Practical Application of Metabolic Profile Test in Reproductive Management of Japanese Black Breeding Herds

（黒毛和種雌牛群の繁殖管理における代謝プロファイルデータの臨床応用）

The United Graduate School of Veterinary Science

Yamaguchi University

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March 2015

Practical Application of Metabolic Profile Test in Reproductive Management of Japanese Black Breeding Herds

ACADEMIC DISSERTATION

Presented to

The United Graduate School of Veterinary Science

Yamaguchi University, Japan

By

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In partial fulfillment of requirements for the degree of

Doctor of Philosophy

In

Veterinary Medicine

March 2015

THE UNITED GRADUATE SCHOOL OF VETERINARY SCIENCES
YAMAGUCHI UNIVERSITY

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ABSTRACT

In successful reproductive management of beef cattle, calving-to-conception interval is minimized and a cow produces one calf per year in order to increase productivity. However, reproductive problems including low conception rates and longer calving intervals are arising on a global scale in the dairy and beef cattle industry. The reproductive performance of beef cattle is affected by various factors, such as duration of suckling, reproductive disorders, genetic problems, and maternal nutritional status, all of which affect postpartum reproductive performance. One of the major factors contributing to a prolonged calving-to-conception interval is inadequate nutrition, which often causes sub-clinical and/or clinical metabolic diseases. Prepartal and postpartal nutritional status is related to the interval between calving and first ovulation. It was assumed that certain metabolic parameters, which reflect the energy and protein status of cows during prepartum and postpartum, are associated with reproductive performance of breeding cattle herds. Metabolic profile test (MPT) is a blood test used to assess metabolic status and diagnose metabolic disorders in dairy herds and to objectively evaluate the nutritional status of breeding cattle herds consuming feed of various compositions. We used metabolic profile test to monitor herd health and to determine the nutritional status and reproductive status of our regional Japanese Black cattle herds.

In the first series of experiments, we isolated 10 reared cows of a Japanese Black cattle herd in Kagoshima prefecture, Japan, with extremely low concentration of blood urea nitrogen (BUN) (2.6 ± 0.6 mg/dL). Examination of dietary feed nutrition and relevant content of pastureland soil suggested a correlation with crude protein deficiency or

unbalanced nutritional dietary feeds. Thirteen months after the introduction of a dietary remedial measure (bean cake supplementation), BUN, total cholesterol, and albumin concentration of the same five cows increased significantly and the postpartum open day period significantly decreased. The abnormally low BUN levels in the cattle herd may be due to inadequate dietary nutritional content, primarily from the imbalance of the total digestible nutrition (TDN) and crude protein (CP) in the feed and the far-lower-than-average CP value. In conclusion, routine examination of serum biochemical parameters in Japanese Black breeding cattle may be a useful strategy for determining subclinical metabolic failure of cattle herds, and consequently, its effect on reproductive performance of the herd.

The objectives of the second set of experiments were (1) to confirm the effects of nutritional improvement in prepartal and postpartal periods, monitored using the serum MPT and reproductive performance, and (2) to clarify regional characteristics of the MPT results within our region by using the MPT database. Among 42 breeding cattle herds that were mainly fed home-pasture roughage, three experimental herds showing subnormal BUN levels were selected and compared with one representative of the herd with normal BUN levels. Dietary remedial measures were implemented in each herd. The BUN concentration in all three herds increased significantly, and the postpartum open days in two of the herds significantly reduced, compared with values before the dietary supplementation. Additionally, 37 herds within our region were grouped into three categories (Area 1, 2, and 3) by location and soil condition of the pastureland. The MPT and reproductive performance in cows whose blood samples were collected prepartum

(60–20 days before calving) and postpartum (30–90 days after calving) were compared among the three areas. Significant regional differences were found in prepartal albumin, total cholesterol, BUN, and glucose and in postpartal BUN, glucose, and open days ($P < 0.05$). Overall, the MPT (especially BUN) are useful for determining the metabolic nutritional status of breeding cattle herds, particularly those fed home-pasture roughage. Additionally, poor reproductive performance of beef breeding cattle herds probably reflects nutritionally inadequate diet, possibly arising from regional pastureland differences.

The objectives of the third series of experiment, the retrospective study, were (1) to evaluate the relationship between pre- and postpartum metabolic parameters and postpartum reproductive performance, and (2) to infer seasonal characteristics of metabolic parameters from the MPT database of Japanese Black breeding herds. The MPT databases of multiparous cows whose blood samples were collected prepartum and postpartum were divided into two groups according to the calving interval, and each MPT parameter was analyzed (evaluation 1). The same MPT database was divided into two groups according to the sampling period, from July through October (summer; mean air temperature, 26.3°C) and the rest of the year (non-summer; mean air temperature, 14.6°C) (evaluation 2). The main dietary roughage feed in the summer included barnyard grass silage from digitaria, and in the non-summer months, it included Italian ryegrass and oats hay. Significant differences were found in the prepartal total protein and postpartal γ-glutamyltransferase (evaluation 1), and in prepartal and postpartal total protein, albumin/globulin ratio, and glucose (evaluation 2). Clear seasonal differences in MPT results emphasize the usefulness of the MPT in breeding cattle herds fed home-pasture roughage and suggest that unsatisfactory reproductive performance during hot periods reflects inadequate nutritional content of the diet and possibly reduced feed intake due to heat stress.

GENERAL INTRODUCTION

In the reproductive management of beef cattle, two factors are identified to increase productivity, the importance of minimizing the calving-to-conception interval and production of one calf per year per cow. However, reproductive problems including low conception rates and longer calving intervals are arising on a global scale not only in dairy industry (Butler, 2000; Dochi et al, 2010) but also in the beef cattle industry (Burns et al., 2010). Japan is no exception; the average calving interval for Japanese Black breeding cows in Japan is 416 days (Wagyu Registry Association, 2013), and the interval for herds located within the Soo Agriculture Mutual Aid Association (SAMAA) is 403 days (personal com.). The calving interval among SAMAA herds is shorter than Japan's national average, but it is much longer than the target of "one calf per year". In addition, according to the classification of disease types derived from our field database, 46.8% of the diseases among SAMAA within Japanese Black breeding cattle between April 2011 and March 2012 were reproductive diseases, of which most were reproductive failure. In the midst of such issues, SAMAA has been performing regular genital diagnosis of Japanese Black breeding cattle herds in order to improve reproductive performance, but we are still a long way from achieving the target of one calf per year.

Various factors influence the reproductive performance of cattle. In beef cattle, the duration of suckling, reproductive disorders, genetic problems, and maternal nutritional status have all been reported to affect postpartum reproductive performance (Stagg et al, 1998; Short and Adams, 1988; Canfield et al, 1990). Furthermore, the estrous behavior of cows is influenced by many factors such as the season, temperature, number of times the herd is moved, number of feedings, and material of the cowshed floor; concrete cowshed floors have been reported to clearly reduce estrous behavior (Britt et al, 1986; Sakaguchi, 2010). One of the major factors contributing to a prolonged calving-to-conception interval is inadequate nutrition, which often causes sub-clinical and/or clinical metabolic diseases (Hess et al., 2005; Oliveira Filho, et al., 2010). Prepartal and postpartal nutritional status is also related to the interval between calving and first ovulation in beef cattle (Oliveira Filho et al. 2010). It was assumed that certain metabolic parameters, which might reflect the energy and protein status of cows during prepartum and postpartum, are associated with reproductive performance of the breeding cattle herds. Therefore, since many studies indicate that nutrition management is fundamentally important in the reproductive management of breeding, we decided to use metabolic profile test in health monitoring of the herd to determine the nutritional status and reproductive status of SAMAA Japanese Black cattle herds.

Metabolic profile tests (MPT) are blood tests that were first established by Payne et al. (1970) as a tool for assessing metabolic status and aiding to the diagnosis of metabolic disorders in dairy herds. Currently the metabolic profile is considered an extremely useful tool for objective nutritional evaluation of dairy herds in Japan (Kida, 2002). Today, the metabolic profile testing is considered to be a method for objective evaluation of the nutritional status of breeding cattle herds, including Japanese Black cattle, consuming feed of various compositions (Shibano et al., 2009).

While conducting reproductive monitoring using the metabolic profile testing, we discovered a herd that had low blood urea nitrogen (BUN) concentration likely caused by low levels of crude protein in home pasture-roughage feed. The calving-to-conception interval in that herd was greatly reduced by improving the composition of its diet. We report this case as an excellent example of practical application of MPT in Chapter 1. In Chapter 2, we discuss the metabolic profile of Japanese Black cattle. We increased the number of tested herds that exhibited low BUN and further verified the effects of improved diet composition on reproductive performance applying the MPT in farm conditions. Moreover, using the past results of metabolic profile tests of SAMAA Japanese Black breeding cattle herds, we tested for regional differences in MPT parameters. In addition, in light of the results presented in Chapter 1 and Chapter 2, we conducted a retrospective study using the results of the past metabolic profile tests to evaluate the MPT parameters between SAMAA herds with superior reproductive performance and those with poor reproductive performance. The results were used to determine the factors affecting reproductive performance after calving and to assess seasonal differences in MPT parameters and they are presented in Chapter 3.

Chapter 1

A Japanese Black breeding herd exhibiting low blood urea nitrogen: A metabolic profile study examining the effect on reproductive performance

Abstract

Ten reared cows of a Japanese Black cattle herd in Kagoshima prefecture, Japan, exhibited extremely low blood urea nitrogen (BUN) concentration (2.6±0.6 mg/dL). Examination of dietary feed nutrition and relevant pastureland soil content suggested a correlation with crude protein deficiency or unbalanced nutritional dietary feeds. Thirteen months after the introduction of a dietary remedial measure (bean cake supplementation), BUN, total cholesterol, and albumin concentration of the same 5 cows increased significantly compared with values of before the dietary remedy. The postpartum day open period was significantly lower after the dietary remedial measure than that before it. The abnormally low BUN levels of the cattle herd may be due to inadequate dietary nutritional content, primarily from the imbalance of TDN and CP of the feed and far-lower-than-average CP value. In conclusion, routine examination of serum biochemical parameters in Japanese Black breeding cattle may be a useful strategy for determining subclinical metabolic failure of cattle herds, and consequently, its effect on reproductive performance of the herd.

Introduction

The period from calving until conception is the most critical period in a cow's reproductive cycle. Minimizing this period is important for increasing the reproductive potential of breeding cattle. Several factors, including degree of calf suckling, reproductive failure, genetic variation, and level of maternal nutrient intake, are known to affect the postpartum reproductive efficacy of beef cows (Stagg et al., 1998, Sullivan et al., 2009, Oliveira Filho et al. 2010). Inadequate nutrition is a major contributing factor to a prolonged calving-to-conception interval in female beef cattle (Oliveira Filho et al. 2010).

Studies of the metabolic profiles of Japanese Black breeding cattle herds under various feeding management conditions (Yonai et al., 1995, Okada et al., 1999) have revealed a significant relationship between nutritional metabolic status and the occurrence of metabolic diseases or postpartum reproductive performance within each herd. It has been suggested that crude protein (CP) alone in the dietary feeds may reduce the reproductive performance of beef cattle (Sasser et al., 1988). In addition to identifying nutritional problems pertaining to a particular herd, such studies may assist in improving the reproductive efficacy of the herd, when the dietary feed is properly supplemented (i.e., with an order-made therapeutic remedy). To the best of our knowledge, few reports describe the relationship between the occurrence of subclinical metabolic disorders of the herd and the dietary remedial measures designed to manage these disorders, as viewed from the perspective of improvements in reproductive performance in Japanese Black breeding herd at the farm level.

Here, we report on one Japanese Black breeding herd with extremely low blood urea nitrogen (BUN) concentration, presumably originally resulting from imbalanced TDN and CP values in the dietary feed, revealed during our routine examination of the metabolic profiles of 82 cattle herds for a 3-year period. Both BUN concentration and reproductive performance were considerably improved in dietary remedy-treated cattle.

Materials and Methods

Animals and herd:

During our metabolic examinations of 82 cattle herds on a farm within the region of Soo Veterinary Clinical Center, Kagoshima, Japan, we detected 10 cows mean age (±SD) 5.7±3.3 years (range, 2–12 years) and reproductive history of 3.8±2.7 times, without any visible signs of clinical disease. The chief complaint of the herd owner was the poor reproductive performance of the herd. All cows were housed indoors within a pen (5m × 15m), except for a 2-month period before and after parturition, when the cows were housed separately within a smaller pen (3m × 1.5m). All cows were fed with roughages collected from the herd's pastureland and supplemented with 2 concentrated commercial feeds (Concentrate for Breeding Cows and Nidogami, JA Kagoshima, Kagoshima, Japan) twice a day (in the morning and the evening), and the residues of the dietary feed were generally observed in the herd. Feed compositions are detailed in Table 1. Additionally, reproductive records of the herd, especially postpartum day open periods, were inspected to evaluate the reproductive performance of the herd (whether less than 85 days or not) concomitant with the number of treatments for postpartum reproductive disorders.

Blood sampling and serum biochemical analysis of metabolic parameters:

Blood samples from the jugular vein were collected from all 10 cows 2–3 h after morning feeding. Serum was separated within 2 h after collection and stored in a refrigerator until processing. To assess energy balance, the mean body condition score (BCS) of the herd on a scale of 1 (too thin) to 5 (obese) was determined by 2 skilled veterinarians (Kida, 2002).

Biochemical analysis was performed to determine the following parameters: glucose (Glu), free fatty acid (FFA), and total cholesterol (T-Cho) for evaluation of energy metabolism; total protein (TP), serum albumin (Alb), and BUN for protein metabolism; and serum aspartate aminotransferase (AST) and γ-glutamyltransferase (GGT) for liver function (measured on a Labospect 7080 autoanalyzer; Hitachi, Tokyo, Japan). The plasma IGF-1 concentration was determined by enzyme immunoassay using the biotin-streptavidin amplification technique (Kawashima et al., 2007).

Nutritional evaluation of the roughage samples:

Based on the results of serum biochemical examinations, roughage samples foraged by the herd were collected concomitant with the soil samples for the nutritional evaluation of the herd. The nutrient compositions of the roughage, including crude protein (CP), crude fat (CFa), nitrogen-free extract (NFE), crude fiber (CFi), and crude ash (CA) of the roughage, were determined by near-infrared reflecting spectroscopy (NIRS6500, Nireco Corporation, Tokyo, Japan); mineral content [e.g., calcium (Ca), magnesium (Mg), and potassium (K)] was determined by atomic absorption spectrophotometer (HITACHI Z-6100, Hitachi) and phosphate (P) content was determined by a spectrophotometer (UV-1800, Shimadzu Corp., Kyoto, Japan), which was evaluated according to the Japanese Feeding Standard (2008).

Soil examination of the concerned pastureland:

Five soil samples from different pasturelands used by the herd were collected. Soil pH (glass electrode method; Horiba, Kyoto, Japan), electrical conductivity (EC; platinum electrode method; Horiba, Kyoto, Japan), and Mg and Ca concentrations (MgO and CaO; soil plant general analyzer SFP-3; Fujihira Industry Co., Tokyo, Japan) were determined and compared with the normative guidelines on soil diagnosis of Kagoshima Prefecture (2010).

Dietary remedy for the herd based on the examinations and second blood examinations:

Based on the overall results of the biochemical analyses (Radostits et al., 2000) and the nutritional evaluation of the dietary feeds for metabolic parameters of the herd, dietary remedial measures aimed at improving both energy and protein intakes were strongly recommended. We speculated that the reason why the herd had low serum BUN levels might be due to high TDN values (fullness rates; normal periods, 194%; perinatal periods, 184%) of the dietary feeds, concomitant with crude protein deficiency due to low CP value of the feed roughage. The herd owner was advised to modify the feed compositions supplementing the feed diet with bean cake (0.6 kg) shown in Table 1. The bean cake was chosen based on the consideration of both nutritional concerns primarily from the supplementation of protein, and economical aspect for estimating the feed supplementation for long-term periods. Thirteen months after the dietary remedial measure was introduced, the serum biochemical parameters and reproductive performance of the herd were re-examined to control for the seasonal effects of the dietary roughages. Since 5 cows were culled from the herd due to illness or other reasons, blood samples were collected from the remaining 5 cows (mean age±SD, 5.2±2.2 years; range, 3–8 years). In the present study, the results of the blood biochemical examinations were compared within the same 5 cows obtained at the first blood samplings.

Statistical analysis:

The results of serum biochemical analysis, postpartum day open period and frequency of clinical treatment collected before and after the dietary remedial measures were compared within the same 5 cows using the Student's *t* test. P values less than 0.05 were considered to indicate a statistically significant difference.

Results

Serum biochemical analysis, body condition score, and reproductive performance at the first examination of the herd:

The results of mean serum biochemical analyses at the first blood sampling (pre-dietary feed remedy; n=10) are as follows; TP, 7.3±0.3 g/dL; Alb, 3.3±0.2 g/dL; AG, 0.8±0.1; BUN, 2.6±0.6 mg/dL; FFA, 45.9±9.3 μEq/L; T-Cho, 101.6±12.1 mg/dL; Glu, 53.3±3.8 mg/dL; AST, 64.2±5.2 IU/L; GGT, 19.0±7.3 IU/L; IGF-1, 69.9±21.1 ng/ml; Ca, 9.5±0.4 mg/dL; Mg, 1.8±0.2 mg/dL; iP, 4.8±0.7 mg/dL; VA, 110±12 IU/dL; and VE, 363±57 IU/dL. The mean values of all biochemical parameters were within normal ranges, except for the extremely low BUN (2.6±0.6 mg/dL; normal range, 6.0–27.0 mg/dL) and FFA (45.9±9.3 μEq/L; normal range, 150–350 μEq/L) levels.

The mean BCS of the herd was approximately 3.0–3.5, with no clinically remarkable differences compared with that observed in other herds. The day open periods of the herd were 102.7 days, and the postpartum frequencies of treatment for reproductive failure were 8 times per 10 cows.

The nutritional evaluation of the roughage samples and soil of the concerned pastureland were followed by the aforementioned examinations of the herd.

Nutritional evaluation of the roughage samples:

Nutritional analysis of roughage collected at the first sampling is shown in Table 3. Minerals (Ca, Mg, P, K) and nutritional contents (CP and CFa) of the roughage were below the reference range of the Japanese Feed Standard (2008), except for NFE (56.2% as dry matter) and CFi (35.7% as dry matter).

Soil examination of the concerned pastureland:

Results of the soil examination of the pasture of the concerned herd are shown in Table 4. The concentrations of all measured parameters were below the range of the normative guidelines for Kagoshima prefecture.

Serum biochemical analysis, body condition score, and reproductive performance before and after the dietary remedy of the herd:

The results of serum biochemical analyses before and after the dietary feed remedy derived from the same 5 cows are shown in Table 2. After 13 months, the mean concentrations of both BUN (8.8 mg/dL) and T-Cho (134.6 mg/dL) increased significantly compared with values determined at the first blood sampling (2.2 mg/dL and 99.0 mg/dL, respectively; $p<0.01$). Similarly, a significant increase in the mean concentrations of both Alb (3.5 mg/dL) and FFA (68.9 μEq/L) compared with values determined at the first blood sampling (3.3 mg/dL and 43.3 μEq/L, respectively; $p<0.05$) was evident. No significant differences were observed in the other metabolic parameters, including mean serum IGF-1 concentrations, which were within the normal reference ranges.

The mean BCS of the herd at the second blood sampling was statistically comparable to the first blood sampling (approximately 3.0–3.5 for both). In contrast, the reproductive performance of the herd, derived from the herd record of the same 5 cows, was significantly improved with dietary supplementation, where the postpartum day open period following the dietary remedial measure was significantly lower than that observed before the introduction of the dietary remedial measure (47.8±5.7 days versus 82.4±12.1 days, respectively; $p<0.05$). The frequency of treatment for reproductive failure following the dietary remedial measure was 0 times per 5 cows (compared with the frequency of postpartum treatments for reproductive failure of 3 times per 5 cows before the remedy).

Discussion

In the present study, all 10 cows of the herd exhibited abnormally low BUN concentrations before the introduction of the dietary remedial measure, although no clinical symptoms were observed prior to the study period. The present study clearly supports the practical efficacy of our routine examinations of metabolic profiles for breeding herd management, with the potential of detecting subclinical metabolic conditions that may affect the reproductive performance of Japanese Black breeding herds in our region. The significantly improved reproductive performance observed upon use of dietary remedies may suggest a possible strategy for reducing the excessive usage of hormones for treatment of reproductive failures in the cattle breeding industry.

Based on our results of the examination of serum metabolic parameters and nutritional examination of the dietary feeds, we hypothesized that cows in the herd suffered from a subclinical metabolic disorder originating from unbalanced dietary feeds, specifically from (1) an unbalance of TDN/CP ratio [i.e., the fullness rate of CP (106%) of the dietary feed before the feed remedy was approximately half the ratio compared with TDN (194%)] and from (2) the poor nutritional quality of the roughage fed to the herd (i.e., the relatively low levels of CP and dry matter compared with each reference levels of Japanese Feed Standard).

Examinations of serum metabolic parameters before and after dietary remedy clearly showed a significant increase in mean levels of BUN and Alb in the herd within the normal range of cattle. Although the total mean volume of the dietary feeds after the remedial measure decreased from the initial pre-remedial volume, the results of BCS of the herd were not significantly different between the two time points. It has been shown that BUN level is a sensitive indicator of the balance between available digestible CP and energy fed to a ruminant (Kenny et al., 2002), which can assist in the measure of the efficiency of protein utilization (Manston et al., 1975, Kohn et al., 2002). Therefore, the results of the present study strongly support the findings of previous reports concerning the existence of a strong relationship between the protein/energy metabolism and serum BUN concentration.

In the present study, the high normal BUN concentration observed after introduction of the feed remedy could be due to increased CP content in the feed (pre-remedy; 106%, post-remedy; 134%). It has also been suggested that the nutritional status is reflected in changes in body weight and BCS, including the levels of metabolites and hormones in blood, which may affect the relationship between energy balance and postpartum reproductive physiology (Astessiano et al., 2012). Moreover, the FFA level after the dietary remedy increased (68.8 μEq/L) significantly compared with the FFA level before the remedy (43.3 μEq/L), although both were below the normal range of FFA concentration. Since it has been reported that FFAs are the blood metabolites most directly associated with energy balance as a negative signal (Herdt, 2000, Hess et al., 2005), the reason for this increased FFA concentration after the dietary remedy is not clear from the present results. Further studies are required to clarify these contradictory observations.

IGF-1 is suggested to play a pivotal role in cattle fertility, acting as a monitoring signal that allows reproductive events to occur when the nutritional conditions for successful reproduction are fulfilled (Velazquez et al., 2008). In a previous study, nutritional status was not implicated in the regulation of plasma IGF-1 levels during the estrous cycle of Japanese

Black cows (Kawashima et al., 2007). In the present study, the serum IGF-1 concentration of the cows pre- and post-supplementation were statistically comparable, suggesting a lack of IGF-1 metabolic monitoring in Japanese Black cattle. Further investigation is required to complete this evaluation.

To supplement our finding of metabolic profiles, with the normative guidelines on soil diagnosis of Kagoshima prefecture used as a reference, we also examined the soil conditions of the pasturelands that were foraged by the herd. Soil analysis revealed that the soil parameter values were below the reference value, especially the pH, EC, and mineral contents. Soil pH affects the availability of plant nutrients and the solubility of soil minerals, and declining pH is generally a sign of inefficient nitrogen consumption where ammonia-based fertilizers are used (Smith and Doran, 1996, USDA, 2001). Additionally, it is generally accepted that soil EC is a measurement that correlates with soil properties affecting crop productivity. The EC measurement detects the amount of cations or anions in the soil, and the useful relationship between the EC readings and soil nitrate (NO_3^-) concentrations has been established (Smith and Doran, 1996, USDA, 2001). Because the soil NO_3^- is a form of inorganic nitrogen that is available for use by plants, we presume that the low pH value of the pasture in the present study may have resulted in minimal absorption of both NO_3^- and another minerals from the soil to the Italian ryegrass, which is clearly reflected in the low EC value, affecting crop productivity. Moreover, the fertility of the examined pastureland was too poor for the suitable growth of Italian ryegrass, which was reflected in the low CP and mineral contents of the roughage samples consumed by the present herd. The present study strongly suggests the importance of including soil parameter analysis of pastureland concurrent with the examination of metabolic profiles of the cattle herd fed by roughage from the same pastureland.

Finally, there is strong interest in determining which factors or interventions influence herd pregnancy rates. Pre- and postpartum nutritional levels are the most important variables for reducing the interval between parturition and first ovulation (Short and Adams, 1988, Hess et al., 2005). Weight gain and body condition during these periods is extremely important to reduce postpartum anestrus in beef cattle (McSweeney et al., 1993, Hess et al., 2005). Several studies have reported a direct negative effect of high BUN on fertility derived from changes in the uterine environment due to alterations of uterine pH (Elrod and Butler, 1993a, Elrod et al., 1993b, Ferguson et al., 1993) and changes in the concentration of urea, Mg, K, P, and Zn in uterine fluid (Jordan et al., 1983). Moreover, a prior study has reported a reduction in reproductive performance occurring as a result of CP deficiency in beef cattle (Sasser et al., 1988). In the present study, improved reproductive performance concomitant with increased BUN levels probably reflected a restoration of adequate protein intake by the cattle with subclinical metabolic failure, suggesting a relationship between protein/energy balance and reproductive efficacy of beef cattle herd.

In conclusion, the results of our field investigation indicated that routine examination of metabolic profiles on Japanese Black breeding cattle herd, especially for herds fed on roughage from their own pastureland, may be a useful strategy for determining the subclinical metabolic conditions of cattle herd with normal BCS under the saturated feed volumes conditions. We confirmed that the inadequate nutritional balance/content of the dietary feeds, possibly arising from the conditions relating to a particular soil type of the pastureland, may considerably affect the reproductive performance of beef breeding cattle herd; therefore, the soil-plant-animal relationship must be taken into account for herd management of Japanese Black breeding cattle.

Table 1. Composition of feed provided to the Japanese Black breeding herd at first blood sampling (before remedy of dietary feeds) and second blood sampling (13 months after the first sampling)

	Usual period		Perinatal period	
Feeds	Before remedy	After remedy	Before remedy	After remedy
Italian ryegrass silage (kg)	12	7	12	7
Concentrate 1 (kg)	1.8	0	2	0
Concentrate 2 (kg)	0	2	2	3
Bean cake (kg)	0	0.6	0	0.6
Fullness rate *				
Dry Matter (%)	144	106	147	105
TDN (%)	194	140	184	129
CP (%)	106	134	115	116
NFE (%)	272	163	227	145

Concentrate 1: Concentrate for breeding cows (JA Kagoshima, Kagoshima, Japan)

Concentrate 2: Concentrate for breeding cows (Nidogami; JA Kagoshima, Kagoshima, Japan)

TDN: Total digestible nutrients, CP: Crude protein, NFE: Nitrogen-free extract

*: Fullness rate in the case of 100% dry matter intake

Table2. Results of the serum biochemical analyses (mean±SD) of the same 5 cows before and after the remedy of the dietary feeds

Parameters for protein metabolism			Parameters for energy metabolism		
	Before (n=5)	After (n=5)		Before (n=5)	After (n=5)
TP (g/dL) (Range)	7.2±0.2 (6.8–7.4)	7.5±0.2 (7.3–7.8)	FFA (μEq/L)	43.3±8.4[c] (33.0–53.1)	68.8±21.7[d] (39.8–92.4)
Alb (g/dL)	3.3±0.2[c] (3.1– 3.5)	3.5±0.2[d] (3.4–3.8)	T-Cho (mg/dL)	99.0±13.5[a] (83.1–116.1)	134.7±16.8[b] (114.1–163.1)
AG	0.8±0.1 (0.7–1.0)	0.9±0.1 (0.8–1.1)	Glu (mg/dL)	53.5±3.7 (48.8–58.4)	55.3±0.7 (54.5–56.2)
BUN (mg/dL)	2.2±0.5[a] (1.6–3.0)	8.8±1.0[b] (7.9–9.8)			

Liver functions			Liver metabolism		
	Before (n=5)	After (n=5)		Before (n=5)	After (n=5)
AST (IU/L)	65.6±5.6 (56.9–71.3)	68.3±11.6 (49.1–79.9)	IGF-1 (ng/mL)	77.7±26.0 (42.6–113.4)	52.5±6.9 (45.9–62.4)
GGT (IU/L)	21.9±10.3 (16.0–40.1)	21.7±9.3 (14.8–40.1)			

Minerals			Vitamins		
	Before (n=5)	After (n=5)		Before (n=5)	After (n=5)
Ca (mg/dL)	9.6±0.5 (8.8–10.0)	9.7±0.5 (8.9–10.3)	VA (IU/dL)	111.8±10.3 (102.0–126.0)	123±13 (113–146)
Mg (mg/dL)	1.8±0.1 (1.7–1.9)	1.8±0.1 (1.6–1.9)	VE (IU/dL)	348.0±63.1 (258.0–412.0)	470±123 (336–642)
iP (mg/dL)	5.2±0.6 (4.6–6.1)	4.7±0.3 (4.2–5.0)			

a-b; $p<0.01$, c-d; $p<0.05$

Reference range: TP 5.7–8.1; Alb 2.1–3.6; BUN 6–27; AST 45–110; GGT 11–25; FFA 150–350; T-Cho 100–180; Glu 45–75; Ca 9.7–12.4; Mg 1.8–2.3

Table 3. Results of nutritional examination of roughage of the herd

	Dry rate	TDN (%)	DCP (%)	CP (%)	CFa (%)	NFE (%)	CFi (%)	CA (%)	Ca (%)	P (%)	Mg (%)	K (%)
Wet matter	40.9	38.5	1.5	2.2	1.2	33.3	21.1	1.4	0.10	0.11	0.07	1.23
Dry matter	59.1	65.1	2.6	3.7	1.9	56.2	35.7	2.4	0.17	0.18	0.12	2.08
Reference (dry matter)*		60.6	8.9	12.7	4.1	37.7	32.5	13.0	0.42	0.27	0.22	3

TDN: Total digestible nutrients, DCP: Digestive crude protein, CP: Crude protein, CFa: Crude fat, NFE: Nitrogen-free extract,

CFi: Crude fiber, CA: Crude ash

*: From the Japanese Feeding Standard (2008)

Table 4. Results of the soil examination of the pasture of concerned herd

Soil No.	pH	EC (mS/cm)	CaO (mg/100g)	MgO (mg/100g)
1	5.1	0.08	119	17
2	5.8	0.08	149	21
3	5.6	0.09	88	24
4	5.5	0.07	103	24
5	5.4	0.07	36	9
Reference range*	6.0–6.5	0.3–0.5	231–252	30–45

EC: Electric conductivity, CaO: Calcium, MgO: Magnesium

*: From the normative guidelines on soil diagnosis of Kagoshima prefecture (2010).

Chapter 2

Metabolic Profile of Japanese Black Breeding Cattle Herds: Usefulness in Selection for Nutrient Supplementation to Enhance Reproductive Performance and Regional Differences

Abstract

The study aims were (1) to confirm the effects of nutritional improvement in prepartal and postpartal periods, monitored using the serum metabolic profile test (MPT) and reproductive performance, and (2) to clarify regional characteristics of the MPT results within our region by using our MPT database. Experiment 1: Among 42 breeding cattle herds in our region mainly fed home-pasture roughage, 3 experimental herds showing subnormal BUN levels were selected and compared with 1 representative excellent herd. Dietary remedial measures were implemented from feed analysis in each herd. Blood urea nitrogen (BUN) concentration in all 3 herds increased significantly, and open days postpartum in 2 of the herds were significantly reduced, compared with values before dietary supplementation. Experiment 2: Thirty-seven herds within our region were grouped into 3 categories (Area 1, 2, and 3) by location and soil condition of the herd pastureland. The MPT and reproductive performance in cows whose blood samples were collected at both prepartum (60–20 days before calving) and postpartum (30–90 days after calving) were compared among the 3 areas. Significant regional differences were found in prepartal albumin, total cholesterol, BUN, and glucose and postpartal BUN, glucose, and open days ($P<0.05$). Overall, the MPT (especially BUN) might be useful for determining the metabolic nutritional status of breeding cattle herds, particularly those fed home-pasture roughage. Additionally, poor/unsatisfactory reproductive performance of beef breeding cattle herds probably reflects inadequate nutritional content of the diet, possibly arising from regional pastureland differences.

Introduction

For optimal beef breeder production, a calving interval of 1 year must be maintained. Thus, the first postpartum estrus must occur within 80 to 85 days after parturition, and this is closely associated with the postpartum interval to first ovulation (Sullivan et al., 2009). Several factors, such as the extent of calf suckling, reproductive failure, genetic variation, and level of maternal nutrient intake, affect postpartum reproductive performance of beef cows (Sasser et al., 1988; Short and Adams, 1988; Stagg et al., 1998). Among them, one of the major factors contributing to a prolonged calving to conception interval is inadequate nutrition, which often causes subclinical and/or clinical metabolic diseases (Hess et al., 2005; Oliveira Filho, et al., 2010). Prepartal and postpartal nutritional statuses are also related to the interval between calving and first ovulation in beef cattle (Oliveira Filho et al. 2010). Thus, it was assumed that certain metabolic parameters, which might reflect the energy and protein status of cows during prepartum and postpartum, would be associated with reproductive performance of breeding cattle herds.

Payne et al. (1970) first established the metabolic profile as a tool for assessing metabolic status and aiding the diagnosis of metabolic disorders in dairy herds, and currently the metabolic profile is considered an extremely useful tool for objective nutritional evaluation in dairy herds in Japan (Kida, 2002). Though some researchers have indicated that the results of the metabolic profile test (MPT) such as total cholesterol (T-Cho) and blood urea nitrogen (BUN) determined from serum biochemical analysis may be used to improve feeding management, detect subclinical health problems, and prevent production problems of the Japanese Black breeding herds (Yonai et al., 1995; Okada et al., 1999), its practical application has mainly focused on the management of dairy cattle. This may be due to the lack of reference values for each metabolite based on blood biochemical data derived from a reliable number of samples and/or the wide variations in feeding management among herds in the same region/locality, which could ultimately affect the results of the MPT. On the other hand, in rural areas of Japan, such as in the study area included in the current study (the Osumi Peninsula, Kagoshima Prefecture, Japan), most of the breeding beef cattle herds are fed hays collected from their home pastureland. Recently, the importance of this beef-forage production system of Japanese Black cattle has been re-recognized because it contributes to improved nutrient utilization (ecological aspect) and reduced cost of commercial feeds (economical aspect) (Tabata et al., 2009; Sithyphone, et al., 2011). However, no evaluative study has been conducted to investigate the nutritional adequacy of forage collected from the home pastureland. Therefore, it was hypothesized that the MPT might be useful for detecting nutritional inadequacies reflected as subclinical metabolic and reproductive disorders in the respective herds.

Recently, during our routine assessments of metabolic profiles of farms in our region, we detected one herd in which all reared cows exhibited abnormal BUN levels (mean, 2.2 mg/d*l*; range, 1.6–4.0 mg/d*l*) without any clinical symptoms. It was hypothesized that this observation might be due to crude protein (CP) deficiency in the roughage (Watanebe et al., 2012). Therefore, regional differences of the MPT due to nutritional quality of fed roughage harvested from the home pastureland were assumed to exist.

In the present study, to confirm the result of our previous report (Watanabe et al., 2012a) with the inclusion of additional herds, the effect of nutritional improvement on the prepartal and postpartal MPTs and reproductive performance in each herd was evaluated. Furthermore, we used our database to evaluate the metabolic profile of the herds according to their location in our region–Kanoya, Soo, and Shibushi Cities; Kagoshima Prefecture; Japan.

Materials and Methods

Experiment 1

Evaluation of the usefulness of metabolic profiles for breeding herd management

Animals and herds

Private Japanese Black breeding herds under the region of Soo Veterinary Clinical Center, Soo Agriculture Mutual Aid Association, Kagoshima Prefecture, Japan, were identified for this study. From our database, the metabolic profiles of 42 Japanese Black breeding cattle herds collected from June 2010 through May 2012 were evaluated. The cows of all herds were mainly fed roughage harvested from the home pasture of each herd and available commercial concentrates supplemented with certain vitamins and minerals. We previously reported the usefulness of metabolic profiles in 1 herd in which all reared cows exhibited abnormally low BUN levels (Watanabe et al., 2012a). Therefore, in the present investigation, to validate the results of our MPT system, we selected 3 herds (experimental herds) in which the metabolic profile, particularly BUN concentration, and reproductive performance, such as number of open days postpartum and frequency of treatments for reproductive disorders, were suggestive of subclinical metabolic disorder during the sampling period. Additionally, 1 herd was selected as a representative model of herd excellence on the basis of the same selection parameters for comparison with the experimental herds. These herds were located in the same area (Soo City, Kagoshima Prefecture) and selected on the basis of standard normal reference ranges for the serum biochemical parameters (Radostits et al, 2000).

Blood sampling and serum biochemical analysis of metabolic parameters:

In all cases, blood sampling and serum biochemical analysis were conducted according to our previous study protocol (Watanabe et al., 2012a), which depended on herd size. All cows were sampled in herds in which less than 20 cows were fed, while approximately 10 cows were samples from each of the prepartal and postpartal periods in herds in which more than 20 cows were fed. Briefly, blood samples were collected from the jugular vein of selected cows of each herd after morning feeding, and serum was separated within 2 hr after collection and stored in a refrigerator until processing. Biochemical analysis was performed to determine the following parameters: glucose (Glu), free fatty acids (FFA), and T-Cho for evaluation of energy metabolism and total protein (TP), serum albumin (Alb), albumin/globulin ratio (A/G), and BUN for protein metabolism (measured on a Labospect 7080 autoanalyzer; Hitachi, Tokyo, Japan). In the present study, only the cows whose blood samples were collected at both prepartum (between 60 and 20 days before parturition) and postpartum (between 30 and 90 days after parturition) were selected for data analysis of Herds 2, 3, and 4 because the nutritional condition of breeding cattle during both prepartum and postpartum may have marked effects on the duration of the postpartum interval from calving to first estrus (Oliveira Filho et al. 2010).

To assess energy balance, the mean body condition score (BCS) of the herd on a scale of 1 (too thin) to 5 (obese) was also determined by 2 skilled veterinarians (Kida, 2002).

Nutritional evaluation

At the time of blood sampling, roughage samples foraged by each herd as well as any fed concentrates were collected for nutritional evaluation of the selected herds. The nutrient compositions of the roughage were measured according to our previous report by near-infrared reflecting spectroscopy (NIRS6500; Nireco Corporation, Tokyo, Japan) (Watanabe et al., 2012a), which was verified according to the Japanese Feeding Standard (Agriculture, Forestry and Fisheries Research Council Secretariat 2008), and nutrient ingredients in the roughage were calculated as dry matter (DM), total digestible nutrients (TDN), and CP.

Dietary recommendations for the herds based on the biochemical and nutritional analyses and repeat blood tests

On the basis of the biochemical analyses and nutritional evaluation of the dietary feeds of the 4 herds, dietary remedial measures aimed at improving both energy and protein intakes were implemented according to our previous report (Watanabe et al., 2012a). Briefly, the herd owners were advised to modify the feed composition by supplementing the diet with bean cake (0.6 kg).

Several months after this dietary remedial measure was introduced, the serum biochemical parameters and reproductive performance of the herds were reexamined as described above. Again, only the blood samples collected during the prepartal and postpartal stages were selected for the evaluation of serum biochemical parameters. Additionally, reproductive performance, such as the frequency of clinical treatments for reproductive disorders and open days, of the selected herds before and after the introduction of the remedial measure was evaluated.

Experiment 2

Evaluation of the metabolic profile of breeding herds according to location in our practice region

Japanese Black breeding herds and cows

A total of 37 private Japanese Black breeding herds under the region of Soo Veterinary Clinical Center, Soo Agriculture Mutual Aid Association, Kagoshima Prefecture, Japan, were identified for this study. Cows included in this study were of different ages and in different states of the reproductive cycle without any visible signs of clinical disease. All cows were housed indoors and fed roughage collected from the home pastureland and supplementary concentrate purchased from various feed companies; however, feeding and management varied among herds. The herds were grouped into 3 categories (Area 1, Kanoya City; Area 2, Soo City; and Area 3, Shibushi City) according to the soil characteristics and the geographic location of the property within our region (Figure 1). Briefly, the pasturelands for cattle herds in Area 1 were cultivated lands for growing crops on which roughages were grown after harvesting, while the pasturelands of herds in Area 2 and 3 were used only for growing roughages; thus, the pasturelands in Area 1 were considered well-managed fertilized soil (with the use of both compost and slack or dolomitic lime) compared with pasturelands of Area 2 and 3 (with the use of compost only).

Blood collection, metabolic profile parameters, and data analytical methods

According to the request of the herd owners, blood collections were scheduled to incorporate all seasons from October 2010 through December 2011. Blood samples were collected from the jugular vein in plain vacuum tubes as described above. Serum was separated from the blood within 2 hr after collection and stored in a refrigerator until processing. The following biochemical parameters were determined: Glu, FFA, and T-Cho for evaluation of energy metabolism; TP, Alb, AG, and BUN for protein metabolism; and serum aspartate aminotransferase (AST) and γ-glutamyltransferase (GGT) for liver function (measured on a Labospect 7080 autoanalyzer; Hitachi). For the same reason as described above, cows whose blood samples were collected at both prepartum (between 60 and 20 days before calving) and postpartum (between 30 and 90days after calving) were selected for evaluation of the metabolic status of each herd by using the results of serum biochemical analysis.

Additionally, reproductive performance data were obtained from each herd record at the same time as blood sampling; data included postpartum open days during the postpartal period to the time of pregnancy confirmation in individual cows. BCS of each cow was also determined to assess energy balance at the time of blood sampling of the herd.

Statistical Analysis

The results of serum biochemical analysis, open days postpartum, and frequency of clinical treatments before and after implementation of the dietary remedial measure were compared using the Student's *t* test and *Chi*-square test. The results obtained for each herd are expressed as the mean ± SD. The results of serum biochemical analysis collected both in the prepartal and in the postpartal periods were compared among sampling areas by ANOVA using the General Linear Model function of SAS. P values less than 0.05 were considered to indicate a statistically significant difference, while P values less than 0.1 were considered to indicate a significant tendency.

Results

Experiment 1

Body condition score, serum biochemical analysis and reproductive performance before and after dietary supplementation in each herd

Four herds from the same area were selected for this evaluation, including 1 herd with excellent reproductive performance (Herd 1: No. of breeding cows, n=55) and 3 herds as experimental herds (Herd 2, n=21; Herd 3, n=115; and Herd 4, n=20), according to baseline metabolic profiles. The compositions of feeds provided to the 4 herds both before and after dietary supplementation are shown in Table 1, and the results of serum biochemical analysis and reproductive performance in each herd before and after dietary supplementation are shown in Table 2.

The results of biochemical analyses of Herd 1, the representative excellent herd, were all within the normal ranges, with a BUN value of 18.7 mg/dl. The open days postpartum was 47.2, and the number of treatments for reproductive disorders during the postpartum period was only 1 in the herd of 6 cows. The mean BCS of Herd 1 was 3.0–3.5; further, the mean BCS at the second blood sampling was statistically comparable to that at the first blood sampling in the 3 experimental herds (approximately 3.0–3.5 for both).

In Herd 2, BUN concentration was significantly increased after dietary supplementation (before, 8.6 mg/d*l*; after, 12.5 mg/d*l*), but no significant differences were observed in the other serum biochemical parameters. The reproductive performance of Herd 2 was significantly improved; the number of open days postpartum following the dietary remedial measure (57.9 days) was significantly decreased compared with that observed before the introduction of the dietary remedial measure (73.5 days). Additionally, the frequency of treatments for reproductive failures following the dietary remedial measure (3 times per 14 postpartum cows) tended to decrease significantly (P=0.053) compared with that before the introduction of the dietary remedial measure (8 times per 14 cows).

In Herd 3, regarding the parameters for protein metabolism, significant increases were observed in Alb (before, 3.3 mg/d*l*; after, 3.5 mg/d*l*) and BUN (before, 10.6 mg/d*l*; after, 13.6 mg/d*l*) and regarding the parameters for energy metabolism, significant increases were observed in T-Cho (before, 100.8 mg/d*l*; after, 145.1 mg/d*l*) and Glu (before, 49.3 mg/d*l*; after, 61.3 mg/d*l*) and a significant decrease was observed in FFA (before, 232.4 μEq/*l*; after, 64.3 μEq/*l*). Although no significant differences were found, the reproductive performance of Herd 3 was improved; the open days postpartum following the dietary remedial measure (64.0 days) was decreased compared with that observed before the introduction of the dietary remedial measure (76.2 days). Additionally, the frequency of treatments for reproductive failures following the dietary remedial measure (1 time per 8 postpartum cows) tended to decrease significantly (P=0.06) compared with that before the introduction of the dietary remedial measure (5 times per 9 cows).

In Herd 4, regarding the parameters for protein metabolism, significant decreases were observed in TP (before, 7.7 mg/d*l*; after, 7.3 mg/d*l*) and Alb (before, 3.6 mg/d*l*; after, 3.4 mg/d*l*) and a significant increase was observed in BUN (before, 6.5 mg/d*l*; after, 9.8 mg/d*l*) after remedy supplementation. Regarding the parameters for energy metabolism, a significant increase was observed in FFA (before, 73.1 μEq/*l*; after, 114.4 μEq/*l*) but T-Cho (before, 138.2 mg/d*l*; after, 111.1 mg/d*l*) was significantly decreased after supplementation. The reproductive performance of Herd 4 was significantly improved; the open days postpartum following the dietary remedial measure (77.3 days) was significantly decreased compared with that observed before the introduction of the dietary remedial measure (122.5 days). Additionally, the frequency of treatments for reproductive failures following the dietary remedial measure (2 times per 13 postpartum cows) was reduced without significance compared with that before the introduction of the dietary remedial measure (4 times per 13 cows).

Experiment 2

Serum biochemical analysis and open days postpartum of cattle herds derived from 3 different regions

Data from the cows whose samples were collected at prepartum

The results of serum biochemical analyses derived from cattle herds in the 3 regions whose samples were collected at prepartum are shown in Table 3. Significant differences were observed in Alb (Area 1, 3.4 g/d*l*; Area 2, 3.2 g/d*l*), T-Chol (Area 2, 103.8 mg/d*l*; Area 3, 121.4 mg/d*l*), BUN (Area 1, 11.3 mg/d*l*; Area 2, 8.4 mg/d*l*; and Area 3, 9.6 mg/d*l*) and Glu (Area 1, 54.2 mg/d*l*; Area 2, 47.6 mg/d*l*; and Area 3, 49.5 mg/d*l*). The mean open days postpartum did not differ significantly among the 3 regions.

Data from the cows whose samples were collected at postpartum

The results of serum biochemical analyses derived from cattle herds in the 3 regions whose samples were collected at postpartum are in Table 4. Significant differences were observed in BUN (Area 1, 10.4 mg/d*l*; and Area 2, 8.3 mg/d*l*) and Glu (Area 1, 59.0 mg/d*l*; and Area 2, 52.8 mg/d*l*). A significant difference was observed in the open days postpartum between Area 1 (75.9 days) and Area 2 (113.4 days).

Discussion

Results of the present study suggest that (1) nutritional supplementation (mainly bean cake in the present study) based on the results of the MPT might help to improve the reproductive performance of breeding herds with energy/CP deficiency compared with excellently managed herds in our region and (2) nutritional metabolic profile parameters differ according to area in breeding cows in our region.

In the present study, we investigated the usefulness of the MPT to enhance reproductive performance. It has been reported that prepartal and postpartal nutritional levels are the most important factors for decreasing the interval between parturition and first ovulation (Short and Adams, 1988; Hess et al., 2005), and weight gain and body condition during these periods are extremely important to decrease postpartum anestrus in beef cattle (McSweeney et al., 1993; Hess et al., 2005). The results of the present study comprising 3 herds (Herds 2, 3, and 4), in which we evaluated the MPT results before and after dietary supplementation, clearly indicated the shortening of the postpartal open-day period in each herd, concomitant with not only increased BUN concentration but also decreased FFA concentration, except for Herd 4, which probably reflected an upturn of adequate protein intake and positive energy balance of the cows. Additionally, improved reproductive performance with decreased treatments for reproductive failure was observed in the present study, even though calving intervals were less than 1 year before the dietary remedy was implemented in all the experimental herds. Although hormonal treatments for reproductive failure of cattle are important tools to control breeding or remedy reproductive failure and proper use of hormones does not have any known negative effect on animal welfare or public health to date, it must be emphasized that animal health and fertility should be improved by good management rather than by extensive use of hormones (Refsdal, 2000). Therefore, on the basis of the results from the albeit limited number of herds together with our previous report (Watanabe et al., 2012a), we concluded that metabolic profile examination for detecting subclinical metabolic failure of breeding herds together with appropriate nutritional supplementation is an effective and essential tool for on-farm breeding cattle herds.

Although reports exist regarding the application of examination of metabolic profiles for Japanese Black breeding cattle (Yonai et al., 1995; Okada et al., 1999), most of the studies were conducted in intensive herds or under experimental controlled feeding management conditions and, therefore, might not be representative of commercial beef herds reared under various feeding and grazing management systems, especially in Japanese Black breeding cattle herds fed home-pasture roughage. We previously reported one Japanese Black breeding herd, in which all cows exhibited abnormal BUN concentrations (mean, 2.2 mg/d*l*; range, 1.6–4.0 mg/d*l*), and indicated that the routine examination of metabolic profiles in breeding cattle herds, especially those fed roughage from their home pastureland, might be a useful tool for identifying subclinical metabolic failure in the herd. Additionally, we suggested that poor reproductive performance of the breeding herd is caused by inadequate nutritional content of the roughage arising from the conditions relating to a particular soil type of the pastureland (Watanabe et al., 2012a). BUN has been shown to be a sensitive indicator of the balance between amount and availability of digestible CP and energy fed to ruminants (Kenny et al., 2002), and BUN helps measure efficiency of protein utilization (Manston et al., 1975; Kohn et al., 2002). On the other hand, some studies have reported a negative effect of high BUN on fertility in beef cattle, while others have indicated reduced conception risks when milk urea nitrogen was either very low or very high in dairy cattle (Pehrson et al., 1992; Carlsson and Pehrson, 1993). Although we did not examine both roughage and soil conditions of the pastureland of each herd in this study, biochemical analyses from breeding herds in our region may reflect regional differences of protein/energy metabolism of each cattle herd possibly due to nutritional differences of the roughage harvested from the home pasturelands. Additionally, although the herds from all 3 areas included cows with abnormally low BUN and Glu levels, approximately half of the examined herds in Area 2 included cows with abnormally low BUN (<6 mg/d*l*) and Glu (<45 mg/d*l*). Therefore, the present study also indicates the practical usefulness of our MPT during both the prepartal and the postpartal periods to detect herds with subclinical metabolic problems. Further epidemiological investigation for clarifying the relationship among the serum metabolic profile, nutritional content of the fed roughage, and soil quality according to the geographic area of our region in the Osumi Peninsula in Kyushu, Japan, is warranted.

In conclusion, the results of our field investigation confirmed that the routine examination of metabolic profiles, especially BUN, of breeding cattle herds particularly fed on roughage from home pastureland, is a useful tool for determining subclinical metabolic disorders of the cattle herd with normal BCS under saturated feed volume conditions. Additionally, it was strongly suggested that the occurrence of poor/unsatisfactory reproductive performance of the beef breeding cattle herd might be due to inadequate nutritional content of the diet, possibly arising from regional differences of pastureland conditions; thus, appropriate feeding management based on objective evaluation of the dietary feeds, especially the roughage from home pastureland, may improve reproductive performance of the breeding cattle herds in our region.

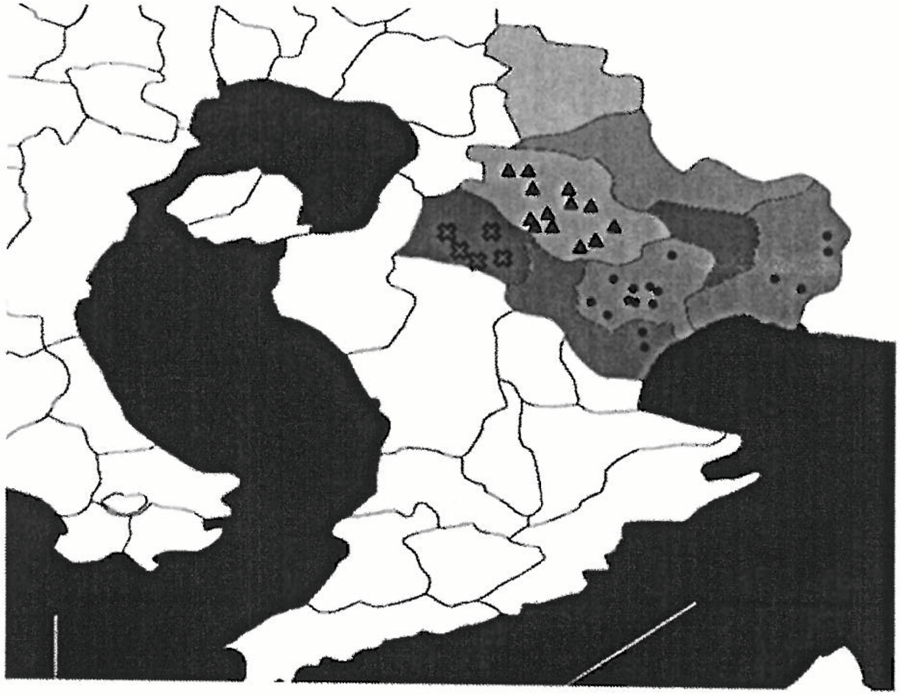

Figure 1. The 3 regions of the sampled herds in Osumi Peninsula, Kagoshima Prefecture, Japan.

×: Area 1, △: Area 2 and ●: Area 3

Table 1. Composition of feed provided to the Japanese Black cattle herds kept for breeding purposes

Herd		Forage feed	Concentrate feed					Fullness rate#		
		kg	Total kg	Bran %	Cereal %	Oil seed meal, %	Other %	DM %	TDN %	CP %
1	Before*	Italian ryegrass + oats hay 8	2	15	24	5	56	101	120	151
2	Before	Italian ryegrass 6	2	33	47	15	5	96	78	60
	After	Italian ryegrass 6	2	33	47	15	5	100	99	105
3	Before	Italian ryegrass 8.9	0.5	41	43	12	4	99	96	106
	After	Italian ryegrass 8.9	0.5	41	43	12	4	110	110	145
4	Before	Barnyard grass silage 8 + Bermuda straw 2.5 + sorghum 2.4	2	15	24	5	56	112	122	130
	After	Same as before	2	15	24	5	56	117	132	153

*: before the dietary remedial measure, **: after the dietary remedial measure

DM: dry matter, TDN: total digestible nutrients, CP: crude protein

#: fullness rate in the case of 100% dry matter intake

Table 2. Summaries of serum biochemical analyses and reproductive records (mean ± SD) of selected Japanese Black herds in the same area before and after dietary supplementation

	Herd1	Herd 2		Herd 3		Herd 4	
Main diagnoses of feed analysis	ND	TDN and CP insufficient for demand		TDN and CP insufficient for demand		CP insufficient for demand	
Follow-up period		2 months		6 months		6 months	
	Before*	Before	After**	Before	After	Before	After
No. of cows	5	6	4	5	4	13	14
TP (g/dL)	7.0±0.3	7.9±0.9	7.7±0.6	7.4±0.5	7.3±0.7	7.7±0.3[a]	7.3±0.4[b]
Alb (mg/dL)	3.5±0.4	3.5±0.2	3.7±0.3	3.3±0.1[a]	3.5±0.1[b]	3.6±0.2[a]	3.4±0.2[b]
AG	1.0±0.1	0.8±0.1	0.9±0.1	0.8±0.2	1.0±0.2	0.9±0.1	0.9±0.2
FFA (mmol/L)	98.8±30.9	142.7±93.3	68.8±13.0	232.4±132.8[a]	64.3±51.0[b]	73.1±32.3[a]	114.4±57.9[b]
T-Cho (mg/dL)	123.2±14.8	114.9±30.2	91.9±5.1	100.8±20.5[a]	145.1±17.3[b]	138.2±33.8[a]	111.1±16.2[b]
BUN (mg/dL)	18.7±1.6	8.6±2.3[a]	12.5±1.6[b]	10.6±2.1[a]	13.6±1.2[b]	6.5±1.0[a]	9.8±1.3[b]
Glu (mg/dL)	52.3±3.0	45.0±2.4	44.5±4.6	49.3±4.2[a]	61.3±3.0[b]	57.7±4.1	56.8±3.5
Days open (days)	47.2	73.5±16.0[a]	57.9±15.9[b]	76.2±15.4	64.0±9.6	122.5±56.6[a]	77.3±48.4[b]
Treatment for repro disorder (time/head)	1/6	8/14[c]	3/14[d]	5/9[c]	1/8[d]	4/13	2/13

Herd 1; representative of an excellent cattle herd, Herds 2, 3, and 4; experimental herds selected according to the results of serum biochemical analysis.

*: before the dietary remedial measure, **: after the dietary remedial measure

a-b: significantly different within the same herd (P<0.05), c-d: significantly different within the same herd (P<0.1)

Table 3. Serum biochemical analyses (mean±SD) and postpartum open days of the herds derived from different regions of Our region in the Osumi Peninsula whose blood samples were collected during the prepartum

	Area 1	Area 2	Area 3
No. of herd	9	4	19
No. of cows	30	16	64
TP (g/dL)	7.3±0.5	7.1±0.5	7.3±0.3
Alb (mg/dL)	3.4±0.2[a]	3.2±0.4[b]	3.3±0.3
AG	0.9±0.1	0.8±0.2	0.8±0.2
GOT (U/L)	53.2±11.4	53.7±11.5	53.5±10.0
GGT (U/L)	15.9±3.5	17.1±3.1	16.9±3.8
FFA (mmol/L)	208.7±254.9	206.9±153.9	206.2±164.2
T-Cho (mg/dL)	113.1±21.9	103.8±20.1[a]	121.4±27.5[b]
BUN (mg/dL)	11.3±3.1[a]	8.4±4.4[b]	9.6±3.5[b]
Glu (mg/dL)	54.2±6.5[a]	47.6±8.0[b]	49.5±8.0[b]
Day open (days)	70.6 (n=20)	78.8 (n=15)	98.0 (n=41)

Prepartum: blood sampling was conducted between 60 and 20 days before calving

a-b: significant difference within the same row ($P<0.05$)

Table 4. Serum biochemical analyses (mean±SD) and postpartum open days of the herds derived from different regions of our region in the Osumi Peninsula whose blood samples were collected during the postpartum

	Area 1	Area 2	Area 3
No. of herd	10	7	19
No. of cows	36	39	75
TP (g/dL)	7.7±0.7	7.5±0.5	7.5±0.5
Alb (mg/dL)	3.5±0.3	3.4±0.4	3.4±0.3
AG	0.8±0.1	0.9±0.2	0.8±0.2
GOT (U/L)	63.6±22.9	60.6±9.7	61.8±13.9
GGT (U/L)	18.7±4.0	20.2±6.3	19.9±8.4
FFA (mmol/L)	102.7±121.1	139.7±95.7	128.3±103.4
T-Cho (mg/dL)	123.7±41.0	120.2±35.4	123.2±29.3
BUN (mg/dL)	10.4 ± 3.6^{a}	8.3 ± 4.1^{b}	8.9±3.1
Glu (mg/dL)	59.0 ± 9.5^{a}	52.8 ± 10.1^{b}	56.5±6.6
Days open (days)	75.9^{a} (n=27)	113.4^{b} (n=27)	82.6 (n=43)

Postpartum: blood sampling was conducted between 30 and 90 days after calving

a-b: significant difference within the same row ($P<0.05$)

Chapter 3

Retrospective Surveillance of Metabolic Parameters Affecting Reproductive Performance of Japanese Black Breeding Cows

Abstract

This retrospective study was conducted (1) to confirm the relationship between pre- and postpartum metabolic parameters and postpartum reproductive performance and (2) to clarify seasonal characteristics of the metabolic parameters by using our metabolic profile test (MPT) database of Japanese black breeding herds. In Evaluation 1, MPT databases of blood samples from multiparous cows collected prepartum and postpartum were divided into two groups according to calving interval, and each MPT parameter was compared. In Evaluation 2, the same MPT databases used in Evaluation 1 were divided into 2 groups according to the sampling period. Significant differences were found in the prepartal total protein and postpartal γ-glutamyltransferase in Evaluation 1. In Evaluation 2, significant differences were found in the prepartal and postpartal total protein, albumin/globulin ratio, and glucose. Clear seasonal differences in MPT results emphasized the usefulness of the MPT in breeding cattle herds fed home-pasture roughage and suggest that unsatisfactory reproductive performance during hot periods reflects inadequate nutritional content of the diet and possible reduced feed intake due to heat stress.

Introduction

For optimal beef breeder production, a calving interval of 1 year must be maintained. Several factors including degree of calf suckling, reproductive failure, genetic variation, and level of maternal nutrient intake are known to affect postpartum reproductive efficacy of beef cows (Oliveira Filho et al., 2010; Stagg et al., 1998; Sullivan et al., 2009). Among them, one of the major factors contributing to a prolonged calving to conception interval is inadequate nutrition, which often causes subclinical and/or clinical metabolic diseases (Hess et al., 2005, Oliveira Filho et al., 2010). Prepartal and postpartal nutritional statuses are also related to the interval between calving and first ovulation in beef cattle (Oliveira Filho et al., 2010). Thus, it was assumed that certain metabolic parameters, which might reflect the protein and energy status of cows during prepartum and postpartum, would be associated with reproductive performance of breeding cattle herds.

We recently reported one Japanese Black breeding herd with extremely low blood urea nitrogen (BUN) concentration (mean, 2.2 mg/d*l*; range, 1.6–4.0 mg/d*l*), presumably due to nutritional quality of fed roughage harvested from the home pastureland, without any clinical symptoms (including body condition scoring); both BUN concentration and reproductive performance of the herd were considerably improved after dietary supplementation of crude protein to the herd (Watanabe et al., 2013a). Our follow-up study reconfirmed the usefulness of our metabolic profile test (MPT) parameters, particularly BUN and glucose (Glu), not only for determining the metabolic nutritional status of the breeding cattle herds in our region but also for selecting the dietary remedy for each herd to improve reproductive performance, and additionally indicated regional differences in the MPT results, which reflected inadequate nutritional content of the roughage possibly arising from regional pastureland differences (Watanabe et al., 2013b). Our previous results led to the hypothesis that MPT parameters, such as BUN and Glu may be suitable for evaluating the metabolic nutritional status of Japanese Black breeding cattle, which may greatly affect reproductive performance, at least within our region.

In the present study, retrospective surveillance was conducted to confirm the relationship between metabolic parameters collected at pre- and postpartum periods and postpartum reproductive performance by using our MPT database collected from Japanese Black breeding herds in our region. Furthermore, we also evaluated the relationship between metabolic parameters and seasonal conditions, which might vary according to seasonal change of dietary roughage and possible heat stress during the summer period in our region.

Materials and Methods

Animals were cared for according to the Guide for the Care and Use of Laboratory Animals (Joint Faculty of Veterinary Medicine, Kagoshima University).

Japanese Black breeding herds and cattle

Private Japanese Black breeding herds under the region of Soo Veterinary Clinical Center, Soo Agriculture Mutual Aid Association, Kagoshima Prefecture, Japan (latitude, 31°36′ N; longitude, 131°00′ E) were identified for this study. Multiparous cows included in this study were of different ages and in different stages of the reproductive cycle, without any visible signs of clinical disease. All cows were housed indoors and fed roughage mainly collected from the home pastureland, as well as supplementary concentrate purchased from various feed companies; however, feeding and management varied among herds.

Blood collection, metabolic profile parameters, and analytical methods

At the request of the herd owners, blood collections were scheduled to include all seasons, and MPT data generated from samples collected from 2247 cows in 95 herds between June 2010 and May 2012 (minimum and maximum temperatures during this period, −2.9°C and 34.7°C, respectively, Japan Meteorological Agency, Japan) were used in the present study. Blood samples were collected from the jugular vein in plain vacuum tubes, and transported to the laboratory. Serum was separated from the blood within 2 h of collection and stored in a refrigerator at -20°C until processing. The following biochemical parameters were determined using a Labospect7080 autoanalyzer; (Hitachi, Japan): Glu, free fatty acid (FFA), and total cholesterol (T-Cho) for evaluation of energy metabolism; total protein (TP), albumin (Alb), albumin/globulin (A/G) ratio, and BUN for protein metabolism; serum aspartate aminotransferase (AST) and γ-glutamyltransferase (GGT) for hepatic injury. Additionally, samples were analyzed for vitamins A and E (VA and VE, respectively) by high-performance liquid chromatography (Shimadzu, Japan). In the present study, only cows whose blood samples were collected at both the prepartum (between 80 and 21 days before calving; mean ± SEM, 37.7 ± 1.5 days) and postpartum (between 30 and 80 days after calving; mean ± SEM, 55.1 ± 2.1 days) period were selected for evaluation of the metabolic status of each examined group based on the results of serum biochemical analysis reported previously (Oliveira Filho et al., 2010; Watanabe et al., 2013a,b).

Reproductive performance data were also obtained from each herd record at the same time as blood sampling, and data included the postpartum open-day period of each cattle herd.

Evaluation 1-Evaluation of the metabolic profiles of breeding cattle in our region according to reproductive performance (postpartum open-day period)

The selected database of cattle derived from the 46 breeding cattle herds with blood samples collected at pre- and postpartum periods was divided into two groups according to the postpartum open-day period of each cow according to a previous standard (Rae, 1999); less than 80 days (good; calving interval < 12 months; n = 58 in prepartum, mean age, 5.2 years; n = 71 in postpartum, 5.6 years) or more than 110 days (poor; calving interval > 13 months; n = 19 in prepartum, 5.8 years; n = 22 in postpartum, 5.5 years). Each metabolic parameter during the pre- and postpartum sampling periods was compared between groups. Additionally, each metabolic parameter was compared between pre- and postpartum periods in good and poor groups to clarify the physiological alterations of metabolic profiles of each parameter between pre- and postpartal periods in each group.

Evaluation 2-Evaluation of the metabolic profiles of breeding herds in our region according to seasonal changes in dietary roughage and possible heat stress

In our region, most farmers use barnyard grass silage from digitaria as dietary roughage for breeding cattle during the summer period to save the cost of general dietary grass such as Italian ryegrass and oats hay; accordingly, farmers believe that reduced conception rates occur in cattle inseminated during these periods. In this evaluation, the databases of the metabolic profile in pre- and postpartum periods were divided into two groups: July through October (summer) or other months (non-summer). Each metabolic parameter during the pre- (summer, n = 31, mean age 5.2 years; non-summer, n = 64, 5.4 years) and postpartum (summer, n = 26, 5.9 years; non-summer, n = 66, 5.0 years) sampling period was compared between groups. Because cows without data regarding postpartum reproductive performance were included in Evaluation 2, the total number of the examined cows in both the pre- and postpartum periods were different from that in Evaluation 1.

Statistical analysis

The results obtained for each herd are expressed as the mean ± SD, as in previous reports (Alberghina et al., 2010). The results of biochemical analysis of serum collected in the prepartal and postpartal periods were compared by analysis of variance (ANOVA) using the General Linear Model function of SAS for Windows, ver. 9.1 (SAS Institute Japan). The statistical model used in Evaluation 1 included the sampling period, type of reproductive performance, and two-way interactions. For Evaluation 2, the statistical model included the sampling period, season, and two-way interactions. If the sampling period interactions were significant, these were excluded from the model. p values < 0.05 were considered to indicate a statistically significant difference, while p values less than 0.1 were considered to indicate a significant tendency.

Results

Evaluation 1

The results of biochemical analysis of serum from each group (good and poor) examined during the pre- and postpartum periods are shown in Table 1. Upon evaluation of serum biochemical parameters, a significant sampling period × type of reproductive performance interaction was observed for GGT ($p < 0.05$). However, no significant sampling period × type of reproductive performance was observed for the other parameters.

The results of biochemical analysis in both good and poor groups were all within the normal ranges. In the prepartum period, there was a significant tendency in TP (7.19 g/dL and 7.61 g/dL) between good and poor groups, respectively. In the postpartum period, there was a significant difference in GGT (17.8 IU/L and 21.4 IU/L) between good and poor groups, respectively. No significant differences were observed in Alb, A/G ratio, BUN, AST, FFA, T-Cho, Glu, VA, and VE between good and poor groups.

Comparison of metabolic parameters between the pre- and postpartum periods revealed significant differences between TP (prepartum 7.19 g/dL, postpartum 7.51 g/dL), Alb (prepartum 3.30 g/dL, postpartum 3.45 g/dL), AST (prepartum 51.3 IU/L, postpartum 60.8 IU/L), FFA (prepartum 173.0 mmol/L; postpartum 104.5 mmol/L), and Glu (prepartum 50.2 mg/dL; postpartum 57.0 mg/dL) in the group with good reproductive performance; however, no significant differences were observed between pre- and postpartal parameters in the group with poor performance.

Evaluation 2

The results of serum biochemical analysis derived from cattle whose samples were collected both pre- and postpartum are shown in Table 2. No significant sampling period × season was observed for any examined parameters.

Significant differences were observed in TP (summer, 7.54 g/dL; non-summer, 7.07 g/dL in prepartum; and summer, 7.90 g/dL; non-summer, 7.34 g/dL in postpartum), A/G ratio (summer, 0.83; non-summer, 0.94 in prepartum; and summer, 0.75; non-summer, 0.92 in postpartum), and Glu (summer, 45.2 mg/dL; non-summer, 54.7 mg/dL in prepartum; and summer, 47.7 mg/dL; non-summer, 57.9 mg/dL in postpartum); however, no significant differences were observed in Alb, BUN, AST, GGT, FFA, T-Cho, VA, and VE between summer and non-summer periods in pre- and postpartum.

Discussion

The main objectives of this retrospective surveillance were to investigate and clarify the blood metabolic parameters within our database, which might be associated with not only reproductive performance but also seasonal differences in Japanese Black breeding cattle herds in our region. Our results suggest that protein and energy metabolisms, as well as hepatic cytotoxicity status during perinatal periods affect reproductive performance of breeding cattle in our region. Additionally, results revealed that metabolic statuses of protein and energy of breeding cattle might be affected by seasonal differences, which might manifest as differences in dietary harvested grasses, particularly in our region, or reduced dry matter intake due to heat stress during summer time.

A significant tendency in TP (P=0.054) with no differences in Alb, A/G ratio and BUN were observed between the good and poor groups in Evaluation 1 suggesting increased globulin level in the poor group. Globulins are a heterogeneous group of proteins that includes antibodies and other inflammatory molecules, as well as hemostatic and fibrinolytic proteins (Alberghina et al., 2010). Therefore, it was assumed that certain inflammatory factors within the body, with no accompanying clinical symptoms or increased protein intake, during the prepartum period might affect postpartum reproductive performance. Regarding the data of the postpartum period, significant difference in GGT activity was observed. GGT is a membrane-bound enzyme that is found in cells with high rates of secretion or absorption. Although GGT activity is present in many tissues, it is primarily considered a serum marker for diseases of the hepatobiliary system associated with cholestasis (Kaneko et al., 1997).

Thus, although the cause of the increased GGT in our study herds was not determined, *Fasciola hepatica* infection common in cattle in our region is a possible cause, and such an infection might have affected reproductive performance.

Interestingly, when we compared each parameter between pre- and postpartum periods in the good and poor reproductive performance groups, significant differences were observed in TP (increase), Alb (increase), AST (increase), Glu (increase) and FFA (decrease) only in the good and not in the poor group. It has been suggested that metabolism of protein plays an important role in the regulation of physiological functions during the perinatal period (Djuricic et al., 2011; Kaneko et al., 1997). Additionally, the activity of aminotransferases in blood is very important for the metabolism of amino acids and carbohydrates; thus, the increased aminotransferase activity in the blood (reflected as increased AST in our study) might have been a consequence of increased activity within normal reference values in the cells or cell structural damage as previously reported (Djuricic et al., 2011; Kaneko et al., 1997). Therefore, the results of these comparisons between good and poor groups may indicate essential patterns in our established MPT system for achieving improved reproductive performance in the breeding cattle herds in our region. Further study with an expanded MPT database is warranted.

Unsatisfactory reproductive performance was acknowledged by most farmers after artificial inseminations during the periods in which cattle were fed barnyard grass silage (digitaria) as dietary roughage in our region, which raised the hypothesis that these types of long-term feeding customs by the farmers may be affecting reproductive performance of the herd in our region. Following this hypothesis/speculation, we conducted a preliminary evaluation that aimed to clarify seasonal effects (change of dietary roughage from Italian ryegrass to barnyard grass silage and possible heat stress in summer time) on MPT values in our region. Significant differences both in TP and A/G ratio were observed between the 2 seasonal groups both in the pre- and postpartum, implying that these differences must be derived from differences in globulin concentrations. As mentioned above, globulins are heterogeneous group of proteins that includes antibodies and other inflammatory molecules as well as hemostatic and fibrinolytic proteins (Alberghina et al., 2010); thus, although the underlying cause of the difference in globulin concentrations was not evident from our results, the above finding, together with the result of Evaluation 1 indicating a relationship with reproductive performance of the herd, indicate that occurrence of inflammation without clinical symptoms may affect reproductive performance in breeding cattle herds during the summer period in our region.

In the present evaluation, significant differences in Glu between the 2 seasonal groups both in the pre- and postpartum periods were observed. It has been suggested that blood Glu has more potential as an indicator of carbohydrate status and dietary starch content than as an indicator of energy balance, illustrating that at the same degree of negative energy balance, cows fed with a higher proportion of grain had higher blood Glu concentrations than those receiving a lower proportion of grain (Herdt et al., 1981; Herdt, 2000). Therefore, the difference in Glu in the present study might have reflected differing energy and/or carbohydrate status of the roughage fed between the 2 seasonal terms, and suggests that Glu may be a useful parameter for monitoring the energy status of breeding cattle in our region.

Pre- and postpartum nutritional levels have been reported the most important variables for reducing the interval between parturition and first ovulation (Hess et al., 2005; Short and Adams, 1988). Additionally, it has been shown that BUN level is a sensitive indicator of the balance between available digestible crude protein and energy fed to a ruminant (Kenny et al., 2002), which can assist in the measure of the efficiency of protein utilization (Kohn et al., 2002; Manston et al., 1975). Recently, we confirmed these findings by revealing improved reproductive performance in Japanese Black breeding cattle herds with use of a dietary remedial measure based on evaluations of MPT parameters, particularly BUN and Glu, in each breeding herd in our region (Watanebe et al., 2013a; Watanabe et al., 2013b). These results led to the hypothesis of a possible relationship between protein/energy balance reflected as BUN and Glu levels, and reproductive efficacy in each breeding cattle herd within the MPT database in our region. Interestingly, in the present study, no relationships emerged between BUN concentration and reproductive performance (evaluated by number of open days after calving) in the samples collected in the pre- and postpartum periods. The reason for these results in the present study is obscure. It has been reported that BUN concentration reflects current dietary protein intake, and concentration of Alb is affected by long-term protein status because of its long half-life (Herdt, 2000; Manston et al., 1975). Therefore, possible reasons for our study findings are that all databases were evaluated without considering regional differences in BUN concentration, as previously reported (Watanabe et al., 2013b), or that the differences in half-life between BUN and Alb together with wide variations in pre- and postpartal sampling periods (approximately 2 months) might have affected the relationship with reproductive performance in our region.

In conclusion, the present results of retrospective surveillance of our database of metabolic parameters indicated importance of both increased protein and energy metabolisms during perinatal periods for improving the reproductive performance in breeding cattle fed with roughage from home-pastures in our region. In addition, seasonal effects manifesting as changes in roughages or possible heat stress might have affected MPT values; thus, the study parameters/factors may facilitate detection of seasonal effects in breeding cattle in our region. The retrospective surveillance findings suggest that dietary supplementary remedies such as protein and energy based on MPT results may be an alternative strategy for reducing excessive usage of hormones for treatment of reproductive failures in the cattle breeding industry. Further field evaluations/experiments are warranted for evaluating the relationship between the metabolism of protein and/or energy and reproductive performance in breeding cattle within our region.

Table1. Results of serum biochemical analysis (mean±SD) in Japanese Black breeding cattle with both good and poor reproductive performances, whose blood samples were collected during pre- and postpartum definite periods

Sampling	Prepartum period		Postpartum period	
	Reproductive performance		Reproductive performance	
	Good (n=58)	Poor (n=19)	Good (n=71)	Poor (n=22)
Days open (days)	61.3±15.3	171.5±48.1	63.7±13.1	160.2±77.6
TP (g/d*l*)	7.20 ± 0.44[e,*]	7.61 ± 0.58[f]	7.51 ± 0.62[*]	7.65 ± 0.63
Alb (mg/d*l*)	3.30 ± 0.29[*]	3.31 ± 0.25	3.45 ± 0.26[*]	3.46 ± 0.34
A/G ratio	0.87 ± 0.16	0.79 ± 0.15	0.87 ± 0.16	0.84 ± 0.16
BUN (mg/d*l*)	9.5±3.7	8.7±3.2	8.7±3.5	7.9±3.5
AST (IU/*l*)	51.3±9.3*	56.3±13.4	60.8±10.1*	60.6±11.3
GGT (IU/*l*)	17.1±3.6	16.1±2.9	17.8±4.9[a]	21.4±7.8[b]
FFA (mmol/*l*)	173.0±131.4*	197.4±145.4	104.5±84.6*	163.7±106.1
T-Cho (mg/d*l*)	116.1±23.9	129.6±37.1	127.0±35.0	127.4±39.1
Glu (mg/d*l*)	50.2±8.1*	51.0±9.0	57.0±8.7*	53.8±8.9
VA (IU/d*l*)	97.6±11.4	96.6±14.9	94.1±15.9	90.1±16.5
VE (IU/d*l*)	322.6±96.4	389.6±139.7	339.6±132.3	331.1±135.0

[a-b]$p < 0.01$; [e-f]$0.05 < p < 0.1$ (mean ± SD): between the good and poor groups in prepartum or postpartum. [*]Significant difference ($p < 0.05$) between pre- and postpartum periods within good or poor groups. [†]Because of a lack of data, the numbers of examined cows displaying good reproductive performance were 55 during prepartum and 68 in postpartum. [‡]Because of a lack of data, the numbers of examined cows displaying good and poor reproductive performance were 54 and 19 in prepartum and 65 and 21 in postpartum, respectively. Reference ranges reported by Watanabe *et al.* (2013a) are as follows: total protein (TP), 5.7 ~ 8.1; albumin (Alb), 2.1 ~ 3.6; albumin/globulin (A/G) ratio, 0.8 ~ 1.3; blood urea nitrogen (BUN), 6 ~ 27; aspartate aminotransferase (AST), 45 ~ 110; γ-glutamyltransferase (GGT), 11 ~ 25; free fatty acid (FFA), 150 ~ 350; total cholesterol (T-Cho), 100 ~ 180; glucose (Glu), 45 ~ 75; vitamin A (VA), > 80; vitamin E (VE), > 105.

Table2. Results of serum biochemical analysis (mean±SD) of Japanese Black breeding cattle whose blood samples during pre- and postpartum definite periods were divided according to sampling period

	Prepartum period		Postpartum period	
Blood sampling months	7, 8, 9, 10* (Summer)	Other (Non-summer)	7, 8, 9, 10 (Summer)	Other (Non-summer)
	n=31	n=64	n=26	n=66
TP (g/d*l*)	7.54 ± 0.46[a]	7.07 ± 0.61[b]	7.90 ± 0.70[a]	7.34 ± 0.54[b]
Alb (mg/d*l*)	3.37 ± 0.24	3.36 ± 0.27	3.33 ± 0.25	3.47 ± 0.31
A/G ratio	0.83 ± 0.13[c]	0.94 ± 0.23[d]	0.75 ± 0.13[a]	0.92 ± 0.18[b]
BUN (mg/d*l*)	10.1±3.2	10.2±3.0	9.1±2.9	8.7±2.8
AST (IU/*l*)	57.1±10.5	61.9±44.5	60.0±8.8	61.5±10.6
GGT (IU/*l*)	15.6±3.4	17.8±8.8	18.4±4.2	18.6±6.2
FFA (mmol/*l*)	182.9±118.6	195.9±186.5	148.5±101.2	127.8±99.8
T-Cho (mg/d*l*)	105.2±24.1	119.2±27.6	122.0±30.2	133.8±42.0
Glu (mg/d*l*)	45.2±6.2[a]	54.7±5.5[b]	47.7±8.3[a]	57.9±7.6[b]
VA (IU/d*l*)	108.7±24.8	100.9±22.3	99.4±17.9	92.2±16.2
VE (μg/d*l*)	277.6±144.9	350.3±119.7	342.1±160.6	379.2±189.9

[a-b] $p < 0.01$; [c-d] $p < 0.05$ (mean ± SD) between good and poor groups in prepartum or postpartum. *From June through October, most farmers within our region feed the cattle barnyard grass silage from digitaria as roughage. †Because of a lack of data, the numbers of examined cows in summer and non-summer were 29 and 59 in prepartum and 25 and 62 in postpartum, respectively. ‡Because of a lack of data, the numbers of examined cows in summer and non-summer were 29 and 59 in prepartum and 23 and 62 in postpartum, respectively.

OVERALL DISCUSSION AND CONCLUSION

Reducing the calving-to-conception interval and keeping the calving period to 365 days are two important factors for a successful reproductive management of beef cattle. The average calving interval for breeding beef cattle in Japan is presently 416 days, whereas in the herds of the Soo Agriculture Mutual Aid Association (SAMAA) it is 403 days, which indicates a problematic decline in reproductive performance. While facing such a decline, SAMAA regularly monitors the reproductive health of the herds, but we have not yet been able to solve this problem. Therefore, as maternal nutritional status is a known factor that affects the reproductive performance of beef cattle, we decided to introduce metabolic profile testing as part of our routine reproductive health monitoring.

In the course of genital diagnosis with metabolic profile testing, we tested a herd of 10 Japanese Black breeding cattle that outwardly appeared to be healthy but had poor reproductive performance and found that blood urea nitrogen (BUN) concentration was low (2.6 ± 0.6 mg/dl) in all of those cattle. The analysis of the nutritional composition of the herd's home-pasture roughage feed revealed that the feed had an abnormally low level of crude protein. In addition, the soil of the field planted with that roughage feed had low pH and low conductance. These results indicated that protein deficiency caused the low BUN. In light of these findings, a new feed composition was calculated and cattle were given 600 g of bean cake per day to improve their protein intake. As a result, BUN concentration improved and the calving-to-conception interval was shortened.

To further verify these results, we conducted reproductive monitoring of the herds that outwardly appeared to be healthy but had poor reproductive performance and low BUN inferred from the metabolic profile test results. We also studied the effects of improved nutritional composition on reproductive performance. In addition, as soil quality influences the crude protein content of home-grown roughage feed, which consequently affects reproductive performance, we tested for regional differences in MPT parameters for SAMAA Japanese Black breeding cattle herds. The results of the additional verification study showed that the BUN concentration increased after nutrition improved and the calving-to-conception interval decreased. Furthermore, concentrations of BUN and glucose (GLU), which are considered indicators of protein and energy intake, were found to vary between SAMAA herds in different regions.

Next, to determine what are the factors that affected reproductive performance in these past studies, we conducted a retrospective study using results of the past metabolic profile tests to compare MPT parameters between SAMAA herds with superior reproductive performance and those with poor reproductive performance. In addition, we also test for seasonal variation in metabolic profile test items of SAMAA Japanese Black breeding cattle herds due to seasonal changes in home-pasture roughage feed. Relative to prepartum levels, postpartum levels of total protein, albumin, and GLU significantly increased, whereas that of free fatty acids significantly decreased among the herds with superior reproductive performance. However, there was no significant difference between prepartum and postpartum levels among herds with poor reproductive performance. In addition, compared to the hot season, the albumin to globulin ratio and GLU were significantly higher and TP was significantly lower in all other seasons in both prepartum and postpartum cows.

The above findings indicate that metabolic profile testing is a practical method for evaluating metabolic and nutritional problems that are not reflected in the herd's appearance, particularly for herds given home-pasture roughage feed, and that reproductive performance improves when nutrition is improved based on metabolic profile test results. Furthermore, there was regional variation in the metabolic profile test results of SAMAA Japanese Black breeding cattle herds, which suggests that differences in soil properties determine the nutritional value of home-pasture roughage feed produced in each area, which subsequently influences metabolic profile test results of each herd. In addition, the results of a retrospective study using a database containing results of the past metabolic profile tests suggested that protein and energy metabolism is associated with the degree of reproductive performance in both prepartum and postpartum cows. Furthermore, the metabolic profile test results of SAMAA Japanese Black breeding cattle herds varied between the hot season and other seasons, which could have been caused by seasonal changes in home-pasture roughage feed or decreased feed intake and increased energy expenditure due to heat stress.

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ACKNOWLEDGEMENT

I would like to thank the following people: My supervisor, Dr. Mitsuhiro Takagi, Associate Professor of Kagoshima University, for the precious opportunity he has given to me. It would have been impossible for me to obtain my doctoral degree without his consistent support and guidance.

My sincere gratitude also goes to Dr. Osamu Yamato, Professor of Kagoshima University, Dr. Takeshige Otoi, Professor of Yamaguchi University, Dr. Eisaburo Deguchi and Dr. Chikara Kubota, Professors of Kagoshima University, for their valuable guidance and coaching they have undertaken during my whole study period.

I would also like to thank Mr. Yukio Kakinoki, Dr. Etsuro Kyuutoku and Dr. Koji Okamoto of the Soo Agriculture Mutual Aid Association for all of their help, understanding and patience until I reached here.

I would also like to extend my sincere appreciation to all staff of the Soo Agriculture Mutual Aid Association and Japanese Black Breeding Cattle Farmers in our area/region for their assistance in providing me sampling and data collections.

Finally I would like to thank my husband and my parents for their endless love, warm support and encouragement, and patience, of course throughout my study.

Urara Shinya

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March, 2015