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Original Article

Retrospective surveillance of metabolic parameters affecting reproductive performance of Japanese Black breeding cows

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This retrospective study was conducted to confirm the relationship between pre- and postpartum metabolic parameters and postpartum reproductive performance and 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 two 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.

Keywords: Japanese Black cattle, metabolic parameters, reproductive performance, retrospective surveillance

Introduction

A calving interval of 1 year must be maintained for optimal beef breeder production. 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 [12,15,16]. Among these, one of the major factors contributing to prolonged calving due to conception interval is inadequate nutrition, which often causes subclinical and/or clinical metabolic diseases [5,12]. Prepartal and postpartal nutritional statuses are also related to the interval between calving and first ovulation in beef cattle [12]. Thus, it is assumed that certain metabolic parameters that 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 a Japanese Black breeding herd with extremely low blood urea nitrogen (BUN) concentration (mean, 2.2 mg/dL; range, 1.6～4.0 mg/dL), presumably due to the nutritional quality of fed roughage harvested from the home pastureland. This herd had no clinical symptoms (including body condition scoring); however, both BUN concentration and reproductive performance of the herd were considerably improved after dietary supplementation with crude protein [17]. 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. Additionally, our findings identified regional differences in the MPT results, which reflected the inadequate nutritional content of the roughage, possibly arising from regional pastureland differences [18]. Our previous results suggested that MPT parameters such as BUN and Glu may be suitable for evaluation of the metabolic nutritional status of Japanese Black breeding cattle, which may greatly affect reproductive performance, at least within our region.

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

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 [17]. 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 [12,18].

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. 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 [13]; 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. 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 [1]. 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

Table 1. Results of serum biochemical analysis in Japanese Black breeding cattle with both good and poor reproductive performance based on blood samples collected during pre- and postpartum definite periods

	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/dL)	7.20 ± 0.44^{e,*}	7.61 ± 0.58^{f}	7.51 ± 0.62*	7.65 ± 0.63
Alb (g/dL)	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/dL)	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 ± 7.8b
FFA (mmol/L)	173.0 ± 131.4*	197.4 ± 145.4	104.5 ± 84.6*	163.7 ± 106.1
T-Cho (mg/dL)	116.1 ± 23.9	129.6 ± 37.1	127.0 ± 35.0	127.4 ± 39.1
Glu (mg/dL)	50.2 ± 8.1*	51.0 ± 9.0	57.0 ± 8.7*	53.8 ± 8.9
VA (IU/dL) †	97.6 ± 11.4	96.6 ± 14.9	94.1 ± 15.9	90.1 ± 16.5
VE (µg/dL) ‡	322.6 ± 96.4	389.6 ± 139.7	339.6 ± 132.3	331.1 ± 135.0

$^{a\text{-}b}p < 0.01$; $^{e\text{-}f}0.05 < p < 0.1$ (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.* [17] 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.

Table 2. Results of serum biochemical analysis of Japanese Black breeding cattle based on blood samples during pre- and postpartum definite periods 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/dL)	7.54 ± 0.46[a]	7.07 ± 0.61[b]	7.90 ± 0.70[a]	7.34 ± 0.54[b]
Alb (g/dL)	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/dL)	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/dL)	105.2 ± 24.1	119.2 ± 27.6	122.0 ± 30.2	133.8 ± 42.0
Glu (mg/dL)	45.2 ± 6.2[a]	54.7 ± 5.5[b]	47.7 ± 8.3[a]	57.9 ± 7.6[b]
VA (IU/dL)[†]	108.7 ± 24.8	100.9 ± 22.3	99.4 ± 17.9	92.2 ± 16.2
VE (μg/dL)[‡]	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.

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 of our database were to investigate and clarify the blood metabolic parameters that might be associated with reproductive performance and reflect seasonal differences in Japanese Black breeding cattle herds in our region. Our results suggest that protein (TP and Alb) and energy (FFA and Glu) metabolisms, as well as hepatic cytotoxicity status, affect reproductive performance of breeding cattle in our region during perinatal periods. Additionally, results revealed that metabolic statuses of protein and energy of breeding cattle might be affected by seasonal differences, which could manifest as differences in dietary harvested grasses, particularly in our region, or reduced feed 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 levels in the poor group. Globulins comprise a heterogeneous group of proteins that includes antibodies and other inflammatory molecules, as well as hemostatic and fibrinolytic proteins [1]. Therefore, it was assumed that certain inflammatory factors within the body during the prepartum period, with no accompanying clinical symptoms or increased protein intake, might affect postpartum reproductive performance. The data describing the postpartum period revealed a significant difference in GGT activity. GGT is a membrane-bound enzyme 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 [6]. Thus, although the cause of increased GGT in our study herds was not determined, *Fasciola hepatica* infection was common in cattle in our region, indicating that it is a possible cause, and 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) in the good group, but not 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 [2,6]. 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 [2,6]. 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. Accordingly, further study with an expanded MPT database is warranted.

Unsatisfactory reproductive performance has been acknowledged by most farmers after artificial inseminations during periods in which cattle were fed barnyard grass silage (digitaria) as dietary roughage in our region, suggesting that these long-term feeding customs of the farmers may be affecting reproductive performance of the herds in our region. Therefore, we conducted a preliminary evaluation to clarify the effects of seasonal change in dietary roughage from Italian ryegrass to barnyard grass silage and possible heat stress during summer on MPT values in our region. Significant differences in both TP and A/G ratio were observed between groups during both pre- and postpartum, implying that these differences must be derived from differences in globulin concentration. As mentioned above, globulins are a heterogeneous group of proteins that include antibodies and other inflammatory molecules, as well as hemostatic and fibrinolytic proteins [1]; thus, although the underlying cause of the difference in globulin concentration was not evident from our results, the above findings, together with the results of evaluation 1, indicate that the occurrence of inflammation without clinical symptoms may affect reproductive performance in breeding cattle herds during summer in our region.

In the present study, significant differences in Glu between seasonal groups were observed in both the pre- and postpartum periods. 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 [3,4]. Conversely, Katamoto *et al*. [7] previously examined the influence of seasonal differences in VE concentration in Japanese Black breeding cattle and reported significant decreases in VE during summer, which might have been related to decreased feed intake due to high ambient temperature. Additionally, Matsumoto *et al*. [11] reported that subclinical health status might have affected consumption and resultant VE concentration. In the present study, no significant differences were observed in VA and VE concentrations between summer and non-summer periods in samples collected during the pre- and postpartum periods. This lack of seasonal effect might have been caused by vitamin supplementation of the breeding cows by farmers in our region throughout the study period. The difference in Glu in the present study might have reflected differing energy and/or carbohydrate status of the roughage fed between seasons, or reduced feed intake due to high ambient temperature. Thus, Glu may be a useful parameter for monitoring the energy status of breeding cattle herds that do not show seasonal differences in vitamin concentrations or clinical symptoms during summer.

Pre- and postpartum nutritional levels have been reported to be the most important variables for reducing the interval between parturition and first ovulation [5,14]. Additionally, BUN level has been shown to be a sensitive indicator of the balance between available digestible crude protein and energy fed to a ruminant [8], which can facilitate measurement of the efficiency of protein utilization [9,10]. We recently confirmed these findings by revealing improved reproductive performance in Japanese Black breeding cattle herds through the use of dietary remedial measures based on evaluations of MPT parameters, particularly BUN and Glu, in each breeding herd in our region [17,18]. The results suggested 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 the number of open days after calving) in samples collected in the pre- and postpartum periods. The reason for these results is not clear. 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 [3,10]. Therefore, possible reasons for our study findings are that all databases were evaluated without considering regional differences in BUN concentration, as previously reported [18], 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 retrospective surveillance findings suggest that dietary remedies such as protein and energy supplementation 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 to evaluate the relationship between the metabolism of protein and/or energy and reproductive performance in breeding cattle within our region.

Conflict of interest

There is no conflict of interest.

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