Effects of humine compounds on iodine utilisation and retention and on the function of the thyroid gland

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ABSTRACT: Effects of sodium humate (HuNa), containing 61.9% of humic acids in dry matter, on utilisation and retention of dietary iodine were investigated in balance experiments carried out in pigs. A control group was fed a commercial diet containing 0.1 mg of iodine per kg. The daily intake of iodine was 129.2 µg per animal or 4.08 µg per kg live weight. The recommended intake of dietary iodine is 0.25 to 0.30 mg per kg at 88% dry matter. The experimental group (HuNa) was fed the same diet supplemented with 3% of sodium humate which contained 0.5 mg of iodine per kg at 90.04% dry matter. The supplementation increased the daily intake to 141.4 µg per animal, or 4.45 µg per kg live weight. The supplementation of HuNa increased significantly the amount of iodine excreted in faeces (53.2 ± 11.0 vs. 43.0 ± 6.47 µg per animal per day; $P < 0.05$) and nonsignificantly the urinary iodine excretion (31.8 ± 8.93 vs. 29.0 ± 11.3 µg per animal per day). Differences between the HuNa and the control groups in blood serum iodine concentrations (18.8 vs. 18.1 µg per litre) and urinary iodine concentrations (32.5 vs. 34.4 µg per litre) were nonsignificant. It is evident from the concentrations that the pigs suffered from a serious iodine deficiency. Compared with controls, the HuNa group showed significantly higher triiodothyronine concentration ($P < 0.05$) and nonsignificantly lower thyroxine and iodine concentrations in blood serum. Dietary iodine utilisation was lower in the HuNa than in the control group (62.3 vs. 66.6%). The results show that utilisation of dietary iodine was limited and that sodium humate (humic acids) reduced iodine utilisation and retention and affected also other parameters indicating their goitrogenic effects.

Keywords: swine; humine acids; triiodothyronine; thyroxine; urine; faeces

INTRODUCTION

Humines rank with the most widely occurring organic substances to be found in soil and drinking and sea water. They are products of chemical and biological degradation of dead plant and animal tissues and of microbial activity and bind with metals and inorganic and organic substances including toxic pollutants to form chelates (Huang *et al*., 1994; Schulten, 1994). Oral intake of humines can result in the formation of metaloorganic complexes which affect the absorption of minerals and trace elements from the gastrointestinal tract (Spencer and Nichols, 1983; Livens, 1991; Lind and Glynn, 1999).

Reduction, oxidation, or microbial degradation of humines give rise to resorcinol and other phenol 7-carboxyl compounds, which, along with other degradation products, such as orcinol, floroglucinol, pyrogallol, and 3,4- and/or 3,5-dihydroxybenzoic acids, can play important roles in the aetiology of endemic goitre. Goitrogenicity of resorcinol as a drinking water contaminant has been demonstrated by Cooksey *et al*. (1985).

Humines inhibit thyroid peroxidase and are regarded as natural goitrogens (Delange, 1988; Huang and Fung, 1991). Higher prevalence of goitre has been recorded in areas with increased humine concentrations in surface water and are as rich in peat (Chang *et al*., 1991; Huang *et al*., 1994; Seffner, 1995; 1996; Cher *et al*., 1995). Goitre is often observed in some areas of the USA that are rich in coal (Cooksey *et al*., 1985) in spite of iodine supplementation. Humine acids effectively bind iodine and can reduce its biological availability (Huang *et al*., 1994) and inhibit hepatic T_4 5-monodeiodinase; humic and fulvo acids are known to inhibit oxidoreductase. Diets with a low iodine content supplemented with humines induced a significant decrease of T_4 and a significant increase of T_3 in blood serum of rats (Huang *et al*., 1994). Humines alone did not induce goitre, but could have increased goitrogenic effects of low-iodine diets. No increase in thyroid gland weight was observed after oral intake of humines.

Increased faecal secretion of iodine was observed by Herzig *et al*. (2000) in balance experiments carried out in pigs receiving iodine humate. Iodine utilisation was sig-

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nificantly lower (67.9%) than in pigs fed diets supplemented with potassium iodide, or ethylenediamine dihydroiodide. This difference may have been due to a poorer release of iodine from the carrier humines resulting in a lower absorption rate.

The objective of our balance experiments, in which the effects of HuNa or humic acid supplementation on dietary iodine utilisation and retention were investigated, was to test antithyroid effects of humines.

MATERIAL AND METHODS

The experiment was carried out on eight Large White × Landrace crosses with initial live weights of 31.6 ± 4.79 kg (V = 15.2%) in the control group and 31.8 ± 4.53 kg (V = 14.2%) in the experimental group.

Control pigs were fed a diet prepared according to our own formula containing an iodine-free mineral supplement (Table 1). Experimental pigs were fed the same diet enriched with 3% of HuNa. The pigs were fed twice a day (07.00 a.m. and 04.00 p.m.) and the amounts of consumed and nonconsumed feeds were recorded. Both the feed and HuNa were analysed for the contents of nutrients, energy (Czech Standard ČSN 46 7092, 1986; Notice No. 222 of the Ministry of Agriculture of Czech Republic, 1996) and iodine. The results are shown in Table 2. The content of humic acids in HuNa was 61.9% in dry matter.

The pigs were kept in metabolic cages which allowed weighing of consumed and nonconsumed feeds and separate collection of urine and faeces from the individual animals. The daily output of faeces and urine was stored in dark ground-neck bottles at 12°C. The amount was measured and 10% portions were collected for analyses after 24 h. The length of the balance experiment was 5 days.

Table 1. Composition of feed mixture

Components	%
Wheat	69.3
Barley	20.0
Extracted soy meal	10.0
Dicalcium phosphate	0.5
Salt – iodine-free	0.2

The experiment was carried out in facilities of the Veterinary Research Institute. All principles regulating the design and execution of balance experiments (Notice No. 194 of the Ministry of Agriculture of the Czech Republic, 1996) were observed. The experiment was biphasic with a treatment change after a 15-day period as shown in the following scheme.

Pigs Nos.	1–4	5–8
Experiment A Preparatory period 10 days	HuNa	0
Balance period 5 days	HuNa	0
Experiment B Preparatory period 10 days	0	HuNa
Balance period 5 days	0	HuNa

0 = controls; HuNa = 3% of sodium humate in the diet

Iodine concentrations in blood serum, urine, faeces and feeds were determined after alkaline ashing spectrophotometrically according to Sandell-Kolthoff (Bednář *et al*., 1964). The principle of the method consists in reduction of Ce^{4+} to Ce^{3+} in the presence of As^{3+} and catalytic action of iodine. The samples were processed by dry alkaline ashing at 600°C. This method determines both inorganic and protein-bound iodine. The results of the analyses were used for the calculation of iodine balance. Triiodothyronine (T_3) and thyroxine (T_4) concentrations were determined by radioimmunoanalysis (Humalab, Košice, Slovak Republic). The results were processed using the statistic and graphic software STAT Plus (Matoušková *et al*., 1992).

RESULTS AND DISCUSSION

The only source of dietary iodine were components of the feed mixture used in the experiment (Table 1), which contained 0.1 mg of iodine per 1 kg. The daily intake of

Table 2. Contents of nutrients, metabolisable energy and iodine per 1 kg of feed and sodium humate

		Feed		Sodium humate	
		original dry matter	absolute dry matter	original dry matter	absolute dry matter
Dry matter	g	877.0	1000.0	900.4	1000.0
Crude protein	g	183.2	208.9	63.9	71.0
Fat	g	15.7	17.9	3.4	3.8
Fibre	g	25.7	29.3	6.2	6.9
Ash	g	29.9	34.1	338.5	375.9
NFE	g	622.5	709.8	488.4	542.4
Organic matter	g	847.1	965.9	561.9	624.1
TDN	g	772.2	880.5	–	–
MEp	MJ	13.57	15.47	–	–
Iodine	mg	0.100	0.114	0.500	0.555

iodine in the control group was 129.2 µg per animal or 4.08 µg per 1 kg live weight. Experimental pigs were fed the same diet supplemented with 3% of sodium humate containing 0.5 mg of iodine per 1 kg at 90.04% of dry matter (Table 2). Thus the daily intake of iodine in the HuNa group was increased by 4.45 µg per 1 kg live weight to 141.4 µg per animal. The difference in iodine intake in the HuNa group was due to differences in feed consumption and in iodine content in HuNa. The necessary concentrations of iodine in feed mixtures for standard swine breeds are estimated to 0.30 mg per kg and 0.25 mg per kg for the starter period and the first phase of the fattening period, respectively.

Feeding of the diet supplemented with 3% of HuNa increased significantly the amount of iodine excreted in faeces (53.2 ± 11.0 vs. 43.0 ± 6.47 µg per animal per day; $P < 0.05$). This difference was not due to the higher intake of iodine only. While the intake increased by 9.4%. the excretion was higher by 23.7%.

The increase in urinary excretion of iodine (Table 3) in the HuNa group was nonsignificant (31.8 ± 8.93 vs. 29.0 ± 11.3 µg per animal per day). A similar relation was observed after conversion to 1 litre volume (34.4 vs. 32.5 µg per animal per day). No significant difference between the HuNa and the control group (18.1 ± 5.74 vs. 18.8 ± 6.0 µg per litre) was observed in iodine blood serum concentrations either (Figure 1). The blood serum and urinary concentrations of iodine were indicative of a very low intake; in terms of the ICCIDD system, the animals suffered from a serious iodine deficiency.

Dietary iodine utilisation in the HuNa group was lower than in the control group by 4.3% (62.3% vs. 66.6%); the difference was nonsignificant (Table 3). These data contradict the assumption of Anke *et al.*

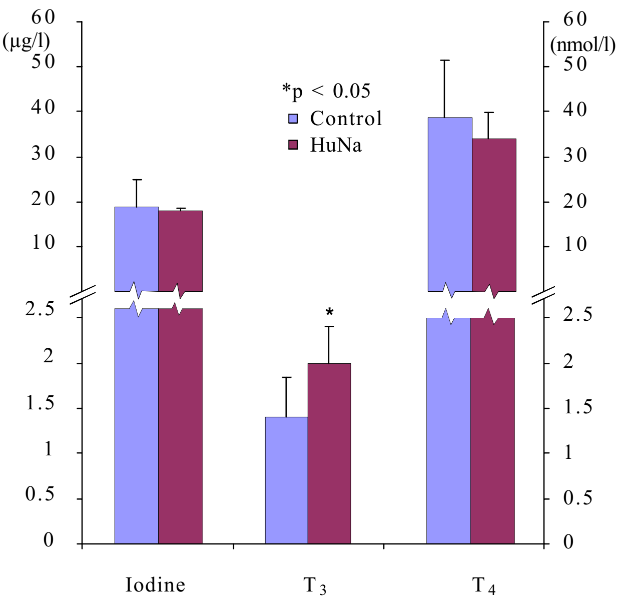

Figure 1. Triiodothyronin, thyroxin and iodine in blood serum concentrations of pigs

Table 3. Iodine balance

Group	Control	HuNa
n	8	8
Iodine intake (µg/animal/day)		
Mean	129.2	141.4
± *s*	13.1	20.1
V (%)	10.1	14.2
Faecal excretion (µg/animal/day)		
Mean	43.0	53.2*
± *s*	6.47	11.0
V (%)	15.0	20.7
Urinary excretion (µg/animal/day)		
Mean	29.0	31.8
± *s*	11.3	8.93
V (%)	39.1	28.1
Retained from intake (%)		
Mean	44.3	39.6
± *s*	9.80	7.40
V (%)	22.1	18.7
Retained from absorbed (%)		
Mean	66.3	63.5
± *s*	12.3	9.51
V (%)	18.5	15.0
Utilisation rate (%)		
Mean	66.6	62.3
± *s*	4.82	5.73
V (%)	7.24	9.20

$^*P < 0.05$

(1993) that iodine contained in plant feeds is absorbed almost completely. Received iodine retention rates in the HuNa and the control groups were 39.6% and 44.3%, respectively. The corresponding values for absorbed iodine retention were 63.5% for the HuNa and 66.3% for the control group.

In addition to iodine utilisation and retention, sodium humate, and humic acids, were found to influence also other indexes implying goitrogenic activity. The HuNa group showed significantly higher concentration of triiodothyronine (2.00 vs. 1.41 nmol/l; $P < 0.05$) and nonsignificantly lower thyroxine and iodine concentrations in blood serum (Figure 1). This decrease in blood serum concentrations of T_4 and iodine accompanied by an increase in T_3 concentration is indicative of an insufficient supply of iodine to the thyroid gland (Janssen, 1994) which then prefers the synthesis of the less iodine-demandingT_3 at the expense of T_4. This findings indicates possible anti-thyroid activity of HuNa and supports the data of Huang *et al.* (1994) who demonstrated a significant increase in T_3 and a decrease in T_4 concentrations in blood serum of rats fed a low-iodine diet containing humine substances.

Goitrogenic effects of humine substances in farm animals were a rather neglected problem in the past. The increasing number of goitrogenic factors in animal nutrition makes us to pay more attention to possible presence of increased amounts of humine substances in surface or drinking water in some regions, particularly those that are rich in peat (Seffner, 1996), which can affect the function of the thyroid gland (Huang *et al*., 1994; Seffner, 1995, 1996). At risk under local conditions may be grazed cattle watered from surface sources.

The absorption of iodine present in HuNa and bound in an organometallic complex is limited and increased faecal excretion further reduces the utilisation of dietary iodine. Similar effects were observed in animals receiving cadmium (Herzig *et al*., 1994) or other elements (Kerndorff and Schnitzer, 1980). The increase in faecal excretion of iodine observed in our experiments is consistent with our earlier results (Herzig *et al*., 2000) and can be associated with goitrogenic effects of humates as described by Huang and Fung (1991), Huang *et al*. (1994) and Seffner (1996).

It can be concluded that the utilisation of iodine present in feed components is limited and that sodium humate, or humine substances, affect utilisation and retention of dietary iodine and induce changes of parameters indicating their goitrogenic activity.

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