Changes of amino acid concentrations in Polish Merino sheep between 21 and 150 days of life

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ABSTRACT: The aim of this study was to evaluate plasma amino acid profiles and their interrelationships in male Polish Merino sheep at two different stages of their systemic development – at the neonatal monogastric stage and as a fully grown ruminant. Male lambs (n = 12, singles) were kept indoors in pens under standard rearing conditions and weaned at the age of 10 weeks of life. Blood samples were collected on day 21 and 150 of life. Free amino acids were determined in plasma using ion-exchange chromatography. There was a significant reduction in plasma amino acid concentrations between neonatal and functioning ruminants for threonine, serine, glutamate, proline, citrulline, tyrosine, tryptophan, lysine, histidine and arginine. Significant increases in the plasma concentrations of alpha-amino-butyrate and isoleucine were observed in 150 day old lambs. Except for glutamine in 21 day old sheep which was significantly negatively correlated with serine, glutamate, proline and tyrosine concentrations in plasma, the obtained results in younger and older groups of lambs showed 47 and 22 significant positive correlations between the evaluated amino acids. This study shows that decreases in plasma amino acid concentrations in 150 day old sheep with physiologically developed forestomachs are dominant in comparison to 21 day-old animals receiving maternal milk as a basic diet. Our results address the possibility of utilising improved amino acid supply for growing lambs with developed forestomachs.

Keywords: amino acid; blood plasma; ion-exchange chromatography; Polish Merino sheep; weaning

The quantity and quality of ingested food are important determinants of whole body protein metabolism and systemic growth and development. Proteins are the major structural and functional components of all cells in the body. From a nutritional perspective, the important aspect of a protein is its amino acid composition (Koletzko et al. 2005). With respect to pre-ruminant and monogastric animals, it was documented that supply and requirements must be expressed for individual amino acids rather than their aggregate – total protein. Thus, commercial diets enriched for a selected single amino acid may lead to improved economic effectiveness of production procedures and better animal performance (Lapierre et al. 2006). In contrast to pre-ruminants and non-ruminants where intake represents supply and deficiencies can be reversed with simple addition of any individual amino acid directly to the diet, in ruminants dietary ingredients are partially digested and utilised for microbial growth during food passage through the reticulorumen. In ruminants with developed forestomachs, the absorbed nutrients differ from those present in the diet. Furthermore, free amino acids are rapidly degraded in the rumen environment and the simple addition of a required amino acid to the diet may not be effective in increasing amino acid flow to the duodenum (Velle et al. 1997; Volden et al. 1998).

Both the colostrum and milk in mammals provide the suckling with amino acids and proteins, energy, vitamins and macro- and micronutrients as well as numerous regulatory substances such as hormones, biologically active peptides, growth factors, immunoglobulins and glycoproteins. All these biologically active factors possess identical structures and comparable biological activities to those produced physiologically in organisms; however, their concentrations in colostrum or milk vastly exceed the values measured in maternal or offspring plasma (Koldovsky et al. 1995, 1998; Xu 1998). Furthermore, the concentration of these biologically active factors is species-specific and changes during the lactation period (Jaeger et al. 1987; Westrom et al. 1987; Raaberg et al. 1990; Read et al. 1994; Blum and Hammon 2000). Similar changes may occur in plasma amino acid concentrations during systemic growth of sheep. These developmental changes in amino acid concentrations in plasma may be the result of age-differentiated metabolic requirements for several amino acids as well as altered gastrointestinal digestibility and absorption. It is noteworthy that maternal milk contains all necessary ingredients for the proper growth and development of new-borns. In contrast to ruminants with fully developed forestomachs where microbial degradation processes limit nutrient absorption and subsequent utilization, all these nutrients are easy absorbable in the small intestines of new-borns. Although some experimental studies have been performed in sheep during pregnancy and lactation, little data are available on developmental changes in plasma amino acid concentrations during postnatal life (Masters et al. 1993; Kwon et al. 2003). Thus, the aim of this study was to evaluate free plasma amino acid profiles and their interrelationships in male Polish Merino sheep at two different stages of their systemic development – at the monogastric neonatal stage and as a fully-grown ruminant.

MATERIAL AND METHODS

The experimental procedures used throughout this study were approved by the Local Ethics Committee on Animal Experimentation of the Medical University of Lublin, Poland.

Experimental design and sampling procedure. The study was performed on 12 healthy Polish Merino male lambs (singles) sired by the same ram and born physiologically to ewes. Lambs and ewes were kept indoors in pens under standard rearing conditions with free access to drinking water. Ewes were fed a standard diet recommended for this physiological stage. From the 22^{nd} day of life, lambs were fed a commercial concentrate and hay *ad libitum* and the diet consisted of 16% crude protein, as recommended for growing sheep. The weaning of lambs from ewes was performed at the age of 10 weeks of life. Blood samples were collected into heparinised tubes at the age of 21 and 150 day of life after 2 h of food deprivation.

Analysis of amino acids in plasma. The blood samples were centrifuged at 2500 g for 15 min to obtain plasma which was stored at –25 °C until analysis. Before the measurements, 1 ml of each plasma sample was deproteinised with 1 ml of 6% sulphosalicylic acid in the lithium-citrate buffer (pH 2.8) and centrifuged at 12 000 g for 15 min. Free amino acid concentrations were determined in the obtained supernatant using ion-exchange chromatography (Ostion LG FA column) with five lithium-citrate buffers (pH: 2.8, 3.1, 3.35, 4.05, 4.95) on a INGOS AAA automatic analyser (Ingos, Prague, Czech Republic). The separation of acidic, neutral and alkaline amino acids was performed at the temperatures of 38 °C, 50–65 °C and 74 °C, respectively.

Statistical analysis. Statistical analysis was performed using Statistica software (version 6.0). All the data are presented as means ± SEM. The differences of mean values determined in 21 and 150 day old lambs were tested for statistical significance using the paired Student's *t*-test. Pearson's correlation coefficient (*r*) was determined between all the evaluated amino acids. Differences with $P < 0.05$ were considered as statistically significant.

RESULTS

Plasma amino acid concentrations in 21 and 150 day old ram lambs are shown in Table 1. Significant decreases in plasma amino acid concentrations in 150 day old lambs were noted for arginine, tryptophan, threonine, proline, tyrosine, lysine, citrulline, glutamate, histidine and serine, when compared to the values obtained in 21 day old animals ($P \leq 0.01$). Plasma concentrations of phenylalanine showed the strong tendency to be lower in 150 day old sheep ($P = 0.06$). Determinations of plasma concentrations of cysteic acid, taurine, aspartate, glutamine, glycine, alanine, valine, leucine and ornithine did not show significant changes between age-differentiated groups of animals ($P > 0.05$). However, concentrations of alpha-aminobutyrate and isoleucine were significantly increased (119.6% and 36.9%, respectively) in animals with functioning rumens as compared to neonates.

Table 1. Free amino acid concentrations (nmol/ml) in the plasma of male Polish Merino sheep (n = 12) at the age of 21 and 150 days of life

Amino acid	21 days of life	150 days of life
Cysteic acid	15.1 ± 2.1	12.3 ± 1.6
Taurine	84.2 ± 9.1	89.2 ± 6.3
Aspartate	34.8 ± 2.4	29.6 ± 1.0
Threonine	260.3 ± 35.6	124.7 ± 8.4*
Serine	123.3 ± 10.8	96.3 ± 5.2*
Glutamate	412.2 ± 33.0	297.3 ± 12.6*
Glutamine	197.1 ± 59.3	281.3 ± 28.5
Proline	248.1 ± 19.8	148.8 ± 8.8*
Glycine	475.2 ± 37.8	566.2 ± 35.5
Alanine	237.7 ± 17.4	230.3 ± 7.7
Citrulline	216.3 ± 19.3	155.6 ± 8.6*
Alpha-amino-butyrate	5.1 ± 1.0	11.2 ± 0.9*
Valine	250.8 ± 15.7	243.7 ± 14.2
Isoleucine	67.0 ± 5.3	91.7 ± 5.6*
Leucine	141.7 ± 7.4	133.8 ± 6.3
Tyrosine	88.7 ± 7.2	60.7 ± 5.6*
Phenylalanine	65.6 ± 5.1	55.2 ± 2.7
Tryptophan	65.6 ± 7.7	25.5 ± 3.2*
Ornithine	93.0 ± 7.3	81.7 ± 5.4
Lysine	173.9 ± 9.0	122.3 ± 5.7*
Histidine	149.0 ± 14.5	114.3 ± 6.6*
Arginine	327.9 ± 78.1	97.6 ± 25.0*

Values are expressed as mean ± SEM
*$P \leq 0.01$

The values of Pearson's correlation coefficient between all the evaluated amino acids in the plasma of 21 day old ram lambs are shown in Table 2. Significant positive correlations were found between taurine and cysteic acid as well as between aspartate and glutamine ($P < 0.05$). The plasma concentration of treonine in 21 day old ram lambs was positively correlated with isoleucine, valine, tyrosine and histidine ($P < 0.05$). Plasma levels of serine were found to be positively correlated with the values obtained for glutamate, proline, valine, leucine, tyrosine and ornithine ($P < 0.05$). Positive correlations were found between glutamate and plasma concentrations of alanine, valine, leucine, isoleucine, tyrosine and phenylalanine; however, glutamine was negatively correlated with glutamate, serine, proline and tyrosine ($P < 0.05$). Plasma concentrations of proline were positively correlated with valine, leucine and tyrosine, while glycine was found to be positively correlated with alanine and phenylalanine ($P < 0.05$). Alanine concentrations were positively correlated with leucine, isoleucine, tyrosine, phenylalanine and ornithine ($P < 0.05$). Significant positive correlations were also found between valine and plasma concentrations of isoleucine, leucine, tyrosine, ornithine, lysine and histidine ($P < 0.05$). Plasma concentrations of isoleucine were positively correlated with the levels of leucine, tyrosine, ornithine and histidine ($P < 0.05$). Positive correlations were observed between leucine and plasma levels of tyrosine, phenylalanine, ornithine and histidine ($P < 0.05$). Phenylalanine and ornithine were also positively correlated with plasma concentrations of tyrosine ($P < 0.05$). Significant positive correlations were found between tryptophan and plasma concentrations of ornithine and lysine as well as between ornithine and lysine ($P < 0.05$).

The values of Pearson's correlation coefficient between all the evaluated amino acids in the plasma of 150 day old sheep are shown in Table 3. Significant positive correlations were found between taurine and plasma concentrations of glycine and phenylalanine ($P < 0.05$). Plasma concentrations of aspartate in 150 day old males were found to be positively correlated with the values obtained for glutamine, alanine, isoleucine and lysine ($P < 0.05$). While plasma levels of glutamate were positively correlated with citrulline, evaluation of glutamine showed positive correlations with glycine and isoleucine ($P < 0.05$). Plasma concentrations of proline were positively correlated with tryptophan and lysine ($P < 0.05$). Significant positive correlations were found between glycine and alpha-amino-butyrate concentrations ($r = 0.85$; $P < 0.05$). While alanine concentrations in plasma were positively correlated with isoleucine, phenylalanine and lysine, citrulline were found to be positively correlated with tryptophan and histidine ($P < 0.05$). Positive correlations were found between valine and plasma concentrations of isoleucine and leucine as well as between isoleucine and leucine ($P < 0.05$). Lysine was also positively correlated with plasma concentrations of leucine and phenylalanine ($P < 0.05$).

DISCUSSION

Sheep are a common animal model used to study the relationship of regulatory processes of

Table 2. The values of Pearson's correlation coefficient estimated for all the evaluated amino acids in the plasma of 21 day old ram lambs

Amino acid	Cysteic acid	Taurine	Aspartate	Threonine	Serine	Glutamate	Glutamine	Proline	Glycine	Alanine	Citrulline	α-Amino-butyrate	Valine	Isoleucine	Leucine	Tyrosine	Phenylalanine	Tryptophan	Ornithine	Lysine	Histidine	Arginine
Cysteic acid		0.68*	0.06	−0.21	0.04	0.31	0.10	−0.25	0.20	−0.04	−0.09	0.46	0.02	0.02	0.11	−0.18	−0.06	−0.21	−0.23	−0.12	−0.02	−0.24
Taurine	0.68*		0.43	−0.50	−0.37	−0.18	0.44	−0.54	0.33	−0.13	0.13	0.07	−0.26	−0.29	−0.19	−0.52	−0.15	0.01	−0.22	0.10	−0.54	0.03
Aspartate	0.06	0.43		−0.43	0.40	0.40	0.80*	−0.45	0.39	0.15	0.12	0.29	−0.22	−0.01	−0.05	−0.37	−0.06	−0.28	−0.21	−0.10	−0.11	−0.13
Threonine	−0.21	−0.50	−0.43		0.40	0.41	−0.37	0.42	−0.08	0.17	−0.17	−0.32	0.74*	0.64*	0.55	0.69*	0.33	0.27	0.54	0.59	0.68*	−0.23
Serine	0.04	−0.37	0.40	0.40		0.79*	−0.71*	0.91*	0.27	0.59	0.31	0.44	0.81*	0.63	0.86*	0.79*	0.58	0.50	0.65*	0.32	0.60	0.22
Glutamate	0.31	−0.18	0.40	0.41	0.79*		−0.67*	0.52	0.51	0.74*	−0.13	0.45	0.69*	0.73*	0.76*	0.77*	0.71*	0.41	0.59	0.32	0.50	0.31
Glutamine	0.10	0.44	0.80*	−0.37	−0.71*	−0.67*		−0.71*	−0.08	−0.29	0.17	0.04	−0.41	−0.22	−0.38	−0.73*	−0.58	−0.43	−0.38	−0.16	−0.16	−0.46
Proline	−0.25	−0.54	−0.45	0.42	0.91*	0.52	−0.71*		0.01	0.36	0.36	0.20	0.70*	0.44	0.69*	0.76*	0.46	0.42	0.53	0.24	0.54	0.21
Glycine	0.20	0.33	0.39	−0.08	0.27	0.51	−0.08	0.01		0.81*	−0.10	0.35	0.32	0.46	0.50	0.35	0.76*	0.45	0.48	0.41	−0.06	0.43
Alanine	−0.04	−0.13	0.15	0.17	0.59	0.74*	−0.29	0.36	0.81*		0.05	0.52	0.58	0.78*	0.72*	0.66*	0.80*	0.45	0.69*	0.40	0.35	0.48
Citrulline	−0.09	0.13	0.12	−0.17	0.31	−0.13	0.17	0.36	−0.10	0.05		0.24	0.29	0.10	0.26	−0.14	−0.28	0.37	0.38	0.29	0.09	−0.05
α-Amino-butyrate	0.46	0.07	0.29	−0.32	0.44	0.45	0.04	0.20	0.35	0.52	0.24		0.20	0.40	0.43	0.12	0.16	−0.19	0.06	−0.29	0.36	−0.07
Valine	0.02	−0.26	−0.22	0.74*	0.81*	0.69*	−0.41	0.70*	0.32	0.58	0.29	0.20		0.86*	0.95*	0.79*	0.54	0.56	0.83*	0.71*	0.74*	−0.05
Isoleucine	0.02	−0.29	−0.01	0.64*	0.63	0.73*	−0.22	0.44	0.46	0.78*	0.10	0.40	0.86*		0.86*	0.75*	0.58	0.31	0.74*	0.51	0.76*	0.01
Leucine	0.11	−0.19	−0.05	0.55	0.86*	0.76*	−0.38	0.69*	0.50	0.72*	0.26	0.43	0.95*	0.86*		0.78*	0.64*	0.50	0.77*	0.56	0.73*	0.04
Tyrosine	−0.18	−0.52	−0.37	0.69*	0.79*	0.77*	−0.73*	0.76*	0.35	0.66*	−0.14	0.12	0.79*	0.75*	0.78*		0.83*	0.39	0.64*	0.45	0.60	0.31
Phenylalanine	−0.06	−0.15	−0.06	0.33	0.58	0.71*	−0.58	0.46	0.76*	0.80*	−0.28	0.16	0.54	0.58	0.64*	0.83*		0.44	0.55	0.42	0.20	0.57
Tryptophan	−0.21	0.01	−0.28	0.27	0.50	0.41	−0.43	0.42	0.45	0.45	0.37	−0.19	0.56	0.31	0.50	0.39	0.44		0.83*	0.76*	−0.01	0.43
Ornithine	−0.23	−0.22	−0.21	0.54	0.65*	0.59	−0.38	0.53	0.48	0.69*	0.38	0.06	0.83*	0.74*	0.77*	0.64*	0.55	0.83*		0.84*	0.39	0.22
Lysine	−0.12	0.10	−0.10	0.59	0.32	0.32	−0.16	0.24	0.41	0.40	0.29	−0.29	0.71*	0.51	0.56	0.45	0.42	0.76*	0.84*		0.17	0.11
Histidine	−0.02	−0.54	−0.11	0.68*	0.60	0.50	−0.16	0.54	−0.06	0.35	0.09	0.36	0.74*	0.76*	0.73*	0.60	0.20	−0.01	0.39	0.17		−0.36
Arginine	−0.24	0.03	−0.13	−0.23	0.22	0.31	−0.46	0.21	0.43	0.48	−0.05	−0.07	−0.05	0.01	0.04	0.31	0.57	0.43	0.22	0.11	−0.36	

*$P < 0.05$

Table 3. The values of Pearson's correlation coefficient estimated for all the evaluated amino acids in the plasma of 150 day old ram lambs

Amino acid	Cysteic acid	Taurine	Aspartate	Threonine	Serine	Glutamate	Glutamine	Proline	Glycine	Alanine	Citrulline	α-Amino-butyrate	Valine	Isoleucine	Leucine	Tyrosine	Phenylalanine	Tryptophan	Ornithine	Lysine	Histidine	Arginine
Cysteic acid		053	0.25	0.03	0.32	0.34	0.02	0.01	0.09	0.07	0.06	0.10	0.03	0.11	0.06	0.32	0.44	0.06	0.41	0.23	0.08	0.14
Taurine	0.53		0.16	0.27	0.01	0.08	0.42	0.43	0.72*	0.03	0.31	0.54	0.17	0.33	0.02	0.13	0.58*	0.44	0.11	0.01	0.03	0.44
Aspartate	0.25	0.16		0.39	0.01	0.09	0.70*	0.41	0.25	0.71*	0.06	0.01	0.39	0.59*	0.45	0.13	0.45	0.36	0.20	0.60*	0.45	0.14
Threonine	0.03	0.27	0.39		0.34	0.50	0.08	0.51	0.01	0.04	0.56	0.20	0.34	0.08	0.12	0.26	0.15	0.53	0.21	0.11	0.56	0.12
Serine	0.32	0.01	0.01	0.34		0.03	0.15	0.09	0.30	0.38	0.25	0.23	0.29	0.37	0.46	0.26	0.23	0.03	0.12	0.30	0.15	0.24
Glutamate	0.34	0.08	0.09	0.50	0.03		0.22	0.25	0.24	0.32	0.62*	0.03	0.10	0.38	0.29	0.09	0.13	0.48	0.23	0.20	0.11	0.04
Glutamine	0.02	0.42	0.70*	0.08	0.15	0.22		0.02	0.58*	0.57	0.34	0.28	0.46	0.79*	0.50	0.38	0.41	0.06	0.27	0.31	0.04	0.20
Proline	0.01	0.43	0.41	0.51	0.09	0.25	0.02		0.36	0.44	0.53	0.43	0.07	0.02	0.13	0.21	0.24	0.79*	0.01	0.63*	0.55	0.04
Glycine	0.09	0.72*	0.25	0.01	0.30	0.24	0.58*	0.36		0.12	0.43	0.85*	0.42	0.51	0.01	0.24	0.34	0.37	0.01	0.09	0.14	0.22
Alanine	0.07	0.03	0.71*	0.04	0.38	0.32	0.57	0.44	0.12		0.09	0.03	0.32	0.59*	0.37	0.34	0.63*	0.43	0.14	0.59*	0.39	0.30
Citrulline	0.06	0.31	0.06	0.56	0.25	0.62*	0.34	0.53	0.43	0.09		0.14	0.03	0.40	0.34	0.26	0.16	0.60*	0.25	0.04	0.59*	0.42
α-Amino-butyrate	0.10	0.54	0.01	0.20	0.23	0.03	0.28	0.43	0.85*	0.03	0.14		0.47	0.30	0.21	0.01	0.13	0.23	0.07	0.38	0.19	0.26
Valine	0.03	0.17	0.39	0.34	0.29	0.10	0.46	0.07	0.42	0.32	0.03	0.47		0.76*	0.60*	0.30	0.18	0.22	0.54	0.29	0.04	0.28
Isoleucine	0.11	0.33	0.59*	0.08	0.37	0.38	0.79*	0.02	0.51	0.59*	0.40	0.30	0.76*		0.82*	0.10	0.53	0.21	0.25	0.56	0.16	0.09
Leucine	0.06	0.02	0.45	0.12	0.46	0.29	0.50	0.13	0.01	0.37	0.34	0.21	0.60*	0.82*		0.17	0.37	0.21	0.28	0.73*	0.17	0.18
Tyrosine	0.32	0.13	0.13	0.26	0.26	0.09	0.38	0.21	0.24	0.34	0.26	0.01	0.30	0.10	0.17		0.29	0.21	0.09	0.35	0.29	0.07
Phenylalanine	0.44	0.58*	0.45	0.15	0.23	0.13	0.41	0.24	0.34	0.63*	0.16	0.13	0.18	0.53	0.37	0.29		0.12	0.27	0.66*	0.21	0.17
Tryptophan	0.06	0.44	0.36	0.53	0.03	0.48	0.06	0.79*	0.37	0.43	0.60*	0.23	0.22	0.21	0.21	0.21	0.12		0.27	0.22	0.42	0.01
Ornithine	0.41	0.11	0.20	0.21	0.12	0.23	0.27	0.01	0.01	0.14	0.25	0.07	0.54	0.25	0.28	0.09	0.27	0.27		0.10	0.40	0.40
Lysine	0.23	0.01	0.60*	0.11	0.30	0.20	0.31	0.63*	0.09	0.59*	0.04	0.38	0.29	0.56	0.73*	0.35	0.66*	0.22	0.10		0.35	0.04
Histidine	0.08	0.03	0.45	0.56	0.15	0.11	0.04	0.55	0.14	0.39	0.59*	0.19	0.04	0.16	0.17	0.29	0.21	0.42	0.40	0.35		0.54
Arginine	0.14	0.44	0.14	0.12	0.24	0.04	0.20	0.04	0.22	0.30	0.42	0.26	0.28	0.09	0.18	0.07	0.17	0.01	0.40	0.04	0.54	

*$P < 0.05$

systemic growth and development with physiological, metabolic and nutritional factors, both in prenatal and postnatal life (Masters et al. 1993; Blum and Hammon 2000; Kwon et al. 2003; Tatara 2008). However, little is known about physiological changes in plasma free amino acid concentrations as well as their relationships during postnatal life and after the development of the forestomach. This study showed significant changes in plasma concentrations of 12 amino acids in sheep between 21 and 150 day of life. In the case of five essential and five non-essential amino acids, significant decreases in concentration were observed. The highest percentage decreases were found in 150 day old lambs for plasma concentrations of essential amino acids such as arginine (70.2%), tryptophan (61.1%) and threonine (52.1%), while lysine and histidine levels were reduced by 29.7% and 23.3%, respectively. With respect to the non-essential group of amino acids, the highest decreases in plasma concentrations were observed for proline (40.0%), tyrosine (31.6%), citrulline (28.1%), glutamate (27.9%) and serine (21.9%). In contrast to these results, two amino acids – isoleucine and alpha-amino-butyrate increased by 36.9% and 119.6% in the older group of sheep. Thus, it is clear that decreases in plasma amino acid concentrations in 150 day old sheep predominate when compared to amino acid status determined in 21 day old animals that received the maternal milk diet. The observed changes may be determined by various factors among which the most important are the remarkable modification of the diet after weaning, the development of forestomachs and altered microbial colonisation of the gastrointestinal tract and its influence on nutrient digestibility and absorption. It is possible that age-related changes in amino acid concentrations in plasma may be the result of varied metabolic requirements at different stages of systemic development and also may be due to different growth rates (Velle et al. 1997, 1998). The results obtained in this study are in accordance with the arterial plasma amino acid concentrations reported by Prior et al. (1981) where enhanced protein diets in sheep with developed forestomachs led to levels of serine rising by 110% and of glycine by 86%. Furthermore, a change in the diet from alfalfa hay to corn and soybean concentrate from 28 days in those lambs resulted in a significant decrease in the plasma concentration of isoleucine by 39%. It must be underlined here that in contrast to our study where protein content in the diet was reduced between 21 and 150 days of life of sheep, in the studies of Prior et al. (1981) the opposite manipulation of dietary proteins was carried. However, Prior and colleagues did not report significant effects of increased protein content in the diet on amino acid concentrations in the blood of the portal vein (Prior et al. 1981).

Our results also showed interrelationships between the concentrations of several amino acids in the blood plasma of 21 and 150 day old sheep. Except for glutamine in 21 day old sheep which was significantly negatively correlated with serine, glutamate, proline and tyrosine, all the obtained correlations were positive. Obtained results in younger and older groups of lambs showed 47 and 22 significant positive correlations between the evaluated amino acids. Among non-essential amino acids in the plasma of 21 day old animals, tyrosine, glutamate, ornithine, serine and alanine reached the highest number of significant correlations with other evaluated amino acids, while in the case of cysteic acid, taurine and aspartate single correlations were found. Furthermore, citrulline and alpha-amino-butyrate were neither positively nor negatively correlated. Except for arginine in 21 day old lambs, all the evaluated essential amino acids showed significant correlations with the other amino acids in plasma. In the group of the essential amino acids, the highest number of correlations was reached for valine, leucine and isoleucine. Results obtained in 150 day old lambs showed that the highest amount of correlations in the group of non-essential amino acids was reached for aspartate, alanine, glutamine, glycine and citrulline, while no correlations were found for cysteic acid, serine, tyrosine and ornithine. Excluding arginine and threonine, essential amino acids showed numerous positive correlations with the highest number reached for isoleucine, lysine, leucine and phenylalanine. We also found significant positive correlations between plasma concentrations of all branch-chained amino acids (BCAA) in both age groups of animals. Similarly, significant positive correlations of alanine with isoleucine and phenylalanine as well as glutamine with aspartate were found. The obtained results correspond with other studies concerning BCAA (Shimomura et al. 2004). In studies on turkeys and pigs, where dietary alteration of amino acid metabolism was induced, plasma concentrations of valine, leucine and isoleucine were altered analogously in response to the applied nutritional manipulations (Tatara et al. 2006, 2008; Tatara 2009). The balance between BCAA and aromatic amino acids such as phenylalanine and tyrosine is known as Fischer's ratio, and is routinely utilised as an important diagnostic marker for monitoring health status and for studying the progression of liver diseases which influence amino acid metabolism (Noguchi et al. 2008). In this study, positive correlations between aromatic amino acids were also found in 21 day old sheep. Okada et al. (2001) reported that valine, leucine and isoleucine show abnormal profiles in animals and humans suffering from chronic renal failure (Okada et al. 2001). The obtained correlations of BCAA with glutamine or its metabolite glutamate and alanine in 21 and 150 day old sheep are in accordance with the data reported by Holeck (2002) which indicate that BCAA serve as essential donors of nitrogen in the synthesis of both these amino acids. Data reported by Tapiero et al. (2002) also confirm the reversible conversion of glutamine to glutamate as well as its conversion to other amino acids such as proline. This may explain the negative correlations of glutamine with plasma levels of proline and glutamate obtained in this study.

In conclusion, this study reveals physiological and age-dependent changes in amino acid concentrations in ram lambs. Decreases in plasma amino acid concentrations predominate in 150 day old sheep with physiologically developed forestomachs in comparison to these metabolic indices determined in 21 day old animals receiving maternal milk as a basic diet. Thus, the change in diet as the result of the weaning of lambs has long-term consequences for amino acid digestion, absorption, metabolism and plasma concentrations. Our results address the possibility of utilising improved amino acid supply for growing lambs with developed forestomachs. Further exploration of this potential intervention should be the challenge of further studies with this experimental model.

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