Acta Veterinaria (Beograd), Vol. 43, No. 4, 219—224, 1993.

UDK 619:612.398.192:636-085.13

THE EFFECTS OF PROTEIN INTAKE ON DIFFERENCES BETWEEN NAA AND EAA IN GUT CONTENT OF RAT JEJUNUM AND ILEUM AFTER SHORT AND LONG TERM DIETARY TREATMENT

SUZIĆ, S.*, RADUNOVIĆ, LJ*, PETROVIĆ, G.** and JANKOVIĆ, V.*

*Institute of Physiology, ** Institute for Medical Research, Medical, Faculty P. Box 783, Belgrade, Yugoslavia

(Received, 3 June 1993)

Young Wistar rats were fed isoenergetic diets with 0.5, 15 and 50% casein ad libitum for 7 and 21 days, respectively. Differences between molar proportions of nonessential (NAA) and essential amino acids (EAA) in jejunal and ileal gut contents under postprandial conditions , were measured. The results showed that the differences between NAA and EAA were more expressed in the ileum than in the jejunum especially after the longer treatment due to the higher values of NAA than EAA, regardless of the protein intake. At the same time, there was a tendecy for the NAA/EAA differences to decrease after the longer treatment, especially in the jejunum. These frindings support the concept of intraluminal homeostasis of amino acids in the jejunum after different protein intakes for longer periods as well as a possible greater accumulation of NAA than EAA in ileal gut content.

Key words: protein, gut, casein, amino acids

INTRODUCTION

During the passage of food through the alimentary tract, considerable quantities of endogenous protein are added in the form of digestive secretions, desquamated mucosal cells, bacterial proteins etc. The precise magnitude of this contribution has not been ascertained (Lewis and Hill, 1983). Nasset (1965), for example, suggested that it was several times greater than protein content of the diet but subsequent data (e. g.Coring, 1975, Silk, 1980. Guilloteau et al., 1986) indicate that the amounts may be considerably smaller. The endogenous protein is itself partly digested and absorbed, and this complicated the assessment of dietary protein effects. Accordingly, ingestion of different quantities of protein results in a mixture of amino acids in the small intestine that comprises unknown proportions of endogenous and exogenous origin with differeces between nonessential amino acids (NAA) and essential amino acids (EAA) which are still not quite defined (Rerat, 1984). With the purpose not to engage in consideration of the origin of the amino acids but changes under different nutritional conditions, we investigated the influence of protein intake

and the duration of dietary treatment (7 or 21 days) on molar differences between the sum of nine NAA and the sum of eight EAA in jejunal and ileal gut content.

MATERIAL AND METHODS

The experiment was performed using male and female 60 day old rats of the Wistar strain weighing 160-180 g. They were housed individually in a room with constant temperature regulation (22-24° C) and fed stock laboratory diet until their body masses had plateaued. After that the animals were divided into four dietary groups offered *ad libitium* isoenergetic diets containing 0%, 5%, 15% (control) or 50% casein protein (groups CHO, CH5, CH15, HP50), for 7 and 21 days respectively. After determined periods the rats were anesthetized with ether between 8 and 9 a. m. (postprandial state). Following laparatomy, the entire small intestine was removed withligatures at the pylorus and ileocecal junction. The excised small intestine was chilled immediately on ice and a ligature was placed to mark its midpoint. The contents of the proximal (jejunum) and distal (ileum) half of the small intestine were washed out with about 15 ml cold isotonic glucose solution and then hydrolized with dilute HCL, as described by Nasset and Ju (1975). Amino acid composition in the final filtrate of gut contents was determinet by ion-exchange chromatography utilizing a 119 CL Beckman Amino Acid Analyser.

One way analysis of variance with the least significance difference test was used for statistical analysis of the data. For the purposes of this presentation, the total content of free amino acids found in the jejunum vs. ileum under the different dietary treatments, was used to compute the molar ratios of individual amino acids (mol/1000) where the sum of NAA plus the sum of EAA equals 1000.

RESULTS

The jejunal and ileal compositions of NAA and EAA (mol/1000) expressed as NAA/EAA diferences are given in tables 1-3. As can be seen, after the short dietary treatment (7 days), there were significant NAA/EAA differences in both parts of the small intestine between of the majority of the dietary groups examined and especially between group HC5 and the other experimental groups of animals (table 1). While these differences between the groups were either maintained or intensified in the contents of the ileum, they completly disappeared in the jejunum after 21 days of the treatment, regardless of the protein intake (table 1).

Date in table 2 showed clearly that amino acid differences were much more expressed in the ileum than in the jejunum after the shorter as well as after the longer dietary treatment, regardless of the protein intake, except in group HC5 where these large differences were maintained in both parts of the small intestine.

The effects of duration of dietary treatment on NAA/EAA differences are presented in table 3. Amino acid differences were maintained from the shorter

to the longer dietary treatment only in the control group of rats (HC15) in both jejunal and ileal content and in group HCO only in the jejunum, respectively. At the same time, with prolongation of the treatments, some groups showed an increase of NAA/EAA differences (group HCO-ileum, group HP50-ileum and jejunum) while the other group (HC5) had the opposite tendency in both halves of the small gut.

TABLE 1. effects of protein intake on the difference between naa and eaa (mol/1000) in gut content (n=7–10).

	NAA/EAA (X+SEM)							
	JEJUNUM				ILEUM			
	1	2	3	4	1	2	3	4
7 DAYS	46±19	12±16	164±29^{O}	54±27^{+*}	146±24	94±21	192±28^{O}	31±23^{+*}
		(p<0.01)				(p<0.01)		
21 DAYS	12±12	20±20	40±22	60±13	124±10	179±23*	96±23^{O}	176±13^{+}
		(NS)				(p<0.01)		

Significantly different from diet 1.*
Significantly different from diet 2.°
Significantly different from diet 3.+

DIETS

1 — HC15 (hypercarbohydrate 15% protein)
2 — HCO (hipercarbohydrate 0% protein)
3 — HC5 (hypercarbohydrate 5% protein)
4 — HP50 (hiperprotein 50%)

DISCUSSION

For the purpose of emphasizing the role of the digestive tract in amino acid homeostasis in postprandial conditions in rats fed high or low protein (casein) diets for 7 or 21 days, respeactively, we analysed the differences between NAA and EAA in the contents of the jejunum vs. ileum (table 1-3). Since most feedstuffs used for monogastric animals are highly digestible, the amounts of amino acids determined analytically are generally fully available (Bondy, 1987). It is desirable that the EAA composition of the diet should approximate the requirements of animals and the ratio of NAA to EAA should be about 1:1. Since the amino acid requirements of growing animals which we used (see Material and Method) are closely related to tissue amino acid composition, the amino acid contents of most animal protein feeds such as casein resemble the amino acid composition required for growth (Edozien and Switzer, 1978, Eggum, 1985), With regard to this, we were interested in whether alterations occured in the amino acid mixture from the proximal to the distal content of the small intestine, during a period of there weeks. Taking into consideration the influence of various endogenous sources which fairly modified the gut content (see Introduction), we expected to find different spectra of amino acid gut

patterns with the different protein diets examined. However, it was found that after 21 days of the treatment the NAA/EAA diferences disappeared completely in the jejunum, regardless of the protein intake (see Table 1). According to Nasset and Ju (1975) a single test meal fed to adequately nourished fasting animals yielded a mixture of amino acids in the gut lumen that was qualitatively relative by constant. These results that a preapsortive step in amino acid homeostasis of jejunal content is attained by hydrolysis of a mixture of proteins of various origins, especially proteins provided by gastric and pancreatic secretions (Nasset, 1974) in the protein deficient groups —HCO, HC5.

TABLE 2. Jejunal/ileal differences off naa and eaa after both dietary treatments

7 DAYS				21 DAYS			
1	2	3	4	1	2	3	4
46/146 ($p<0.01$)	-12/94 ($p<0.01$)	164/192 (NS)	-54/31 ($p<0.01$)	12/124 ($p<0.01$)	20/179 ($p<0.01$)	40/96 (NS)	60/176 ($p<0.05$)

TABLE 3. Effect of duration of dietary treatment on the difference between naa/eaa (mol/1000) in jejunal and ileal gut content

7/21 DAYS							
JEJUNUM				ILEUM			
1	2	3	4	1	2	3	4
46/12 (NS)	-12/20 (NS)	164/40 ($p<0.01$)	-54/60 ($p<0.01$)	146/124 (NS)	94/179 ($p<0.01$)	192/96 ($p<0.01$)	31/176 ($p<0.01$)

At the same time, NAA/EAA differences were expressed in the content of the ileum (table 1 and 2) with significantly higher values of NAA than EAA after both dietary treatments regardless of the protein intake. It is possible that under the condition of complete exogenous protein deficiency for 21 days (HCO group) and/or deficiency for 7 days (HC5), the large availability of carbohydrates in these diets could maintain an adequate of NAA in both halves of the gut (Suzić et al., 1987, 1990) This is probably due to the active metabolic role of the intestinal mucosa in the synthesis of NAA which pass in both directions through the enterocytes (Steven, 1984). At the same time, it is known that some amino acids were absorbed much more rapidly than others from the mixture present during digestion and that the composition of the mixture changed with time and transfer along the gut (Nasset, 1964, Linder, 1985). The amino acid mixture became impoverished in the more rapidly absorbed acids such as EAA approaching the ileum. Conversely, the more slowly absorbed amino acids like some NAA should tend to accumulate. Therefore, the overall accumulation of NAA with simultaneously decreasing molar ratios of EAA in the ileum after both dietary treatmens in all groups (table 2 and 3) could be the result of (i) slower disappearance of NAA than EAA from gut content (ii) accumlation of some NAA (Gly, Pro, Arg etc) of endogenous origin (Holmes, 1974) some of which constitute large fractions of mucoproteins (Bella and Kim, 1972), pancreatic juice (Corring and Jung, 1972) and mucus (Degand et al,

1972), and (iii) the enormous availability of NAA some of which were synthetized de novo by the intestinal mucosa especially in the protein deficent groups (Rerat, 1984, Suzić, 1990). It was found however that the amino acid patterns in portal plasma were characterized by significantly higher molar ratios and/or concentrations of NAA than EAA (Nasset and Ju, 1975, Suzić et al., 1986, 1990).

In conclusion, it is necessary to underline the important contribution of the digestive tract in the maintainence of "intraluminal" homostasis of amino acids in the jejunum after the period of three weeks with simultaneous acumulation of NAA in the distal half of the small intestine. In conseideration of our investigations on the effects of ingestion different quantity of protein on amino acid differences only at the small gut level, further work is required to establish parallel occurrences also at the levels of different vascular beds and tissues.

REFERENCES

1. *Bella, A. and Kim, Y.* 1972. Rat small intestinal mucin: isolation and characterization of a water soluble mucin fraction, Archs. Biochem. *Biophys., 150,* 679.
2. *Bondy, A.* 1987. Proteins and their Metabolism in Monograstric Animals. In: N*utrition, John Wiley and Sons. pp.* 107
3. *Corring, T. and Jung, J.* 1972. The amino acid composition of pig pancreatic juice, *Nutr. Rep. Int., 6,* 187.
4. *Coring, T.* 1975. Apport de proteins d'origine endogene par la secretion du pancreas exocrine chez le porc, Annls. Biol. anim. Biochim. *Biophys., 15,* 115.
5. *Degand et al* 1972. Definition biochemique des sulfomucines et des sialomucines de l'intestin grele du porc, C.r. Seanc. Soc. *Biol., 166,* 622.
6. *Endozien, J. and Switzer, B.* 1978. Influence of diet on growth in the rat, *J. Nutr., 108,* 282.
7. *Eggum, B.* 1985. A study of certain factors influencing protein utilization in rats and pigs, Bertn. *Forsoglasb., 406,* 173.
8. *Guilloteau, P. et al.* 1986. Digestion of milk, fish and soya-bean protein in the preruminant calf: flow of digesta, apparent digestibility at the end of the iluem and amino acid composition of ileal digesta, *Br. J. Nutr., 55,* 571
9. *Holmes, J. et al.* 1974. Digestion of protein in small and large intestine of the pig. *Br. J. Nutr., 32,* 479.
10. *Lewis, D. and Hill, K.* 1983. The provision of nutrients. In: Rook, J. and Thomas. P. Nutritional Physiology of farm animals, Longman, *London and New York pp. 3.*
11. *Linder, M.* 1985. Nutrition and metabolism of proteins. In: Linder, M. Nutritional Biochemistry and Metabolism. Elsevier, New York, Amsterdam, *Oxford, pp. 51*
12. *Nasset, E.* 1965. The role of the digestive system in protein metabolism, Fend. Proc. Fend. Am. Soc. exp. *Biol., 24,* 953.
13. *Nasset, E.* 1972. Amino acid homeostasis in the gut lumen and its nutritional significance, *W. Rev. Nutr. Diet., 14,* 134. (Karger Basel).
14. *Nasset, E. and Ju, J.* 1975. Amino acid in gut contents and blood plasma of rats as affected by dietary amino acid imbalance, *J. Nutr., 105,* 69.
15. *Rerat, A.* 1984. Digestion and transport of dietary components. In: Batt, R. and Lawrence, T. Function and dysfunction of small intestine, Liverpool Univ. Press, pp. 21.
16. *Silk, D.* 1980. Digestion and absorption of dietary protein in man, Proc. *Nutr. Soc. 39,* 61.
17. *Stevens, B. et all.* 1984. Intestinal transport of amino acids and sugars. Advances using membrane vesichles, *Ann. Rev. Physiol., 46,* 417.
18. *Suzić, S. et al.* 1987. Effects of protein-free diet in amino acid homeostasis in rat bllod plasma and gut contents. *Febs. Letts., 216,* 287.

19. *Suzić, S. et al.* 1990. Amino acid (GAS:BCAA) ratios in plasma and gut contents of short-term protein depleted rats. *Physiol. Bohemoslov., 39,* 532.

EFEKAT PROTEINSKOG UNOSA NA RAZLIKE IZMEĐU NEESENCIJALNIH I ESENCIJALNIH AMINOKISELINA (NAK-EAK) U CREVNOM SADRŽAJU JEJUNUMA I ILEUMA PACOVA NAKON KRAĆEG I DUŽEG DIJETETSKOG TRETMANA

SUZIĆ, S., RADUNOVIĆ, LJ., PETROVIĆ, G. i JANKOVIĆ. V.

SADRŽAJ

Mladi pacovi Wistar soja hranjeni su isoenergetskim dijetama sa 0, 5, 15 i 50% kazeina ad libitum u trajanju od 7 i 21 dan. Ispitivane su razlike u molarnim proporcijama između neesencijalnih (NAK) i esencijalnih aminokiselina (EAK) u crevnom sadržaju jejunuma i ileuma u postprandijalnim uslovima. Rezultati su pokazali da su razlike između NAK i EAK više istražene u ileumu nego u jejunumu i to naročito posle dužeg tretmana, zbog viših vrednosti NAK od EAK i to bez obzira na količinu unetih proteina. U isto vreme u jejunumu je postojala tendencija snižavanja razlika NAK/EAK nakon dužeg tretmana. Ovim nalazima može se podržati koncept o intraluminalnoj homeostazi aminokiselina u jejunumu kod različitog proteinskog unosa u uslovima dužeg dijetetskog tretmana kao i moguće veće nagomilavanje NAK u odnosu na EAK u sadržaju ileuma.