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

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Running head: *Reproductive efficacy of Japanese Black cows*

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.

Key words: blood urea nitrogen, crude protein, Japanese Black cow, metabolic profile, reproductive performance

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 jurisdiction 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 jurisdiction. 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., in press). 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, 1993, Elrod et al., 1993, 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.

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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)