

## Temporal Changes in Concentrations of Branched-Chain Amino Acids in Plasma on Healthy Mares and Foals from Birth to 24 Weeks of Age

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**ABSTRACT.** The concentrations of branched-chain amino acids (BCAA; valine, leucine, isoleucine) were determined in plasma of 7 healthy thoroughbred mares and their foals from birth (0 week) to 24 weeks of age, using automated high-performance liquid chromatography. In foals, the concentrations of plasma valine were significantly high ( $p<0.05$ ) at 16, 20 and 24 weeks. The concentrations of plasma leucine were significantly high ( $p<0.05$ ) at 1 and 3 weeks. The concentrations of plasma isoleucine were significantly high ( $p<0.05$ ) from 1 to 24 weeks. In mares, the concentrations of plasma valine were significantly high ( $p<0.05$ ) at 16 and 24 weeks. The concentrations of plasma leucine and isoleucine were significantly high ( $p<0.05$ ) at 16 weeks. It was clear that the concentrations of plasma BCAA in foals and mares were at different levels at various times after birth. Since mares and foals were kept in health during this study, we could get the base data of the concentrations of BCAA in plasma of healthy foals and mares from birth to 24 weeks.

**KEY WORDS:** branched-chain amino acid, foal, mare, plasma.

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Because the concentrations of amino acids in plasma reflect the quantity or quality of food intake, changes in their levels have been used to determine protein nutritional status, quality of ingested protein and the rate-limiting amino acid for growth [15, 23, 26]. Neonatal foals with septicemia or horses with hepatic disease have shown significant differences in the concentrations of several amino acids in their plasma, compared with concentrations from healthy horses [13, 42]. These data suggest that the concentration of plasma amino acids reflects the performance status of horse.

Amino acids are essential nutritional elements for growth, because they are synthesized into proteins that compose the body. In the group that compose branched-chain amino acids (BCAA), valine, leucine and isoleucine are among the essential amino acids. They account for ~35% of the essential amino acids in muscle proteins ~40% of the preformed amino acids required by mammals [14]. In the neonate, the feeding-induced stimulation of protein synthesis occurs in virtually all tissues, but it is most pronounced in skeletal muscle [9, 27, 28]. Low plasma concentrations of BCAA have been observed in causes of malnutrition and have been used as a biochemical sign of poor nutrition in humans [21]. Indeed, in the case of fetuses small for gestational age, concentrations of BCAA were significantly low in humans [35]. This suggests that BCAA are very important nutrients for growth.

Especially after birth, various aspects concerning growth change heavily. So, the temporal changes in concentrations

of amino acids in plasma during the immediate neonatal period have been measured a few species, such as human beings [10, 20, 22], pigs [4, 7], rats [11, 30, 31], and horses [40, 41]. However, previous reports measured only the first month after birth. To our knowledge, there are few investigations of plasma concentrations of amino acids of foals over a long time.

On the other hand, mares need much protein for lactation after parturition. Mares protein mobilization is an adaptive response that enables a high level of milk production under conditions of poor nutrient supply from studies using rats and dogs [1] and sows [18]. Maternal protein mobilization is related to overall milk protein production and is proportional to the number of offspring suckled in sows [3]. And in sow, as lactation progressed, tissue free BCAA levels decreased [6]. It is therefore BCAA play the key role for mares during lactation. Though the concentrations of plasma amino acids of mares at parturition were measured by Rogers *et al.* [33], Zicker and Rogers [40] and Young *et al.* [39], there are few investigations of concentrations of amino acids in plasma of mares over a long time after parturition.

Since amino acid metabolism is very implication and still being studied, we selected just BCAA to clarify the condition or problem in horses. Therefore, we measured the concentrations of BCAA in the plasma of healthy foals and mares from birth to 24 weeks of age to investigate the time that their BCAA metabolism will become unstable.

### MATERIALS AND METHODS

**Animals:** Seven healthy Thoroughbred mares and their

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foals were studied. This investigation was conducted on a farm which has a normal feeding system in Hidaka region of Hokkaido. Mares aged  $12 \pm 6$  (mean  $\pm$  SD) years. This study was done from birth to 24 weeks. All foals were born from February 19 to March 27.

All foals suckled colostrum within 24 hr after birth. Each pair of broodmares and their foals was put out to pasture in a separate paddock of 0.5-ha for 3 or 4 hr of a day until at 1 week after birth. Later, each pair was put out to pasture in a good condition grazing land about 4-ha with other pairs within 6 pairs. All pairs were grazed for 7 hr (09:00–15:00 hr) until May. After June, they were grazed for 19 hr (13:00–08:00 hr). Besides grazing, each foal was kept in his stall with his mare.

Mares were fed 2 kg of oats, 0.5–1 kg of mixed grain (A-mare®, NOSAN corporation, Japan), 7 kg of timothy hay and 0.25 kg of soybeans daily, during late pregnancy. Crude protein content of feedstuffs was shown in Table 1. After birth, they were fed 4.5 kg of oats, 2.25 kg of mixed grain, 7 kg of timothy hay and 0.25 kg of soybeans daily. After June, they were fed 2.25 kg of oats, 1.5 kg of mixed grain, 7 kg of timothy hay and 0.25 kg of soybeans daily, and they were grazed for 19 hr. In their stalls, we installed boarding fences in front of troughs of mares to prevent foals from feed Mare's feed. All foals were fed 300 g of alfalfa hay, 200 g of oats and 10 g of vitamins and minerals to ease into feed after 2 months of age. We put each trough of foal in the space that was divided in the corner of the stall by vinyl chloride pipe to prevent his mare from entering. All pairs were allowed free access to water in their stalls.

**Samples:** All blood samples were collected before suckling (0 week), and when foals were 1, 3, 5, 7, 8, 12, 16, 20 and 24 weeks old. Blood samples were collected from 8 AM to 10 AM. Blood samples were collected aseptically via jugular venipuncture into evacuated, heparinized tubes and immediately centrifuged for 15 min at 4,000 rpm. Plasma was harvested and stored at  $-80^{\circ}\text{C}$  for later analysis.

**Amino acids analysis:** The individual plasma samples (1 ml) were deproteinised with 0.6 ml sulphosalicylic acid (12% w/w), and centrifuged for 15 min at 13,000 rpm at  $4^{\circ}\text{C}$ . The supernatants were filtered pore size 0.45  $\mu\text{m}$ , and it was used to the sample for the analysis. Those were harvested and stored at  $-80^{\circ}\text{C}$  for later analysis. The concentrations of amino acids were assayed by automated high performance liquid chromatography (L-8800, Hitachi, Tokyo). In addition, BCAA to tyrosine ratio (BTR) was determined to investigate their hepatic function.

**Statistical analysis:** Differences between concentrations of amino acids in plasma from 1 week to 24 weeks and those at birth (0 week) were analyzed with one-way ANOVA and multiple comparisons (Tukey-Kramer). Significance for all of the aforementioned determinations was established as  $p < 0.05$ .

## RESULTS

**The concentrations of BCAA in plasma of foals:** The

Table 1. Crude protein content (%) of feed stuffs

feed name	crude protein (%)
Oats	8.6
Timothy Hay	13.4
Soybean	39.5
Alfalfa Hay	19.5
Mixed feed	12.0

changes of concentrations of BCAA in plasma of foals were shown in Fig. 1. Each value was shown as mean value  $\pm$  SD. The concentrations of valine in plasma at 16 ( $273 \pm 51$ ), 20 ( $276 \pm 68$ ) and 24 ( $286 \pm 58$ ) weeks were significantly higher than that at birth ( $180 \pm 35$ ) ( $p < 0.05$ ). The concentrations of leucine in plasma at 1 ( $209 \pm 39$ ) and 3 ( $199 \pm 46$ ) weeks were significantly higher than that at birth ( $137 \pm 32$ ) ( $p < 0.05$ ). The concentrations of isoleucine in plasma at 1 to 24 weeks ( $84 \pm 24$ – $102 \pm 19$ ) were significantly higher than that at birth ( $41 \pm 13$ ) ( $p < 0.05$ ). The concentrations of BCAA in plasma tended to reduce from 5 to 8 weeks after birth, after that, increased gradually. The average level of BTR was 4.8–7.5.

**The concentrations of BCAA in plasma of mares:** The changes of concentrations of BCAA in plasma of mares were shown in Fig. 2. Each value was shown as mean value  $\pm$  SD. The concentration of BCAA in plasma decreased at 1 week compared with that at birth, and increased after 12 weeks. At 16 weeks, the concentrations of plasma BCAA (valine:  $455 \pm 124$ , leucine:  $236 \pm 71$ , isoleucine:  $129 \pm 39$ ) were significantly higher than that at parturition (valine:  $231 \pm 58$ , leucine:  $121 \pm 32$ , isoleucine:  $63 \pm 20$ ) ( $p < 0.05$ ). Valine in plasma also increased significantly at 24 weeks ( $413 \pm 139$ ) ( $p < 0.05$ ). The rage average of BTR was 4.6–10.4.

## DISCUSSION

**The concentrations of BCAA in plasma of foals:** The concentrations of BCAA in plasma of foals at birth was measured by Zicker *et al.* [41, 42], and was similar to our measurement values. The concentration of BCAA in plasma of foals increased after suckling, and decreased gradually. Zicker *et al.* [41], the concentrations of BCAA in plasma neonatal foals significantly increased after suckling, and the concentration of leucine in plasma had no significant differences at 27 days, compared with birth. The gut of foals is able to readily absorb whole proteins within the first 24 hr of life [16]. And total amino acid concentrations of equine decline substantially between colostrums and mature milk [9]. So, it might reflect the changes in the concentrations of BCAA in plasma of foals.

The concentrations of valine were decreased at 7 and 8 weeks, and leucine decreased 8 and 12 weeks. After that, the concentration of BCAA in plasma increased gradually, and those of valine were significantly high at 16, 20 and 24 weeks. After 8 weeks, the amino acid intake and the com-

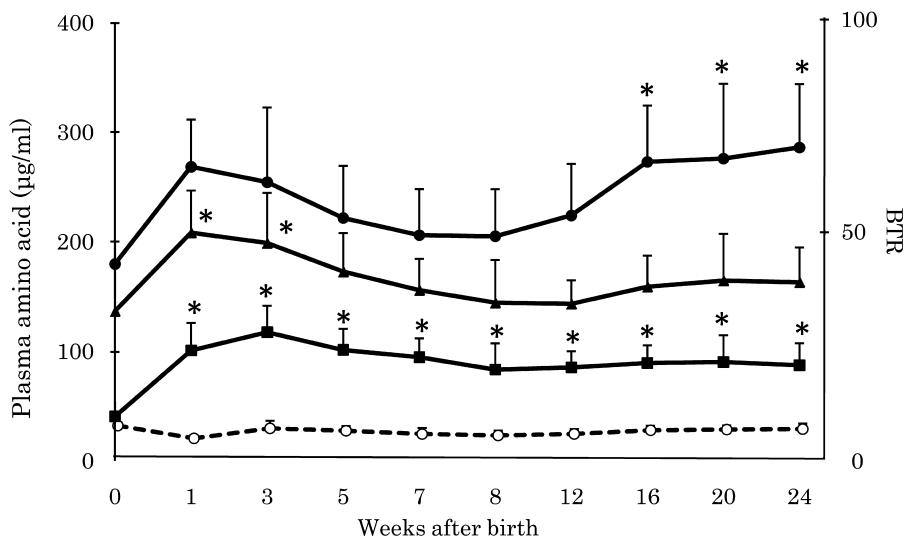


Fig. 1. Mean concentrations ( $\mu\text{mol/l}$ ) + SD of valine (●, n=7), leucine (▲, n=7) and isoleucine (■, n=7) in plasma and mean  $\pm$  SD of value of BTR (○, n=7) in foals. Left axis showed the concentration of amino acid ( $\mu\text{mol/l}$ ). Right axis showed the BTR. Differences between the concentrations of plasma BCAA from 1 week to 24 weeks and those of birth (0 week) were analyzed with one-way ANOVA. \*: Significance was established as  $p<0.05$ . The concentrations of plasma valine at 16, 20 and 24 weeks were significantly higher than that at birth. The concentrations of plasma leucine at 1 week and 3 weeks were significantly higher than that of birth. The concentrations of plasma isoleucine at 1 week to 24 weeks were significantly higher than that at birth.

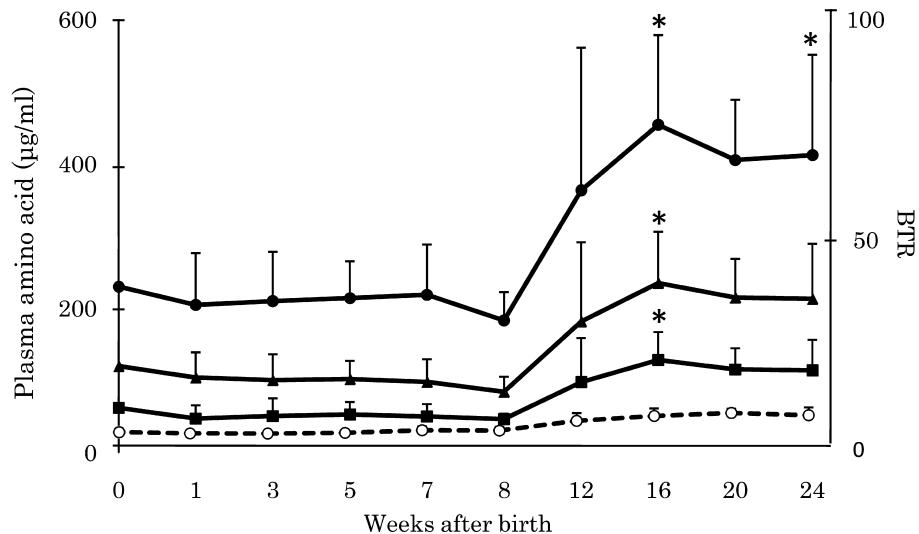


Fig. 2. Mean concentrations ( $\mu\text{mol/l}$ ) + SD of valine (●, n=7), leucine (▲, n=7) and isoleucine (■, n=7) and mean value  $\pm$  SD of BTR (○, n=7) in mares. Left axis showed the concentration of amino acid ( $\mu\text{mol/l}$ ). Right axis showed the BTR. Differences between the concentrations of plasma BCAA from 1 week to 24 weeks and that at birth (0 week) were analyzed with one-way ANOVA. \*: Significance was established as  $p<0.05$ . The concentrations of plasma BCAA at 16 week after birth were significantly higher than those at birth. Plasma valine also increased significantly at 24 weeks.

position of foals might have changed, because foals were fed the weaning food and the whole-day grazing was started after June. In humans, gastric pepsin activity is low during the first 3 post-natal months of life, and trypsin activity in

neonates is low and reaching adult values at approximately 1 year of age [19]. Though there is few studies about absorptive function of horses, Roberts *et al.* [32] revealed acid beta-galactosidase showed a decrease in activity in the

first three months of life. So, the absorptive function of foals changes dramatically. These data imply that the changes of feedstuff and absorptive function reflected the concentrations of BCAA in plasma of foals. But, the cause of change for concentration of BCAA in plasma was not determined. Therefore, the more study about the relationship between the amount of intake or coefficient of digestibility and the concentrations of BCAA in foals at the time of start weaning food or whole-day grazing was necessary.

*The concentrations of BCAA in plasma of mares:* The concentrations of plasma or serum BCAA of mares at parturition were measured [33, 39, 40]. Rogers *et al.* [33] used blood serum of 10 Quarterhorses. Zicker and Rogers [40] used blood plasma of 2 Arabians, 2 Quarterhorses, 2 Thoroughbreds, 2 Arabians × Quarterhorses, and 9 Thoroughbreds × Quarterhorses. Their values were twice as high as ours at parturition and similar to ours at 12 weeks from parturition. Meanwhile, the values of Young *et al.* [39] showed were approximately the same as ours. And, Johnson and Hart [17] determined the concentrations of plasma amino acid of mature geldings of 1 Quarterhorse, 1 Arabian and 4 Morgans. We also got similar measurements.

The protein supplement did not influence foal intakes of milk and milk nutrients, milk composition, and the growth rate of the foals [24]. In our test, though the concentrations of BCAA in plasma of mares were different from the values of Zicker and Rogers [40], the values of foals were similar. So, it indicates if the concentrations of BCAA in plasma of mares are normal range, they don't influence those of foals.

The concentrations of plasma amino acid are influenced by quantity or quality of intake amino acid. Rogers *et al.* [33] showed the concentrations of most serum essential and non-essential amino acids tended to increase from 12 weeks prepartum to 7 weeks prepartum in methionine-supplemented mares compared with those receiving other treatments. Zicker and Rogers [40] used 2 kg of mixed grain containing about 14% crude protein and 10 kg alfalfa hay containing about 17% crude protein daily during last 3 months of gestation. On the other hand, Young *et al.* [39] did not show the feed data. In our test, mares were fed about 13% crude protein feed, It was low protein feed compared with the feed which Zicker and Rogers [40] used. These data implied that different compositions of feed may influence the concentrations of plasma amino acids of mares at parturition.

In growing horses, it is known that horses took the digestible energy of 1 Mcal per hour by pasturing, as the amount of food intake and exercise increased, when the grazing time will be extended from 7 to 17 hr [2]. In our study, mares and foals 2 or 3 months after birth were grazed for 19 hr (1300–0800 hr) after June. This was about the same time when the BCAA concentrations in mares increased significantly. The amount of oats and the grained mixture decreased while the pasture intake in mares increased as the grazing time increased. In general, crude protein content of oats or wheat ration is 96–100 (g/kg), but that of grass is 136–220 (g/kg) [8]. Since, crude protein of grass is higher, after the whole-

day grazing, the crude protein intake might have increased. These suggested that an increase of the protein intake of mares may lead to an increase in the concentrations of BCAA in plasma after 12 weeks.

In the rat, it is known that branched-chain aminotransferase that is one of the BCAA metabolic enzymes is remarkably caused in mitochondria of the mammary gland epithelial cells during late pregnancy and lactation [37]. Therefore the metabolism of BCAA of mares may accelerate during lactation. And, since foals were fed weaning food after 2 month from birth, foal's dependence on maternal milk reduced gradually. Maternal protein mobilization is related to overall milk protein production [3]. Total amino acid concentrations of equines decline substantially between colostrums and mature milk [9]. These data implied maternal protein requirement was critical within 2 months after parturition.

*Hepatic function:* It is known that concentrations of plasma BCAA with hepatic disease are low level, and Fisher's ratio, it is the molar ratio of BCAA to aromatic amino acids, has been frequently used to evaluate hepatic function [34]. But these days, the BTR, it is the molar ratio of BCAA to tyrosine, has been used as a more simpler method. In our study, we calculated the BTR of mares and foals. The normal range of BTR in humans is 4.40–10.05 [36]. In our data, hepatic function of both mares and foals did not have problem.

From our research, we got the base dated of the concentrations of plasma BCAA on healthy mares and their foals from birth to 24 weeks of age in Japan. It has attracted the interest of many athletes as an important element, since it inhibits proteolysis and stimulates photosynthesis. And, BCAA infusion improves respiratory function and is associated with a reduction in apnea in the premature infant [5]. Besides, glutamine availability may influence risk of infection [12]. Parry, *et al.* [29] indicated that BCAA prevented an exercise-induced decline in glutamine. Therefore, the base data of concentrations of plasma BCAA on thoroughbred is very useful to improve horse management.

Six mares we used got pregnant during our study. Pregnancy in the factor causing an increase in protein requirements of mares. And decreases in concentrations of essential and nonessential amino acids in the fetus may be a mechanism whereby maternal dietary protein restriction results in fetal growth retardation [38]. So, the changes of concentrations of plasma BCAA on mares need to be determined during pregnancy. On the other hand, weaning is very stress for foals [25]. Therefore, the changes of concentrations of plasma BCAA on foals need to be determined after weaning.

From this study, the concentrations of plasma BCAA of mares might be reflected from parturition, lactation or the whole-day grazing. In foals, sucking colostrum, weaning period or grazing might influence their concentrations of plasma BCAA. Therefore, it is necessary to check the concentrations of plasma BCAA in foals, for example, at 1 week, 8 weeks and 24 weeks after birth to ascertain whether

they obtained colostrum appropriately or whether weaning period and feeding system during grazing were adequate was good. In mares, it is necessary to checked those to ascertain the condition of feed system in the prepartum or grazing period. On the other hand, it is unknown how much did the change of feed influenced the concentrations of BCAA in plasma in this study, because an actual amount of the pasture intake in the whole-day grazing was not measured. And we also didn't measure the concentrations of BCAA in milk. Therefore, it needs to be examine the feed intake, the elements of the pasture and the concentrations of BCAA in milk.

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