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The effect of feeding on the hippuric acid content of cow's urine

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Abstract. The urinary hippuric acid contents of dairy cows on a purified, protein-free feed (0-feed, 0-cows), as well as the effect of benzoic acid and aromatic amino acid supplements, and silage and hay supplements, on the urinary hippuric acid content, were followed in the present study. The hippuric acid contents of the urine of 0-cows were compared with those of a cow on low-protein, urea-rich feed containing hemicellulose (ULP-cow), and of normally-fed cows (NorP-cows).

The urinary hippuric acid content of the 0-cows varied between 0.1 and 0.6 g/l ($n = 8$). The proportion of hippuric acid nitrogen of the total urinary nitrogen was 0.2—0.9 %. The presence of hippuric acid in the urine of the 0-cows indicates an endogenic hippuric acid production within the cow. 0-feed is deficient in all exogenic precursors of benzoic acid. Addition of benzoic acid to the feed of 0-cow caused temporary rise in the hippuric acid contents of the urine, of aromatic amino acids the addition of tyrosine, besides benzoic acid, raised the urinary hippuric acid content by a highly significant amount.

The urinary hippuric acid content of the ULP-cow was on average 6.7 g/l ($n = 42$) and the proportion of hippuric acid on the total urinary nitrogen 5.2 %.

The urinary hippuric acid contents of the NorP-cows were on average 11.9 g/l ($n = 14$), and the proportion of hippuric acid nitrogen of the total urinary nitrogen 10.7 %. The difference in the urinary hippuric acid contents with 0- and NorP-cows is significant, as is also the difference in the proportions of hippuric acid nitrogen of the total urinary nitrogen with these cows.

The difference in the hippuric acid contents of 0- and ULP-cows is also significant ($P < 0.01$). The same regards ULP- and NorP-cows ($P < 0.01$).

Introduction

In the urine of herbivorous animals, particularly ruminants, hippuric acid, a metabolic product of benzoic acid and its derivatives, is excreted. Only very little information is available about the contribution of the different feed components to the amount of hippuric acid excreted in the urine, though the effect of fibre, carbohydrates and proteins has been studied to some extent (Nehring et al 1965, Martin 1969 a, b, 1970, 1973, 1975, Scott et al. 1964, Schiemann et al. 1965, Ely at al. 1953). The results, obtained mainly in experiments performed with sheep, are contradictory.

In purified, protein-free feed (Virtanen 1966, 1971) there are no benzoic acid precursors, i.e. exogenic benzoic acid sources. This makes it possible to demonstrate any endogenic hippuric acid production by the dairy cow. The benzoic acid metabolism of the 0-cow which had been on a long-term purified protein-free feed differs from that of the ULP-cow, that is one on low-protein, urea-rich feed (Kreula et al. 1978).

In the present study the hippuric acid excretion by a 0-cow is compared with the corresponding excretion by a ULP-cow and normally-fed cows. Also the effect of a range of benzoic acid and aromatic amino acid feed supplements, as well as the effect of silage, on the excretion of hippuric acid are reported.

Materials and methods

Test cows and their feeding

The studies reported in this article were performed with 0-cows Oona, Voona and Tila, and with ULP-cow Euru. The feeding of these cows has been reported in detail in previous publications (Virtanen 1966, Virtanen et al. 1971, Ettala and Kreula 1976 a). For comparison, the urine of cows on normal feed (NorP-cows) was also studied. Of these cows 11 were on pasture feed and 3 on indoor feed. All were milk-producing cows.

Feed supplements

57 urine samples were taken from 0-cow Oona during a period of six months. The following feed supplements were used during this time: benzoic acid (E. Merck AG p.a.), starting at 2 g/day and being raised to 10 g over a period of a month, and continuing at 10 g for five months, 3—10 g/day L-tyrosine (E. Merck AG, f.b. Zw. and Fluka, puriss), L-tryptophan (Fluka, puriss) and DL-phenylalanine (E. Merck AG, f.b. Zw.). At their highest the daily supplements were 10 g benzoic acid, 10 g tyrosine, 3 g tryptophan and 10 g phenylalanine (Table 1).

Urine samples

2 samples were taken from the urine of 0-cow Voona. From the urine of 0-cow Tila 2 samples were taken when the cow was on 0-feed, and a daily sample for 15 days when 30 % of the 0-feed was replaced by silage and hay (5 kg silage/day + 1 kg hay). 42 samples were taken from the urine of ULP-cow Euru during a period of 11 months.

14 urine samples were taken from the NorP-cows.

All urine samples were collected in the morning, stored in 200 ml polyethylene flasks at + 2 — + 4° C overnight and analysed the following day.

Methods of analysis

Hippuric acid contents were determined in the urine by the paper chromatographic method of Gaffney et al. (1954) modified by Nehring and Zelck (1965). The absorbance of the methanol eluates was measured with a Perkin-Elmer UV-Vis 139 spectrophotometer at 460 nm. On each sample

Table 1. Urinary hippuric acid content of 0-cow Oona fed purified, protein-free feed during supplementation with benzoic acid and aromatic amino acids.

Period	Number of samples	Supplements in the feed, g/day: Benzoic acid	Tyrosine	Tryptophan	Phenyl-alanine	Hippuric acid g/l urine: Mean	Standard deviation
13. 06 — 26. 06	4					0.5[1]	0.1
27. 06 — 04. 07	8	2—6				0.5	0.2
05. 07 — 20. 07	11	7				1.0[2]	0.4
25. 07 — 05. 08	5	8—10				0.7	0.5
05. 08 — 10. 08	1	10	0— 2			0.1	0
15. 08 — 19. 08	3	10	6—10			1.7[3]	0.2
20. 08 — 03. 09	8	10	10	3		0.6	0.3
08. 09 — 03. 10	10	10	10	3	1—10	0.5	0.3
04. 11 — 30. 11	7	10				0.4	0.2
13. 06 — 30. 11	57					0.7	0.4

1) Normal level of hippuric acid excretion from the 0-cow.

2) Almost significant rise from the normal level.

3) Highly significant rise from the normal level.

2—7 parallel determinations were made. The relative deviation of the analytical method, on the basis of 10 parallel analyses, was 5.6 %, when the sample contained 0.5 g/l hippuric acid.

Total nitrogen determinations were also performed on the urine samples (Ettala and Kreula 1976 b).

Urinary hippuric acid contents of the different cow groups were tested with the Mann-Whitney test (Conover 1971).

Results and discussion

The hippuric acid content of the urine of test cows on purified, protein-free feed, i.e. 0-feed, varied between 0.1 and 0.6 g/l. The mean of the hippuric acid contents of the urine samples of ULP-cow Euru, which had received low-protein, urea-rich feed, was 6.7 g/l (range 1.1—12.8 g/l), and of a NorP-cow 11.9 g/l, range 4.8—23.5 g/l (Table 2). The urinary hippuric acid contents in NorP-cows on pasture and indoor feeding may also differ. Owing to the small number of samples it is not possible to make further conclusions. The variation in the contents of hippuric acid in the urine samples of the NorP-cows was much greater than that obtained by Nehring et al. (1965) in the urine of young bulls. Here the proportion of the urinary hippuric acid nitrogen of the total urinary nitrogen was consistently 4—6 %. The range in the NorP-cows was 4.1—23.7 %.

The difference in hippuric acid levels between 0- and NorP-cows is significant ($P < 0.01$), as is also the difference in the proportions of hippuric acid nitrogen of the total urinary nitrogen ($P < 0.01$) with these cows. The urinary hippuric acid contents with 0-cows can be regarded as indicative of a production of endogenic hippuric acid in the cow, as all benzoic acid precursors are absent from the feed.

Table 2. Average hippuric acid contents of the urine of 0-, ULP- and NorP-cows.

Animal	Number of samples	Feed	Hippuric acid g/l Mean	Standard deviation	Hippuric acid N % of total urinary N Mean	Standard deviation
Oona	4	0-feed	0.5	0.1	0.9	0.2
Voona	2	0-feed	0.1		0.2	
Tila	2	0.-feed	0.2		0.3	
Tila	15	70 % 0-feed 30 % silage + hay	3.2	1.9		
Euru	42	ULP-feed	6.7	2.4	5.2	1.9
NorP-cows	11 3	pasture indoor	$11.9 \begin{cases} 10.5 \\ 17.4 \end{cases}$	$6.1 \begin{cases} 4.4 \\ 9.4 \end{cases}$	$10.7 \begin{cases} 8.9 \\ 17.5 \end{cases}$	$6.2 \begin{cases} 3.7 \\ 9.4 \end{cases}$

When 2—6 g benzoic acid daily was added to the feed of 0-cow Oona, no rise was observed in its urinary hippuric acid contents (Table 1). When the supplement was increased to 7 g per day, an almost significant rise was observed. If the amount of urine produced by 0-cow Oona is taken as 15 kg per day (Ettala and Kreula 1976 b), it follows that the benzoic acid fed was excreted quantitatively. No statistically significant rise was observed when the supplement of benzoic acid was raised to 8—10 g.

When 2 g tyrosine was first fed in addition to benzoic acid the excretion of hippuric acid was at its lowest. The benzoic acid given on the same day as the first tyrosine supplement was not excreted in the urine. When the tyrosine supplement was 6—10 g/day, the benzoic acid supplement being 10 g/l, the hippuric acid content rose during a period of one week. After this the hippuric acid content dropped to the normal level, and the continuing addition of aromatic amino acids to the feed no longer affected the urinary hippuric acid contents (Table 1). The addition of tyrosine to the feed raised also the tyrosine content of the blood plasma. The addition of the aromatic amino acids of milk did not have a corresponding effect (unpublished data).

On the basis of the above results and those obtained in this laboratory earlier with ^{14}C-labelled benzoic acid it is evident that the excretion of benzoic acid in the urine of the 0-cow is retarded. It is possible that benzoic acid is metabolised in the 0-cow to other compounds besides hippuric acid. This suggestion is supported by a definite adaptation effect when benzoic acid is fed. In the ^{14}C feeding experiment it was observed that of the activity in the milk (0.5 % of the amount fed) more than half was in the soluble fraction and the rest in the milk protein. With the ULP-cow also, the only milk component which was labelled was the protein (Kreula et al. 1978). The rumen microorganisms may convert benzoic acid to 3-phenyl or 4-hydroxy phenyl fatty acids, and in this way benzoic acid can be utilised for the biosynthesis of the carbon skeleton of aromatic amino acids (Kristensen 1974). It is also possible that some rumen bacteria decompose it (Dagley and Patel 1957).

When 30 % of the feed of 0-cow Tila was replaced by silage and hay, the urinary hippuric acid content rose from 0.2 g to 3.2 g per litre and the rise was permanent (Table 2). This shows that the 0-cow is capable of a normal conjugation of glycine and benzoic acid. Whether this is due to the plant-derived precursors of benzoic acid or to other components of grass fodder remains an unanswered question.

The low hippuric acid content in the urine of the 0-cows is a clear indication of the small amounts of aromatic amino acids and other aromatic compounds in these cows as compared with cows on ULP- and NorP-feeds; this scantiness is not made good solely by the addition of benzoic acid and aromatic amino acids to the feed.

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SELOSTUS

Ruokinnan vaikutuksesta lehmän virtsan hippuurihappopitoisuuteen

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Proteiinittomilla, puhdistetuilla rehuilla ruokituilla lypsylehmillä (0-ruokinta, 0-lehmät) on tutkittu virtsan hippuurihappopitoisuutta sekä rehuihin lisättyjen nousevien bentsoehappo- ja aromaattisten aminohappolisäyksien sekä säilörehu- ja heinälisäyksien vaikutusta virtsan hippuurihappopitoisuuteen. 0-lehmien virtsan hippuurihappopitoisuuksia on verrattu vähän proteiinia ja runsaasti ureaa tavanomaisten rehujen ja hemiselluloosan ohella saaneiden lehmien (ULP-ruokinta, ULP-lehmät) sekä normaalisti ruokittujen lehmien (NorP-lehmät) virtsan hippuurihappopitoisuuksiin.

0-lehmän virtsan hippuurihappopitoisuus vaihtelee 0,1—0,6 g/l (n = 8). Hippuurihappotypen osuus virtsan kokonaistypestä on ollut 0,2—0,9 %. 0-lehmien virtsan hippuurihappopitoisuuksien voidaan katsoa vastaavan endogeenista hippuurihappotuotantoa lehmällä. 0-ruokinnasta puuttuvat kaikki eksogeeniset bentsoehappoprekursorit. Bentsoehapon lisäys 0-lehmän rehuihin on aiheuttanut tilapäistä nousua virtsan hippuurihappopitoisuuksissa. Aromaattisista aminohapoista tyrosiinin lisäys bentsoehapon ohella on nostanut virtsan hippuurihappopitoisuutta erittäin merkitsevästi usean päivän ajaksi.

ULP-lehmän virtsan hippuurihappopitoisuus on ollut keskimäärin 6,7 g /l (n = 42) ja hippuurihappotypen osuus virtsan kokonaistypestä 5,2 %.

NorP-lehmien virtsan hippuurihappopitoisuudet ovat olleet keskimäärin 11,9 g/l (n = 14), hippuurihappotypen osuus virtsan kokonaistypestä vastaavasti 10,7 %.

Ero 0-lehmien ja NorP-lehmien virtsojen hippuurihappopitoisuuksissa on ollut merkitsevä, niin kuin ero ko. lehmien hippuurihappotypen osuuksissa virtsan kokonaistypestä. Ero 0- ja ULP-lehmien hippuurihappopitoisuuksissa on myös merkitsevä ($P < 0,01$), samoin vastaavasti ero ULP- ja NorP-lehmien välillä ($P < 0,01$).