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Original article

Portal absorption of ^{15}N and amino nitrogen in the growing pig after ingestion of labelled milk, yogurt or heat-treated yogurt

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Abstract — The aim of this experiment was to study ^{15}N and amino-nitrogen (AN) portal absorption in the growing pig after ingestion of uniformly (0.2509 APE) labelled ^{15}N milk (M), yogurt ingested just after manufacturing (Y0), yogurt stored for 21 d at 4 °C (Y21) and heat-treated yogurt (HY). The highest porto-arterial differences (PAD) in ^{15}N and AN were found in the period between 30 min and 90 min after ingestion. The absorption of nitrogen from M and HY mainly occurred during the 0–120 min time period (about 70% for M and 67% for HY). For Y0 and Y21, a larger displayed absorption period over the 0–240 min time period was observed. Y0 and Y21 presented a quite similar portal absorption profile. The ^{15}N absorption rate was close to 80% for each studied milk product, suggesting that under our experimental conditions, dairy products (M, Y0, Y21 and HY) deliver nearly the same amounts of nitrogen to the organism. AN absorption rates were around 78% with a higher variability between the milk products. These results also indicate that most of the proteins were absorbed within the 240 min postprandial period.

absorption / milk / yogurt / pig

Résumé — Cinétiques d'absorption portale de ^{15}N et d'azote aminé chez le porc en croissance suite à l'ingestion de lait, de yaourt et de yaourt thermisé enrichis en ^{15}N (0,2509 APE). Les différences porto-artérielles de ^{15}N et d'azote aminé (AN) suite à l'ingestion de 1000 mL de lait (L), de yaourt ingéré à l'issue de la fabrication (Y0), de yaourt stocké 21 jours à 4 °C (Y21) et de yaourt thermisé (YT) mettent en évidence des dynamiques distinctes d'absorption de l'azote des produits laitiers étudiés. L'azote du lait et du yaourt thermisé est absorbé principalement durant les 2 premières heures postprandiales (70 % et 67 % respectivement) tandis que l'azote du yaourt (Y0 et Y21) se

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caractérise par une apparition portale plus étalée sur les 3–4 heures qui suivent le repas. Les profils d'absorption portale ont été similaires pour Y0 et Y21. Les taux d'absorption de ^{15}N ont été proches de 80 % pour les quatre produits laitiers, ce qui signifie que dans nos conditions d'étude les produits laitiers (L, Y0, Y21, YT) apportent à l'organisme des quantités équivalentes de ^{15}N. Les taux d'absorption d'AN ont été plus variables entre les produits laitiers avec une moyenne de 78 %. Ces résultats indiquent aussi que la majeure partie des protéines est absorbée dans les quatre premières heures postprandiales.

absorption / lait / yaourt / porc

1. INTRODUCTION

Milk and yogurt constitute a major source of dietary protein. Their nutritional value can be determined either by methods measuring nutrient disappearance in the digestive tract or nutrient uptake by the portal vein. Protein digestion and absorption are well illustrated by amino acid uptake in portal blood [25]. The nutritional value of dietary proteins is linked to subsequent postprandial amino acid availability in the portal blood [25]. Portal absorption of nutrients cannot be studied in humans, but pigs provide a valid model for studying protein digestion in humans [30].

Since stable isotopes are suitable to distinguish the exogenous from the endogenous protein fraction in the intestinal lumen, intrinsic isotopic labelling of milk proteins has been considered as one of the most interesting techniques for nutritional studies [8, 9, 17]. Recently, the use of ^{15}N labelled milk proteins made it possible to distinguish exogenous from endogenous N fractions in the human intestine after ^{15}N milk or ^{15}N yogurt ingestion [8]. These authors pointed out that ^{15}N nitrogen jejunal flow was different for milk and yogurt. It is known that milk proteins and lactose undergo preliminary hydrolysis during lactic fermentation [43]. It is also suggested that lactic fermentation enhances the nutritional value of milk proteins [14, 45].

The present study was designed to examine the postprandial portal absorption of ^{15}N and amino nitrogen in the growing pig after ingestion of milk (M), yogurt ingested just after manufacturing (Y0), yogurt ingested after a storage period of 21 days at 4 °C (Y21) and heat treated yogurt (10 min at 80 °C, HY).

2. MATERIALS AND METHODS

2.1. Preparation of ^{15}N labelled milk, yogurt and heat-treated yogurt

^{15}N labelled cow milk was produced by oral administration of ^{15}N ammonium sulphate in lactating cows [23]. Cows received three successive daily oral doses (300, 150 and 150 g) of $(^{15}NH_4)SO_4$ (10 atom percent isotopic enrichment). The administration of repeated doses of ^{15}N ammonium sulphate resulted in a milk enrichment plateau from 36 h to 84 h (0.2509 APE) after the beginning of the labelled product administration [2]. Milk from this period was collected and dehydrated (Danone, Steenvorde). High ^{15}N incorporation was demonstrated in all casein amino acids [2]. ^{15}N milk (M) was obtained by dilution of 150 g ^{15}N milk powder per litre of water. ^{15}N yogurt was produced using reconstituted ^{15}N milk and specific yogurt starters (Hansen, YB3, 43 °C, 70° dornic acidity). We studied two different yogurts in order to detect a potential effect of storage on the portal absorption of yogurt proteins (Y0: yogurt ingested on the day of manufacturing, and Y21: yogurt stored for 21 d at 4 °C before ingestion). Indeed, during storage at 4 °C, lactic acid

bacteria may pursue their metabolic activities and, particularly, extracellular proteases may hydrolyse milk proteins [13, 20]. Heat-treated ^{15}N yogurt (HY) resulted from fresh yogurt heated at 80 °C for 10 min [42].

2.2. Animals and diets

The animal protocol was in accordance with the French Animal Care Guidelines. Four castrated male Large White pigs (body weight 43–47 kg) from a commercial farm herd (EARL BIMA, 54160 Pulligny, France) were used. For one week before the experiment the animals were kept in our laboratory facilities and received a well balanced diet (800 g per meal) based on wheat and soybean to meet the maintenance and growing needs of animals [12]. Each animal was fitted with two catheters, one of which was placed in the portal vein and one in the brachiocephalic artery [38]. Anaesthesia was induced with sodium thiopentone (10 to 15 $mg \cdot kg^{-1}$) and maintained with fluothane inhalation (0.5 to 1.5% as required). The animals were intubated with a cuffed endotracheal tube; and the lungs were mechanically ventilated at a minute volume of 150 $mL \cdot kg^{-1}$. Surgery was performed under very strict aseptic conditions. The animals began to eat on the day after the operation and rapidly recovered their normal growth rate (400 $g \cdot d^{-1}$). To prevent obstruction by blood clots the cannulae were rinsed daily with a heparinised (100 $IU \cdot mL^{-1}$) NaCl solution (9 $g \cdot L^{-1}$). This was done under aseptic conditions to avoid any risk of infection. The experimental period began when the pigs had completely recovered from surgery (5–6 d). Throughout the experimental period, they were kept in individual cages allowing easy access to the cannulae.

2.3. Experimental measurements

At 10, 15, 20 and 25 d postsurgery, 1000 mL of either M, Y0, Y21 or HY were given to the animals after a fasting period of 12 h. The milk products were given to the animals according to a Latin square design as described in Table I.

Table I. Experimental design.

	Pig 1	Pig 2	Pig 3	Pig 4
Day 10	M	Y0	Y21	HY
Day 15	Y21	M	HY	Y0
Day 20	HY	Y21	Y0	M
Day 25	Y0	HY	M	Y21

For each experimental day, portal and arterial blood samples (10 mL) were collected simultaneously prior to the milk product distribution and at 30, 60, 90, 120, 150, 180, 210 and 240 min after ingestion. Blood was replaced by equal amounts of sterile heparinised NaCl (9 $g \cdot L^{-1}$) solution. The packed cell volume ranged from 27–34% without any apparent influence of the sampling on the relative part of plasma in whole blood.

^{15}N and amino nitrogen (AN) were the two parameters used to assess milk protein absorption. For measurements of ^{15}N and AN in deproteinised blood, 1 mL aliquots of blood were mixed with 3 mL of a solution containing 65 $g \cdot L^{-1}$ sulfosalicylic acid and 6 $g \cdot L^{-1}$ thiodiglycol and centrifuged (3000 *g*, 10 min, 4 °C). This treatment ensured erythrocyte break-up and blood deproteinisation, making both intracellular and extracellular circulating free amino acids available. The deproteinised blood samples were immediately frozen at –80 °C and then stored at –20 °C. AN was determined using the T.N.B.S. colorimetric method [22, 26]. As was demonstrated by these authors, AN is indicative of the free amino acids and small peptides in the blood. ^{15}N enrichments as well as total nitrogen content were determined by IRMS (Delta E Finningan MAT, INRA Champenoux) on aliquots of freeze-dried deproteinised blood samples (Tab. II). All the chemical determinations were performed in duplicate.

Table II. Composition of the studied milk products (n = 4, mean ± standard deviation).

	M	Y0	Y21	HY
^{15}N enrichment (APE)	0.2509	0.2509	0.2509	0.2509
^{15}N content (mg·L^{-1})	4046	4046	4046	4046
Amino nitrogen (mg·L^{-1})	4543 ± 144	4581 ± 136	4691 ± 165	4573 ± 122

2.4. Portal absorption calculations

Postprandial kinetics of each parameter in the portal vein and arterial blood was determined as well as postprandial kinetics of porto-arterial concentration differences. Portal absorption of ^{15}N and AN were calculated as: "porto-arterial differences (PAD) * blood flow". ^{15}N and amino nitrogen meal absorption rate was calculated as: "portal absorption of ^{15}N or AN / ^{15}N or AN content in the meal". Blood flow per min and per kg body weight could be estimated from many references using growing pigs [10, 11, 15, 16, 26–29, 32, 38, 46]. In fact, it is known that meal ingestion is followed by a small rise in portal blood flow during the first 1–2 postprandial hours and individual variations in pig portal blood flow have been established to be between 2.8 and 5.7% [36, 37]. Several authors have found relatively constant blood flow values after ingestion of the meal [10, 11, 15]. It can easily be assumed that portal blood flow variations in the present work were similar for all the animals and that they consequently interfered in the same way for all four dietary products, all the more since the experimental design was a 4 × 4 Latin Square Design. In this study, we calculated portal absorption with a constant blood flow value of 41 mL·min^{-1}·kg^{-1} body weight, which corresponds with the mean value from observations of different authors (Tab. III) and with mean PAD values for M, Y0, Y21 and HY for the 0–240 min studied time period.

Table III. Blood flow references in the growing pig.

Body weight (kg)	Blood flow·min^{-1}·kg^{-1} (mL·min^{-1}·kg^{-1})	Reference
61	39.7	[11]
65	37.6	[10]
8	44.7	[24]
45	41	[27]
45	37.9	[28]
40	49.9	[29]
22	45.2	[32]
64	42	[38]
57	32.8	[40]
Mean value	41.2	

2.5. Statistical analysis

Statistical analysis [41] involved calculating of the mean and standard error as well as analysing the Latin square design. Variance analyses were performed using the SAS statistical software general linear model (GLM) procedure (ANOVA, SAS Institute, Cary, NC). The Student t-test and the Newman-Keul test were used to compare the means at a significant level of 0.05. Data are presented as mean ± standard error.

3. RESULTS

Figure 1 indicates postprandial PAD in ^{15}N in deproteinised portal blood samples after ingestion of 1000 mL M, Y0, Y21 and HY. These ^{15}N PAD kinetics show the

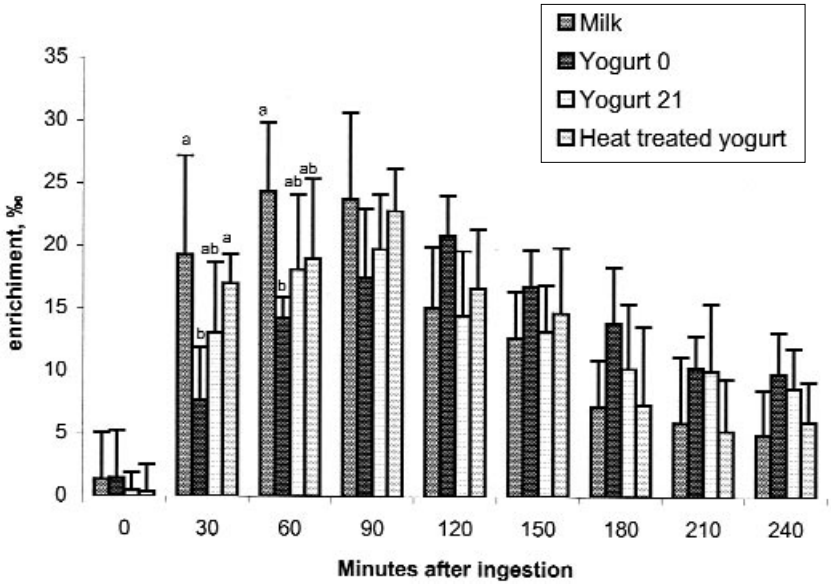

Figure 1. Porto-arterial differences of ^{15}N after ingestion of 1000 mL labelled milk, yogurt 0*, yogurt 21** or heat-treated yogurt in the growing pig (mean + standard error, $n = 4$).
[a, b] At a time point, means with a different letter are signifiantly different ($P < 0.05$).
* Yogurt ingested just after manufacturing.
** Yogurt ingested after a storage period of 21 d at 4 °C.

^{15}N absorption profiles for the four studied milk products. Statistical analysis of the ^{15}N kinetics parameters indicated significant differences between M and Y0 during the first hour (Fig. 1). PAD in milk nitrogen were high very soon after ingestion and decreased after 90 min. For Y0, Y21 and HY ^{15}N, PAD kinetics revealed a later absorption peak (around 90 min). During the 90–240 min period, ^{15}N PAD decreased more slowly with yogurt (Y0 and Y21) than with M or HY, revealing a persistence of ^{15}N absorption for Y0 and Y21.

PAD in AN are presented in Figure 2. Two main steps in the absorption profile of AN can be distinguished: the first 90 min following ingestion and the period after 90 min. For the four milk products, the postprandial PAD of AN rose very rapidly. This high absorption rate already reached 30 min after the start of the oral administration, indicating a very rapid intestinal transit and digestion of milk proteins. The highest porto-arterial differences were observed in the period between 30 min and 90 min (Fig. 2). For the first 60 min following ingestion, no significant differences could be detected between milk, yogurt and heat-treated yogurt. At 90 and 120 min, PAD in AN were significantly higher for Y0 or Y21 than for M or for HY (Fig. 2): PAD generally remain higher for yogurt (Y0 and Y21) during the 120–240 min period.

Figures 1 and 2 thus demonstrate a different digestion and absorption profile of proteins from M, Y0, Y21 and HY. The two studied parameters, ^{15}N and amino nitrogen, indicate a rapid absorption of milk proteins mainly during the 0–120 min period and a larger displayed absorption period for Y0 and Y21. HY digestion and absorption

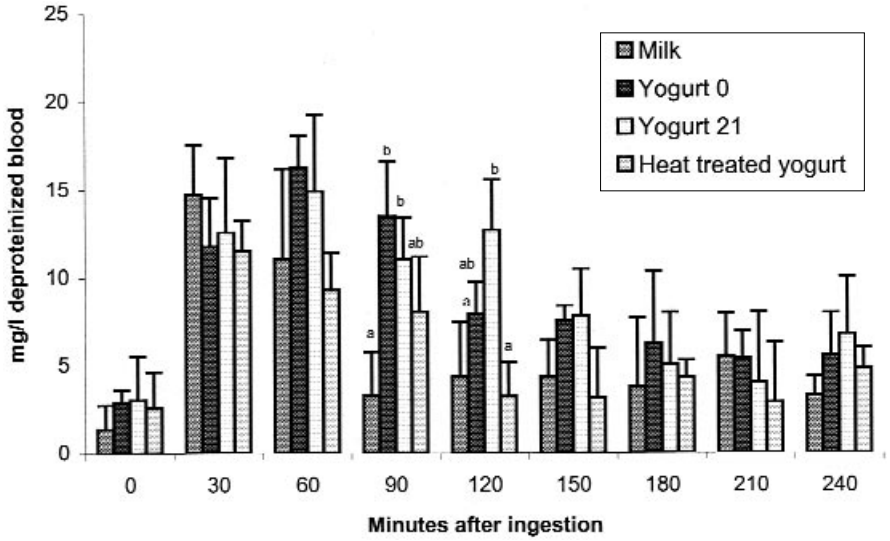

Figure 2. Porto-arterial differences of amino nitrogen after ingestion of 1000 mL milk, yogurt 0*, yogurt 21** or heat-treated yogurt in the growing pig (mean + standard error, $n = 4$).
[a, b] At a time point, means with a different letter are signifiantly different ($P < 0.05$).
* Yogurt ingested just after manufacturing.
** Yogurt ingested after a storage period of 21 d at 4 °C.

behaviour appears to be intermediate between M and Y0 or Y21.

Portal absorption of ^{15}N and AN estimated as "porto-arterial differences * blood flow" as well as ^{15}N and AN absorption rates evaluated as "portal absorption of ^{15}N or AN / ^{15}N or AN content in the meal" are presented in Table IV. According to our calculations, most of the absorption occurred during the 0–240 min time period.

The ^{15}N absorption rate was close to 80% for each studied milk product, suggesting that M, Y0, Y21 and HY delivered nearly the same amounts of ^{15}N to the organism. Although no great differences were observed for the absorption rate of the four milk products, it is interesting to note that within the 0–240 min time period, M and HY had a similar behaviour and so did Y0 and Y21. ^{15}N portal absorption shows that the main absorption of nitrogen from M and HY occurred during the 0–120 min time period (about 70% for M and 67% for HY). For Y0 and Y21, portal absorption of ^{15}N is mostly displayed over the whole 0–240 min studied period.

For amino nitrogen, the absorption rate was between 63% and 89.9% (Tab. IV), with higher values for Y0 and Y21 than for M or HY. When compared to ^{15}N absorption rates, AN absorption rates were quite high for Y0 and Y21 and rather low for M and HY. The lower values found for M and HY are correlated to the low AN PAD for M and HY during the 60–120 min period (Fig. 2) such that global portal absorption of amino nitrogen from M and HY is less than that of Y0 and Y21 (Fig. 2).

Both studied parameters, ^{15}N and AN, demonstrated quite a similar portal absorption profile for Y0 and Y21 (Tab. IV). Thus storage of yogurt at 4 °C did not modify the nutritional characteristics of yogurt.

Table IV. Portal absorption of ^{15}N and amino nitrogen after ingestion of 1000 mL labelled milk, yogurt 0*, yogurt 21** and heat-treated yogurt in the growing pig.

	^{15}N (mg)				Amino nitrogen (mg)			
	M	Y0	Y21	HY	M	Y0	Y21	HY
0–60 min	1305.7	657.4	934.0	1074.5	1484.9	1555.5	1524.7	1213.6
60–120 min	1158.8	1060.7	1020.3	1176.9	591.4	1184.5	1314.6	791.6
120–180 min	593.1	914.8	699.0	657.8	578.3	762.7	711.8	468.6
180–240 min	324.6	600.9	557.7	430.4	487.4	609.5	601.2	428.8
0–240 min (a)	3382.2	3233.9	3210.9	3339.6	3142.0	4112.3	4152.3	2902.7
meal content (b)	4046.0	4046.0	4046.0	4046.0	4543.0	4581.0	4691.0	4573.0
% absorption rate (a/b)	83.6	79.9	79.4	82.5	69.2	89.8	88.5	63.5

M: Milk.
* Y0: Yogurt ingested just after manufacturing.
** Y21: Yogurt ingested after a storage of 21 d at 4 °C.
HY: Heat-treated yogurt.

4. DISCUSSION

The aim of this investigation was to study ^{15}N and AN portal absorption after ingestion of 1000 mL of labelled M, Y0, Y21 and HY. Measuring these events is of great physiological importance since it enables to determine the specific absorption profile of nutrients for the tested milk products. The postprandial PAD of ^{15}N and AN were already high 30 min after the start of milk product ingestion (Figs. 1 and 2). These results can be related to previous observations. A rise in portal plasma amino acids in the guinea pig, beginning 5 or 10 min after the start of a duodenal infusion of casein hydrolysate, has been observed [40]. Some authors [4, 5, 39] observed a net portal appearance of amino nitrogen and amino acids as soon as 30 min after feeding a diet containing casein as the exclusive protein source. Other authors [18] noted that the exogenous nitrogen appeared during the first 20 min after ingestion of casein.

For both studied nutrients and the four milk products, the highest PAD values were found between 30 min and 90 min (Figs. 1 and 2). Recently, the absorption of milk and yogurt was calculated from its net disappearance (ingested minus recovered exogenous nitrogen) in the lumen [7, 8]. These authors particularly noticed that the highest exogenous jejunal nitrogen contents were measured 20 min after milk ingestion and 60 min after yogurt ingestion. Thus they suggested a slower intestinal emptying rate for yogurt. The obtained ^{15}N and AN PAD kinetics in deproteinised portal blood (Figs. 1 and 2) are in agreement with this hypothesis. In fact, the ^{15}N and AN PAD kinetics demonstrate that yogurt nitrogen is differently absorbed than milk nitrogen.

Regarding ^{15}N and AN portal absorption or absorption rate we conclude that they were rather high for each milk product. These results mean that M, Y0, Y21 and HY nitrogen was of high nutritional quality as was also demonstrated with other models by [1, 6, 21, 31, 34, 44, 45]. Furthermore, they also reveal (Tab. IV) that most of the nitrogen was absorbed within the 0–180 min time period. Total portal absorption of ^{15}N as well as global absorption rate (Tab. IV) indicate that no quantitative differences could be found within the four milk products. For AN, absorption rates appeared less homogeneous between the milk products. Absorption rates of M and

HY were particularly low because of the very low AN PAD value for M and HY for the 90–120 min time period (Fig. 2).

Y0 and Y21 presented a quite similar portal absorption profile. This result means that even the lactic acid bacteria exerted a metabolic activity through extracellular proteases during storage at 4 °C [13, 20], the effect of which is too weak to modify nitrogen absorption from yogurt. Proteolytic activity of *L. bulgaricus* during fermentation and storage at 4 °C before ingestion did not change digestion and absorption of yogurt nitrogen.

M and HY portal absorption showed nearly the same nutrient delivery to the organism: the main absorption of nitrogen (about 70%) occurred during the 0–120 min period (Fig. 1). In fact, heat treatment destroys most of the natural body and viscosity of yogurt [42], it can therefore be explained that the absorption profile of M and HY presents many similarities (Figs. 1 and 2, Tab. IV). Although it is known that Maillard reactions during heating may reduce the availability of free amino acids, peptides or proteins [23], 15N and AN portal absorption was in the same range for the two products (Tab. IV).

M and HY are principally absorbed during the first 2 hours after ingestion whereas Y0 and Y21 absorption is mostly displayed over the postprandial period. This result is undoubtedly related to the different intestinal emptying rates between milk, yogurt and heat treated yogurt as has been suggested by several authors [7, 8, 33, 35]. Since the viscosity differences between milk and live yogurt result in a slower gastric emptying rate for yogurt [33], it is understandable that the portal absorption profile of ^{15}N and AN is for live yogurt (Y0 and Y21) when compared to M and HY. Thus each milk product can be characterised by its specific nutritional behaviour.

Regarding parameters ^{15}N and AN, used to assess portal absorption of proteins from milk products, ^{15}N appears a valuable marker since it allows to establish the specific absorption profile of nitrogen from milk and milk products. The main positive aspects relating to AN are the quite easy and cheap analysis for a general assessment of the global amino acids pool in the blood [22]. This AN parameter, and more precisely the T.N.B.S. colorimetric method, however present a variable sensibility for the different amino acids [47] and could explain some of the differences found between M or HY and Y0 or Y21. It would be interesting in further studies to add parameters such as small peptides or amino-acids. In fact, recent studies have demonstrated the high nutritional value of di- and tri-peptides which can be directly absorbed through the intestinal epithelium [3]. Several authors [3, 19] pointed out that small peptides obtained by casein hydrolysis are absorbed and can be detected in plasma. It thus appears interesting to investigate more precisely the relation between proteolysis during fermentation and absorption.

This study demonstrates how valuable is the pig as an animal model to investigate the nutritional behaviour of milk products. The catheters placed in the portal vein and the brachiocephalic artery made it possible to establish and analyse porto-arterial differences in terms of nutrient absorption.

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