Changes in Serum Metabolites and Growth Characteristics of Korean Native Steers Fed Alcohol-fermented Feeds*

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ABSTRACT : This study was carried out to assess whether feeding of alcohol-fermented feeds (AFF) affects the nutritional metabolism and growth characteristics of Korean native steers. Ten steers were randomly assigned to one of two treatment groups. The dietary treatments were AFF (50% commercial beef cattle feed+30% alcohol-fermented soybean curd dregs+20% rice straw) and control (80% commercial beef cattle feed+20% rice straw). The change of serum metabolites and growth characteristics were measured every two months during the whole twelve months experimental period and the relationships between serum metabolites and growth characteristics were simultaneously analyzed. Four hours after feeding AFF, serum alcohol concentration reached its peak with a significantly higher value than that after control feeding (11.9 and 4.9 mg/dl, respectively). Serum glucose and inorganic phosphorus (IP) concentrations (63.1 and 8.4 mg/dl, respectively) of steers fed AFF were higher than those (56.6 and 7.0 mg/dl) fed the control diet. In both treatments, the serum glucose concentration rapidly increased when body weight (BW) of the steer reached about 600kg, while IP concentrations were rapidly diminished at that BW. Lower concentrations of both blood urea nitrogen (BUN) and cholesterol were observed in steers fed AFF up to 450 kg of BW. The IP concentration was correlated with concentrations of BUN, cholesterol and glucose in AFF fed cattle but not in the cattle fed control diets. Average daily gain was higher in steers fed AFF than steers fed control, particularly during the growing stage of cattle. These findings indicated a capability of AFF to improve BW gain of Korean native steers by decreased protein degradation as well as increased fat synthesis. *(Asian-Aust. J. Anim. Sci. 2004. Vol 17, No. 5 : 648-654)*

Key Words : Alcohol-fermented Feeds, Korean Native Steers, Serum Metabolites, Growth Characteristics

INTRODUCTION

Alcohol is known to produce NADH through oxidation by alcohol dehydrogenase (ADH) in the liver. This pathway, in turn, suppresses Krebs cycle activity and oxidations of glucose and fatty acids via the Krebs cycle, but stimulates the hepatic syntheses of fatty acids and triglyceride (Day and Yeaman, 1994; Lieber, 1994). Thus, the addition of an adequate amount of alcohol to beef cattle diets has been known to be desirable to improve feed efficiency and carcass traits. But its use has been limited due to its off-flavor, refusal of eating by the animal, and difficulty of preparing alcohol included feedstuffs. Shin (1995) solved these problems by adding yeasts that induce alcohol fermentation to feedstuffs. Later, Yan (1998) found beneficial effects of alcohol- fermented feeds for improving the marbling score of beef. However, a logical explanation for the improved meat quality by dietary alcohol has not been reported.

In addition to alcohol, organic acids such as lactic acid are also produced during alcohol fermentation of feedstuffs, which resulted in low pH of the diets. Lin (2001) observed there is a clear difference in alcohol related metabolism between alcohol-added feed and alcohol-fermented feed. Total volatile fatty acid (VFA) production increased whereas propionic acid production decreased with alcohol-added feed but *vice versa* with the alcohol-fermented feeds. This result indicates that the effect of alcohol-fermented feeds on meat quality is not only due to alcohol itself, but also due to the change in rumen fermentation metabolism.

Alcohol increases the concentrations of serum glucose and fatty acids through the Krebs cycle in which the oxidation of glucose and fatty acids is suppressed by alcohol in single stomach animals (Erkki et al., 1998). In ruminants, energy metabolism and serum metabolites can be affected by alcohols, because most alcohols are absorbed through the rumen wall into the blood (Burning and Yokoyama, 1988; Anbarasu et al., 2002; Chen et al., 2002). Sano (1997) reported increased blood urea nitrogen (BUN) concentration in beef cattle fed alcohol-added diet. Higher concentrations of serum glucose and cholesterol in Korean native steers fed alcohol-fermented feeds were also reported by Yan (1998). Serum inorganic phosphorus (IP) is also closely related to energy metabolism of beef cattle (Wata,

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Table 1. Chemical composition of experimental diet

Item	Commercial feed	AFS	Rice straw
DM, %	86.6±0.1	52.4±1.1	83.1±2.0
	% of dry matter		
Crude protein, %	13.2±0.3	15.5±0.1	5.1±0.1
Ether extract, %	4.2±0.1	3.7±0.2	4.2±0.1
Crude fiber, %	3.0±0.2	10.0±0.4	30.4±1.2
NDF, %	30.7±0.7	40.2±0.4	64.7±0.2
ADF, %	10.7±0.1	14.2±0.4	41.7±0.2
ADL, %	2.0±0.6	2.8±0.1	8.5±0.3
Ash, %	7.0±0.1	6.8±0.2	6.5±0.1
Alcohol, %	-	3.2±0.4	-
Lactate, %	-	3.3±0.5	-
Ammonia, mg/100 g	-	65.2±2.0	-
pH	-	4.7±0.01	-

- Not detected. AFS: Alcohol-fermented soybean curd dreg, NDF: Neutral detergent fiber, ADF: Acid detergent fiber, ADL: Acid detergent lignin

1990; Chandramoni et al., 2001). These results implied that alcohol related metabolism in beef cattle could be evaluated by examining the change of serum metabolites.

So far, only a limited number of studies has been carried out to evaluate the effectiveness of alcohol feeding in terms of serum metabolites as well as carcass quality and BW of Korean native steers. Therefore, the objectives of this study were to evaluate the effectiveness of feeding alcohol-fermented feeds on nutritional metabolism and growth characteristics in steers. To understand the metabolism, serum alcohol concentration, serum metabolites and their interrelationships were also analyzed.

MATERIALS AND METHODS

Experimental design and feeding management

A feeding experiment was carried out for 12 months on a beef farm located in Jung Sun, South Korea. Ten Korean native steers, weighing average 328.8 kg in BW were fed one of two diets, control and alcohol-fermented feeds (AFF) diet. Chemical composition of experimental diet ingredients is shown in Table 1. Alcohol-fermented soybean (AFS) curd dregs were prepared by mixing 50% commercial beef cattle feed and 50% soybean curd dregs that had already been supplemented with 2% molasses and 0.5% yeast. Then the whole mixture was fermented at 30°C for 72 h. The AFF diet was prepared by mixing 50% commercial beef cattle feeds+30% AFS+20% rice straw, whereas the control diet was prepared by mixing 80% commercial beef cattle feeds and 20% rice straw. Ten steers were assigned randomly to one of two experimental groups and experimental diets were given daily at approximately 20 g per kg of BW. Experimental diets were given twice (9 a.m and 6 p.m) a day, and the amount of daily feed gradually increased as BW increased. The steers were managed according to commercial feeding practices recommended by the Animal Farming Association of Kangwon Province, Korea.

Measurements and analytical procedures

Body weight of steers was measured every 2 months and average daily gain (ADG) was calculated from BW gains divided by number of feeding days. Every 2 months, blood samples of each steer were collected 0, 2, 4 and 6 h after feeding by 10 ml Vaccutainer (Becton Dickinson Co, USA) and serum was separated by centrifugation (3,000 rpm for 15 min at 4°C) of whole blood. Serum alcohol concentration was measured using a glucose analyzer (YSI 2700, USA). Three replicates of each serum sample were frozen at -25°C until analysis, then BUN, calcium, creatinine, cholesterol, glucose, and IP were analyzed using an automatic blood serum analyzer (Express Plus, USA). The chemical composition of experimental diets was analyzed by AOAC methods (1990). The AFF samples for alcohol determination were obtained after dissolution at 4°C for 30 min followed by 5 X dilutions with distilled water. Then, the samples were centrifuged at 3,000 rpm for 5 min and supernatants were collected. Alcohol concentration in the supernatant was analyzed by glucose analyzer (YSI 2700, USA) with YSI 2386 alcohol oxidase membrane. The ammonia content of the samples was determined according to the method of Chaney and Marbech (1962) whereas lactic acid content was analyzed by UV spectrophotometer (Hitachi, Japan).

Statistical analyses

The data were analyzed using the ANOVA procedure of SAS (SAS, 1990) with the following model:

$$Y_{ij}=\mu+T_i-e_{ij}$$

where Y_{ij}=dependent variable, μ=overall mean, T_i=effect of $_i$th treatment, e_{ij}=residual error. The correlation among blood metabolites, BW and ADG was calculated using the Pearson correlation with the following model:

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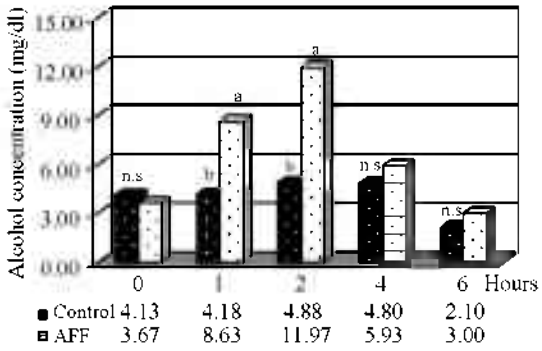

Figure 1. Post feeding changes in serum alcohol concentrations of the steers fed either AFF or control diet. AFF: alcohol-fermented feeds. [a, b] Mean value with different superscript in the same hour differ significantly ($p<0.05$). [n s] Not significant.

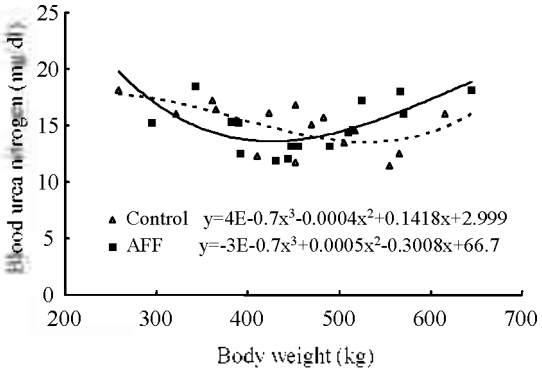

Figure 2. blood urea nitrogen concentrations of the steers fed either AFF or control diet by increasing body weight. AFF: alcohol-fermented feeds.

$$r = \frac{\mathrm{Cov}\ X_{ij}}{\sqrt{\mathrm{Var}\ X_i} \times \sqrt{\mathrm{Var}\ X_j}}$$

where : r= Correlation coefficient

Cov X_{ij}=Sample covariance between $_i$th and $_j$th treatments

Var X_i=Sample variance of $_i$th treatment

Var X_j=Sample variance of $_j$th treatment

RESULTS

Change in serum alcohol concentration

Effects of AFF feeding on the alcohol concentration of serum are presented in Figure 1. Serum alcohol concentration was higher in steers fed AFF, with a greater difference in the serums obtained 1 and 2 h after feeding. The highest alcohol concentration in the serum, obtained 2 h after feeding, resulted from the peak absorption of alcohol through the rumen wall during that period. The concentration of serum alcohol rapidly decreased 4 and 6 h after feeding AFF.

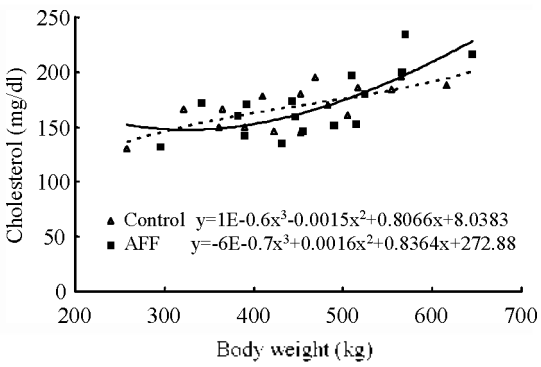

Figure 3. Serum cholesterol concentrations of the steers fed either AFF or control diet by increasing body weight. AFF: alcohol-fermented feeds.

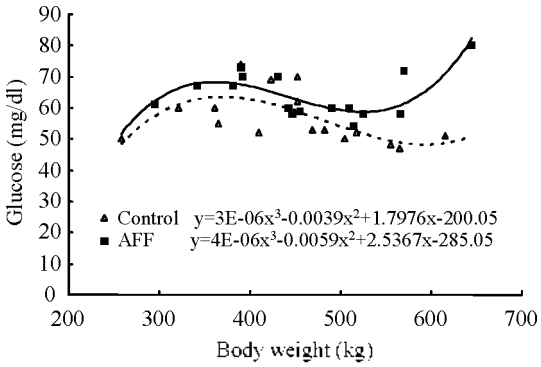

Figure 4. Serum glucose concentrations of the steers fed either AFF or control diet by increasing body weight. AFF: alcohol-fermented feeds.

Change in serum metabolites

BUN concentrations (Figure 2) decreased as BW of steers increased up to 450 kg whereas cholesterol concentration (Figure 3) proportionally increased with increasing BW. However, above 450 kg BW, concentrations of both BUN and cholesterol in both treatments increased along with BW, with relatively higher values in steers fed AFF than steers fed control diets.

Changing pattern of both serum glucose and IP concentration was similar between steers fed AFF and those fed the control diet. Serum glucose concentration (Figure 4) increased as BW of the steer increased up to approximately 400 kg, then decreased during 400-600 kg BW and finally increased when BW exceeded 600 kg. With over 600 kg of BW, the higher concentration of serum glucose was remarkable in the steers fed AFF. Serum IP concentration

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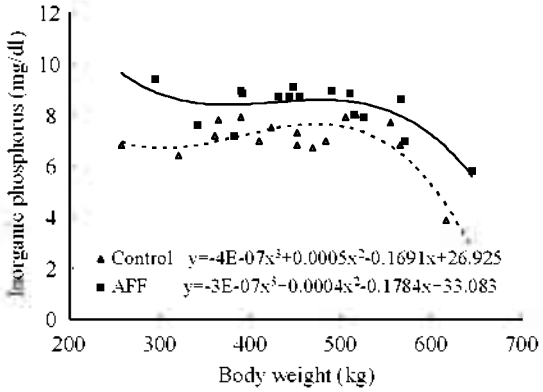

Figure 5. Serum inorganic phosphorus concentrations of the steers fed either AFF or control diet by increasing body weight. AFF: alcohol-fermented feeds.

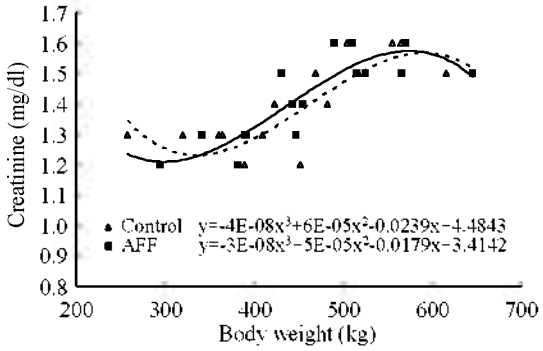

Figure 6. Serum creatinine concentrations of the steers fed either AFF or control diet by increasing body weight. AFF: alcohol-fermented feeds.

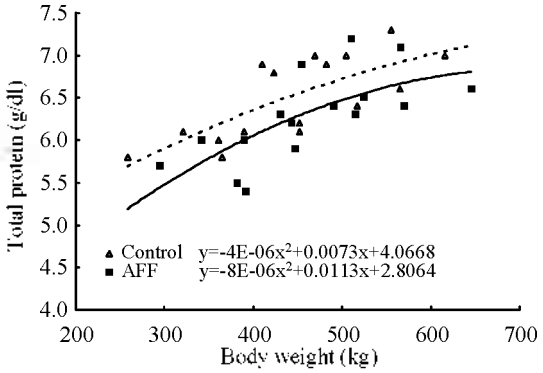

Figure 7. Serum total protein concentrations of the steers fed either AFF or control diet by increasing body weight. AFF: alcohol-fermented feeds

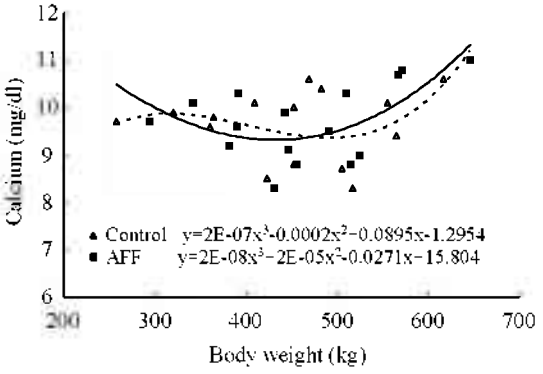

Figure 8. Serum calcium concentration of the steers fed either AFF or control diet by increasing body weight. AFF: alcohol-fermented feeds.

(Figure 5) was higher in steers fed AFF than control diet regardless of BW. However, the serum IP values in both treatments rapidly decreased with increasing BW once it approached the fattening stage.

No statistical difference was observed in serum creatinine concentrations (Figure 6) between the two treatments, but those values were increased as BW of steers increased up to 600 kg. Total protein (TP) concentration (Figure 7) in the serum increased as BW increased regardless of the experimental diets. But the concentration was always less in the steers fed AFF than control steers during the whole experimental period.

The serum calcium (Ca) concentrations (Figure 8) in steers fed either AFF or control diets tended to decrease at early growing stage until approximately 450 kg BW, but then increased during the rest of feeding period. There was no difference in serum Ca concentrations due to the type of diet.

During the growing stage, steers fed AFF diet grew significantly ($p<0.05$) faster than steers fed control diet, while ADG of steers fed AFF tended to be less than steers fed control diet after the growing stage (Figure 9). The above findings indicated that the AFF feeding resulted in a decrease in concentrations of serum BUN, cholesterol and Ca but an increase in ADG during growing stage of steers. However, these effects were reversed during the fattening stage of the steers.

Correlations between serum metabolites and body weights (Table 2)

Regardless of the diet, concentrations of serum cholesterol, creatinine and TP were highly ($p<0.01$) correlated with the BW ($r=0.72$, $r=0.79$ and $r=0.69$ for AFF; $r=0.72$, $r=0.69$ and $r=0.73$ for control feeding, respectively). But there were differences due to dietary treatments in the degree of correlation between BW and serum metabolites. Serum IP was correlated ($p<0.05$) with BW of steers fed AFF diet but not with steers fed control diet. BUN was only

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correlated with BW of steers fed control diet. In general, no significant correlations were observed between serum metabolites and ADG with the exceptions of serum cholesterol (r=-0.68) and Ca (r=0.61) that were correlated with ADG. These results showed that AFF affects not only fat metabolism but also BW gain in the steers.

BUN was correlated (r=-0.51, p<0.05) with serum TP in control, but not in AFF treatment. Higher correlation (r=-0.60, p<0.05) between serum BUN and IP in steers fed AFF diet indicated that protein metabolism in the liver can be changed by AFF feeding. Concentrations of both serum cholesterol (r=0.52, p<0.05) and Ca (r=0.50, p<0.05) were also correlated with BUN in steers fed AFF diet.

In steers fed AFF diet, a significant correlation (r=-0.64, p<0.01) was observed between serum cholesterol and serum IP, but there was no such correlation in steers fed control diet. While serum glucose and creatinine were highly correlated (r=-0.71, p<0.01) in steers fed control diet, no such relationship was observed in steers fed AFF diet. High correlation (r=-0.63, p<0.01) between serum IP and serum glucose was observed only in steers fed AFF diet.

DISCUSSION

Alcohol, introduced in the rumen by diets, is known to be only partially (about 20%) transformed to VFA by the rumen microorganisms (Andree et al., 1991). Most alcohol is known to be absorbed through the rumen wall (Burning and Yokoyama, 1988). Since a higher alcohol concentration was observed in steers fed AFF diet, it was speculated that there was a significant amount of alcohol absorption from AFF diet into the blood via the rumen wall. This result also indicates that the alcohol-fermented feed can be equally effective as alcohol-added diet in terms of the absorbed energy to steers.

Changes in the concentrations of serum metabolites and the degree of correlations among serum metabolites were greater in steers fed AFF diet than control diet. The net concentrations of both serum IP and glucose and the magnitude of relationship between serum glucose and creatinine were also more remarkable in AFF treatment compared to the control. This is probably related to the decreased Krebs cycle activity induced by alcohol from AFF diet. Increase in serum glucose concentration is known to be responsible for the decreased activity of pyruvate dehydrogenase via NADH which, therefore, prevents glucose from entering to Krebs cycle (Wata, 1990; Lieber, 1994; Day and Yeaman, 1994; Erkki et al., 1998). Increase in serum IP concentration is also closely related to the increase in body fat synthesis. Yu and Cronholm (1997) reported that triacylglyceride (TAG) concentration was

Table 2. Correlations among serum metabolites, body weight and average daily gain of steers

Items		BUN	Chol	Crea	Glu	IP	TP	Ca	ADG
BW	AFF	0.30	0.72**	0.79**	-0.19	-0.53*	0.69**	0.37	-0.60*
	Control	-0.54*	0.72**	0.69**	-0.36	-0.28	0.73**	0.04	0.17
BUN	AFF	-	0.52*	0.04	0.28	-0.60*	-0.22	0.50*	-0.49
	Control	-	-0.36	-0.39	0.33	-0.16	-0.51*	-0.09	-0.12
Chol	AFF		-	0.51*	0.35	-0.64**	0.42	0.79**	-0.68**
	Control		-	0.57*	-0.43	-0.24	0.57*	0.18	0.38
Crea	AFF			-	-0.03	-0.20	0.77**	-0.18	-0.47
	Control			-	-0.71**	-0.08	0.73**	-0.05	0.19
Glu	AFF				-	-0.63**	-0.17	0.46	-0.21
	Control				-	0.22	-0.41	-0.29	-0.05
IP	AFF					-	-0.07	-0.39	0.37
	Control					-	-0.18	-0.57*	0.41
TP	AFF						-	0.18	-0.42
	Control						-	0.19	-0.16
Ca	AFF							-	-0.61*
	Control							-	-0.38

BW, body weight. TP, total protein. BUN, blood urea nitrogen. Ca, calcium, Chol, cholesterol, Glu, glucose, Crea, creatinine. ADG, average daily gain from previous blood collection day to current blood collection day, IP, inorganic phosphorus, AFF, alcohol-fermented feeds.
* 0.01<p<0.05, ** p<0.01

increased in the liver with increasing GTP, for which NADH was used through the oxidation of alcohol. Although we could not confirm the report because TAG concentration was not analyzed in this study, Yan (1998) had already reported that TAG concentration had significantly increased in steers fed alcohol-fermented feeds.

Adenosine triphosphate, required as the constant energy source for the muscle contraction-relaxation cycle, can be generated by glycolysis and oxidative phosphorylation of either creatine phosphate or two ADP molecules (Murray et al., 2003). The concentration of serum creatinine is closely related to the exploitation of serum glucose in muscle, even though some creatine phosphate is excreted as creatinine in urine after its degradation into phosphate and creatinine. This was confirmed by this study since a significant correlation ($r = -0.71$, $p<0.01$) between serum creatinine and glucose was observed in the control steers. The correlation between the above two metabolites, however, was not observed in steers fed AFF diet. These results enable us to speculate that the energy source for creatine phosphate synthesis may not be generated from the oxidation of serum glucose. Therefore, it is believed that the feeding of AFF diet can increase fat synthesis in Korean native steers by decreasing the oxidative phosphorylation of glucose through the Krebs cycle.

Change in correlations among the concentrations of BUN, cholesterol, TP and IP after feeding AFF diet implies the possible alteration of protein metabolism in the body. Since BUN is generated during the excretion of ammonia in urine through the urea cycle (David et al., 1983), the lower correlation between serum BUN and TP in steers fed AFF could primarily result from the increase of BUN and the decrease of protein synthesis (Charles, 1999). No significant difference was observed between treatments in serum TP concentrations, although the concentration in steers fed AFF diet was generally higher throughout the entire experimental period. This may suggest that steers fed AFF diet were able to grow faster due to enhanced fat synthesis by increased ATP, which originated from the increased Krebs cycle activity upon degradation of amino acid.

The strong correlation between hepatic serum glucose and serum IP in AFF treatment could result from decreased glucose utilization, increased glucose synthesis and increased ATP production through the Krebs cycle. Yan (1998) evidenced the higher proportion of rumen propionic acid that was induced by the increased glucose concentration that resulted from alcohols provided by AFF diet. The results may actually be due to both increased activity in the sympathetic nervous system and increased cortisol secretion (Grogan and Kochar, 1994), although it could not be confirmed in this study. The higher serum IP concentration in AFF treatment is thought to be induced by the dietary alcohol that can increase fat synthesis in the body (Wata, 1990).

From 450 to 600 kg of BW, body protein deposition gradually decreased, whereas the body fat deposition increased. This is consistent with the increase of BUN and decrease of serum glucose during the above fattening stage. Body growth rate decreased as both serum BUN and glucose increased but as the serum IP decreased. This seems to be related to the decrease of both protein and fat metabolism as well as to the increase of energy metabolism for maintenance. Generally, the growth of steers was accelerated by AFF feeding compared to control diet feeding, with greater effects during the growing stage. These results reveal that AFF feeding can be applied to modulate the nutritional metabolism and growth characteristics of Korean native steers.

CONCLUSIONS

Results of this study indicate that the AFF feeding can not only improve weight gain of Korean native steers but also change the concentrations of serum metabolites. Relatively stronger correlations between serum metabolites such as creatinine, total protein, cholesterol, inorganic phosphorus and BW of steers were observed in steers fed AFF diet. The improvement in ADG of steers fed AFF diet can be explained both by the decrease in protein degradation and by the increase in fat synthesis. Further research is needed to confirm whether AFF affects the hormonal metabolism that is responsible for the improvement of BW gain in Korean native steers.

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