

AGE-DEPENDENT CHANGES OF AMINO ACID CONCENTRATION IN THE BLOOD PLASMA OF EARLY-WEANED PIGLETS

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Abstract

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Blood plasma amino acid (AA) concentration was determined in 10 Large White × Landrace piglets weaned on the 1st postnatal day onto a liquid diet prepared from Selasan, a commercial milk feed mixture. Blood samples were obtained at 6, 9, 14, 17, 25 and 25 d of age from the external jugular vein using catheters.

During the period under study the blood plasma concentrations of some AA, namely serine, glycine, alanine, tyrosine and valine, showed a significant rapid decrease and that of glutamic acid, proline and phenylalanine a non-significant decrease, particularly between 9 and 14 d so that their contribution to total amino-plasma, which also decreased with age, was reduced.

The concentrations of threonine, histidine, arginine, leucine, tryptophan and isoleucine, on the other hand, showed a significant increase during the same period, particularly between 9 and 14 d.

No major age-dependent changes were recorded for aspartic acid, cystine and glutamine.

These fluctuations in blood plasma concentration of the individual AA resulted in changes of their mutual ratios. The ratio of branched to aromatic AA (Fischer's index), e. g., increased with advancing age from 1.27 (at 6 d) to 2.97 (at 25 to 26 d), but even so remained relatively low.

Since the environmental rearing conditions and the feeding regimen were the same during the whole 3-week period, the changes in the AA concentrations could result only from internal changes occurring with advancing age in the piglets themselves.

Age, pig, neonatal development, liquid diet, aromatic and branched amino acids

Amino acid (AA) concentrations in the blood plasma of piglets have been studied both at the end of intra-uterine life (Ježková et al. 1974, 1977) and in the first postnatal hours and days (Čuperlovič 1967, Bengtsson 1971, Chavez and Bayley 1977). Consideration has also been given to the effects of nutrition, e. g. AA composition of sows' colostrum (Čuperlovič 1967, Bengtsson 1972). However, data on age-dependent changes in the aminoplasmia of piglets during their following postnatal development have been scarce (Baranyiová and Holub 1989). Consequently, its interaction with various rearing practices and feeding regimens remained undisclosed. The present study was designed to provide further information along this line.

Materials and Methods

Ten Large White × Landrace littermate piglets (6 males and 4 females) were removed from their clinically healthy mother, kept under conventional conditions in a large-scale production unit, at the end of the 1st day after birth and transferred to the laboratory where they were weighed and then reared individually in cages in a thermoneutral environment (Holub 1968; Kotrbáček et al. 1979).

They were fed a commercial milk diet Selasan (Jihočeské mlékárny, závod Jindřichův Hradec; ON 57 0836, 1974) sterilized by radiation (Olbrichová et al. 1984). They were offered the liquid diet (1 part of Selasan per 4 parts of water) for sucking from bottles nine times a day between 6 and 22 h (Holub 1963, 1964ab, 1967; Holub and Komárek 1964; Baranyiová and Holub 1989; Holub and Baranyiová 1989).

Their blood plasma AA concentrations were measured at 6, 9, 14, 17, 25 and 26 d after birth, invariably at 7 h in the morning. The blood samples were obtained by means of catheters (Cavafix, Braun, GFR and Delmed I-Cath, USA) or cannulas (Chiraflex, ČSFR) implanted in the external jugular vein (Nickel et al. 1976), tunnelled under the skin and exteriorized behind the ear. The implantations were performed aseptically under 48 h prior to blood sampling. The catheters were perfused with heparinized saline (10 U · ml⁻¹) once daily.

Heparinized blood samples were centrifuged for 10 min at 12 000 g, frozen immediately afterwards and stored at -20 °C until processing.

The AA content of the diet (Table 1) was determined after acid (Liška 1976, 1980) and alkaline hydrolysis with 4 mol · l⁻¹ NaOH (Šimová 1972) on an AAA 881 analyzer; that of the blood plasma was determined after deproteination with 5% sulphosalicylic acid on a T 339 (Mikrotechna, ČSSR) amino acid analyzer.

The relative growth of the piglets was expressed according to Brody (1945). The significance of the results was assessed by Student's *t*-test and their variability was expressed in terms of standard errors of the means.

Results

The largest contributions to the blood plasma AA concentration of the piglets were by glycine (12.0 to 20.9 %) and alanine (9.1 to 13.5 %) followed by proline, serine, tyrosine, valine and lysine, each of which accounted for more than 5 per cent of the AA concentration (proline, 6.9 to 10.3 %; serine, 7.8 to 9.8 %; tyrosine, 2.8 to 9.3 %; valine, 2.3 to 8.2 %; and lysine 5.1 to 6.0 %). Thus glycine and alanine constituted as much as one third, and together with the other five AA almost two thirds to three quarters, of total aminoplasmia. The contributions by the remaining AA were smaller.

The concentrations of the individual AA changed with age, though not in the same way. The concentrations of serine, glycine, alanine, tyrosine and valine,

Table 1
Amino acid content of the diet

Amino acid	mmol · l ⁻¹	Amino acid	mmol · l ⁻¹
Aspartic acid	27.0	Methionine	8.0
Threonine	15.1	Isoleucine	17.5
Serine	22.1	Leucine	34.3
Glutamic acid	68.9	Tyrosine	11.7
Proline	32.0	Phenylalanine	14.5
Glycine	12.4	Histidine	8.8
Alanine	17.1	Tryptophan	10.1
Cystine	1.2	Lysine	22.6
Valine	22.4	Arginine	8.6

Table 2

Concentrations of individual amino acids in the blood plasma of piglets ($\mu\text{mol} \cdot \text{l}^{-1}$) \pm \pm S.E.M.

Amino acid	Age of piglets - days				
	6	9	14	17	25-26
Taurine	124 \pm 18	45 \pm 4	29 \pm 7	90 \pm 19	191 \pm 14
Phosphoethanolamine	25 \pm 1	—	5 \pm 5	25 \pm 2	—
Aspartic acid	84 \pm 8	104 \pm 11	118 \pm 18	103 \pm 5	84 \pm 9
Threonine	210 \pm 14	246 \pm 30	408 \pm 51	277 \pm 13	258 \pm 34
Serine	604 \pm 44	599 \pm 66	783 \pm 87	604 \pm 27	439 \pm 44
Glutamic acid	212 \pm 30	281 \pm 26	262 \pm 22	209 \pm 29	169 \pm 19
Glutamine	103 \pm 20	117 \pm 4	—	131 \pm 6	108 \pm 8
Proline	617 \pm 54	418 \pm 47	596 \pm 162	553 \pm 36	497 \pm 68
Glycine	1 008 \pm 45	1 272 \pm 80	1 289 \pm 102	1 019 \pm 60	576 \pm 45
Alanine	809 \pm 102	826 \pm 61	772 \pm 127	640 \pm 42	438 \pm 17
Cystine	63 \pm 11	75 \pm 11	—	90 \pm 6	84 \pm 29
Valine	480 \pm 32	400 \pm 46	640 \pm 83	579 \pm 51	112 \pm 22
Methionine	155 \pm 13	104 \pm 19	157 \pm 35	134 \pm 13	100 \pm 23
Isoleucine	221 \pm 14	147 \pm 18	297 \pm 36	229 \pm 30	245 \pm 23
Leucine	209 \pm 21	144 \pm 23	387 \pm 29	251 \pm 31	323 \pm 47
Tyrosine	593 \pm 42	322 \pm 21	387 \pm 59	238 \pm 19	133 \pm 21
Phenylalanine	125 \pm 6	157 \pm 16	170 \pm 15	127 \pm 9	97 \pm 21
Ornithine	154 \pm 16	137 \pm 23	260 \pm 4	170 \pm 17	144 \pm 28
Histidine	117 \pm 8	150 \pm 10	159 \pm 1	121 \pm 8	202 \pm 11
Tryptophan	58 \pm 15	26 \pm 11	100 \pm 25	60 \pm 9	82 \pm 29
Lysine	343 \pm 44	310 \pm 67	547 \pm 26	335 \pm 26	290 \pm 63
Arginine	88 \pm 17	129 \pm 14	213 \pm 31	119 \pm 8	122 \pm 13

e. g., were significantly lower at the end of the experiment than at its start; in the two last-named AA the decrease was by more than three quarters, in the remaining ones by one fifth to a half. This downward trend was most pronounced between 17 and 25 to 26 d and in the case of tyrosine also between 6 and 9 d. The concentrations of some other AA, namely lysine, glutamic acid, proline, methionine and phenylalanine, also declined with age but the decrease was not significant.

Other AA, namely threonine, histidine, arginine, leucine, tryptophan and isoleucine, on the other hand, increased with age. Significant differences between the initial and final concentrations were recorded only for the two first-mentioned AA; in threonine, arginine, leucine, tryptophan and isoleucine the increase occurred between 9 and 14 d. The concentrations of threonine, histidine, arginine and leucine, however, fluctuated temporarily showing a significant fall between 14 and 17 d or between 17 and 25 to 26 d.

No major age-dependent changes were recorded for the remaining two AA, namely aspartic acid and glutamine.

These age-dependent changes also affected the mutual relations between the individual AA and their groups. For example, the ratio of branched AA (leucine + isoleucine + valine) to aromatic AA (phenylalanine + tyrosine) (Fischer's index) was 1.27 in the youngest piglets and then rose with advancing age, amounting to 2.97 at 25 to 26 d.

Discussion

The largest contributions to aminoplasma in newborn piglets have been ascribed to alanine but also to glutamic acid, serine, valine (Čuperlovič 1967) or proline, lysine, leucine (Bengtsson 1971) or also to glycine and aspartic acid (Chavez and Bayley 1977). The differences between the published data are considerable. They can be accounted for by different sites of blood withdrawal: v. jugularis (Čuperlovič 1967), v. umbilicalis (Bengtsson 1971), sinus orbitalis (Chavez and Bayley 1977).

Another possible cause of the differences may be seen in various rearing and feeding practices. At first piglets suck colostrum in which proline accounts for almost one fifth of all AA, and leucine and also alanine, serine, glutamic acid and glycine each account for one tenth of all AA (Bengtsson 1972) so that these six AA make up almost three quarters of its total AA content.

After ingestion of colostrum by newborn piglets their blood plasma concentrations of all AA increase (Čuperlovič 1967), after fasting for 6 to 9 h, on the other hand, the concentrations of all of them, except isoleucine, decrease, with interrelationships being demonstrated between the changes in the concentrations of individual AA; most of them were shown by threonine and leucine (Chavez and Bayley 1977).

Affected by the developmental changes are also the levels of branched (leucine, and valine) and aromatic (tyrosine and phenylalanine) AA, each of the two groups in a different way so that their ratio, Fischer's index, is shifted. As computed by us from the available data, Fischer's index in the plasma of newborn piglets reached 1.40 upon blood withdrawal from the vena jugularis (Čuperlovič 1967); 3.45, from the vena umbilicalis (Bengtsson 1971); and 1.69, from the orbital sinus (Chavez and Bayley 1977). After fasting it increased to 1.55 (Čuperlovič 1967) or to 1.56 (after 6 h) and to 2.77 (after 9 h) (Chavez and Bayley 1977). In 3-day-old piglets it reached 2.92 (Bengtsson 1971).

Piglets in our experiments, however, were fed from the 1st day after birth a diet in which the predominant AA were glutamic acid (accounting for almost 20 %) and leucine and proline (each accounting for about 10 % of all AA). These three AA, together with aspartic acid and serine, made up more than half of the total molar AA concentration. With advancing age the aminoplasma increased to almost three times the original level. In piglets over 20 kg in body mass, i. e. twice to three times older than our experimental animals at the end of the experiment, the increase was still higher and rose further after fasting for 24 and 36 h, amounting to 2.68 and 3.34, respectively (Typpo et al. 1970).

The results of our experiments in piglets in the 2nd to the 4th postnatal week are in keeping with the data reported by other investigators except Bengtsson (1971). They are also in agreement with the data on AA levels in the plasma obtained by blood withdrawal from the vena portae in 8- to 10-day-old piglets suckled by the sows *ad libitum*; however, in the blood plasma obtained by blood withdrawal from the cranial vena cava the AA concentration was about 50 % lower (Shimada and Zimmerman 1973).

The live body mass of the piglets increased as described previously (Baranyiová and Holub 1989).

Also the relative growth of the piglets was in keeping with the observations described in previous reports (Holub 1964ab, 1968; Baranyiová and Holub 1989).

In the early postnatal period the hierarchy of physiological functions in piglets undergoes a change characterized by distinct developmental periods (Holub 1968, 1988) conditioned by a number of both external and internal signals. In consequence, changes also occur in the blood plasma concentration of AA which are not only substrates of the metabolism but also integrative elements (Harper 1983).

In our experiments we studied the aminoplasma in piglets subjected to a brutal intervention — weaning — on the 1st day after birth. Thus they were separated not only from the mother but also from one another. Afterwards the field of their external signals did not change any further. The stereotype of rearing remained the same. Nevertheless, their aminoplasma did undergo a change. Therefore its changes observed between 6 and 25 to 26 d could result only from internal signals. These led, after an about one-week rise in aminoplasma between 14 and to 26 d, not only to its significant decrease (Baranyiová and Holub 1989) but also to a shift in the ratio of individual AA.

Věkové změny koncentrace aminokyselin v krevní plazmě u raně odstavených selat

U 10 selat, kříženců plemene bílého ušlechtilého a landrace, odstavených 1. dne života na tekutou dietu připravovanou z mléčné krmné směsi Selasan, jsme ve věku 6, 9, 14, 17, 25 a 26 dní stanovovali z krvi získavané katetry z v. jugularis externa plazmatickou koncentraci aminokyselin (AMK).

Během sledovaného údobí hladina některých AMK, serinu, glycinu, alaninu, tyrosinu a valinu průkazně, a dalších, kyseliny glutamové, prolinu a fenylalaninu, statisticky neprůkazně, rychle klesala, a to zvláště mezi 9. a 14. dnem, takže jejich podíl na celkové, rovněž se snižující aminoplazmii, se zmenšoval.

Koncentrace dalších AMK, threoninu, histidinu, argininu, leucinu, tryptofanu a isoleucinu, se v téže době naopak průkazně zvyšovala, a to zvláště mezi 9. a 14. dnem.

U kyseliny asparágové, cystinu a glutaminu jsme významné věkové změny koncentrace nezaznamenali.

Toto kolísání plazmatické hladiny jednotlivých AMK vedlo ke změnám jejich vzájemného vztahu. Například, poměr větvených a aromatických AMK (Fischerův index) se s věkem selat postupně zvyšoval z 1,27 (6. d) na 2,97 (25.—26. d); i tak však zůstával relativně nízký.

Protože vnější podmínky odchovu selat, tedy i nutriční režim, zůstávaly neměnné, mohou být ve sledovaném třítydenním údobí popisované změny koncentrace AMK jen výsledkem věkových proměn selat samých.

Возрастные изменения концентрации аминокислот в кровяной плазме у преждевременно отлученных от матки поросят

У 10 поросят, помеси белой породистой породы и ландрас, отлученных от матки на первые сутки жизни и переведенных на жидкую диету, состоящую из молочной кормовой смеси Селасан, в возрасте 6, 9, 14, 17, 25 и 26 суток определяли из крови полученной катетерами из наружной яремной вены плазматическую концентрацию аминокислот (AMK).

V ходе исследуемого периода уровень некоторых АМК, серина, глицина, аланина, тирозина и валина явно, других – глутаминовой кислоты, пролина и фенилаланина статистически невыразительно, быстро понижался, в особенности между 9 и 14 сутками, следовательно, их доля в общей, также понижающейся аминокислоте понижалась.

Концентрация следующих АМК треонина, гистидина, агринина, лейцина, триптофана и изолейцина в то же время наоборот явно увеличивалась, в особенности между 9 и 14 сутками.

У аспарагиновой кислоты, цистина и глутамина существенных возрастных изменений не отмечали.

Данное колебание плазматического уровня отдельных АМК вылилось в изменение их взаимных отношений. Например, соотношение разветвленных и ароматических АМК (индекс Фишера) с возрастом поросят постепенно увеличивалось с 1,27 (6 сутки) до 2,97 (25 – 26 сутки), однако, несмотря на это, оно оставалось относительно низким.

Так как внешние условия содержания поросят и, следовательно, режим питания оставались без изменений, в исследуемый период продолжительностью трех недель могут стать приводимые результаты концентрации АМК лишь результатом возрастных перемен собственно поросят.

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