT was conducted in 343 commercial dairy herds in various districts of Hokkaido between January 1998 and December 1999. A total of 4,679 Holstein-Friesian multiparous cows were selected from cows at all lactation stages and the dry period, except cows just after dry off and calving. The cows were separated into cows in the dry period (467 cows, 10 to 39 days after dry off), early lactation (514 cows, 22 to 49 days in milk; DIM), peak lactation (1,331 cows, 50 to 109 DIM), mid lactation (1,185 cows, 110 to 209 DIM), and late lactation (1,182 cows, 210 to 309 DIM). The average number of calving was 3.4, and average milk production was 32.2 kg/cow/day (range 5 to 79 kg/cow/day) and 9,216 kg/cow/305-day (range 5,167 to 13,746 kg/cow/305-day).

Blood collection, MPT components and analytical methods: Blood was analyzed on the farm in a vehicle equipped with automatic biochemical analyzers. Blood samples were collected from the jugular vein in plain vacuum tubes between 09:00 and 11:00 hr, 2–4 hr after morning feeding about 07:00 hr. Heparinized micro-capillary tubes were immediately filled for determination of the hematocrit (Ht). Just after blood collection, to avoid a drop in glucose, the serum was separated by centrifuging (750 × g) for 5 min. As fibrin appeared in the serum layer, a small quantity of plastic granules, serum separator (Blut-Z, TOHSHIN CHEMICAL Co., Ltd.), was then added to the sample tubes and further centrifuged (1,600 × g) for 5 min. Within 1–2 hr after the blood was drawn, serum components were analyzed in an automatic biochemical analyzer (TBA-40FR, TOSHIBA MEDICAL SYSTEMS Co., Ltd.) and an automated electrophoresis system (CTE-700, JOKOH Co., Ltd.).

The MPT components and analytical methods were as follows: indicators used for protein metabolism were Ht (Micro capillary), albumin (Alb, Electrophoresis) and blood urea nitrogen (BUN, Urease-GLDH), for energy metabolism, glucose (Glc, HK-G6PDH), total cholesterol (Cho, Choresterol oxidase), nonesterified fatty acids (NEFA, Enzymatic colorimetric) and body condition score (BCS,1: too thin to 5: too fat [15, 17, 37]), and for mineral metabolism, serum calcium (S-Ca, O-CPC), serum inorganic phosphorus (S-iP, Enzymatic UV) and serum magnesium (S-Mg, Enzymatic UV). Results of the MPT were diagnosed on the basis of the individual cow's deviation from the reference mean [23, 24].

Feeding evaluation: At the time of MPT, the quantity of daily feed given to the cows was measured and the condition of feed bunk was observed to estimate feed refuse. Based on the NRC nutrient requirements of dairy cattle [28], the rate of nutrient intake to the requirement of cows with average body weight and milk production were calculated for each stage of lactation and the dry period. Since most of the cows were fed concentrate in accordance with milk production or days after calving and forage ad lib, dry matter intake (DMI) was estimated as cows ate up the concentrate and forage ad lib until the DMI requirement was fulfilled. Calculated nutrients were total digestible nutrients (TDN), crude protein (CP), calcium (D-Ca), phosphorus (D-P), magnesium (D-Mg), acid detergent fiber (ADF) and forage (Forage). Forage requirement, which was not shown in the nutrient requirement table of the NRC, was defined as DMI multiplied by constants - 65% in dry cows and 50% in milking cows, which was generally recommended. The chemical composition of concentrates was taken from the feed composition tables of the Japanese feeding standard [11, 12] or the NRC [28]. Those of the concentrate mix were taken from the publications of the feed companies. Chemical compositions of all forage were determined by near infrared reflecting spectroscopy at a commercial laboratory.

Milk production: The kilogram yield of solids-corrected milk (SCM) [40] was calculated based on milk production, which was recorded within a month before the MPT, as shown below.

SCM (kg) = 12.3 × Fat (kg) + 6.56 × Solids non-fat (kg) - 0.0752 × Milk (kg)

Statistical analysis: According to the diagnosis by 10-day criteria [23, 24], the deviation from the reference value of each MPT component and milk production was calculated as shown below.

Deviation = (actual value - reference mean value) / reference standard deviation value

Stepwise regression analysis, in which the F value was less than 4.0, was used to evaluate the relationships of the results of MPT to feeding and milk production. As predictors, significant explanatory variables were determined by forward set-up selection, from the deviated values for the MPT components to predict the dependent variables, i.e., milk production and feeding, in each or all lactation stages. The goodness-of-fit of each model was estimated by adjusted R square (R^2). Significant explanatory variables were also evaluated by means of the standard partial regression coefficient, of which the components are useful for evaluating feeding and milk production. The statistical analysis was conducted with a computer software, "Japanese version of StatView® for Windows Ver. 5.0 (SAS Institute Inc.) [34, 38].

RESULTS

Feed supplied to 343 dairy herds is shown in Table 1. Range and mean values for blood metabolite concentrations, BCS and milk production of cows, are shown in Table 2. The mean value for all cows in each MPT component was quite similar to the reference [24].

The nutrient intake compared to the NRC requirement of specific nutrients is shown in Table 3. Average rates of TDN and CP intake were approximately 100 % and the ranges were 50 to 150 % but the rates of mineral and fiber intake varied among the herds.

Results of the stepwise analysis of the relationship between deviated MPT components and the rates of nutrient intake, in which the chosen explanatory variables are significant predictors of the dependent variables, are shown in Table 4.

The TDN model showed low goodness-of-fit (R^2=0.018, $p<0.0001$) in all-stage. In each stage of the TDN model, only the dry period showed a relatively high relationship (R^2=0.053, $p<0.0001$) with the chosen predictors of NEFA (negative partial regression coefficient: –), S-Ca (positive partial regression coefficient: +) and Glc (+).

In all-stage of the CP model was found the highest goodness-of-fit (R^2=0.072, $p<0.0001$) in all regression models of nutrient intake. In all stages of the CP model, BUN (+) was

Table 1. Feed supplied to 343 dairy herds

Feed	Number of herds
Total mixed ration	56
Grass hay	196
Legume hay[a)]	128
Grass - legume silage	209
Corn silage	120
Pasturing	56
Concentrate mix	280
Soy bean	107
Corn grain[b)]	69
Barley	45
Beet pulp	202
Other by-products[c)]	42
Mineral supplements[d)]	194

a) Alfalfa hay, cubes and pellets.
b) Cracked or steam-flaked corn.
c) Cotton seed, brewers grains and fish meal, etc.
d) Ca 22.6%, P 11.9%, Mg 5.6% on average.

Table 2. Range and mean values of blood metabolite concentrations, milk production and body condition scores of cows

Items	Range	Mean
Hematocrit, %	17–44	31
Albumin, g/m*l*	1.6–5.5	4.0
Blood urea nitrogen, mg/100 m*l*	2.0–30.7	14.2
Body condition score	1.0–5.0	2.8
Glucose, mg/100 m*l*	40.0–95.0	62.0
Cholesterol, mg/100 m*l*	53–417	186
Nonesterified fatty acid, μEq/*l*	17–694	125
Serum calcium, mg/100 m*l*	6.5–12.3	9.9
Serum inorganic phosphorus , mg/100 m*l*	2.6–8.6	5.4
Serum magnesium , mg/100 m*l*	1.5–3.8	2.4
Solids-corrected milk, kg	4.5–68.2	31.1

Table 3. Range and average dietary intake of specific nutrients in cows

Dietary intake[a)]	Range	Average
Total digestible nutrient	59–142	99
Crude protein	45–150	96
Calcium	10–325	100
Phosphorus	45–234	104
Magnesium	43–286	93
Acid detergent fiber	54–215	112
Forage	36–191	119

a) Dietary intakes are shown as a rate(%) of NRC requirements for cows sampled in each stage.

selected as a significant predictor with a high coefficient. Glc (+) was chosen in early to mid lactation of the CP model and BCS (+) in peak to late lactation, also. Furthermore, S-Ca (+) was chosen in the stage of peak, late lactation and the dry period.

Low goodness-of-fit for each mineral model in all-stage was observed: D-Ca (R^2=0.037, p<0.0001), D-P (R^2=0.047, p<0.0001) and D-Mg (R^2=0.046, p<0.0001), respectively.

In each stage of the D-Ca model, goodness-of-fit was relatively high in the late lactation stage (R^2=0.048, p<0.0001) and the dry period (R^2=0.079, p<0.0001). Only in these stages, S-Ca (+) was chosen as a significant predictor of D-Ca. BUN (+), NEFA (+), Alb (+) and Ht (–) were chosen in the late lactation stage, and Glc (+), BCS (+) and BUN (+) in the dry period.

In each stage of the D-P model, goodness-of-fit was relatively high in mid lactation (R^2=0.043, p<0.0001), late lactation (R^2=0.063, p<0.0001) and the dry period (R^2=0.051, p<0.0001). As the predictor of D-P, S-iP (+) was chosen in the stages of mid and late lactation, but not in the dry period. Furthermore, BUN (+) in all stages, Glc (+) in the stage of mid lactation and the dry period, Cho (+) and S-Ca (+) in the stage of late lactation, and S-Mg (–) in the stage of mid and late lactation were determined as the significant predictors of D-P.

In each stage of the D-Mg model, goodness-of-fit was relatively high in the stage of late lactation (R^2=0.048, p<0.0001) and the dry period (R^2=0.062, p<0.0001). In these stages, as predictors of D-Mg, BUN (+), Glc (+) and S-Ca (+) were chosen, but S-Mg was not chosen in any of the stages of the D-Mg models.

In all-stage of the ADF and Forage models, low goodness-of-fit was observed in ADF (R^2=0.016, p<0.0001) and Forage (R^2=0.031, p<0.0001), respectively. In each stage of the ADF and the Forage models, relatively high goodness-of-fit of ADF (R^2=0.043, p<0.0001) and Forage (R^2=0.059, p<0.0001) were observed in the stage of late lactation. In both models, BCS (–), Cho (–) and S-iP (–) were selected as the significant predictors of each dependent variable.

Milk production model was found to be high goodness-of-fit, in which R^2 was 0.214 in all-stage and more than 0.2 in each lactation stage. As the significant predictors in each lactation stage, Cho (+), Glc (–), BUN (+) and S-Mg (+) were selected. Furthermore, Alb (+) in all the lactation stages except late lactation, S-Ca (–) in peak to late lactation and S-iP (–) in peak and late lactation were selected.

DISCUSSION

Protein metabolism: Detection of anemia seldom gives a final answer, it merely alerts the farmer and veterinarian [33]. Assessment should be made based on the other MPT components as well. The BUN concentration reflects the status of microbial utility of ammonia in rumen [30], and has been used as a parameter of ruminal protein metabolism in many studies [4, 5, 14, 21].

In the stepwise analysis of this study, relatively high goodness-of-fit in the CP models was observed. In this model, BUN was chosen as a significant explanatory variable with a high coefficient, but Ht and Alb were not. Manston *et al.* [27] described how the BUN concentration reflects current dietary protein intake, and concentrations of Alb and Ht are affected by the long-term protein status. This is the reason why Ht and Alb were not selected. BUN was

Table 4. Relationships between nutrient intakes and components of metabolic profile test

Nutrients[b]	Standardized partial regression coefficients of chosen explanatory variables[a]											Adjusted	Significance[d]
Stages[c]	Ht	BUN	Alb	BCS	Glc	NEFA	Cho	S-Ca	S-iP	S-Mg	Constant	R^2	
TDN													
D					0.123	–0.148		0.139			108.3	0.053	****
E				0.094							96.0	0.007	0.0338
P													ns
M		0.087		0.079	0.052					–0.077	99.3	0.016	****
L		0.066		0.073	0.062						101.9	0.012	0.0005
All	0.033	0.092	–0.051		0.091						99.8	0.018	****
CP													
D		0.171						0.234			98.1	0.082	****
E		0.124			0.139	0.130					95.3	0.040	****
P		0.233		0.092	0.123			0.063			94.9	0.076	****
M		0.268		0.060	0.094		0.073				97.2	0.093	****
L		0.237		0.092				0.106	–0.064		97.9	0.076	****
All		0.228		0.059	0.089		0.033	0.072			96.7	0.072	****
D-Ca													
D		0.109		0.12	0.148			0.120			93.8	0.079	****
E		0.107									95.1	0.010	0.0148
P	0.057	0.094	0.082			0.076				–0.083	81.8	0.029	****
M		0.126	0.067	–0.055		0.081					101.9	0.028	****
L	–0.084	0.136	0.097			0.099		0.074			105.0	0.048	****
All	0.055	0.123	0.068	–0.052	0.044	0.092		0.055			98.3	0.037	****
D-P													
D	–0.108	0.194			0.115						113.9	0.051	****
E		0.103									97.7	0.009	****
P		0.109			0.073				0.110	–0.056	96.8	0.027	****
M		0.195			0.075				0.053	–0.064	106.7	0.043	****
L		0.215					0.068	0.065	0.062	–0.086	110.5	0.063	****
All		0.181		–0.067	0.085	0.037			0.073	–0.043	105.0	0.047	****
D-Mg													
D	–0.118	0.151			0.144			0.139			106.9	0.062	****
E					0.124	0.127					88.6	0.029	0.0002
P		0.060				0.110	–0.079	0.101			86.9	0.024	****
M		0.128	–0.051	–0.059	0.082	0.072	0.079	0.064			87.8	0.038	****
L		0.166			0.065			0.127	–0.072		104.2	0.048	****
All		0.155	–0.048	–0.068	0.111	0.067		0.087			93.4	0.046	****

(continues)

also chosen as a predictor of the dietary minerals in this study. As for this reason, it is supposed that considerable quantities of legumes, such as Alfalfa, which contain high concentrations of protein and minerals [11, 12] were fed to most of the herds in this study.

Jones *et al.* [19] studied the metabolic profiles of commercial dairy herds differing in milk production. They reported that a high concentration of Alb and Ht was observed in high producing herds, but there was no practical meaning in the interpretation. In this study, positive relationships between Alb and SCM were found in the stage of early to mid lactation, as well as Ht and SCM in peak lactation. These relationships indicate that high producing cows are easy to dehydrate. Because the high producing cows in this study were fed much concentrate, this feeding strategy might induce imbalance in the 'forage and concentrate ratio', resulting in indigestion. Although goodness-of-fit was low, negative relationships between ADF or Forage and Ht or Alb were observed in this study. This can provide additional evidence of dehydration caused by shortage of fiber in the diet. Diarrhea was observed at the time of MPT in such high producing cows.

Energy metabolism: The change in BCS directly reflects the energy status of dairy cows [15, 17, 37]. In this study, this was evidenced by the negative relationship of BCS to milk production in the mid lactation stage, but a relationship between BCS and TDN was hardly observed, probably because the BCS expressed the result of the energy status in a certain past period, whereas feeding was evaluated based on the current energy status; milk production and body weight.

Most high-protein feeds, such as soybean and soybean meal, contain not only protein, but also high energy [11, 12]. Therefore, intake of high-protein feed leads to energy sup-

Table 4. (continued)

Nutrients[b)] Stages[c)]	Standardized partial regression coefficients of chosen explanatory variables[a)] Ht	BUN	Alb	BCS	Glc	NEFA	Cho	S-Ca	S-iP	S-Mg	Constant	Adjusted R^2	Significance[d)]
ADF													
D		–0.161					0.134	–0.096			114.9	0.036	0.0002
E													ns
P	–0.112					–0.081					122.6	0.017	****
M	–0.099					–0.048		0.068	–0.047	–0.066	105.5	0.020	****
L				–0.135			–0.149		–0.059		103.8	0.043	****
All	–0.060					–0.102			–0.040	–0.034	112.3	0.016	****
Forage													
D				–0.131		0.092					105.4	0.020	0.0036
E			–0.119		–0.097						118.8	0.016	0.0062
P	-0.128					–0.116					117.0	0.028	****
M	-0.091			–0.050	–0.049		–0.067	0.080	–0.067		124.2	0.033	****
L				–0.177			–0.149		–0.080		112.0	0.059	****
All	-0.074	–0.044		–0.063	–0.060		–0.064	0.045	–0.067		117.0	0.031	****
SCM													
E		0.084	0.122		–0.099	0.172	0.278			0.091	0.265	0.209	****
P	0.057	0.087	0.081		–0.113		0.313	–0.105	–0.067	0.143	0.331	0.213	****
M		0.114	0.111	–0.050	–0.082		0.353	–0.100		0.120	0.342	0.225	****
L		0.067			–0.107		0.421	–0.064	–0.091	0.067	0.404	0.219	****
All		0.094	0.090		–0.096		0.355	–0.086	–0.058	0.104	0.339	0.214	****

a) The explanatory variables are shown as deviations from the reference mean of Ht: hematocrit, BUN: blood urea nitrogen, Alb: albumin, BCS: body condition score, Glc: glucose, S-Ca: serum calcium, S-iP: serum inorganic phosphorus and S-Mg: serum magnesium.
b) The dependent variables are shown as rates (%) of intake in relation to NRC requirements of TDN: total digestible nutrients, CP: crude protein, D-Ca: dietary calcium, D-P: dietary phosphorus, D-Mg: dietary magnesium, ADF: acid detergent fiber and Forage: forage, and SCM: solids-corrected milk.
c) Cows in the stage of D: dry period, E: early lactation, P: peak lactation, M: mid lactation, L: late lactation and All: all stages are shown.
d) Significances are shown as $P<0.0001$(****), P values or ns: no significance ($p\geq0.05$).

ply simultaneously, so that protein-feed intake can increase both CP and BCS. It was for this reason that a positive relationship between BCS and CP was observed in this study. The positive relationship between Glc and CP can be similarly explained.

BCS showed further negative relationships to ADF and Forage in the late lactation stage. In almost all herds in this study, cows in late lactation were fed a lot of forage, such as grass hay. Due to the low energy and high ADF concentration in grass hay [11, 12], feeding of high forage might reduce the energy supply.

Although blood glucose is one of the important indicators of the energy status in MPT, it is not a reliable indicator of the nutritional status [33]; hypoglycemia was interpreted as an indicator of ketosis and infertility rather than low energy intake. On the other hand, hyperglycemia may be observed when animals are stressed [33] rather than over fed, but in this study, Glc showed a positive relationship to the TDN of the cows in the dry period, and negative to milk production in the all lactation stages. Therefore, it is seemed that Glc can be interpreted as the energy indicator.

Glc had positive relationships to the minerals D-Ca, D-P and D-Mg, especially in the dry period. Most of the dry cows with hypoglycemia were often observed with depression of the left para-lumbar fossa, indicating small rumen volume caused by ‘off feed’. At the same time, these cows had high NEFA, also. Accordingly, it seemed that such ‘off feed’ resulted in the positive relationships between Glc and dietary mineral intake.

NEFA changes reflect body fat mobilization; an increase in NEFA indicates a basic energy deficiency in the diet of the cow [2]. In this study, the negative relationship of NEFA to TDN was observed in the dry cows. It was confirmed that an increase in NEFA directly indicated a negative energy balance. On the other hand, no significant relationship between NEFA and TDN was observed in the milking cows, rather an increase in milk production was explained by high NEFA only in cows in early lactation. Dairy cows in early lactation produce milk with obligate and lose their body weight [29]. In this study, this fat mobilization resulted in a positive relationship between NEFA and SCM in cows in early lactation.

The serum cholesterol is a part of the lipoprotein complex. It is closely related to milk production by accumulating in blood after milk synthesis from lipoprotein [2]. Cho in this study also showed an obvious positive relationship to milk production. Furthermore, on the interpretation of Cho in MPT, Cho is one of the indexes of energy status [33]. The negative relationships of Cho to ADF and Forage in late lactation can be explained in the same way as the relationships of BCS to ADF and Forage as described above; low energy and high ADF content in forage.

Mineral metabolism: The serum calcium concentrations in dairy cows are held under close homeostatic control and are seldom changed under common feeding management [33]. In this study, however, S-Ca indicated significant positive relationship to D-Ca in the stage of late lactation and the dry period. This means that serum calcium changes reflect calcium intake. The negative relationship of S-Ca to SCM indicated that calcium deficiency was accompanied by calcium excretion into milk in high producing dairy cows. The significant positive relationship of S-Ca to D-Mg was observed, but the reason for it is not clear.

The serum inorganic phosphorus concentrations relatively reflect dietary phosphorus intake [33]. From the results on the positive relationship of S-iP to D-P in this study, it can be interpreted that serum inorganic phosphorus concentrations increase with the increase in intake of dietary phosphorus. On the other hand, significant negative relationships of S-iP to Forage and SCM were observed in this study. It was shown that D-P intake might decrease with an increase in forage in a daily total ration and that S-iP might decrease with phosphorus excretion into milk.

The serum magnesium concentration is not under endocrine control, but changes reflecting magnesium intake [8]. No correlation was observed between S-Mg and D-Mg in this study, but a positive relationship of S-Mg to SCM was found. It was indicated that the S-Mg might be important in milk production.

As for the goodness-of-fit in multiple regression equations, the relationships between blood metabolites concentrations and feeding management were sometimes observed to be statistically significant but meaningless as to feeding management [26]. In this study, although almost models of MPT components in feeding were statistically significant ($p<0.0001$), the adjusted R^2 was not so high, indicating low usefulness, but the chosen predictors, MPT components of the models with $R^2>0.04$, could be interpreted rationally from the metabolic and the feeding points of view. These models indicated that blood metabolites and BCS were closely related to feeding and milk production. It was concluded that the values which deviated from the reference values of the MPT components could assess milk production and feeding, and MPT is a practical tool for auxiliary feeding evaluation.

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