Effects of Dietary Supplementation with Branched-chain Amino Acids (BCAAs) during Nursing on Plasma BCAA Levels and Subsequent Growth in Cattle

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ABSTRACT : To determine the effects of short-term dietary supplementation of branched-chain amino acids (BCAAs) during nursing (from 3 to 28 days of age) on plasma BCAA levels and subsequent growths in cattle, 12 nursing male Holstein calves, randomly assigned to control and treatment groups (n = 6 in each group), orally received a daily supplement of essential BCAAs (2 g/kg body weight/day; 1:1:1 of valine, leucine and isoleucine) or not. The plasma BCAA levels increased linearly after the administration. During the treatment period, average daily gain (ADG) was lower in the treatment group (0.43±0.07 kg/day) than the controls (0.71±0.07 kg/day, p<0.05). However, at 2 months of age, ADG was significantly higher in the BCAA-treated group (1.16±0.26 kg/day vs. 0.51±0.06 kg/day, p<0.05). Furthermore, at age 8, 9 and 10 month, ADG in the treated group (1.35±0.23, 1.46±0.07 and 1.60±0.16 kg/day, respectively) showed a linear increase and was significantly higher than that in the control group (0.88±0.14, 0.70±0.21 and 1.11±0.11 kg/kg, respectively, p<0.05). Overall, ADG was 15.6% higher in the treatment group (1.26±0.05 kg vs. 1.09±0.04 kg; p<0.05). The final body weight at slaughter was 14.8% higher in the treatment group (759.5±17.7 kg vs. 661.7±21.2 kg, p<0.01). Thus, the supplementation of BCAAs during nursing improves ADG and carcass weight in cattle and is a useful husbandry technique for beef cattle. ***(Asian-Aust. J. Anim. Sci. 2005. Vol 18, No. 10 : 1440-1444)***

Key Words : Branched-chain Amino Acid, Plasma Concentration, Average Daily Gain, Carcass Weight, Cattle

INTRODUCTION

The ultimate goal of many livestock husbandry systems is to improve the efficiency of protein gain. Enhancement of the efficiency with which plant feedstuffs are converted into the appropriate animal product makes sense in terms of conservation of natural resources and reduction of environmental pollution, and also results in economic gains for both the producer and the consumer.

For protein gain to be efficient, essential amino acids must be continuously available for protein synthesis. Studies of the peculiarities of branched-chain amino acid (BCAA) metabolism indicate that these amino acids play important roles in regulating overall amino acid and protein metabolism. In particular, BCAAs have been shown to promote protein synthesis, reduce protein degradation (Leibholz, 1965; Bergen et al., 1988), and stimulate insulin secretion (Malaisse et al., 1984). Accumulating evidence strongly supports the key roles of BCAAs as signaling molecules in the regulation of protein synthesis by modulation of mRNA translation. However, relatively little research has connected this laboratory evidence with livestock feedlot performance.

In the last decade, the notion that nutrition during the early phase of human development can alter organ function and thereby predispose individuals to adult disease has attracted considerable research interest. The exposure of the fetus to different levels of metabolic substrates can influence insulin secretion and sensitivity; the number, size, and physiological function of fat cells; and the regulation of appetite by the central nervous system in later stages of life (Whitaker and Dietz, 1998). The intrauterine environment resulting from the altered transfer of metabolic substrates from the mother to the fetus affects the development of the structure and function of the fetal organs involved in energy metabolism. Because postnatal nutrition is also implicated in triggering pathologic metabolic programs, it has been suggested that an apt designation of this association would be the "early origins" hypothesis of adult disease (Martorell et al., 2001).

These observations suggest that excessive nutrition in the early developmental phases of an animal's life could contribute to obesity later. However, in ruminants, because dietary proteins and amino acids are extensively metabolized by the ruminal microbiota, it has been considered difficult to increase the plasma levels of amino acids, especially BCAAs, through changes in oral nutrition in pregnant cows or feedlot calves. On the other hand, the digestive system in newborn ruminants is similar to that of monogastric animals. Before the development of rumen fermentation, milk enters the abomasum directly and is subsequently digested by enzymatic processes in the abomasum and intestine in the same way as occurs in monogastric animals. Presently, we do not have sufficient

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Table 1. Composition of experimental diets

Ingredient	Duration					
	1 month old Milk (A)	2-3 month old starter	4-12 month old beef-zenki	13-18 month old beef-goki	From 18 months old slaughter Hol-power	Dry rice straw*
Crude protein (%)	26	20	16	12	13	5
Crude fat (%)	17	3	3	3	4	2
Crude fiber (%)	1	5	6	5	6	28
Crude ash (%)	8	5	73	4	4	15
Total digestible nutrients (%)	-	75	1	75	75	38
Ca (%)	1	1	1	0	0	-
P (%)	0	0		1	0	-
Vitamin A (IU/kg)	80,000	8,000	3,600	3,000	960	-
Vitamin D (IU/kg)	4,000	901	513	304	101	-
Vitamin E (IU/kg)	96	26	18	16	16	-
Carotene (mg/kg)	-	3	5	1	1	-

* Dry rice straw: compiled from data of Standard Tables of Feed Composition in Japan 2001.
The calves were given dry rice straw from 1 month old to slaughter.

data on the effects of the oral administration of amino acids on the plasma and tissue levels of amino acids in nursing calves. Thus, the first objective of the present study was to elucidate the effects of dietary BCAAs on plasma BCAA levels in preruminant calves during the nursing period (from 3 to 28 days of age). Our second objective was to determine whether this early treatment with excess BCAAs produces changes in the subsequent growth performance.

MATERIALS AND METHODS

Animals, diet and experimental design

Twelve male Holstein calves (12 h old) were randomly assigned to control and BCAA-treated groups (n = 6 in each group). The birth weights of the tow groups were 37.7±3.0 and 41.4±3.6 kg, respectively. All animals were trained once or twice to accept bucket-feeding and then received colostrum by bucket for 2 days after birth (10% body weight). Whole milk, was offered in two equal meals at 08:30 and 16:30 from 3 to 5 days after birth. The whole milk was replaced with 25, 50 and 75% skim milk on days 6, 7 and 8, respectively. From 9 to 28 days, the calves were fed 100% skim milk (10% body weight). Subsequently, they were weighed at one-month intervals until 20 months old. Concentrates and dry rice straw (Table 1) were available *ad libitum* starting at age 14 days. At 6 months of age, all the calves were castrated, and then penned together in a pen with a concrete floor covered by wood shavings. During the experiment, daily feed intake was recorded. All procedures were approved by the Institutional Animal Care and Use Committee of the University of Tokyo.

A mixture of essential BCAAs (2 g/kg body weight/day; 1:1:1 of valine, leucine and isoleucine) was added to the whole or skim milk, and the mixture was orally administered in the morning (9:00) from 3 to 28 days of age in the BCAA-treated group. In the control group, an equal volume of whole or skim milk was orally administered.

Plasma amino acid measurement

To investigate the effects of supplemental BCAAs on plasma levels, we measured the changes in plasma BCAA levels at age 7, 14 and 28 days. Briefly, blood samples were drawn of 2-h intervals for 24 h after the oral administration of BCAAs at 09:00. Moreover, blood samples were drawn at 16:30 during the treatment period (from age 3 to 28 days) and 6, 12 and 18 months of age. The plasma samples separated by centrifugation at 3,000 g for 30 min were stored at -20°C prior to use. The plasma proteins were precipitated with 10% trichloroacetic acid (Wako Pure Chemicals, Osaka, Japan) and removed by centrifugation at 15,000 g for 10 min. The supernatant was filtered through a 0.1-micrometer ultrafree-MC centrifugal filter unit (Millipore, Bedford, MA, USA) and used for the amino acid analysis. The free amino acid levels in plasma samples were measured using an automatic amino acid analyzer (L-8500A; Hitachi, Tokyo, Japan) according to the manufacturer's instructions.

Statistical analysis

ANOVA with Fisher's least significant differences test was performed using the StatView IV program (Abacus Concepts, Berkely, CA, USA) on a Macintosh computer. Each value represents the mean±SE. Differences of $p<0.05$ were considered significant.

RESULTS AND DISCUSSION

Body weight and average daily gain (ADG)

The effect of excessive short-term dietary administration of BCAAs on ADG is shown in Figure 1. The average birth weights in the control and BCAA-treated groups were 37.7±3.0 and 41.8±3.6 kg, respectively. Throughout the experiment, a significantly higher body weight in the treatment group was noted (Figure 1), and final body weight was 14.8% higher in the treatment group

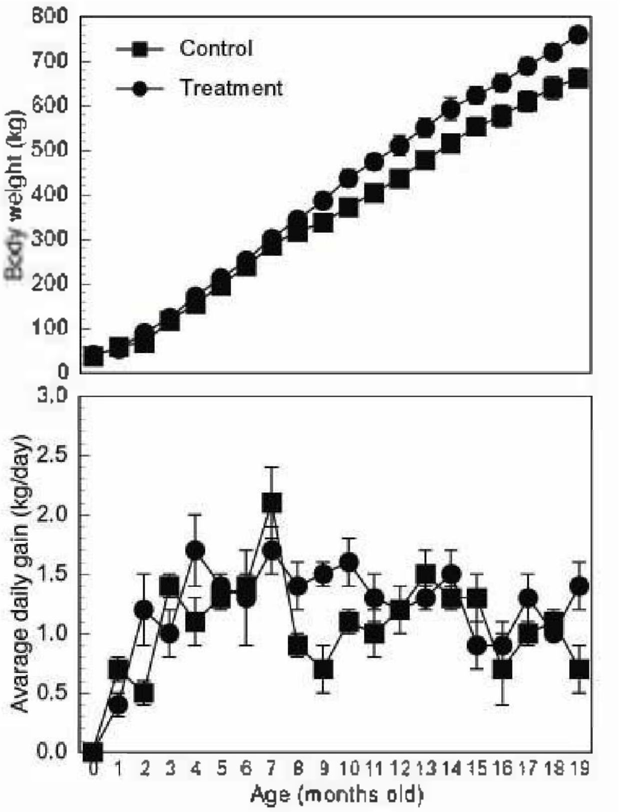

Figure 1. Change in body weight and average daily gain in control and branched chain amino acid (BCAA)-treated groups. Squares and circles indicate control and BCAA-treated groups, respectively.

(759.5±17.7 kg) than control group (661.7±21.2 kg; $p<0.01$).

During the nursing period (from 3 to 28 days of age; treatment period), ADG was higher in the control group (0.71±0.072 kg/day) than treatment group (0.43±0.068 kg/day, $p<0.05$). From age 1 to 2 months, however, ADG was significantly higher in the treatment group (1.16±0.26 kg/day vs. 0.51±0.06 kg/day, $p<0.05$). Moreover, at age 8, 9 and 10 months, ADG was higher in the treatment group (1.35±0.23, 1.46±0.07 and 1.6±0.16 kg/day vs. 0.88±0.14, 0.7±0.21 and 1.11±0.11 kg/day, respectively, $p<0.05$). Overall, ADG was 15.6% higher in the treatment group (1.26±0.05 kg) than control group (1.09±0.04 kg; $p<0.05$).

In nursing lambs, increasing the dietary protein intake results in increases in the plasma levels of essential amino acids, but not those of non-essential amino acids (Bergen and Potter, 1975). Moreover, valine and alanine can be absorbed through the rumen wall (Veresegyhazy et al., 2001). The data indicate that nursing calves rapidly absorbed BCAAs after oral administration, which is consistent with previous findings that indicated that newborn ruminants absorb BCAAs in a manner comparable to that of monogastric animals. The plasma levels of BCAAs in the treated group of nursing calves depended on the level of dietary supplementation and remained elevated until 28 days of age. After the treatment period, no significant differences in the plasma levels of BCAAs were found between the control and treatment groups.

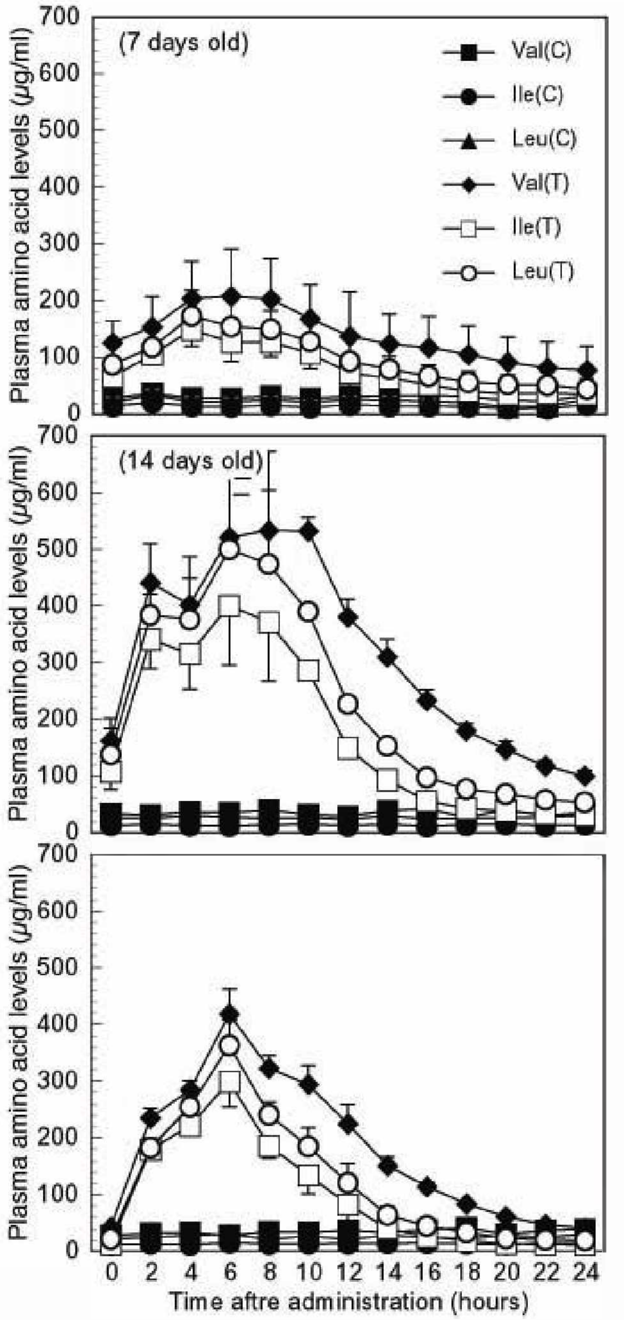

Figure 2. Changes in plasma levels of branched chain amino acids (BCAA; valine, isoleucine and leucine) in control (C) and BCAA-treated (T) groups for 24 h following the administration at 1, 14 and 28 days of age.

Changes in plasma amino acid levels

Changes in plasma levels of valine, isoleucine and leucine at 2 h intervals after the supplementation of BCAAs at 1, 2 and 4 weeks of age are shown in Figure 2. The levels of valine, isoleucine and leucine increased linearly after the

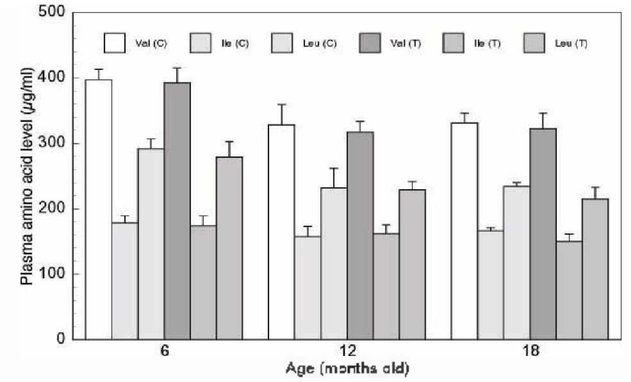

Figure 3. Changes in plasma levels of branched chain amino acids (BCAA; valine, isoleucine and leucine) in control (C) and BCAA-treated (T) groups at 6, 12 and 18 months of age.

administration. At age 6, 12 and 18 months (Figure 3), there was no significant difference in plasma BCAA levels between the control and treatment groups.

During the treatment period in this study, the ADG was higher in the control group than in the treated group. This observation may be attributable to dehydration during the treatment period, as three of the six calves in the treated group had mild diarrhea from which they recovered without medical treatment. The diarrhea was not caused by leucine toxicity, because no symptoms of maple syrup urine disease were detected, and after recovery the calves exhibited good growth capacity. However, after the period of treatment with BCAAs was complete, the ADG in the treated group increased linearly from two to ten months of age, whereas in the control group the ADG varied widely during the same period. Over the duration of the study, the average daily weight gain was 15.6% greater in the treated group than in the control group, and the final average body weight of the treated calves was 14.8% greater than that of the controls. Harris et al. (2004) suggested that higher rates of fetal oxidation of BCAAs appeared to be a contributing factor in poor growth. They found that the activity of branched chain alpha-ketoacid dehydrogenase complex (BCKDC) was dramatically increased and that of its kinase (BDK) was significantly decreased in fetuses that were small for their gestational age. This probably causes a decrease in serum levels of BCAAs that in turn could restrict growth through negative effects upon protein synthesis by inducing less activation of mammalian target of rapamycin (mTOR; a translation initiator) and increased activation of mGCN. The results of the study by Harris et al. (2004) suggest that treatment with excessive BCAAs may induce a dysfunction of the BCKDC system; thus, it is desirable to limit BCKDC activation to lower levels.

On the other hand, over-nutrition during pregnancy in humans, as indicated by high birth weight or gestational diabetes, is associated with subsequent adult obesity (Martorell et al., 2001). The association between birth weight and adult weight suggests that the intrauterine environment has long-lasting effects on the later risk of obesity in humans (Whitaker and Dietz, 1998). Recent studies have established the idea that prenatal life is a critical or sensitive period for the development of obesity. In immature swine, an excess of dietary BCAAs induces different growth rates in different muscle types (Richmond, 1982), and the levels of essential amino acids in the liver are responsive to dietary protein intake (Bergen and Potter, 1975). In rats, leucine supplementation increases protein synthesis in the diaphragm (Buse and Weigand, 1977), perfused hind limbs (Buse et al., 1984), and isolated skeletal muscles (Hong and Layman, 1984). As results showing in Figure 2, the aspect of absorbance ration of amino acids varies from 1 to 4 weeks age. When the calves were 1 week age, plasma level of BCAAs in treatment group was more than 4 times higher than that in control group. In 2 weeks age groups, BCAAs plasma level in treatment group was more than 20 times higher than that in control group. In 4 weeks age groups, BCAAs plasma level in treatment group was more than 10 times higher than that in control group. These findings suggest that the post-natal period is also a critical time during which nutritional conditions, especially BCAA levels, can effect the structural and functional development and growth of the organs involved in energy metabolism. The present study suggests that ADG in calves could be improved by short-term supplementation with excess BCAAs during the early nursing period.

Leucine is unique among the BCAAs because of its ability to promote protein translation, decrease protein degradation, and promote insulin release (Harris et al., 2004). In this study, the calves were treated with a mixture of three branched-chain amino acids, not leucine alone; although valine and isoleucine do not produce the same direct effects that leucine does, the ratios of the BCAAs must be maintained at appropriate levels to observe the beneficial effects of leucine. Further research is required to elucidate the mechanisms responsible for the present findings, including examination of serum levels of hormones (insulin, growth hormone, insulin like growth factor, leptin, etc.), nutrients (glucose, non-volatile fatty acids, etc.), and metabolites (blood urea nitrogen, creatinine, etc.). The control animals in this study exhibited lower ADG at 8, 9, and 10 months of age as compared to the BCAA-treated calves.

In conclusion, dietary supplementation with BCAAs during the nursing period increases the plasma levels of BCAAs and improves subsequent growth performance.

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