Comparison of Blood Metabolites and Enzyme Activities at Different Slaughter Ages of Hanwoo Cattle

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ABSTRACT

This study was conducted to investigate changes of blood metabolites and enzyme activities at different slaughter ages of Hanwoo cattle. Blood samples were taken from six hundred thirty two steers (24~33 months of age) and one hundred twenty eight bulls (17~24 months of age) by venipuncture from caudal vein immediately prior to slaughter. Glucose concentrations were linearly increased ($P<0.001$) with increases of the slaughter age in steers, and blood urea nitrogen (BUN) concentrations were linearly decreased ($P<0.001$) with increases of the slaughter age in steers. Likewise, glucose and albumin concentrations in bulls were linearly decreased ($P=0.009$ and $P<0.001$, respectively) with increases of the slaughter age. BUN, cholesterol, triglycerides, and total cholesterol concentrations were linearly increased ($P<0.001$, 0.018 and 0.002, respectively), and creatinine concentrations were quadratically increased ($P=0.009$) with increases of the slaughter age. Activity of gamma glutamyltransferase (γ -GT) and aspartate aminotransferase (AST) was linearly increased ($P<0.001$) with increases of the slaughter age in both steers and bulls, whereas alanine aminotransferase (ALT) was not affected by increasing slaughter age. In addition, the concentrations of γ -GT and AST were higher ($P<0.05$) for bulls than those of steers at 24 months of age. Increased levels of γ -GT and AST indicate impaired liver function; it may be associated with increases of concentrate level which is a concomitant of the extended fattening periods in Hanwoo feeding. In conclusion, the results in the present study may be one of the useful information for diagnosis of the metabolic disorder in Hanwoo cattle.

(**Key words** : Blood metabolites, Blood enzyme activity, Slaughter ages, Hanwoo cattle)

INTRODUCTION

Recently, Hanwoo industry has been increasingly adopting extended fattening period (more than 30 months of age) in order to produce a high grade of Hanwoo beef, thereby, the fattening period is usually coupled with increased concentrate level (up to 95%) in the diet. Even feeding concentrate only throughout the finishing phase has become a routine practice in Hanwoo bulls (RDA, 2007). Although feeding a high-concentrate diet can be favorable for increasing beef quality, it can drop ruminal pH below 6.0 which causes the ruminal disturbance and increase the risk of acidosis. Consequently, lengthy fattening period may have an adverse effect on animal's health because it could induce acidosis, bloat, and liver abscess.

Changes of blood metabolites in ruminants have been used as a criterion of nutrient status (Russel, 1984) and as an index of metabolic disturbance (Rankins et al., 1991; Puoli et al., 1992). The concentration of specific enzymes in serum serves as indicators of tissue damage or metabolic disorders (Kramer, 1989). Hepatic dysfunction that can be induced by a high-concentrate diet in the ruminants has been documented by elevated serum protein, bilirubin, and enzyme activities such as alanine aminotransferase, aspartate aminotransferase, and gamma-glutamyltransferase (Lechtenberg and Nagaraja, 1991). Accordingly, blood profiling is considered to be one of the significant sources for judging the nutrient status and body condition, and a comparison of some blood metabolites and enzyme activities in ruminants may be used for diagnosis of the metabolic disorder. However, the effects

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of the extended fattening period with a high-concentrate diet on hematologic aspect in Hanwoo cattle is not well established and properly understood. Therefore, this study was conducted to collect the blood data and investigate the changes in blood metabolites and enzyme activities at different slaughter ages of Hanwoo cattle fed a high-concentrate diet.

MATERIALS AND METHODS

1. Experimental procedures

This experiment was conducted at National Livestock Cooperative Federation in Bucheon, South Korea. Of the shipped cattle to the abattoir, six hundred thirty two steers (24–33 months of age) and one hundred twenty eight bulls (17–24 months of age), which were raised with a high-concentrate diet (over 90% of total feed) at the late fattening phase, were randomly selected for blood collection. Blood samples were taken by venipuncture from caudal vein immediately prior to slaughter between 0500 and 0600 in the morning, a day after the cattle shipment to the abattoir. Thus, the cattle were fasted for 24 h prior to blood collection. Blood samples were collected into 10 mL vacuum tubes (BD Vacutainer, Becton & Dickinson, NJ, USA) without any anticoagulant, and allow the blood to clot for 1 h at ambient temperature. Serum samples were separated after centrifugation ($2,000 \times g$ at 4°C for 20 min) and stored (-20°C) until analysis.

2. Blood metabolites and enzyme activities

Blood samples were analyzed by an automatic blood analyzer (Express Plus, Ciba-Corning, CA, USA) for metabolites and enzyme activities. Total protein (TP), glucose, blood urea nitrogen (BUN), albumin, and creatinine concentrations were analyzed according to the biuret method of Flack and Woollen (1984), the hexokinase method of Farrance (1987), the urease method of Rocch-Ramel (1967), the bromocresol green method of Doumas et al. (1971), and the picric acid method of Husdan and Rapoport (1968), respectively.

Total cholesterol (TC), triglycerides (TG), calcium, inorganic phosphorus (IP), and iron concentrations were analyzed using the method of Allain et al. (1974), the method of McGowan et al. (1983), the cresolphthalein complexone method of Moorehead and Briggs (1974), the method of Daly and

Estingshausen (1972), and the method of Higgins (1981), respectively.

Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were analyzed by the International Federation of Clinical Chemistry and Laboratory Medicine method (1986a,b), and serum gamma glutamyltransferase (γ -GT) activities were measured by the method of Rosalki and Tarlow (1974).

3. Statistical analysis

Data obtained from the blood analysis was subjected to statistical analysis using the GLM procedure (SAS Inst. Inc., Cary, NC) according to the model: $Y_{ij} = \mu + A_i + S_j + (A \times S)_{ij} + e_{ij}$, where Y_{ij} , μ , A_i , S_j , $(A \times S)_{ij}$ and e_{ij} are the response, overall mean, mean effect of animal i , mean effect of slaughter age j , and interaction between animal and slaughter age and random residual error, respectively.

An orthogonal contrast was used to assess linear and quadratic relationships between the slaughter age and the dependent variable. Orthogonal coefficients for unequally spaced slaughter ages were acquired using procedure Interactive Matrix Language (SAS Inst. Inc.). Duncan's multiple range test was used to determine significant differences between bulls and steers at the same slaughter age (24 months of age). Effects were declared significant at $P < 0.05$.

RESULTS

Changes of serum metabolites, enzyme activities, and body weight at different slaughter ages in Hanwoo steers are presented in Table 1. Glucose concentrations in steers were linearly ($P < 0.001$) and quadratically ($P = 0.008$) increased with increases of the slaughter age, whereas BUN concentrations were linearly decreased ($P < 0.001$) with increases of the slaughter age. The γ -GT ($P = 0.007$) and AST ($P = 0.001$) activities were linearly increased with increasing slaughter age. However, serum ALT activities were not affected by slaughter age.

Changes in serum metabolites, enzyme activities, and body weight at different slaughter ages in bulls are presented in Table 2. Glucose and albumin concentrations in bulls were linearly ($P = 0.009$, < 0.001 , respectively) decreased with increasing slaughter age, whereas BUN, TG, and cholesterol concentrations were linearly increased with increasing slaughter age ($P < 0.001$, 0.018 , and 0.002 , respectively). The

Table 1. Changes in serum metabolites, enzyme activities, and body weight by different slaughter ages in Hanwoo steers

Items ¹⁾	Slaughter age, months									SEM	Statistical significance ²⁾	
	24	25	26	27	28	29	30	31	33		Linear	Quadratic
No. of steers	42	25	66	27	113	57	257	29	16			
Glucose, mg/dl	84.45	63.35	96.71	94.05	91.20	94.36	90.48	104.91	92.89	3.05	<001	0.008
BUN, mg/dl	19.34	18.22	16.91	14.01	16.62	16.06	15.74	14.08	14.08	0.57	<001	0.067
TG, mg/dl	19.08	18.44	18.64	18.84	16.98	16.75	16.99	18.69	18.88	0.97	0.654	0.123
TC, mg/dl	178.39	172.00	181.56	161.16	177.47	184.95	185.08	174.91	176.95	6.14	0.605	0.893
TP, mg/dl	6.24	6.10	6.52	6.15	6.20	6.28	6.34	6.17	6.35	0.06	0.436	0.752
Creatinine, mg/dl	1.33	1.28	1.27	1.33	1.32	1.30	1.30	1.36	0.35	0.03	0.259	0.386
Albumin, g/dl	4.68	4.56	4.65	4.44	4.57	4.57	4.61	4.46	4.54	0.04	0.069	0.265
Ca, mg/dl	7.95	7.25	7.53	8.20	7.75	7.74	7.45	7.91	7.98	0.16	0.318	0.498
IP, mg/dl	5.27	5.43	5.01	4.78	5.23	4.84	5.05	5.02	4.88	0.16	0.088	0.403
Iron, mg/dl	1.07	1.55	1.06	1.26	1.24	1.08	1.27	1.62	0.91	0.12	0.559	0.050
γ -GT, u/l	24.79	28.64	26.27	27.00	29.86	28.32	28.27	28.52	35.25	1.87	0.007	0.288
AST, u/l	88.72	94.86	93.80	104.25	96.38	112.01	98.96	97.95	113.03	4.01	0.001	0.727
ALT, u/l	18.93	19.83	22.56	18.86	19.66	22.34	20.81	17.63	19.74	0.76	0.651	0.132
BW, kg	603.02	609.20	639.46	635.22	648.06	651.51	665.84	696.76	703.59	8.72	<001	0.124

¹⁾ BUN: blood urea nitrogen; TG: triglyceride; TC: total cholesterol; TP: total protein; IP: inorganic phosphorus; γ -GT: gamma glutamyltransferase; AST: aspartate aminotransferase; ALT: alanine aminotransferase.

²⁾ Statistical significance of linear and quadratic effects were tested by contrast statement of GLM procedure.

Table 2. Changes in serum metabolites, enzyme activities, and body weight by different slaughter ages in Hanwoo bulls

Items ¹⁾	Slaughter age, months					SEM	Statistical significance ²⁾	
	17	20	21	22	24		Linear	Quadratic
No. of bulls	13	41	38	22	14			
Glucose, mg/dl	93.00	90.61	95.45	77.67	81.21	3.14	0.009	0.341
BUN, mg/dl	13.15	13.42	15.91	20.57	18.56	0.82	<001	0.860
TG, mg/dl	9.50	10.98	10.50	15.17	13.43	1.20	0.018	0.950
TC, mg/dl	134.38	153.82	164.95	181.46	181.50	9.48	0.002	0.674
TP, mg/dl	6.14	6.16	6.18	6.31	6.13	0.07	0.699	0.322
Creatinine, mg/dl	1.38	1.30	1.28	1.41	1.47	0.04	0.148	0.009
Albumin, g/dl	4.69	4.65	4.42	4.37	4.35	0.06	<001	0.177
Ca, mg/dl	7.26	7.14	7.11	7.13	7.49	0.20	0.627	0.197
IP, mg/dl	5.95	6.03	5.92	5.72	5.48	0.17	0.104	0.196
Iron, mg/dl	1.70	1.84	1.52	1.50	1.79	0.11	0.879	0.361
γ -GT, u/l	30.31	31.12	33.50	36.73	49.79	3.49	0.002	0.031
AST, u/l	95.02	98.22	107.09	119.23	132.51	7.05	0.001	0.195
ALT, u/l	18.84	18.86	19.94	21.12	19.34	0.92	0.445	0.475
BW, kg	576.92	585.46	606.92	644.82	647.43	10.67	<001	0.423

¹⁾ BUN: blood urea nitrogen; TG: triglyceride; TC: total cholesterol; TP: total protein; IP: inorganic phosphorus; γ -GT: gamma glutamyltransferase; AST: aspartate aminotransferase; ALT: alanine aminotransferase.

²⁾ Statistical significance of linear and quadratic effects were tested by contrast statement of GLM procedure.

Fig. 1. Comparison of serum enzyme activities between Hanwoo steers and bulls at 24 months of age. Error bars are SE.

γ -GT and AST activities were linearly increased ($P=0.002$ and $P=0.001$, respectively) with increasing slaughter age in bulls. However, ALT activities were not affected by aging. Activities of γ -GT and AST were higher ($P<0.05$) for bulls than those of steers at 24 months of age (Fig. 1). However, ALT levels were not significantly different between steers and bulls.

DISCUSSION

Fattening period of Hanwoo cattle has been extending in order to increase beef quality grades, especially marbling score. Feeding a high-concentrate or concentrate diet alone throughout the finishing phase has become a routine practice. It has been well-established that high concentrate diets supported higher total VFA concentrations and higher proportions of propionate relative to acetate in cattle (Hungate, 1966). Propionate is a major glucose precursor in ruminants. Over 80% of propionate absorbed into the portal blood is utilized for glucose synthesis (Steinhour and Bauman, 1988). The results of the present study also indicate that most of the glucose turnover in ruminants is derived from propionate and are in agreement with present findings. Urea is produced in the liver and excreted via the kidneys into urine. Consequently, the circulating levels of urea depend upon protein intake, catabolism, and kidney function. Elevated urea levels can occur with dietary changes, diseases which impair kidney function, liver disease, diabetes, and infection (Burtis and Ashwood, 1994). In Korea, the feeding strategy for producing a quality beef, the CP

content of concentrate in the diet is reduced according to increase in fattening duration (Kim et al., 2005). On the other hand, BUN concentrations in bulls were linearly increased with increasing of the slaughter age, this may be a result of lower nitrogen utilization in digestive tract. Serum cholesterol concentrations were linearly increased ($P=0.002$) with increasing slaughter age in bulls only. Adipose tissue is the cholesterol storage site, whereas tissue cholesterol concentrations were not related to serum cholesterol (Wheeler et al., 1987). The relation of increase cholesterol concentrations in the blood and increased body fat is unclear. The blood cholesterol and triglyceride concentrations varied in ruminants fed fat-rich diets (Skeaff et al., 2004). Meanwhile, the cholesterol levels are important in the diagnosis and classification of hyperlipoproteinaemias. Stress, age, gender, hormonal balance, and pregnancy affect normal cholesterol level (Burtis and Ashwood, 1994). The increase of protein synthesis in the muscle decreases the blood albumin concentrations. Albumin is used for the accumulation of protein to the muscle (Galbraith et al., 1978). Therefore, the decrease of albumin concentrations may be a result associated with aging, which can infer from body weight that was linearly increased with aging in both steers and bulls. In addition, it may be because of increasing concentrate feeding associated with increases of the fattening periods.

The blood metabolites in the present study are in accordance with the findings of Lee et al. (1997) and Ahn et al. (2000) when compared with 25 months of age and 600 kg Hanwoo steers, with TP, TC, albumin, and glucose concentration were reported at 7.8 g/dl, 140.1 mg/dl, 3.7 g/dl, and 57.6 mg/dl, respectively, at 25 months of age, and TG, TC, TP, and glucose in 600 kg Hanwoo steers have range of 17.8–22.0 mg/dl, 144.3–159.0 mg/dl, 6.6–6.9 g/dl, and 63.5–70.5 mg/dl, respectively.

Increase in serum γ -GT levels was confirmed in all forms of liver disease or damage (Kachmar and Moss, 1976). Clinical reports established that serum γ -GT in calves was increased by liver infection where it was 3 to 20 times higher than normal (Anderson et al., 1977). Serum AST is a good indicator of hepatic damage as well, and it is commonly measured clinically as a marker for liver health. Zadnik (2003) reported that the AST activity of healthy cows was 60.2 ± 12.1 IU/L. Ahn et al. (2000) reported that the AST activity with the levels of concentrate diet (84, 87, and 89% of total feed) in Hanwoo steers was 82.75 ± 5.75 , 90.25 ± 15.45 , and 80.40 ± 9.46 u/L, respectively. Damage or disease

to some body tissues such as myocardial infarction, viral hepatitis, liver necrosis, cirrhosis, and muscular dystrophy may result in increased serum or plasma levels of AST (Zilva and Pannall, 1979). In addition, Lechtenberg and Nagaraja (1991) detected that rectal temperature, leukocyte counts, fibrinogen, globulin, bilirubin, γ -GT, and sorbitol dehydrogenase concentrations increased when hepatic abscesses were induced experimentally in steers by inoculating *Fusobacterium necrophorum* via ultrasonography-guided, percutaneous catheterization of the portal vein. Therefore, the increased levels of γ -GT and AST with increases of the slaughter ages may be associated with a high-concentrate diet because extension of fattening period is usually coupled with increase of a high-concentrate diet in Hanwoo industry. In addition, a strategy to quickly increase meat yield by feeding over 95% high-concentrate diet at the late fattening phase before slaughter has become a routine practice in Hanwoo industry. Cattle fed a high-concentrate diet are energetically more efficient for increasing beef quality and yield compared with feeding a high-fiber diet, however, it has been reported that feeding a high-concentrate diet decreased and (or) fluctuated feed intake in finishing phase (Shin et al., 2002). The erratic feed intake in the finishing phase may result from decreased ruminal pH below 6.0 or erratic pH because the decreased and erratic ruminal pH can cause the rumen disturbance and an acidic condition which can increase the risk of ruminal acidosis (Nocek, 1997). Moreover, an acidic condition can damage the ruminal wall which predispose to ruminal abscess by *Fusobacterium necrophorum*. In addition, the bacteria can gain entry to the portal circulation and subsequently causes bacterial emboli in the portal circulation or filters by liver. Therefore, as a result of feeding a high-concentrate diet liver infections and abscess can occur in the cattle.

The γ -GT and AST levels in the present study were higher ($P < 0.05$) for bulls than those of steers at the same age of 24 months. Such difference of serum enzymes level between bulls and steers at the same age may result from differences in the fattening periods, feeding management, and feed intake. The fattening periods of the bull is generally shorter than those of the steer and to increase energy supply for bulls, feeding a concentrate diet only throughout the finishing phase has become a routine practice (RDA, 2007), as mentioned above. Although feed intake was not monitored, it was assumed that normally bulls consume more feed than steers as the body weight of bulls was higher than

that of steers in the present study. The general feeding management of Hanwoo cattle during the finishing phase is feeding concentrate prior to roughage or even all concentrate diet in order to increase concentrate intake. The roughage is restricted below 10% of the total feed intake at the late fattening phase (RDA, 2007). Such concentrate-centered feeding managements would develop an acidic rumen condition. Acid-induced rumenitis and damage of the ruminal wall are associated with a sudden change to high energy diets and other dietary indiscretions such as a change in feeding patterns, allowing cattle to become overly hungry, feeding unpalatable diets, and feeding very low roughage ratio (Elam, 1976).

Although all slaughtered cattle for this study were not raised in the same farm with a same diet, we used cattle for blood collection which were raised with a high-concentrate diet (over 90% of total feed) at the late fattening phase. Therefore, results in the present study may be one of the useful information in hematologic aspect that is linked with a high-concentrate diet and fattening period, and indicate that serum γ -GT and AST may be an important predictor for evaluating hepatic dysfunction in Hanwoo cattle. However, further study that is systematically designed by controlled diet and environment may be needed to provide more accurate indices about correlations among the hepatic dysfunction, the length of fattening period, and feeding a high-concentrate diet in Hanwoo cattle.

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