NOTE *Internal Medicine*

Blood biochemical values in Japanese Black calves in Kagoshima Prefecture, Japan

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ABSTRACT. To obtain blood biochemical basic data of Japanese Black calves in Kagoshima Prefecture, Japan, blood samples were obtained from 582 clinically healthy calves on 27 farms. Calves were divided into three stages: the suckling stage (between 14 and 90 days of age, n=191), the early growing stage (between 91 and 180 days of age, n=200) and the late growing stage (between 181 and 270 days of age, n=191). The mean concentration of total cholesterol, triglyceride, nonesterified fatty acids, calcium and zinc, and the mean activities of γ-glutamyltransferase and alkaine phospatase in the suckling stage were significantly higher than those in the early and late growing stages ($P<0.01$). The mean concentration of total protein, albumin and globulin increased gradually with growing. The mean concentration of beta-hydroxybutyrate in the suckling stage was below 150 μmol/*l*, however, it elevated above 400 μmol/*l* in the early and late growing stages. The mean concentration of copper concentration was above 70 μg/d*l* in all stages. The mean concentration of zinc was between 90 and 110 μg/d*l* in all stages. These results suggest that the blood biochemical values of Japanese Black calves vary with growing stages, and the blood parameters obtained in this study are considered useful as indices for health management of Japanese Black calves.

KEY WORDS: blood biochemical value, growing stage, Japanese Black calf

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The Japanese Black is a breed of beef cattle that originated in and is mainly distributed in Japan [4]. An animal's health can be defined as the absence of diseases determined by clinical examinations combined with various diagnostic tests. Serum biochemical reference values are used to establish normality [3, 13] and to diagnose diseases [14] and development of ruminal function [7, 19]. Although many reference values have been established for dairy calves [5, 8, 13, 19], there have been few published references for beef calves [3, 18, 22]. In particular, the reports for Japanese Black calves have been very scarce. Among them, blood parameters in Japanese Black calves were reported to be different from dairy calves [22]. It is well known that physiological variables, such as weaning, have influence on many blood parameters [5, 19]. To the best of the authors' knowledge, reference values of serum biochemical parameters have not been established for particular stages of Japanese Black calves. In Japan, almost all Japanese Black calves were sold by auction at a market around 8 to 9 months of age. Therefore, the current study was conducted to establish reference values of serum biochemical parameters from the birth up to 8 months dividing into three stages of Japanese Black calves kept on ordinary farms in Kagoshima Prefecture, Japan.

Privately owned Japanese Black herds (27 farms) in Kagoshima Prefecture, Japan, were enrolled in this study. The number of calves in each herd ranged from 40 to less than 300. Five hundred eighty two Japanese Black bulls or steer calves (14 to 270 days of age) on these farms were used, and blood samples were collected once per head by the authors between April 2014 and May 2015. All calves were stayed with their dams generally for five days after birth, fed milk replacer from 5 days of age and weaned at around 90 days of age. All calves were clinically healthy, housed indoor and were castrated between 4 to 5 months of age. Feed consisted of supplemental concentrate purchased from several feed companies and grasses (grown at the farm or purchased), such as rice straws, Italian ryegrass or oats, from 91 to 270 days of age. Although the contents and amounts of supplemental concentrate and grasses were different for each farm, the feed fundamentally met the requirements of the Japanese Feeding Standard for Beef Cattle [1]. About 21 calves on each farm were sampled at random during the suckling stage (between 14 and 90 days of age, n=191), early growing stage (between 91 and 180 days of age, n=200) and late growing stage (between 181 and 270 days of age, n=191). Animals were cared for according to the Guide for the Care and Use of Laboratory Animals of the Joint Factory of the Veterinary Medicine, Kagoshima University.

Blood samples were collected from the jugular vein into plain vacuum tubes between 10 a.m. and noon. Serum was separated within 30 min after blood collection and stored at −30°C until analysis (a centrifuge and a freezer were brought to farms). The following biochemical parameters were determined using a Labospect 7020 autoanalyzer (Hitachi High-Technologies Corporation, Tokyo, Japan): total protein (TP), albumin (Alb), globulin (Glb), Alb/Glb (A/G) ratio, urea nitrogen (UN), creatinine (Cre), total cholesterol (T-Cho), triglyceride (TG), nonesterified fatty acids (NEFA), beta-hydroxybutyrate (βHB), aspartate aminotransferase (AST), γ-glutamyltransferase (GGT), alkaline phosphatase (ALP),

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Table1. Serum biochemical values of Japanese Black calves in different stages

	Suckling stage (n=191)			Early growing stage (n=200)			Late growing stage (n=191)		
	Mean	SD	Normal range*	Mean	SD	Normal range	Mean	SD	Normal range
TP (g/d*l*)	5.79	0.54 a,b)	4.70–6.87	6.23	0.44 a,c)	5.34–7.11	6.41	0.37 b,c)	5.67–7.14
Albumin (g/d*l*)	3.17	0.25 a)	2.68–3.66	3.22	0.21 b)	2.80–3.63	3.35	0.20 a,b)	2.94–3.75
Globulin (g/d*l*)	2.61	0.50 a,b)	1.61–3.61	3.01	0.48 a)	2.05–3.97	3.06	0.36 b)	2.35–3.78
A/G	1.26	0.24 a,b)	0.78–1.74	1.10	0.21 a)	0.69–1.51	1.11	0.15 b)	0.81–1.41
UN (mg/d*l*)	11.0	3.4 a,b)	4.2–17.7	10.7	3.9 a,c)	3.0–18.5	11.3	4.0 b,c)	3.3–19.4
Creatinine (mg/d*l*)	0.94	0.17 a,b)	0.59–1.28	0.79	0.12 a,c)	0.55–1.02	0.80	0.11 b,c)	0.58–1.03
T-Cho (mg/d*l*)	120.7	39.8 a,b)	41.0–200.3	80.2	20.4 a,c)	39.4–121.0	99.9	23.7 b,c)	52.5–147.3
Triglyceride (mg/dl)	19.6	9.4 a,b)	0.9–38.4	17.2	6.4 a)	4.3–30.1	16.6	5.8 b)	4.9–28.3
AST (IU/*l*)	59.0	22.1 a)	14.9–103.1	71.2	18.9 a)	33.4–109.0	61.5	11.2	39.0–83.9
GGT (IU/*l*)	31.6	30.1 a,b)	0–91.7	18.1	6.0 a)	6.1–30.1	16.5	4.8 b)	7.0–26.0
ALP (IU/*l*)	733.6	286.6 a,b)	160.3–1,306.8	569.9	175.3 a,c)	219.3–920.3	492.2	126.8 b,c)	238.7–745.8
CK (IU/*l*)	166.2	68.2 a,b)	29.8–302.5	206.0	91.0 a)	24.0–388.1	189.0	52.0 b)	84.9–293.1
NEFA (μEq/*l*)**	243.4 a,b)		116.5–508.1	183.0 a)		75.5–443.9	169.8 b)		70.1–411.3
βHB (μmol/*l*)**	137.3 a,b)		29.8–632.0	441.8 a)		214.4–910.2	454.3 b)		269.9–764.7
Calcium (mg/d*l*)	10.8	0.7 a)	9.4–12.1	10.4	0.4 a)	9.6–11.2	10.4	0.4	9.5–11.2
iP (mg/d*l*)	8.89	0.97	6.95–10.83	8.82	1.05	6.72–10.91	8.80	0.95	6.89–10.71
Magnesium (mg/d*l*)	1.85	0.26 a,b)	1.34–2.37	2.11	0.27 a)	1.58–2.65	2.08	0.25 b)	1.58–2.59
Copper (μg/d*l*)	72.2	19.8 a)	32.7–111.7	77.2	14.9 a)	47.4–106.9	76.2	20.6	35.0–117.4
Zinc (μg/d*l*)	105.9	23.8 a,b)	58.3–153.4	92.1	15.0 a,c)	62.1–122.0	99.2	15.1 b,c)	68.9–129.5

TP=total protein, A/G=albumin/globulin ratio, UN=urea nitrogen, T-Cho=total cholesterol, AST=aspartate aminotransferase, GGT=γ-glutamyltransferase, ALP=alkaline phosphatase, CK=creatine kinase, NEFA=nonesterified fatty acids, βHB=beta-hydroxybutyrate, iP=inorganic phosphorus. *Normal range=mean ± 2SD. **A log10 transformation was applied to NEFA and βHB. Same letters indicate significant difference in sampling stage within the same parameters (P<0.01).

creatine kinase (CK), calcium (Ca), inorganic phosphorus (iP), magnesium (Mg) and zinc (Zn). Serum copper (Cu) level was analyzed using the AU 480 chemistry system (Beckman Coulter Inc., Tokyo, Japan). The results obtained for each stage were expressed as the mean ± 2SD. As distributions of NEFA and βHB were skewed to the left, statistical analysis was conducted using logarithmic transformation. The values less than the mean −3SD and values more than the mean + 3SD were regarded as outliers. This method was determined based on a study by Kida [12] and Otomaru [16]. Statistical analyses of data were conducted using analysis of variance (One-way ANOVA) followed by the Tukey–Kramer multiple comparison test to determine the difference of biochemical parameters among the stages. All statistical analyses were performed using SPSS Statistics 21 software (IBM, Tokyo, Japan), and P<0.01 was considered statistically significant.

The results of serum biochemical analysis for the three stages are shown in Table 1. The mean concentration of T-Cho, NEFA, Ca and Zn, and the mean activities of GGT and ALP in the suckling stage were significantly higher than those in the early and late growing stages (P<0.01). The mean concentration of TP, Alb and Glb increased gradually with growing. The mean concentration of βHB in the suckling stage was below 150 μmol/*l*, but on the other hand, it was above 400 μmol/*l* in the early and late growing stages. The mean concentration of Cu was above 70 μg/d*l* in all stages. The mean concentration of Zn was between 90 and 110 μg/d*l* in all stages.

The serum TP value is usually used as an indicator of the nutritive status of animals, as it reflects feed intake, metabolism and immunity [6]. The serum Alb value has been reported to be a long-term indicator of available digestible crude protein status because of its long half-life [6], and immunoglobulin, one type of Glb, is produced during humoral immune reaction contributing to acquired immunity. In the present study, the TP, Alb and Glb values increased with growing. Therefore, increased protein in blood might have reflected efficient and increased protein utilization, and development of immunity with growing. These agree with reports from other investigations [8, 13, 18].

The serum T-Cho value is influenced by energy intake [9]. Normal T-Cho level in calves was reported as 80–120 mg/d*l* [10]. Blood T-Cho level in calf decreased after weaning due to decrease in dietary lipid. Prior to weaning, majority of dietary lipid was provided in milk replacer, and thus, lipid intake decreased after weaning [2]. In the present study, the serum T-Cho value was slightly above the normal range, but it also decreased after weaning. Therefore, the tendency of high T-Cho value in the suckling stage might have reflected absorption and metabolism of milk replacer in Japanese Black calves.

Serum GGT represents liver associated enzymes that leak into the bloodstream following liver damage. In addition, serum GGT is used to indicate the colostrum absorption, because colostrum absorption and serum GGT level are closely correlated in neonatal calves [17, 21]. In the present study, the serum GGT value in the suckling stage was higher than that in the other stages. This result might reflect the colostrum intake from their dams in the neonatal period.

In mature animals, serum ALP originates mainly from the liver [10, 11], however, in growing animals, most serum ALP originates from bone tissues [10, 11]. In Shorthorn calves, the serum ALP value from birth to 331 days of age has been reported to be 136–431 IU/*l* [3]. In the present study, the serum ALP value was above 490 IU/*l* in all stages, and ALP in the suckling stage was higher than that in the other stages, but ALP decreased with growing. Therefore, skeletal growth in Japanese Black calves might be higher than Shorthorn calves, and skeletal growth might have decreased with growing. The other researchers also reported the negative relationship between blood ALP and age in calves [3, 18].

βHB produced by ruminal epithelium is a metabolite of butyrate, which is produced by ruminal fermentation of ingested feed. βHB is considered one of an indicator of rumen development in calves [7, 19]. Huge *et al*. [7] and Quigley *et al.* [19] reported the plasma βHB values from 4 to 18 weeks of age and from birth to 112 days of age in dairy calves, and those reports demonstrated increased βHB value after weaning. In the present study, the serum βHB value in the early and late growing stages (between 91 and 180, and between 181 and 270 days of age) was higher than that in the suckling stage, and it reached above 400 μmol/*l*. It suggests the development of the ability of ruminal epithelium to produce and absorb butyrate during the early and late growing stages, where calves were weaned and fed dry feed.

Cu is known as an essential microelement found in all living organisms [23]. It is vital for survival and serves as an important catalytic cofactor in redox chemistry for fundamental biological functions, as well as in growth and development [23]. Normal serum Cu concentration was reported to be above 70 μg/d*l*, and Cu concentration lower than 55 μg/d*l* was considered insufficient [15]. In the present study, the Cu value was above 70 μg/d*l* in all stages. Zn is known to play an important role in regulation of more than 200 enzymes in the animal body and is associated with growth, tissue repair, ossification and the immune system [23]. The normal serum zinc level in calves has been reported to be 80–120 μg/d*l* [20]. In the present study, the Zn value was within normal range in all stages. Therefore, most of calves in Kagoshima prefecture might have no shortage of the serum Cu and Zn values.

Although the data in the present study were obtained only from ordinary farms in one prefecture of Japan, these results suggest that blood biochemical values of Japanese Black calves vary with the growing stage, and the blood parameters obtained in this study are considered useful as indices for health management of Japanese Black calves.

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