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Seasonal Change of Plasma Free Amino Acids with Special Reference to 3-Methylhistidine in Racehorses

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ABSTRACT : Seasonal changes in the concentration of free amino acids were determined monthly in plasma from the jugular blood of 50 thoroughbred racehorses that compete during the night between June and September and during the day between October and January. The concentration of most free amino acids remained relatively constant between June and January. However, those of glutamic acid, alanine, isoleucine and lysine tended to decrease and that of arginine (Arg) significantly decreased between July and September. The concentration of methionine (Met) gradually increased between June and September and significantly decreased thereafter. The concentration of 3-methylhistidine (3-MH) significantly increased between July and September and decreased thereafter. There were significant correlation between 3-MH and Arg. In conclusion, this study provided evidence of significant seasonal change in plasma 3-MH, Arg and Met of racehorses, and this was considered to relate to an environmental effect. Moreover, our study is the first to show 3-MH in plasma of racehorses affected by environmental change. (**Key Words :** Heat Stress, 3-Methylhistidine, Plasma Free Amino Acid, Racehorse)

INTRODUCTION

Protein and amino acid nutrition has not been studied as extensively in racehorses as in other domestic animals such as swine, poultry and cattle, although optimal protein and energy nutrition is very important for racehorses. The determination of free amino acids in blood plasma is generally effective for understanding protein nutrition in many domestic animals.

These domestic animals nutrition related reproduction and productivity strongly, however racehorses related with more exercise and environmental factor. Only few study reported amino acids in plasma of horses influenced by feeding and fasting (Johnson et al., 1974; Russell et al., 1986; Depew et al., 1994). However there are no studies how the exercise and environment affect to amino acids nutritional status of horses even it is important to develop muscle protein. In amino acids we interested in 3-MH status because this is related with muscle protein in other animals, and there is no study about in horses.

Histidine methylation during actin and myosin breakdown is the only source of 3-methylhistidine (3-MH), which is liberated into the plasma amino acid pool. Several studies indicate that 3-MH is a valid index of muscle protein breakdown in rats (Young et al., 1972), rabbits (Nishizawa et al., 1977), cattle (Nishizawa et al., 1979), goats (Nishizawa et al., 1989) and humans (Long et al., 1975), because it is irreversibly excreted mainly into the urine (Young et al., 1978; Harris et al., 1981; Kocen et al., 1982; Nishizawa et al., 1984). Plasma levels of 3-MH in dairy cows at preparturition significantly increase, and this is considered to be due to the increased breakdown of muscle protein for milk protein synthesis (Blum et al., 1985).

3-MH is also recognized in recent study as for protein mobilization from skeleton muscle in dairy cows (Doepel et al., 2002). Although the condition of racehorses tends to worsen, and lose body weight under heat stress, little is known about protein and amino acid nutrition in this animal. Therefore the aim of this study was to investigate the plasma free amino acid seasonal changes of racehorses with special reference to 3-MH metabolism and amino acid nutrition, and how are these influenced by high ambient

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Table 1. Composition and nutrient level of the experimental diet

Ingredient	% of diets
Timothy hay	35.13
Oats	48.88
Wheat bran	15.78
NaCl	0.05
Dicalcium phosphate	0.05
Mineral mixture	0.05
Total	100.00
Nutrient composition[1]	
DE (Mcal/kg)	2.72
CP (%)	9.90
Amino acids composition (%)[2]	
Arginine	0.585
Cystine	0.217
Glycine	0.482
Histidine	0.223
Isoleucine	0.344
Leucine	0.676
Lysine	0.457
Methionine	0.168
Phenylalanine	0.435
Serine	0.425
Threonine	0.349
Valine	0.478

CP = Crude protein. DE = Digestible energy.

[1] Calculated using the analytical value of Oat and Timothy hay, and SFCJ* valuse of wheat bran as dry matter basis.

[2] Calculated using SFCJ values as dry matter basis.

* Standard Tables of Feed Composition in Japan (2001) edited by National Agricultural Research Organization.

temperature in summer.

MATERIALS AND METHODS

Animals and diet

Fifty healthy thoroughbred racehorses (34 males and 16 females aged between 3 and 6 years, weighing 450-500 kg) were bred at Kobayashi Horse Farm that belongs to the Tokyo City Racing Association. The horses were fed with chopped timothy hay, oats and wheat bran, and ration were formulated to meet protein and energy requirements according to the Japanese Feeding Standard for Horses (JFS, 1998) shown in Table 1. Including free timothy hay 9.3 to 14.4 kg diet was given to individual horses divided into three times, at 06.00, 11.00 and 15.00 h. every day. During the year diets composition were same.

In present study, feed intake could not to be measured, however some horses reduced feed intake in summer. Drinking water and timothy hay were available *ad libitum*.

Sampling and measurement

At the beginning of the trial 20 healthy horses were selected, and blood samples were collected monthly. However if the horses transferred or changed due to race plane, canceled or collected from replaced horses. Sampling time was more than two hours after meal, and avoid just after exercises and the day before and after competition. Samples of blood collected from the jugular vein between 13.00-15.00 h once each month between June and January into heparinized vacuum tubes, were immediately placed on ice and then separated by centrifugation at 6,000 rpm for 15 min at 4°C. Plasma was removed and stored at -30°C. Thawed plasma samples (1 ml) were deproteinized with two volumes of 5% trichloroacetic acid, and separated by centrifugation at 10,000 rpm for 15 min at 4°C. The supernatants were then passed through membrane filters (0.45 μm), and free amino acids were measured using an automated amino acid analyzer (Model L-8500A; Hitachi Industries Ltd., Tokyo Japan).

Statistical analysis

All data are shown as means and standard deviation for each month. The significance of difference between means was determined using an analysis of variance. Significance was established at the level of 5% or 1% using Student's *t*-test. Peason's correlation coefficient test was used for 3-MH and Arg relation.

RESULTS AND DISCUSSION

Figure 1 and 2 show changes in the concentrations of individual plasma free amino acids between June and January. These values did not significantly differ between female and male horses. The concentrations of serine, glutamine, glycine and alanine were the highest among all amino acids determined, and ranged from 20 to 60 μmol/100 ml. Those of threonine, asparagine, glutamic acid, citrulline, valine, isoleucine, leucine, tyrosine, phenylalanine, ornithine, 3-methylhistidine (3MH), histidine, lysine and arginine were intermediate and ranged from 4 to 20 μmol/100 ml. Those of aspartic acid, methionine and cystine were the lowest and <5 mol/100 ml. Although there is only few report about amino acids in blood of horse, Johnson et al. (1974) reported amino acids in plasma level after feeding and fasting. DePew et al. (1994) also reported time relative feeding. Comparing to these data and our data, each amino acids range were very similar.

The concentrations of most amino acids remained relatively constant from June to January. However, those of glutamic acid, alanine, isoleucine and lysine tended to be lower and that of arginine decreased significantly between July and September. On the other hand, the 3-MH concentration significantly increased from July to September and decreased thereafter. The interesting things are 3-MH and Arg change inversely through the period. There were significant correlations between these two amino acids (Table 2). The concentration of methionine

gradually increased from June to September and significantly decreased thereafter.

Only one molecule of non-metabolizable 3-MH is generated by histidine methylation per molecule of actin and of myosin, then it is liberated into the plasma amino acid pool and irreversibly excreted mainly into the urine without further metabolism or catabolism in most animals (Young et al., 1978; Nishizawa and Itabashi, 1984). Plasma

Table 2. Correlation coefficient (r) and p-values between 3MH and Arg concentration in blood plasma in all raw data in this study by Peason's test

	r	p-value
Male[1]	-0.83292	p<0.01
Female[2]	-0.76266	p<0.01

[1] n = 96. [2] n = 32.

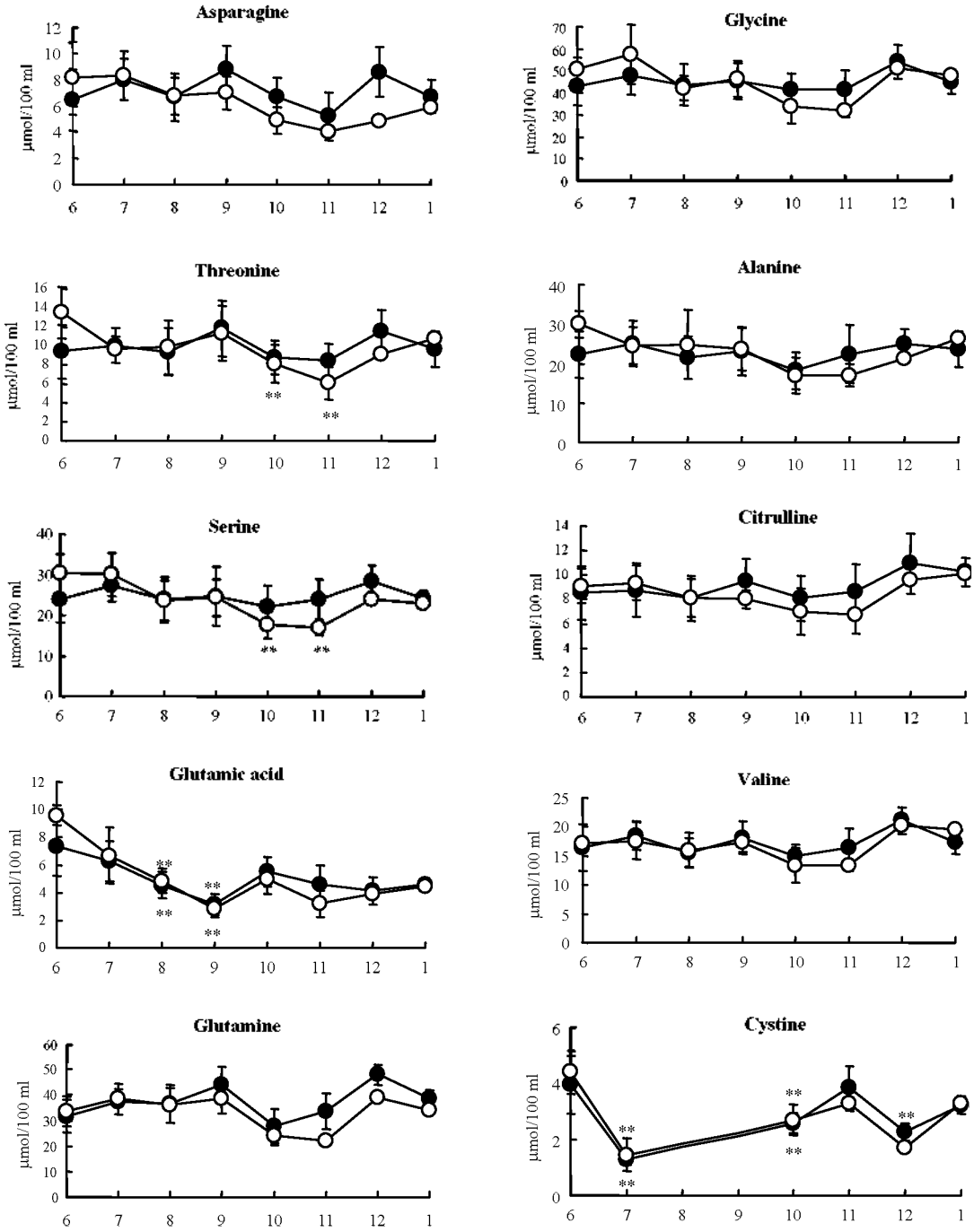

Figure 1. Changes in plasma concentration of asparagine, threonine, serine, gulutamic acid, glutamine, glycine, alanine, citrulline, valine and cystine of male (●) and female (○) horses during June to January. Values are expressed as means±SD. *, ** Significantly different from the value of June (* p<0.05, ** p<0.01).

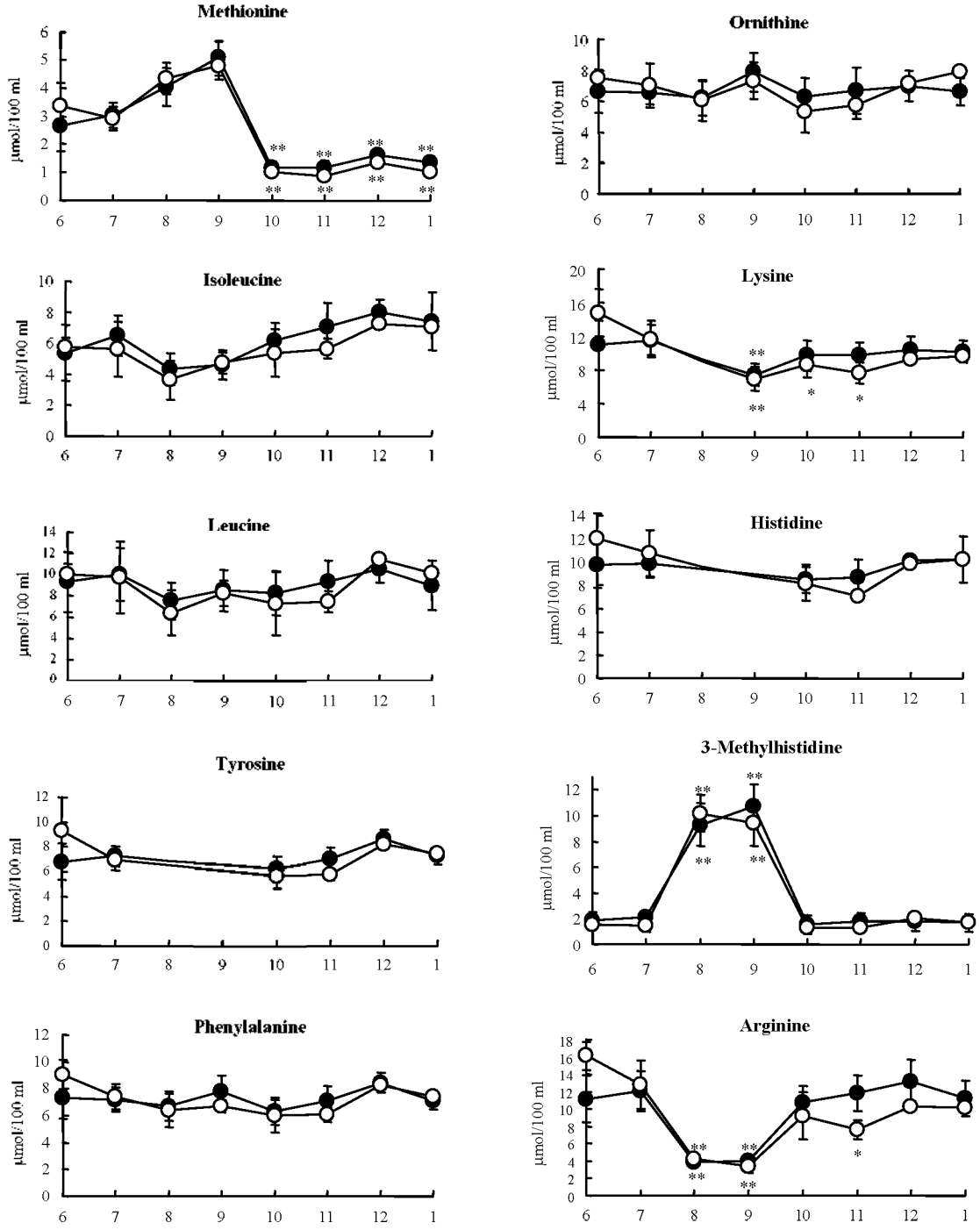

Figure 2. Changes in plasma concentrations of methionine, isoleoucine, leoucine, tyrosine, phenylalanine, ornithine, lysine, histidine, 3-methylhistidine and arginine of male (●) and female (○) horses during June to January. Values are expressed as means±SD. *, ** Significantly different from the value of June (* $p<0.05$, ** $p<0.01$).

3-MH significantly increases just after parturition in dairy cattle because of decreased feed intake and protein deficiency (Motyl et al., 1986). Several studies have indicated that 3-MH is a valid index of muscle protein breakdown in rats (Young et al., 1972), rabbits (Nishizawa et al., 1977), cattle (Nishizawa et al., 1979), goats (Nishizawa et al., 1989) and humans (Long et al., 1975). However, this method is not applicable to pigs and sheep because some 3-MH is metabolized in these animals (Harris et al., 1980; Harris et al., 1981). The relationship between 3-MH and muscle protein metabolism in horses has not been determined.

The present study found that plasma concentrations of 3-MH in racehorses changed seasonally, since levels were

Table 3. Monthly maximum and minimum air temperature around experimental field reported by Japan Meteorological Agency in Chiba

	Month							
	6	7	8	9	10	11	12	1
Maximum (°C)	33.5	34.6	33.8	32.7	26.2	23.9	17.9	12.8
Minimum (°C)	12.4	16.3	18.2	11.2	3.0	1.1	-2.5	-5.4

about 5-fold higher during August and September compared with other months. Racehorses are sensitive to the environment, especially to heat. The horses in this experiment were stabled in a reinforced concrete building, the temperature of which was not well-regulated during the summer. Many horses were affected by heat stress during the summer, and reduced their feed intake.

Blum et al. (1985) studied changes in energy and protein metabolism by measuring plasma 3-MH, feed intake and other indices in transition dairy cows. They found that plasma 3-MH sharply increased at about one week postpartum, and remained elevated for about three weeks postpartum. During this period, the feed intake of these cows was about half of the normal requirement. When feed intake recovered thereafter, plasma 3-MH became normalized. Motyl et al. (1986) also determined urinary 3-MH and plasma free amino acids in transition dairy cows. They found that the onset of lactation was accompanied by intensified muscle protein catabolism expressed as increased urinary 3-MH excretion and a loss of body mass. During the same time, plasma levels of alanine, threonine, proline, citrulline, ornithine, arginine and urea were reduced. When intake satisfied protein requirements during the mid stage of lactation, urinary 3-MH excretion was decreased and plasma levels of urea cycle amino acids such as ornithine, citrulline, and arginine, as well as urea increased. These findings indicated that decreased feed intake and a protein deficiency increased muscle protein breakdown that was reflected in altered levels of plasma free amino acids during the early stage of lactation. Kamiya et al. (2006) reported that high ambient temperature (28°C) lower the energy and protein sufficiency ratio, and plasma 3-MH increase in lactating Holstein cows, and also they confirmed plasma 3-MH increased by high ambient temperature (28°C) alone even feed intake were same. In present study the air temperature at this area in summer was more than 30°C (Table 3). It was suggested by above study and our study the requirement of energy and protein increase by high temperature and promotes myofibilar protein degradation in horses also.

In addition Arg in plasma in this study decreased in summer, Balnave and Oliva (1991) also reported that heat stress might alter gut absorption of arginine in broiler. Brake (1998) identified a significant reduction in sodium-dependent arginine uptake by the intestinal epithelium among heat stressed birds, and this was reflected in a significant reduction in total arginine uptake.

In conclusion the present study provided clear evidence that 3-MH, Arg and Met in plasma of racehorses changed significantly in seasonally, and this is considered high ambient temperature affected because this evidence observed from July to September.

Moreover, our study showed for the first time that 3-MH in plasma of horses level affected by environment. The reason of the strong negative correlation between 3-MH and Arg was not clear in this study, so further studies are necessary to investigate the reason of this evidence. The reason for significant seasonal changes in plasma methionine levels is unclear. In addition, present study suggested that feeding some amino acids to horses during the hot season might effectively overcome heat stress. More studies are required to clarify amino acids nutrition of racehorses during the year.

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REFERENCES

Balnave, D. and A. G. Oliva. 1991. The influence of $NaHCO_3$ and sulfur amino acids on the performance of broilers at moderate and high temperatures. Aust. J. Agric. Res. 42:1385-1397.

Blum, J. W. and T. Reding. 1985. Variations of 3-methylhistidine in blood of dairy cows. J. Dairy Sci. 68:2580-3587.

Brake, J., D. Balnave and J. J. Dibner. 1998. Optimum dietary arginine: lysine ratio for broiler chickens is altered during heat stress in association with changes in intestinal uptake and dietary sodium chloride. Br. Poult. Sci. 39:639-647.

DePew, C. L., D. L. Thompson, J. M. Fernandez, L. S. Sticker and D. W. Burleigh. 1994. Changes in concentrations of hormones, metabolites, and amino acids in plasma of adults horses relative to overnight feed deprivation followed by a pellet-hay meal fed at noon. J. Anim. Sci. 72:1530-1539.

Doepel, L., H. Lapierre and J. J. Kennelly. 2002. Peripartum performance and metabolism of dairy cows in response to prepartum energy and protein intake. J. Dairy Sci. 85:2315-2334.

Equine Research Institute, Japan Racing Association. 1998. Japanese Feeding Standard for Horses 1st Ed. 17-47. Animal Media Ltd. Tokyo.Press, Tokyo, Japan.

Harris, C. I. and G. Milne. 1981. The urinary excretion of N^{τ}-methylhistidine by cattle: validation as an index of muscle protein breakdown. Br. J. Nutr. 45:411-422.

Harris, C. I. 1981. Reappraisal of the quantitative importance of non-skeletal muscle source of N^{τ}-methylhistidine in urine.

Biochem. J. 194:1011-1014.

Harris, C. I. and G. Milne. 1981. The inadequacy of urinary N^{τ}-methyl histidine excretion in the pig as a measure of muscle protein breakdown. Br. J. Nutr. 45:423-429.

Harris, C. I. and G. Milne. 1980. The urinary excretion of N^{τ}-methyl histidine in sheep: an invalid index of muscle protein breakdown. Br. J. Nutr. 44:129-140.

Johnson, R. J. and J. W. Hart. 1974. Influence of feeding and fasting on plasma free amino acids in the equine. J. Anim. Sci. 38:790-794.

Kamiya, M., Y. Kamiya, M. Tanaka, T. Oki, Y. Nishiba and S. Shioya. 2006. Effect of high ambient temperature and restricted feed intake on urinary and plasma 3-methylhistidine in lactating Holstein cows. Anim. Sci. J. 77:201-207.

Kamiya, M., Y. Kamiya, M. Tanaka and S. Shioya. 2006. Milk protein production and plasma 3-methylhistidine concentration in lactating Holstein cows exposed to high ambient temperatures. Asian-Aust. J. Anim. Sci. 19:1159-1163.

Kocen, J. A., J. E. Wohlt and W. L. Foy. 1982. Dietary intake and routes of excretion of 3-methylhistidine in lactating dairy cattle. J. Anim. Sci. 55(Suppl.1):435(Abstr.).

Long, C. L., L. N. Haververg, Y. R. Young, J. M. Kinney, H. M. Munro and J. W. Geiger. 1975. Metabolism of 3-methylhistidine in man. Metabolism: clinical and experimental 24:929-935.

Motyl, T. and W. Barej. 1986. Plasma amino acid indices and urinary 3-methyl histidine excretion in dairy cows in early lactation. Ann. Rech. Vet. 17(2):153-157.

Nishizawa, N. and H. Itabashi. 1984. Measurement of synthetic and catabolic rates of skeletal muscle protein by using 3-methylhistidine method and its application. Proceeding of Japanese Society for Animal Nutrition and Metabolism 28(2):97-110.

Nishizawa, N., M. Shimbo and S. Harayama. 1977. Fractional catabolic rates of myosin and actin estimated by urinary excretion of N^{τ}-methylhistidine: the effect of dietary protein level on catabolic rates under conditions of restricted food intake. Br. J. Nutr. 37:345-353.

Nishizawa, N., Y. Toyoda, T. Noguchi, S. Hareyama, H. Itabashi. and R. Funabiki. 1979. N^{τ}-methylhistidine content of organs and tissues of cattle and an attempt to estimate fractional catabolic and synthetic rates of myofibrillar protein of skeletal muscle during growth by measuring urinary output of N^{τ}-methylhistidine. Br. J. Nutr. 42:247-252.

Nishizawa, N., S. Igarashi, H. Itabashi and S. Harayama. 1989. Urinary excretion of N^{τ}-methylhistidine in goat. Nutrition Report International 40:567-576.

Russell, M. A., A. V. Rodiek and L. M. Lawrence. 1986. Effect of meal schedules and fasting on selected plasma free amino acids in horses. J. Anim. Sci. 63:1428-1431.

Young, V. R. and H. N. Munro. 1978. N^{τ}-methylhistidine (3-methylhistidine) and muscle protein turnover: an overview. Fed. Proc. 37(9):2291-2300.

Young, V. R., S. D. Alexis, B. S. Baliga, H. N. Munro and W. Muecke. 1972. Metabolism of administered 3-methylhistidine. Lack of muscle transfer ribonucleic acid charging and quantitative excretion as 3-methylhistidine and its N-acetyl derivative. J. Biol. Chem. 247:3592-3600.