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Nitrogen compounds and volatile fatty acids absorption from the caecum and colon of sheep

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ABSTRACT

Four rams provided with a permanently isolated of 250 ml capacity caecum and another 4 with an isolated 1 m long loop colon were used in this study. The isolated caecum was filled and colon perfused with solutions of differing urea concentrations (0.01; 0.015; 0.02%) or VFA and ammonia concentrations (43 and 91 mM or 50 and 100 mM). The caecum pouch was filled with a solution of 0.1, 0.15 or 0.3% enzymatic casein hydrolysate.

Urea was not absorbed from the caecum or colon, regardless of its concentration in the solution. The fractional absorption rate of VFA in the caecum was higher (58%) than in the colon (38%). The rate of absorption of butyric acid was highest, and acetic acid lowest, irrespective of the VFA concentration in the solution. Increasing the VFA concentration administered in solution had a significant effect only on the amount of acetic acid absorbed from the caecum and colon ($P \leqslant 0.01$ and $P \leqslant 0.05$, respectively).

Only the net disappearance of aspartic and glutamic acids, threonine and serine from the enzymatic casein hydrolysate in the caecum was significant, but increasing the concentration of amino acids in the solutions did not have a significant effect on their rate of absorption.

KEY WORDS: sheep, absorption, amino acids, urea, VFA, caecum, colon

INTRODUCTION

There is evidence that substantial quantites of microbial and feed protein are digested in the large intestine and that nitrogen is absorbed predominantly in the form of ammonia (Żebrowska, 1975; Dixon and Nolan, 1982; Schmitz et al., 1991; Bochroder et al., 1994). Some reports show that amino acids can be absorbed from the caecum of pigs and mice (Robinson et al., 1973; Olszewski,

1975; James and Smith, 1976; Jarvis et all., 1977), but data is lacking on the absorption of amino acids from the large intestine of sheep. Volatile fatty acids appear in the large intestine digesta, as the products of carbohydrate fermentation, in proportions similar to those found in the rumen (Ørskov, 1970; Owens and Goetsch, 1988) and 65 to 95% of them are absorbed from the ceacum and colon.

The aim of the present study was to measure the extent of ammonia, urea, amino acids and VFA absorption from the isolated caecum and colon of sheep.

MATERIAL AND METHODS

Animals and feeding

The experiment was carried out on 8 one-year-old rams of about 45 kg body weight. Four of them were provided with permanently isolated caecum pouches and 4 with 1 m long loops of colon which were washed with 0.9% NaCl solutions containing antibiotics as described in previous paper (Skiba et al., 1995). The animals were given diet consisting of 700 g of meadow hay and 400 g of barley with mineral mixture supplement containing (% of DM): OM – 94.7; CP – 11.1; crude fibre – 24.8; ether extract – 2.4 and NFE – 56.4). Daily rations were distributed over two equal meals at 8 and 15 h and given for at least two weeks before the experiment started.

Solutions

The following solutions containing 2 g/l PEG 4000 were used:

- urea solution – 0.01, 0.015 and 0.02%
- VFA mixture (molar proportion of C2 – 60%, C3 – 25% and C4 -15%, partly neutralized with ammonium) contained:
  a. 43 mM VFA and 12 mM ammonia or 91 mM VFA and 16 mM ammonia use to measure their absorption from the caecum pouch
  b. 50 mM VFA and 10.3 mM ammonia or 100 mM VFA and 20.7 mM ammonia, used to measure absorption from the colon loop,
- free amino acids as 0.1, 0.15 and 0.30% casein enzymatic hydrolysates used to measure free amino acids absorption from the caecum pouch.

Experimental design

To study the absorption of nitrogenous compounds and VFA the isolated caecum and colon were filled or perfused with each of experimental solutions. The experiments started after the morning meal and were repeated for 5 days.

Urea, ammonia and VFA absorption was measured in both caecum and colon while the amino acids absorption was estimated only in the caecum.

The isolated pouch of the caecum was filled with 250 ml of experimental solutions and samples of 20 ml were withdrawn at 2 and 4 h afterwards. The isolated colon loop was perfused with experimental solutions by means of a peristaltic pump at a rate of 5 ml/min for 5 h. The perfusate, collected in the bottle kept on ice during the last 4 h of perfusion, was measured and sampled. The perfusion procedure was repeated for 5 days. All samples were stored deep frozen for analysis.

Analysis

Total nitrogen, protein-N, urea-N, ammonia-N and PEG were measured as reported in a previous paper (Skiba et al., 1995). Samples for VFA assay were acidified with 0.1 ml 85% formic acid per 1 ml of sample and kept at -18^0C until analyzed for VFA according Ziołecki and Kwiatkowska (1973) using Philips PU 4410 gas chromatography equipment. Samples for amino acids determination were deproteinized with sulphosalicylic acid and analyzed with amino acid analyzer T 339.

Statistical analysis

Statistical analysis was carried out by single variable variance analysis and of Tuckey test using Statgraphics Software, wersion 7.0.

RESULTS

Urea and ammonia absorption

The results given in Tables 1 and 2 show that there was not net absorption of urea in the caecum and colon. The amount of urea-N estimated in the caecum pouch increased with time independent of the urea concentration in the

TABLE 1

The amount of urea-N (mg/250 ml) introduced into the caecum and found after 2 and 4 h (mean ± SD; n = 4)

Urea-N (mg/250 ml)	Urea concentration in the solution, %, (mmol)		
	0.01% (7.0)	0.015% (10.0)	0.02% (14.0)
Introduced to the caecum	22.2 ± 0.5^{a}	33.9 ± 0.5^{a}	42.9 ± 1.3^{a}
Estimated in the caecum after 2 h	23.1 ± 1.1^{ab}	34.9 ± 1.3^{ab}	44.8 ± 1.5^{ab}
Estimated in the caecum after 4 h	24.2 ± 1.7^{b}	36.1 ± 1.2^{b}	46.0 ± 1.8^{b}

in colums: a, b – $P \leqslant 0.05$

TABLE 2

The amount of urea-N (mg/m/h) introduced and estimated in the liquid leaving the isolated colon (mean ± SD; n = 4)

Hours		Urea concentration in the solution		
		0.01% (7.0 mmol)	0.015% (10.0 mmol)	0.02% (14.0 mmol)
1	introduced	27.8 ± 2.6[a]	38.8 ± 3.1[a]	49.5 ± 4.3[a]
	found	21.9 ± 3.5[b]	26.8 ± 3.6[b]	37.3 ± 6.5[b]
2	introduced	27.5 ± 3.0[a]	38.1 ± 1.1[a]	52.1 ± 4.3[a]
	found	28.1 ± 3.5[a]	39.5 ± 2.0[a]	53.4 ± 4.1[a]
3	introduced	26.8 ± 1.7	37.1 ± 2.4	50.8 ± 4.3
	found	27.1 ± 2.2	37.9 ± 2.4	51.5 ± 4.3
4	introduced	26.2 ± 1.6	39.8 ± 3.2	51.6 ± 4.4
	found	25.6 ± 2.1	40.7 ± 3.2	53.9 ± 5.2
5	introduced	26.8 ± 1.7	37.9 ± 2.4	47.9 ± 2.7
	found	25.8 ± 1.7	36.9 ± 2.5	49.2 ± 4.0
mean[x]	introduced	26.8	38.2	50.6
	found	26.8	38.7	52.0

[x] – mean without 1 h

a, b – $P \leq 0.05$ differences between introduced and found amounts

administered solution ($P < 0.05$ after 4 h) (Table 1). The amount of urea-N introduced into the isolated colon loop was similar to the amount leaving the colon loop (Table 2).

Ammonia was absorbed effectively from the isolated caecum and colon (Table 3). Net absorption of ammonia from the caecum at both concentrations equaled 2.2 mM, but when expressed as a relative percentage of the amount introduced, ammonia absorption was lower at higher concentrations (73 and 55%). The amount of absorbed ammonia in the colon was smaller at lower concentrations (1.74 vs. 4.35, $P < 0.01$) but similar when expressed as a relative percentage of the introduced (14 vs. 18%).

Volatile fatty acid absorption

The amount of total, acetic, propionic and butyric acids absorbed was greater ($P < 0.05$) from the solution with the higher VFA concentrations (Table 3). The absorption rate expressed as a percentage of total and individual acids was higher in the caecum (49-79%) than in the colon (26-56%). The fractional absorption rates of VFA from the caecum and colon were in the following order: butyrate > propionate > acetate. The rate of absorption of these acids from the

TABLE 3

Average amount of VFA and ammonia introduced, found after 4h and absorbed in the caecum pouch and in the colon loop

	Caecum pouch							
	VFA 43 mM; ammonia 12 mM				VFA 91 mM; ammonia 16 mM			
	introduced	found	absorbed	absorption, %	introduced	found	absorbed	absorption, %
VFA	10.8	4.5	6.3^{A}	58	22.8	7.7	15.0^{B}	66
acetate	6.6	3.4	3.3^{A}	49	13.7	5.1	8.6^{B}	62
propionate	2.6	0.8	1.8^{a}	69	5.7	1.6	4.1^{a}	72
butyrate	1.5	0.3	1.2^{a}	79	3.4	1.0	2.4^{a}	70
ammonia	3.0	0.8	2.2^{a}	73	4.0	1.8	2.2^{a}	55
	Colon loop							
	VFA 50 mM; ammonia 10.3 mM				VFA 100 mM; ammonia 20.5 mM			
	introduced	found	absorbed	absorption, %	introduced	found	absorbed	absorption, %
VFA	59.9	37.1	22.8^{a}	38	120.3	85.3	35.1^{b}	29
acetate	36.3	25.5	10.8^{a}	30	74.1	55.2	18.9^{b}	25
propionate	14.9	7.8	7.1^{a}	48	28.7	19.2	9.5^{a}	33
butyrate	8.7	3.8	5.0^{a}	56	17.6	10.9	6.7^{a}	38
ammonia	12.1	10.4	1.7^{A}	14	24.7	20.4	4.4^{B}	18

a, b – $P \leqslant 0.05$; A, B – $P \leqslant 0.01$, differences in absorption of component depending on concentration in introduced solution

TABLE 4

Average amount of amino acids introduced into the caecum with different concentrations of hydrolyzed casein, found after 4 h and percentage of absorption (AA mg/250 ml; n = 4)

Amino acids	Concentration of hydrolyzed casein solution								
	0.1%			0.15%			0.3%		
	introduced, mg	found, mg	absorbed, %	introduced, mg	found, mg	absorbed, %	introduced, mg	found, mg	absorbed, %
Asp	10.7^{a}	8.7^{a}	18.7	16.1^{a}	12.9^{a}	19.9	30.6^{A}	18.3^{B}	40.2
Glu	34.6^{a}	30.6^{a}	11.6	50.9^{A}	36.7^{B}	27.9	98.2^{A}	83.0^{B}	15.5
Thr	6.2^{A}	1.7^{B}	76.6	8.7^{A}	2.9^{B}	66.7	17.2^{A}	8.9^{B}	48.3
Ser	8.2^{A}	2.4^{B}	70.7	12.0^{A}	3.3^{B}	72.5	23.0^{A}	11.1^{B}	51.7

a, b – $P \leqslant 0.05$; A, B – $P \leqslant 0.01$, differences in absorption of amino acid for each concentration in introduced solution

colon was lower than from the caecum, and butyrate and propionate had also higher rates of absorption than acetate.

Amino acid absorption from the caecum

Table 4 shows the amount of some amino acids introduced into the caecum and found after incubation in the caecum pouch. Among 16 amino acids assayed, only Asp (19-40%), Glu (28%), Thr (48-73%) and Ser (30-52%) were absorbed from the isolated caecum in significant after 4h. Changes in the amount of other amino acids were small and insignificant. The concentration of amino acids in the experimental solution had no effect on their absorption rate (Table 4).

DISCUSSION

Results of this study show that urea was not absorbed from the isolated caecum and colon of sheep. Under physiological conditions, urea entering the large intestine is rapidly hydrolyzed to ammonia by bacterial urease, therefore it is possible that ability of the large intestinal epithelium to absorb urea did not develop. Data found in the literature concern only disappearance of urea from the large intestine after its degradation to ammonia (Hogan, 1961; Nolan and Leng, 1972; Dixon and Nolan, 1982).

Net absorption of ammonia from the isolated colon rose with increased ammonia concentration at both concentration indicating that the colon has a high absorptive potential of ammonia. Net absorption of ammonia in the caecum was 2.2 mmol at both concentrations, demonstrating that both already exceeded the absorptive potential of ammonia from the caecum.

The concentration of total VFA and the relationship between proportion of individual VFA in the rumen and large intestine depends on the amount and composition of the diet fed, but mainly on the type and amount of carbohydrate in the diet (Murphy et al., 1982; Sutton, 1985). The molar proportion of acetate in the rumen or large intestine digesta rise with increasing amount of roughages in the diet (Parks et al., 1964) when the total VFA concentration is usually higher as the amount readily fermented carbohydrate.

According to Ørskov et al. (1970) the proportions of individual VFA in the caecum content of sheep are similar with these in the rumen. In our experiments absorption of VFA from the solutions of 43 and 91 mM/l in the caecum or 50 and 100 mM/l in the colon were estimated. The molar proportions of acetic, propionic and butyric acids were 60:25:15, close to the values often met in the rumen of animals fed rations containing high proportion of roughages (Owens and Goetsch, 1980).

Contrary to the results of Masson and Phillipson (1951), and Hogan (1961), in the present study the effect of VFA concentration on individual fractional absorption from the caecum and colon was not clear; only absorption of acetic acid depended significantly on the VFA concentration. These results are in agreement with Dijkstra et al. (1993) who showed that absorption of individual VFA not always corresponds to their rumen concentration.

In the present study the absorption of VFA from the caecum of sheep (58-66%) was lower than values found in rabbits (65-95%) (Leng, 1978). This differences could be caused by differences between species or higher concentration of butyric acid (22%) in the study of Leng (1978) than in our study (15%). The intensity of the acetate (30-25%), propionate (47-33%) and butyrate (57-38%) absorption from the colon decreased with increasing VFA concentration and was less than our values for the caecum and results of Argenzio et al. (1975) obtained for the colon of goats (95% of the acetate and propionate was absorbed).

A coefficient of the relative fractional rate of individual VFA absorption in relation to acetic acid absorption may be expressed more precisely by the method of Weigand et al. (1972) in which the number of mmols of acid absorbed is divided by the number of mmols in the initial fluid and this results is divided by the fractional rate of acetic acid absorption. Thus, the coefficient of the relative rate of absorption for acetic acid by definition is 1. The highest relative fractional absorption rate coefficient in the caecum was for butyric acid, 1.83; for propionate this value was lower – 1.55 and acetic acid was – 1.0. The specific absorption rates of VFA in the colon were lower than in the caecum but of the same order of magnitude. Comparable results were presented by Weigand et al. (1972) in a study on calves; specific absorption rate coefficients for acetate, propionate and butyrate were 1.0; 1.45 and 1.84, respectively. This also corresponds with studies by Kowalczyk et al. (1971), Hoover and Heitmann (1972), Thorlacius and Lodge (1973) and Dijkstra et al. (1993) who studied rates of individual VFA absorption from the rumen suggesting that the processes of VFA absorption from the caecum resemble those in the rumen, but VFA absorption from the colon tended to be lower than in the rumen and caecum.

Amino acid absorption from the caecum has not been studied intensively and data avilable are often divergent. Bochroder et al. (1994) reported that the colon epithelium of the horse is not permeable for histidine, lysine and arginine. Schmitz et al. (1991) found arginine absorption from the caecum of cow, pig and horse below 10%. Olszewski showed that only threonine and serine were absorbed from the amino acid mixture introduced into the isolated caecum pouch of pigs. According to Darragh et al. (1994) free lysine and methionine were not absorbed in nutritionally significant amounts infused into the colon of the 15-32 d old piglets. Binder (1970) and Hoover and Heitmann (1975) found that

alanine and glycine were absorbed from the colon of rabbits but they were not from the colon of sheep. Our experiment demonstrated significant net absorption of some amino acids from enzymatically hydrolyzed casein introduced into the isolated caecum of sheep: threonine (48-73%), serine (30-52%), arginine (7-31%), aspartic acid (19-40%) and glutamic acid (11-28%) while the disappearance of remaining amino acids was insignificant.

Despite of data indicating possibility of transporting some amino acids across the colon epithelium the mechanism of that is unclear. Binder (1970) suggested that alanine and glycine are absorbed through simple diffusion as increasing amino acid concentrations provokes a linear increase of transport across the colon epithelium. However, the results of our experiment indicate that increased amino acid concentration not always caused increased absorption. The presence of glucose (Olszewski, 1975) or sodium and potassium ions (Robinson et al., 1973; Lind et al., 1980; Munck, 1981) were found to play important role in the absorption of some amino acids from the caecum or colon of sheep, dog or chicken. Such data support the active or facilitated character of the absorption process.

The above results suggest that, irrespective of the animal species, some amino acids can be absorbed from the large intestine and that the active absorption may be involved in this process similarly as in the small intestine. This is, however, nutritionally insigificant because of relatively small amounts of free amino acids entering the large intestine and high deaminative activity of bacteria.

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STRESZCZENIE

Wchłanianie związków azotowych i LKT z jelita ślepego i okrężnicy u owiec

Doświadczenia przeprowadzono na 4 tryczkach z wyizolowanym jelitem ślepym z kaniulą oraz na 4 tryczkach z wyizolowaną częścią okrężnicy. Izolowane jelito ślepe wypełniano, a okrężnicę przepłukiwano roztorami mocznika o stężeniu 0,01; 0,15; 0.02% oraz roztworami różniącymi się stężeniem VFA – 43 i 91 mM oraz 50 i 100 mM. Jelito ślepe wypełniano także roztworem hydrolizatu enzymatycznego kazeiny o stężeniach 0,1; 0,15 i 0,3% w celu określenia zdolności jelita ślepego do wchłaniania aminokwasów.

Mocznik w podawanych roztworach, niezależnie od jego stężenia, nie uległ wchłanianiu z jelita ślepego i okrężnicy. Intensywność wchłaniania LKT z izolowanego jelita ślepego była większa (58%) niż z pętli okrężnicy (38%). Tempo wchłaniania kwasu masłowego było największe, a octowego najmniejsze, niezależnie od stężenia LKT w podawanym roztworze. Ilość wchłoniętego z jelita ślepego i okrężnicy kwasu octowego zależała istotnie ($P \leqslant 0,01$ i $P \leqslant 0,05$, odpowiednio) od stężenia LKT w roztworze.

Zmiany w ilości aminokwasów oznaczonych w jelicie ślepym w stosunku do ilości podanej były niewielkie z wyjątkiem kwasu asparaginowego i glutaminowego oraz treoniny i seryny, których ubytek był znaczący. Stężenie aminokwasów w roztworze nie wpływało istotnie na tempo ich wchłaniania.