JVC Journal of Veterinary Clinics

Original Article

pISSN 1598-298X • eISSN 2384-0749
J Vet Clin 2022;39:342-352
https://doi.org/10.17555/jvc.2022.39.6.342

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Hematology, Serum Biochemistry, and Acute Phase Proteins in Hanwoo (*Bos taurus coreanae*) Calves with Diarrhea

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Abstract This study was conducted to identify the useful blood variables in diagnosing calf diarrhea in Hanwoo calves and good indicators for calf diarrhea. In 530 Hanwoo calves, fecal scores were recorded on a scale of 0 to 3, and blood samples were collected and analyzed for hematology, serum biochemistry, and acute phase proteins. Among the blood variables, 16 blood variables showed significant differences ($p < 0.01$) according to fecal scores. After reference intervals of these 16 blood variables were calculated, the distributions of calves by calculated reference intervals showed a significant difference ($p < 0.001$) and linear associations ($p < 0.001$) in blood urea nitrogen (BUN), glucose (GLU), blood sodium concentration (Na), blood potassium concentration (K), fibrinogen (Fib), and haptoglobin (Hp). Of 6 blood variables, the optimal cut-off values were calculated for BUN, K, Fib, and Hp, and the area under the curve was 0.5 or more: BUN (9.5 mg/dL, AUC: 0.623), K (5.8 mmol/L, AUC: 0.599), Fib (650.0 mg/dL, AUC: 0.706), and Hp (12.5 mg/dL, AUC: 0.847). These findings could be useful in evaluating calves with diarrhea and making decision of further treatment of calf diarrhea in Hanwoo calves.

Key words hanwoo calves, calf diarrhea, hematology, serum chemistry, acute phase proteins.

Received September 20, 2022 / Revised November 6, 2022 / Accepted November 28, 2022

Introduction

Calf diarrhea is one of the most prevalent diseases in cattle industries. It has affected the morbidity and mortality of neonatal calves and their growth performances and has caused worldwide economic loss (2). In diarrhea, calves lose their body fluids, electrolytes and other substances from the body, which leads to electrolyte imbalance. This, in turn, causes the concentration of ions and other substances remaining in the fluid to rise, which increases the osmotic pressure of the extracellular fluid. This mechanism results in electrolyte imbalances and metabolic changes (5).

To evaluate calves affected by diarrhea, these metabolic changes should be well-evaluated for further treatment. Among various methods, blood analysis is considered to be a one of the most useful methods for diagnosing many organ and systemic diseases in veterinary science (12,20). During analysis, data obtained from the healthy and diseased animals can provide an insight into health and metabolic status. Information on the blood variables of calves with diarrhea has been well-studied and used in the treatment protocols; however, there is not enough data regarding Hanwoo (*Bos taurus coreanae*) cattle, especially with regard to calves (25,27)

Measuring the level of acute phase proteins (APPs) in blood is also considered an effective method for evaluating the condition of the host. The acute phase response is a reaction of the host to homeostatic disturbances caused by infections, tissue injuries, neoplastic growth, or immunological disorders, and the concentration of APPs in blood is altered accordingly (19). In addition, as inflammation is not always followed by an increase in leukocyte population in cattle, APPs are considered to be a sensitive marker that can help evaluate the status of the host (19). Although various APPs have been reported so far, many studies considered fibrinogen (Fib), haptoglobin (Hp), and serum amyloid A (SAA) as major APPs in cattle (8,16). However, there have been a lack of research about the relationship between APPs and calf diarrhea.

Many reports have suggested that hematological and serum biochemistry values are different from various factors including species or growth stages; therefore, it is important to analyze the hematology and serum biochemistry for each species of animals before diagnosing a disease using blood test results (18,24). However, because blood tests show different results depending on the test machines or methods, accurate analysis could be performed for the diagnosis of the disease only if the analyzer has its own reference intervals (RI) for the machines (11). Therefore, this study was conducted to identify useful blood variables in evaluating Hanwoo calves affected by calf diarrhea.

Materials and Methods

All procedures were performed according to ethical guidelines for the use of animal samples, as approved by the Institutional Animal Care and Use Committee (IACUC) in Jeonbuk National University (IACUC No. JBU 2016-00026).

Ten farms in five provinces (Gangwon-do, Chungcheongnam-do, Jeollabuk-do, Gyeongsangbuk-do, and Gyeongsangnam-do) in the Republic of Korea were subjected for this study. The farm province, location and history of vaccination against calf diarrhea were recorded (Table 1). Blood was collected from calves up to 60 days of age in the 10 farms during 2016-2017. Age, farm, and fecal scores were recorded. Ten mL blood was collected from the jugular vein of each calf. Three mL of whole blood was stored in two BD Vacutainer® K2 ethylene diamine tetraacetic acid (EDTA) tubes (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) at 4°C, and 4 mL of blood was allowed to clot at room temperature in BD Vacutainer® serum separator transport (SST) tubes (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) and immediately transported to the laboratory. The fecal consistency scoring system is a 4-level scoring system based on the degree of fluidity of the feces (score 0, normal; score 1, semi-formed; score 2, loose but bedding; score 3, watery) created by the University of Wisconsin-Madison School of Veterinary Medicine (29).

The SST tube and one K2 EDTA tube were centrifuged at 3000 g for 30 min to separate the serum and plasma, respectively. Complete blood count including red blood cell (RBC), hematocrit (HCT), hemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), reticulocyte (RETIC), platelet (PLT), white blood cell (WBC), neutrophil (NE), lymphocyte (LYM), monocyte (MO), eosinophil (EO), and basophil (BA) in the whole blood and blood chemistry [Total serum protein (TP), albumin (Alb), blood urea nitrogen (BUN), glucose (GLU), blood sodium concentration (Na), blood potassium concentration (K), and blood chloride concentration (Cl)] using serum were analyzed with an automatic blood analyzer (IDEXX Procyte^{Dx}, IDEXX Laboratories, Inc., Westbrook, ME, USA). Commercial enzyme-linked immunosorbent assay (ELISA) kits for SAA and Hp (PHASE™ RANGE multispecies SAA ELISA kit, Tridelta Development Ltd., County Kildare, Ireland; PHASE™ RANGE Haptoglobin kit, Tridelta Development Ltd., County Kildare, Ireland) were used to evaluate SAA and Hp in serum, following the manufacturer's instruction. Protein levels in the plasma and serum samples were calculated using an optical clinical refractometer (MASTER-SUR/Nα, ATAGO CO., LTD., Tokyo, Japan). Estimated Fib level was

Table 1. The description of 10 Hanwoo cattle farms investigated for calf diarrhea in 2016-2017

Farms	Provinces	Locations	Vaccination against calf diarrhea	Fecal scores				
				0 (n = 145)	1 (n = 185)	2 (n = 114)	3 (n = 86)	Total (n = 530)
1	Gangwon-do	Heongseong	No	0	12	4	1	17
2	Chungcheongnam-do	Asan	No	29	39	21	16	105
3	Gyeongsangbuk-do	Yeongju	No	44	58	39	31	172
4	Gyeongsangbuk-do	Mungyeong	No	12	9	4	1	26
5	Gyeongsangbuk-do	Sangju	No	2	4	2	1	9
6	Jeollabuk-do	Wanju	No	13	14	5	3	35
7	Jeollabuk-do	Iksan	No	6	1	0	1	8
8	Jeollabuk-do	Samnye	No	25	30	17	12	84
9	Jeollabuk-do	Gimje	No	4	15	22	20	61
10	Gyeongsangnam-do	Sancheong	No	10	3	0	0	13

Table 2. The mean and standard deviation description of blood parameters of Hanwoo calves (n = 530) less than 60 days according to fecal scores

Parameters	Fecal scores				F-value	p-value
	0 (n = 145)	1 (n = 185)	2 (n = 114)	3 (n = 86)		
RBC ($\times 10^6/\mu L$)	$10.1 \pm 2.0^{a,b}$	9.7 ± 1.8^{a}	10.1 ± 2.1^{a}	10.7 ± 2.2^{b}	5.156	0.002
HCT (%)	35.9 ± 8.2^{a}	34.2 ± 8.4^{a}	35.5 ± 8.4^{a}	36.2 ± 8.1^{a}	1.914	0.126
Hb (g/dL)	$11.5 \pm 2.3^{a,b}$	11.0 ± 2.0^{a}	$11.5 \pm 2.5^{a,b}$	12.0 ± 2.2^{b}	5.511	0.001
MCV (fL)	35.4 ± 4.1^{a}	35.0 ± 5.0^{a}	35.5 ± 5.6^{a}	34.3 ± 5.7^{a}	0.978	0.403
MCH (pg)	11.4 ± 1.3^{a}	11.4 ± 1.8^{a}	11.5 ± 1.6^{a}	11.4 ± 1.6^{a}	0.190	0.903
MCHC (g/dL)	32.4 ± 2.5^{a}	32.4 ± 3.8^{a}	$33.0 \pm 2.8^{a,b}$	33.6 ± 3.5^{b}	3.399	0.018
RETIC ($\times 10^3/\mu L$)	2.1 ± 2.1^{a}	3.3 ± 4.3^{b}	$2.2 \pm 1.7^{a,b}$	$2.5 \pm 2.8^{a,b}$	4.867	0.002
WBC ($\times 10^3/\mu L$)	10.8 ± 3.3^{a}	11.0 ± 4.2^{a}	$11.8 \pm 5.1^{a,b}$	12.6 ± 8.1^{b}	3.100	0.026
NE ($\times 10^3/\mu L$)	4.5 ± 2.6^{a}	5.1 ± 3.2^{a}	5.4 ± 4.2^{a}	5.6 ± 6.1^{a}	1.900	0.129
LY ($\times 10^3/\mu L$)	5.2 ± 1.7^{a}	4.9 ± 1.9^{a}	5.3 ± 1.8^{a}	5.8 ± 4.2^{b}	2.849	0.037
MO ($\times 10^3/\mu L$)	$0.9 \pm 0.6^{a,b}$	0.7 ± 0.6^{a}	$0.9 \pm 0.6^{a,b}$	1.0 ± 1.0^{b}	4.171	0.006
EO ($\times 10^3/\mu L$)	0.2 ± 0.2^{a}	0.2 ± 0.3^{a}	0.1 ± 0.2^{a}	0.2 ± 0.3^{a}	1.487	0.217
BA ($\times 10^3/\mu L$)	0.0 ± 0.1^{a}	0.0 ± 0.0^{a}	0.0 ± 0.1^{a}	0.0 ± 0.1^{a}	1.206	0.307
PLT ($\times 10^3/\mu L$)	711.3 ± 331.1^{a}	711.2 ± 298.2^{a}	794.0 ± 292.3^{a}	754.0 ± 312.7^{a}	2.547	0.055
TP (g/dL)	6.0 ± 0.8^{a}	5.9 ± 0.8^{a}	6.1 ± 0.7^{a}	5.9 ± 0.8^{a}	1.306	0.272
Alb (g/dL)	$2.7 \pm 0.3^{a,b}$	2.6 ± 0.3^{a}	2.8 ± 0.3^{b}	2.7 ± 0.4^{b}	5.179	0.002
BUN (mg/dL)	8.9 ± 3.5^{a}	10.8 ± 9.5^{a}	15.9 ± 16.4^{b}	15.9 ± 13.9^{b}	12.440	0.000
GLU (mg/dL)	95.7 ± 15.8^{a}	87.6 ± 18.2^{b}	88.7 ± 15.9^{b}	76.1 ± 21.7^{c}	22.117	0.000
Na (mmol/L)	139.2 ± 4.1^{a}	$138.6 \pm 5.0^{a,b}$	137.0 ± 7.2^{b}	133.8 ± 7.9^{c}	16.801	0.000
K (mmol/L)	5.7 ± 0.7^{a}	5.7 ± 0.7^{a}	6.2 ± 1.3^{b}	6.5 ± 1.8^{b}	15.953	0.000
Cl (mmol/L)	99.9 ± 3.6^{a}	$99.4 \pm 2.8^{a,b}$	$98.6 \pm 3.7^{b,c}$	97.5 ± 4.5^{c}	8.618	0.000
Fib (mg/dL)	546.2 ± 106.1^{a}	588.1 ± 224.0^{a}	753.5 ± 288.2^{b}	802.3 ± 289.8^{b}	35.113	0.000
SAA (mg/L)	168.4 ± 123.4^{a}	256.8 ± 146.0^{b}	230.9 ± 128.0^{b}	250.7 ± 142.6^{b}	12.105	0.000
Hp (mg/dL)	5.9 ± 6.7^{a}	7.7 ± 11.8^{a}	15.6 ± 9.7^{b}	31.2 ± 22.9^{c}	76.631	0.000

RBC, red blood cell; HCT, hematocrit; Hb, hemoglobin; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; RETIC, reticulocyte; PLT, platelet; WBC, white blood cell; NE, neutrophil; LYM, lymphocyte; MO, monocyte; EO, eosinophil; BA, basophil; TP, total serum protein; ALB, albumin; BUN, blood urea nitrogen; GLU, glucose; Na, blood sodium concentration; K, blood potassium concentration; Cl, blood chloride concentration; Fib, fibrinogen; SAA, serum amyloid A; Hp, haptoglobin. [a,b,c] Tukey HSD lists different letters between groups whose means that are statistically significant. Those with same letters means no significant difference among their means.

calculated by subtracting the plasma protein from the serum protein.

All blood variables were divided by fecal scores. All variables were compared by fecal scores using one-way analysis of variance (ANOVA) and Tukey's test for post hoc comparisons. The p values < 0.05 were considered statistically significant. RI was calculated using the Reference Value Advisor (v.2.1) freeware, a set of macroinstructions for Microsoft Excel (9), according to the guidelines recommended by the American Society for Veterinary Clinical Pathology (7). The validity of blood variables for the diagnosis of calf diarrhea was decided when the distribution of calves according to RI and fecal consistency showed significant difference in the linear-by-linear association and Pearson's chi-squared test. Next, the receiver operating characteristic (ROC) curve and the area under the curve (AUC) for fecal score 3 were calculated to determine whether blood variables could be effectively used for the predicting the process of calf diarrhea. For the interpretation of AUC, $0.5 < AUC \leq 0.7$ was considered to indicate less diagnostic accuracy and $0.7 < AUC \leq 0.9$ was considered moderate diagnostic accuracy (17). All statistical analyses were performed using SPSS (Version 25.0; IBM Corp., Armonk, NY), and all graphical analyses were performed using GraphPad Prism 6 (GraphPad Software, San Diego, CA).

Results

Blood samples were collected from 544 Hanwoo calves at 3 to 60 days of age (median: 29 days old) raised on 10 farms. Among 544 calves, 14 calves were not included since blood samples in K2 EDTA tubes were clotted. A total of 530 calves were categorized into 145 calves in fecal score 0, 185 calves in fecal score 1, 114 calves in fecal score 2, 86 calves in fecal score 3. Fecal samples were previously tested for the presence of common diarrhea-causing pathogens, such as *Escherichia coli* K99, *Salmonella* spp., *Cryptosporidium* spp., bovine coronavirus, bovine rotavirus, and bovine viral diarrhea virus and all pathogens except *Salmonella* spp. were detected in feces (1).

To determine the blood variables to calculate RI, all blood results were compared according to the fecal scores. The mean and standard deviation of blood variables for each fecal score are described in Table 2. Among the variables, RBC, Hb, MCHC, RETIC, WBC, LYM, MO, Alb, BUN, GLU, Na, K, Cl, Fib, SAA, and Hp showed significant differences ($p < 0.05$) according to the fecal scores (Table 2); thus, additional analysis was performed.

To compare the distribution of 530 Hanwoo calves according to RI in blood variables shown significantly different by fecal scores, the RI for each blood variable was calculated for blood variables that were significantly different according

Table 3. Reference intervals (RI) for blood parameters of healthy Hanwoo calves (n = 145)

Parameters	Descriptive statistics		95% RI	
	Mean ± SD	Median	Lower limit & CI (90%)	Upper limit & CI (90%)
RBC ($\times10^6/\mu L$)	10.1 ± 2.0	10.3	6.3 (5.9-6.7)	14.0 (13.5-14.4)
Hb (g/dL)	11.5 ± 2.3	11.9	7.0 (6.5-7.4)	16.0 (15.4-16.4)
MCHC (g/dL)	32.4 ± 2.5	0.8	27.3 (26.5-28.0)	37.5 (36.8-38.3)
RETIC ($\times10^3/\mu L$)	2.1 ± 2.1	1.8	0.0 (0.0-0.0)	9.5 (6.8-12.5)
WBC ($\times10^3/\mu L$)	10.8 ± 3.3	10.2	4.1 (3.4-4.7)	17.2 (16.2-18.3)
LYM ($\times10^3/\mu L$)	5.2 ± 1.7	4.8	2.5 (1.7-2.7)	9.4 (8.4-10.5)
MO ($\times10^3/\mu L$)	0.9 ± 0.6	0.9	0.0 (0.0-0.0)	2.1 (1.8-5.1)
Alb (g/dL)	2.7 ± 0.3	2.7	2.0 (2.0-2.1)	3.4 (3.3-3.5)
BUN (mg/dL)	8.9 ± 3.5	8.0	3.0 (1.0-4.0)	15.7 (14.7-16.8)
GLU (mg/dL)	95.7 ± 15.8	96.5	64.9 (61.6-68.3)	126.9 (123.4-130.4)
Na (mmol/L)	139.2 ± 41.0	139.0	131.2 (129.8-132.5)	147.2 (145.8-148.9)
K (mmol/L)	5.7 ± 0.7	5.6	4.3 (4.2-4.5)	7.0 (6.8-7.3)
Cl (mmol/L)	99.9 ± 3.6	100.0	92.8 (91.6-94.1)	106.9 (105.7-108.2)
Fib (mg/dL)	546.2 ± 106.1	500.0	328.5 (303.1-354.2)	766.1 (738.7-795.4)
SAA (mg/L)	168.4 ± 123.4	143.7	0.5 (0.0-14.4)	526.2 (389.2-669.4)
Hp (mg/dL)	5.9 ± 6.7	5.0	0.0 (0.0-0.0)	24.0 (16.3-32.0)

RI, reference intervals; CI, confidence interval; RBC, red blood cell; Hb, hemoglobin; MCHC, mean corpuscular hemoglobin concentration; RETIC, reticulocyte; WBC, white blood cell; LYM, lymphocyte; MO, monocyte; Alb, albumin; BUN, blood urea nitrogen; GLU, glucose; Na, blood sodium concentration; K, blood potassium concentration; Cl, blood chloride concentration; Fib, fibrinogen; SAA, serum amyloid A; Hp, haptoglobin.

Table 4. Average ranges of hematology and serum chemistry according to the fecal score of Hanwoo calves (n = 530)

Para-meters	RI group	Fecal scores: 0 (n = 145) Mean ± SD	No. of calves	1 (n = 185) Mean ± SD	No. of calves	2 (n = 114) Mean ± SD	No. of calves	3 (n = 86) Mean ± SD	No. of calves	p-value Pe	Li
RBC ($\times 10^6/\mu L$)	Lower RI	5.0 ± 1.0	2 (1.4%)	5.3 ± 0.8	5 (2.7%)	5.2 ± 1.0	4 (3.6%)	-	0 (0.0%)	0.117	0.076
	Within RI	10.1 ± 1.8	140 (96.6%)	9.8 ± 1.6	177 (95.7%)	10.1 ± 1.7	107 (93.9%)	10.4 ± 1.8	80 (93.0%)		
	Higher RI	14.6 ± 0.4	3 (2.1%)	14.5 ± 0.4	3 (1.6%)	15.3 ± 0.8	3 (2.6%)	15.2 ± 0.9	6 (7.0%)		
Hb (g/dL)	Lower RI	5.8 ± 0.3	2 (1.4%)	6.5 ± 0.3	4 (2.2%)	5.7 ± 0.9	5 (4.4%)	-	0 (0.0%)	0.258	0.978
	Within RI	11.5 ± 2.1	140 (96.6%)	11.0 ± 1.9	180 (97.3%)	11.6 ± 2.0	106 (93.0%)	12.0 ± 2.0	85 (98.8%)		
	Higher RI	16.2 ± 0.1	3 (2.1%)	16.5	1 (0.5%)	18.2 ± 1.5	3 (2.6%)	19.3	1 (1.2%)		
MCHC (g/dL)	Lower RI	25.1 ± 0.6	3 (2.1%)	23.8 ± 6.5	12 (6.5%)	24.7 ± 1.4	3 (2.6%)	26.7 ± 0.1	2 (2.3%)	0.077	0.082
	Within RI	32.3 ± 2.0	136 (93.8%)	32.6 ± 1.9	162 (87.6%)	32.9 ± 2.0	105 (92.1%)	32.8 ± 1.9	74 (86.0%)		
	Higher RI	38.6 ± 1.6	6 (4.1%)	39.8 ± 2.4	11 (5.9%)	39.7 ± 1.5	6 (5.3%)	41.2 ± 1.9	10 (11.6%)		
RETIC ($\times 10^3/\mu L$)	Lower RI	-	0 (0.0%)	-	0 (0.0%)	-	0 (0.0%)	-	0 (0.0%)	0.001	0.244
	Within RI	1.9 ± 1.7	136 (93.8%)	2.4 ± 2.0	155 (83.8%)	2.2 ± 1.7	108 (94.7%)	2.1 ± 2.0	82 (95.3%)		
	Higher RI	11.0 ± 1.5	9 (6.2%)	16.9 ± 6.6	30 (16.2%)	14.0 ± 1.2	6 (5.3%)	12.2 ± 1.1	4 (4.7%)		
WBC ($\times 10^3/\mu L$)	Lower RI	3.6	1 (0.7%)	3.4 ± 0.3	2 (1.1%)	-	0 (0.0%)	-	0 (0.0%)	0.051	0.003
	Within RI	10.4 ± 2.7	138 (95.2%)	10.2 ± 3.0	169 (91.4%)	10.5 ± 3.1	103 (90.4%)	9.8 ± 2.8	72 (83.7%)		
	Higher RI	19.4 ± 1.9	6 (4.1%)	20.3 ± 4.0	14 (7.6%)	23.5 ± 5.8	11 (9.6%)	26.7 ± 11.2	14 (16.3%)		
LYM ($\times 10^3/\mu L$)	Lower RI	2.0 ± 0.5	2 (1.4%)	1.9 ± 0.4	16 (8.6%)	1.7 ± 0.2	3 (2.6%)	2.1	1 (1.2%)	0.009	0.554
	Within RI	5.0 ± 1.4	136 (93.8%)	5.1 ± 1.4	164 (88.6%)	5.1 ± 1.3	105 (92.1%)	5.1 ± 1.5	79 (91.9%)		
	Higher RI	9.5 ± 0.6	7 (4.8%)	11.1 ± 4.3	5 (2.7%)	10.3 ± 1.3	6 (5.3%)	15.8 ± 11.5	6 (7.0%)		

Table 4. Continued

Para-meters	RI group	Fecal scores								p-value	
		0 (n = 145)		1 (n = 185)		2 (n = 114)		3 (n = 86)			
		Mean ± SD	No. of calves	Mean ± SD	No. of calves	Mean ± SD	No. of calves	Mean ± SD	No. of calves	Pe	Li
MO ($\times 10^3/\mu L$)	Lower RI	0.0	1 (0.7%)	0.0 ± 0.0	5 (2.7%)	-	0 (0.0%)	-	0 (0.0%)	0.162	0.234
	Within RI	0.9 ± 0.5	141 (97.2%)	0.7 ± 0.6	175 (94.6%)	0.9 ± 0.5	110 (96.5%)	0.8 ± 0.5	81 (94.2%)		
	Higher RI	3.3 ± 1.6	3 (2.1%)	2.4 ± 0.4	5 (2.7%)	2.5 ± 0.2	4 (3.5%)	3.5 ± 2.0	5 (5.8%)		
Alb (g/dL)	Lower RI	2.0 ± 0.1	4 (2.8%)	2.0 ± 0.1	4 (2.2%)	2.0	1 (0.9%)	1.9 ± 0.1	2 (2.3%)	0.172	0.940
	Within RI	2.7 ± 0.3	135 (93.1%)	2.6 ± 0.3	181 (97.8%)	2.7 ± 0.2	108 (94.7%)	2.7 ± 0.2	81 (94.2%)		
	Higher RI	3.7 ± 0.3	6 (4.1%)	-	0 (0.0%)	3.8 ± 0.6	5 (4.4%)	4.3 ± 0.9	3 (3.5%)		
BUN* (mg/dL)	Lower RI	2.4 ± 0.9	5 (3.4%)	2.8 ± 0.4	6 (3.2%)	3.0 ± 0.0	2 (1.8%)	3.0 ± 0.0	2 (2.3%)	<0.001	<0.001
	Within RI	8.7 ± 2.7	133 (91.7%)	8.7 ± 2.7	155 (83.8%)	9.2 ± 2.8	84 (73.7%)	9.1 ± 2.8	55 (64.0%)		
	Higher RI	17.3 ± 2.2	7 (4.8%)	26.9 ± 19.0	24 (13.0%)	36.9 ± 22.0	28 (24.6%)	29.4 ± 16.6	29 (33.7%)		
GLU* (mg/dL)	Lower RI	55.2 ± 6.5	5 (3.4%)	53.6 ± 11.5	19 (10.3%)	56.9 ± 6.7	8 (7.0%)	52.2 ± 9.6	23 (26.7%)	<0.001	<0.001
	Within RI	96.9 ± 13.6	139 (95.9%)	90.7 ± 13.1	163 (88.1%)	90.7 ± 12.8	105 (92.1%)	82.3 ± 13.9	60 (69.8%)		
	Higher RI	139	1 (0.7%)	137.0 ± 8.0	3 (1.6%)	141	1 (0.9%)	136.7 ± 9.1	3 (3.5%)		
Na* (mmol/L)	Lower RI	129.4 ± 2.6	5 (3.4%)	125.1 ± 6.4	10 (5.4%)	121.9 ± 7.4	13 (11.4%)	123.2 ± 6.4	23 (26.7%)	<0.001	<0.001
	Within RI	139.3 ± 3.0	137 (94.5%)	139.2 ± 3.3	172 (93.0%)	138.3 ± 3.5	96 (84.2%)	138.0 ± 3.8	63 (73.3%)		
	Higher RI	152.7 ± 5.0	3 (2.1%)	149.3 ± 1.5	3 (1.6%)	150.0 ± 1.9	5 (4.4%)	-	0 (0.0%)		
K* (mmol/L)	Lower RI	4.3	1 (0.7%)	4.1 ± 0.1	3 (1.6%)	3.8	1 (0.9%)	2.8	1 (1.2%)	<0.001	<0.001
	Within RI	5.6 ± 0.6	139 (95.9%)	5.7 ± 0.5	174 (94.1%)	5.8 ± 0.6	95 (83.3%)	5.7 ± 0.6	66 (76.6%)		
	Higher RI	7.4 ± 0.3	5 (3.4%)	7.6 ± 0.4	8 (4.3%)	8.7 ± 1.2	18 (15.8%)	9.5 ± 0.9	19 (22.1%)		

Table 4. Continued

Parameters	RI group	Fecal scores								p-value	
		0 (n = 145)		1 (n = 185)		2 (n = 114)		3 (n = 86)			
		Mean ± SD	No. of calves	Mean ± SD	No. of calves	Mean ± SD	No. of calves	Mean ± SD	No. of calves	Pe	Li
Cl (mmol/L)	Lower RI	88.7 ± 4.2	3 (2.1%)	89.5 ± 3.5	2 (1.1%)	88.3 ± 2.9	7 (6.1%)	88.9 ± 3.3	12 (14.0%)	<0.001	0.476
	Within RI	99.8 ± 2.6	136 (93.8%)	99.4 ± 2.4	181 (97.8%)	99.2 ± 2.5	107 (93.9%)	99.1 ± 0.3	74 (86.0%)		
	Higher RI	108.5 ± 2.4	6 (4.1%)	108.0 ± 1.4	2 (1.1%)	-	0 (0.0%)	-	0 (0.0%)		
Fib* (mg/dL)	Lower RI	300.0 ± 0.0	4 (2.8%)	237.0 ± 74.2	27 (14.6%)	175.0 ± 125.8	4 (3.5%)	260.0 ± 54.8	5 (5.8%)	<0.001	<0.001
	Within RI	546.0 ± 90.8	137 (94.5%)	551.8 ± 107.4	112 (60.5%)	580.4 ± 115.1	56 (49.1%)	608.8 ± 79.3	34 (39.5%)		
	Higher RI	800.0 ± 0.0	4 (2.8%)	882.6 ± 87.7	46 (24.9%)	975.9 ± 234.7	54 (47.4%)	1000.0 ± 225.5	47 (54.7%)		
SAA (mg/L)	Lower RI	0.0 ± 0.0	3 (2.1%)	0	1 (0.5%)	0	1 (0.9%)	0	1 (1.2%)	0.045	0.071
	Within RI	160.6 ± 110.5	137 (94.5%)	233.0 ± 114.5	157 (84.9%)	227.4 ± 117.6	98 (86.0%)	233.0 ± 117.1	76 (88.4%)		
	Higher RI	578.9 ± 64.7	5 (3.4%)	598.2 ± 47.8	27 (14.6%)	589.0 ± 35.4	15 (13.2%)	587.6 ± 31.2	9 (10.5%)		
Hp* (mg/dL)	Lower RI	-	0 (0.0%)	-	0 (0.0%)	-	0 (0.0%)	-	0 (0.0%)	<0.001	<0.001
	Within RI	5.5 ± 5.9	143 (98.6%)	5.6 ± 4.8	178 (96.2%)	12.3 ± 7.4	92 (80.7%)	11.9 ± 5.9	36 (41.9%)		
	Higher RI	30.0 ± 2.8	2 (1.4%)	25.5 ± 11.8	7 (3.8%)	29.4 ± 4.4	22 (19.3%)	45.1 ± 20.4	50 (58.1%)		

RI, Reference intervals; SD, standard deviation; No., number; Pe, Pearson's chi-square test; Li, linear by linear association; -, not calculable; RBC, red blood cell; Hb, hemoglobin; MCHC, mean corpuscular hemoglobin concentration; RETIC, reticulocyte; WBC, white blood cell; LYM, lymphocyte; MO, monocyte; Alb, albumin; BUN, blood urea nitrogen; GLU, glucose; Na, blood sodium concentration; K, blood potassium concentration; Cl, blood chloride concentration; Fib, fibrinogen; SAA, serum amyloid A; Hp, haptoglobin. *Means significantly different in linear by linear association and Pearson's chi-square test.

to fecal scores. The described statistics and calculated 95% RI with 90% confidence interval for RBC, Hb, MCHC, RETIC, WBC, LYM, MO, Alb, BUN, GLU, Na, K, Cl, Fib, SAA, and Hp in 145 Hanwoo calves with fecal score 0 are presented in Table 3. After RI calculation, the distribution of 530 Hanwoo calves according to fecal scores and blood variables that were significantly different by fecal scores was investigated (Table 4). Thus, the distributions of calves showed significant difference and linear associations in terms of BUN, GLU, Na, K, Fib, and Hp. The number of calves that showed lower than RI was increased in GLU and Na as fecal scores increased. In contrast, the number of calves that showed higher than RI was increased in BUN, K, Fib and Hp, as fecal scores increased.

To determine the valuable blood variables and their optimal cut-off values of calf diarrhea, ROC analysis was performed. The results of ROC curve analysis, sensitivity-specificity analysis, and determination of cut-off values of BUN, Na, GLU, K, Fib, and Hp are described in Table 5. The predictive accuracy of BUN was low (AUC: 0.623, 95% CI 0.554-0.692, $p < 0.001$) at a BUN cut-off value of 9.5 mg/dL, and the sensitivity and specificity in predicting calf diarrhea were 60.0% and 56.1%, respectively. The predictive ability of GLU was poor (AUC: 0.271, 95% CI 0.207-0.335, $p < 0.001$), and at a GLU cut-off value of 83.5 mg/dL, the sensitivity and specificity in predicting calf diarrhea were 30.2% and 30.6%, respectively. The predictive ability of Na was poor (AUC: 0.366, 95% CI 0.259-0.392, $p < 0.001$) and at a Na cut-off value of 137.5 mmol/L, and the sensitivity and specificity in predicting calf diarrhea were 39.8% and 37.1%, respectively. The predictive ability of K was low (AUC: 0.599, 95% CI 0.526-0.672, $p < 0.001$), at a K cut-off value of 5.8 mmol/L, the sensitivity and specificity in predicting calf diarrhea were 58.3% and 54.2%, respectively. The predictive ability of Fib was moderate (AUC: 0.706, 95% CI 0.644-0.768, $p < 0.001$), at a Fib cut-off value of 650.0 mg/dL, the sensitivity and specificity in predicting calf diarrhea were 68.6% and 61.9%, respectively. The predictive ability of Hp was moderate (AUC: 0.847, 95% CI 0.801-0.893, $p < 0.001$), at a Hp cut-off value of 12.5 mg/dL, the sensitivity and specificity in predicting calf diarrhea were 74.4% and 75.9%, respectively.

Discussion

Hematological changes could give the insights of physiological and pathological changes and also helpful information for the diagnosis, monitoring, and prognosis of diseases (20). In calf diarrhea, various metabolic and inflammatory changes occur in the body of the calf, so it is important to identify these changes during evaluating calf status (22). In this study, among the various methods for evaluating these changes, the analyses of hematology, serum biochemistry, and some acute phase proteins were performed to identify useful blood variables in the diagnosis of calf diarrhea.

As RBC, Hb, MCHC, RETIC, WBC, MO, Alb, BUN, GLU, Na, K, Cl, Fib, SAA, and Hp showed significant difference according to their fecal scores, the RI of these blood variables were calculated. The blood variables related to CBC panels (RBC, Hb, MCHC, RETIC, WBC, MO) showed greater differences than was observed in other reports, however, these differences were considered to be normal physiological changes in young calves (15,18). Moreover, although Fib and SAA in this study were higher than those reported in previous studies, similar Fib and SAA values were also reported in Hanwoo calves of similar ages (3,13). The number of calves for RI calculation was too small to present the exact RI in Hanwoo calves; nevertheless, further analysis was conducted because the RI in this research was considered to be reliable.

After comparing the RI of blood variables with fecal scores, BUN, GLU, Na, K, Cl, Fib, and Hp were found to be useful blood variables in calf diarrhea. In this research, the distribution rates of 530 Hanwoo calves showing higher BUN than

Table 5. The results of receiver operating characteristic curve analysis, sensitivity-specificity analysis, and determination of cut-off values

Blood variables	AUC	SE	95% confidence interval	p-value	Cut point	Sensitivity	Specificity
BUN (mg/dL)*	0.623	0.035	0.554-0.692	<0.001	9.5	0.600	0.561
GLU (mg/dL)	0.271	0.032	0.207-0.335	<0.001	83.5	0.302	0.306
Na (mmol/L)	0.366	0.026	0.259-0.392	<0.001	137.5	0.398	0.371
K (mmol/L)*	0.599	0.037	0.526-0.672	<0.001	5.75	0.583	0.542
Fib (mg/dL)**	0.706	0.032	0.644-0.768	<0.001	650.0	0.686	0.619
Hp (mg/dL)**	0.847	0.023	0.801-0.893	<0.001	12.50	0.744	0.759

AUC, area under the ROC curve; ROC, receiver operating characteristic; SE, standard error; BUN, blood urea nitrogen; GLU, glucose; Na, blood sodium concentration; K, blood potassium concentration; Fib, fibrinogen; Hp, haptoglobin. *mean AUC >0.5; **mean AUC >0.7.

RI (4.8% to 34.9%) significantly increased with increasing fecal scores from 0 to 3 ($p < 0.001$). Higher BUN was commonly reported in calf diarrhea with dehydration and BUN in diarrheic calves that died was significantly higher ($p < 0.01$) than in diarrheic calves that recovered in a previous report (22). Furthermore, BUN was significantly influenced by physical activity, dehydration status, and prognosis of calf diarrhea ($p < 0.001$) (15). These differences could be attributed to the severity of calf diarrhea. Consistent with these results, BUN showed predictive ability in this research, which could prove useful for the predicting the process of calf diarrhea in terms of fecal scores.

Significant changes in GLU levels according to fecal scores were detected in this research. With increasing fecal scores from 0 to 3, the distribution rates of 530 Hanwoo calves showing lower GLU than RI was significantly increased (3.4% to 26.7%) ($p < 0.001$). Hypoglycemia has been reported in diarrheic calves, especially those that were weak, lethargic, or comatose and usually occurs in the terminal stages of the disease (28). Severe hypoglycemia in calf diarrhea was associated with poor survival rates (20.6%), and calves with severe hypoglycemia (less than 40 mg/dL) were extremely dehydrated (23). However, GLU showed no predictive ability in this study, which was also reported in other studies that hypoglycemia showed 3.09 times higher mortality in calves with diarrhea; however, the predictive ability of GLU was poor (AUC = 0.384) according to their ROC analysis (28).

Significant changes in Na and K levels according to fecal scores were detected in the present study. With increasing fecal scores from 0 to 3, the distribution rates of 530 Hanwoo calves showing lower Na than RI was significantly increased (3.4% to 26.7%) ($p < 0.001$). Additionally, with increasing fecal scores from 0 to 3, the rates of Hanwoo calves showing higher K than RI was significantly increased (3.4% to 22.1%) ($p < 0.001$). Electrolytes could vary considerably in calves with diarrhea. Many previous studies reported various changes (lower to higher RI) in electrolytes (Na, K, Cl) during calf diarrhea (15,21,27). These differences were attributed to the severity, duration, and causes of calf diarrhea (5). K showed predictive ability while Na showed no predictive ability in calf diarrhea in this study. Hyperkalemia was most closely associated with dehydration and weakly correlated with venous blood pH levels (4). Moreover, K was more strongly associated with calf diarrhea than Na in other reports (26).

The method to measure Fib in this research was based on the fact that serum does not contain Fib and clotting factors which are the proteins found in plasma. Therefore, plasma protein levels subtracted by serum protein levels would be indirectly correlated to the amount of Fib, which is known as an acute phase protein. The distribution rates of 530 Hanwoo calves showing higher Fib (2.8% to 54.7%) than RI significantly increased with increasing fecal scores from 0 to 3 ($p < 0.001$). The distribution rates of 530 Hanwoo calves showing higher Hp (1.4% to 58.1%) than RI significantly increased with increasing fecal scores from 0 to 3 ($p < 0.001$). To our knowledge, this study is the first to measure the RI of acute phase proteins and its application to calf diarrhea in Hanwoo calves. In addition, Fib and Hp showed moderate predictive ability. These results support the fact that Hp and Fib are major acute phase proteins in cattle. Many researchers have reported the usefulness of quantifying these proteins when monitoring animal health, especially to determine the inflammatory status of the animal (6,19).

In the ROC analysis, the predictive ability of BUN, GLU, Na, K, Fib, and Hp was evaluated. The acceptable predictive ability of BUN (AUC: 0.636), K (AUC: 0.617), Fib (AUC: 0.706), and Hp (AUC: 0.847) was evaluated, and their optimal cut-off values were calculated as follows: BUN (9.5 mg/dL), K (5.8 mmol/dL), Fib (650.0 mg/dL), and Hp (12.5 mg/dL). BUN and K were valuable prognostic indicators for predicting the fate of diarrheic calves according to a previous study, which was consistent with our results (22). Furthermore, although studies evaluating the prognostic value of Hp and Fib are lacking, many studies have reported increased Hp in calf diarrhea and other calf diseases (6,10). The findings in this study would be helpful in the treatment of calf diarrhea. However, this study had a limitation that actual treatment of calf diarrhea was not performed, so further studies might need to evaluate the cut-off values of BUN, K, Fib, and Hp in the actual treatment and prognosis evaluation of calf diarrhea.

In this study, we found that BUN, GLU, Na, K, Fib, and Hp showed significant difference and linear associations in calf diarrhea. Furthermore, after ROC analysis, the optimal cut-off values of BUN, K, Fib, and Hp were calculated. Although many previous studies showed CBC, serum biochemistry, APPs and pro-inflammatory cytokines in diarrheic and non-diarrheic calves, specific values of blood variables were not provided because of not enough number of calves (14). In this study, blood variables that could be useful for calf diarrhea, and ROC analysis to understand the diagnostic and predicting power of some blood variables, and their optimal cut-off values were provided in large number of Hanwoo calves. These findings would be useful for field veterinarians and animal caretakers in diagnosing and predicting the prognosis of calf diarrhea in Hanwoo calves.

Acknowledgements

We would like to thank farm owners and caretakers for helping with collection of samples. This research was supported by a National Research Foundation of Korea (NRF) grant funded by the Korean government (MSIT) (No. NRF2021R1A2C100517111) and partially supported by the Korea Institute of Planning and Evaluation for Technology in Food, Agriculture, and Forestry (IPET) (Grant No. 122017-02-1-HD030).

Conflicts of Interest

The authors have no conflicting interests.

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