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NATURAL OCCURRENCE OF THIOURACIL IN URINE OF LIVESTOCK IN POLAND

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Abstract

Natural occurrence of thiouracil in bovine and swine urine in Poland was investigated. Under the national residue control programme, 537 urine samples were tested. In 77 samples (14.3%) thiouracil was detected above decision limit CCα (0.91 μg L^{-1}), including eight samples over the recommended concentration of 10 μg L^{-1}. Of the bovine urine samples, 95 and 99 percentiles have thiouracil concentration below 4.50 and 14.85 μg L^{-1} ,and of porcine samples below 2.35 and 6.80 μg L^{-1}, respectively.

Key words: cattle, swine, urine, thiouracil, Poland.

Thyreostatic compounds are orally active drugs, which may be used to increase the weight of cattle before slaughter. The growth-promoting effect is mainly due to increased water absorption and retention within an edible tissue as well as filling of the gastrointestinal tract (2). The meat derived from the treated animals can be of a lower quality; moreover, it may contain residues harmful for human health (1). According to the International Agency for Research on Cancer (IARC), some compounds of this group possess carcinogenic and teratogenic properties (9). For this reason, the use of thyreostatic drugs for animal fattening purposes has been banned in the European Union since 1981 (4). In accordance with the Council Directive 96/23/EC (5), thyreostats belong to compounds with anabolic properties (group A2), whose control is mandatory in slaughter animals. In Poland, the examination of thyreostatic drugs in biological material of animal origin has been conducted in the National Veterinary Research Institute since 1990. For almost 20 years, these compounds were not detected in biological samples collected from different animal species. The examinations were performed using mainly a high performance thin layer chromatography, with detection limits at 50 μg L^{-1} (6). A recent great progress in the efficiency of analytical methods for thyreostatic drugs determination has lead to a significant reduction of detection limits (12, 13, 15). As a result, in some European countries, including Poland, one of thyreostatics – thiouracil (TU) is detected in urine samples of slaughtered animals (7). The examination of urine samples was performed by means of a liquid chromatography coupled with a triple quadrupole mass spectrometry - LC-MS/MS (17).

For several years, it has been reported that thiouracil is suspected to have a natural origin. Pinel *et al.* (14) showed a correlation between cruciferous-based animal feed and the identification of TU in urine; concentrations of TU eliminated in urine during the cruciferous diet were low and never exceeded 10 μg L^{-1}. The EURL guidance paper (8) acknowledged this by stating that thiouracil concentrations below 10 μg L^{-1} might have a natural origin deriving from *Brassicaceae* consumption. However, recent reports indicate that a diet containing cruciferae plants may be one of the factors contributing to the presence of thiouracil in urine of animals and humans. The study conducted by Belgian and French researches on a small group of volunteers has shown the presence of thiouracil in human urine at concentrations above 10 μg L^{-1} and the reason for this phenomenon was not connected with a rich cruciferous diet (16). The endogenous occurrence of TU in urine is still not fully explained and it requires a further, more extensive research.

The aim of the study was to establish the natural level of TU in urine of livestock in Poland.

Material and Methods

Urine samples. The urine samples were obtained within the frame of Polish residue control plan. Samples were collected from June 2010 to July 2011. A total of 537 urine samples were taken from cattle (n=279) and swine (n=258). At the laboratory, the samples were stored at temperature below -18 °C and thawed before analysis. To each sample 10 ng of internal standard (DMTU) were added to obtain a

concentration of 10 μg L^{-1}. For all series of analysis, two-spiked samples were added, at recommended concentrations of TU -10 μg L^{-1} and 1.56 μg L^{-1} (CCβ value).

Additionally, urine samples were collected from 20 cows fed feed containing about 15% rapeseed meal. It was a normal balanced feed routinely used for cattle feeding. The samples were taken on the same day, stored in a refrigerator at 0°C-10°C, and analysed the next day.

Sample preparation and LC-MS/MS analysis. The analytical protocol describing the preparation of urine samples and LC-MS/MS analysis has been previously published (17). In brief, TU was extracted from urine samples with diethyl ether after derivatization with 3-iodobenzylbromide in basic medium, and detected and identified by means of LC-ESI (+)-MS/MS. The performance of the method for TU detection in urine was presented in Table 1.

Results

The results of investigations of thyreostats in urine of slaughtered animals conducted in Poland in 2010 and in half of 2011 are presented in Table 2. In 77 urine samples (14.3%) TU was detected above the decision limit of 0.91 μg L^{-1}. In eight samples (seven bovine and one porcine), which represent 1.5% of the analysed samples, the concentration of TU exceeded 10 μg L^{-1}, the recommended concentration for thyreostatic compounds proposed by CRL Guidance Paper (8). In 40 samples (15.5%) from pigs, TU concentration ranged from 0.91 μg L^{-1} (CCα) to 10 μg L^{-1}, in one sample concentration exceed the value of 10 μg L^{-1} (16.6 μg L^{-1}). According to the presented data, 95% and 99% of the porcine urine samples had TU concentration below 2.35 and 6.80 μg L^{-1}, respectively.

In 36 of the bovine urine samples (12.9%), TU concentration ranged from decision limit (CCα – 0.91 μg L^{-1}) to 23.1 μg L^{-1}. In seven samples, the concentration exceeded the recommended concentration of 10 μg L^{-1}, and was in the range of 12.3-23.1 μg L^{-1}.

Designated for all the results, mean concentration (included in the range of 0-CCα) was 0.74 μg L^{-1}. The received set of concentration values has a natural distribution, from which the determined 95 and 99 percentiles were 4.78 μg L^{-1} and 14.87 μg L^{-1}, respectively.

Table 1
Method trueness and precision for thiouracil detection in urine (17)

Parameter		TU
Calibration curves	Correlation coefficient	0.9982
	slope	0.061
	intercept	0.072
Linear range	(μg L^{-1})	0.91 - 50
Decision limit	CCα (μg L^{-1})	0.91
Detection capability	CCβ (μg L^{-1})	1.56
Repeatability	C.V. (%)	9.3
Reproducibility	C.V.(%)	13.8
Recovery	(%)	96
Uncertainty	U (%)	26.1

Table 2
Detailed results of data analysis for thiouracil urinary concentrations in different animal species

Species	Cattle	Swine
Number of samples tested (n)	279	258
<CCα	243	217
>CCα	36	41
CCα - CCβ	11	18
CCβ – 10 μg L^{-1}	18	22
> 10 μg L^{-1}	7	1
Max (μg L^{-1})	23.1	16.6
Mean (μg L^{-1})	0.74	0.43
s.d. (μg L^{-1})	2.78	1.49
95% centile (μg L^{-1})	4.78	2.35
99% centile (μg L^{-1})	14.87	6.80

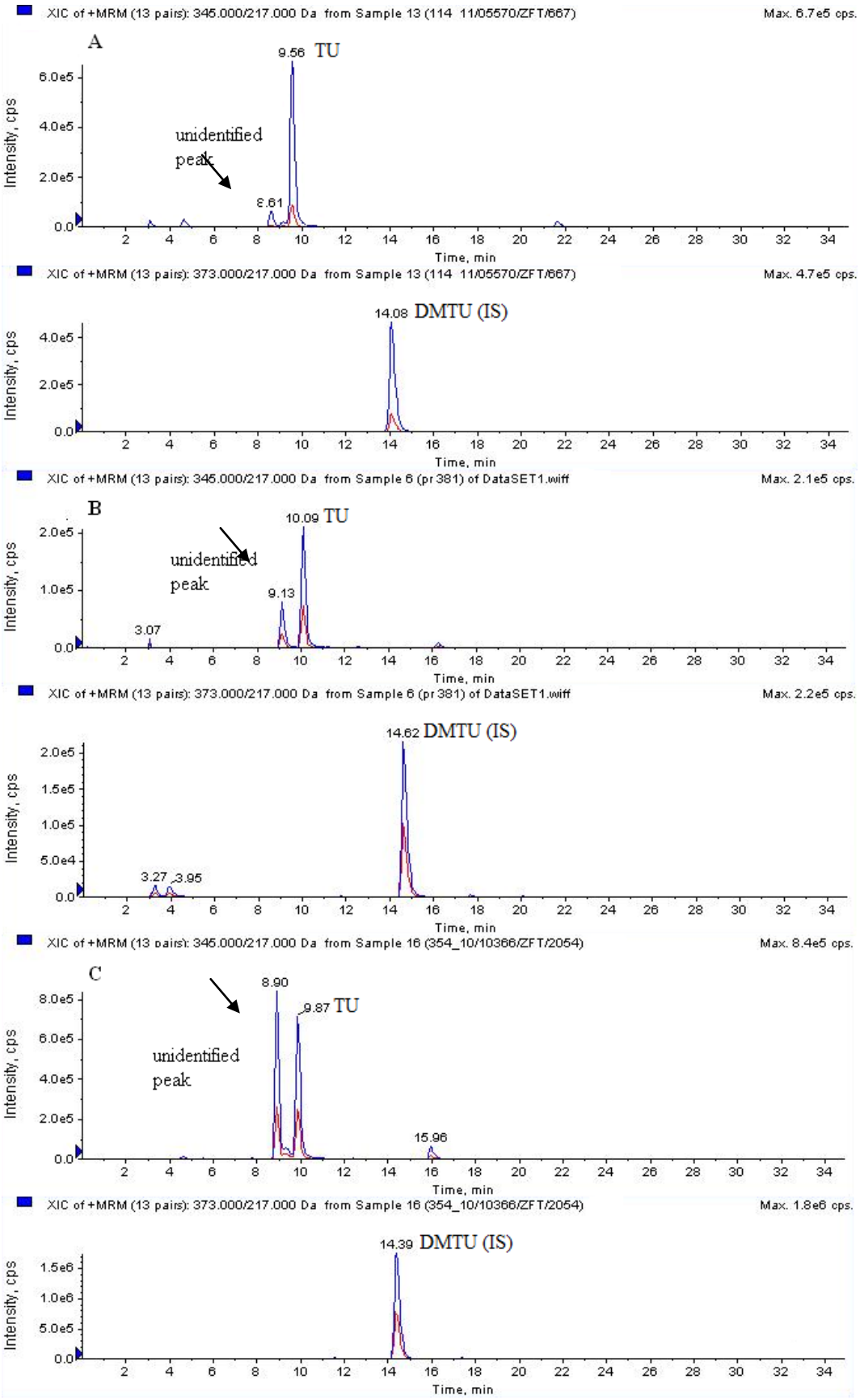


Fig. 1. – LC – MS/MS chromatograms of urine samples with thiouracil:
(A) porcine urine sample confirmed at 16.60 μg L^{-1}, (B,C) bovine urine samples confirmed at 23.10 μg L^{-1} and at 14.15 μg L^{-1}. Differences in the intensities of the peaks: unidentified and originating from TU.

TU was not detected above the CCα concentration in 20 samples of urine collected from cows receiving a rapeseed meal (15%) in diet.

The chromatograms of non-compliant urine samples were presented in Fig. 1. After the analysis of chromatograms of all non-compliant monitored urine samples, it was found that TU was always accompanied by an unidentified peak with a retention time shorter by 1 min regardless of species. In some samples this peak was much larger than the peak of TU, and in terms of TU it exceeded the concentration of CCβ values. The unidentified peak has been never observed in compliant samples.

Discussion

The presence of TU in urine of animals may result from the illegal administration of this compound or an over use of cruciferous plants (*Brassicaceae, Cruciferae*) in animal feeding. The particular reports accessible in the literature confirm that TU can occur endogenously in some animal species, as a consequence of diet containing cruciferous plants, including rape, which is widely used in Poland in feed for livestock.

The investigations carried out by the Veterinary Inspections in Poland did not confirm the administration of thyreostats to animals. It was, therefore, concluded that the detected TU is of an endogenous origin. Furthermore, in the course of investigations conducted by the Veterinary Inspection, additional samples of urine and feed were collected from farms on which TU was found, and the compound was detected three times in concentrations exceeding 10 μg L^{-1} in urine samples taken from one farm in different intervals. In the samples of feed supplied at the same time, there was no presence of TU in concentrations above 3.5 μg kg^{-1} (CCα of applied method), but the analysis of the feed with a modified microscopic method (3) showed the presence of about 40% of rapeseed meal in it. Thus, elevated concentrations of TU in the urine samples from this farm (12.33, 15.45, 14.7 μg L^{-1}) were probably associated with a high content of rapeseed meal in feed.

The present study is a comprehensive overview of the natural occurrence of TU in a large number of urine samples (537) collected from cattle and pigs over a 1.5 year period in Poland. TU was detected in 14.3% of urine samples. In similar studies conducted by French scientists on a larger population of animals (n>1,300), TU was found in 70% of samples tested (11). The reason for the difference may be the fact, that the CCα of TU in our method (0.91 μg L^{-1}) is higher than in the French method (<0.71 μg L^{-1}). The natural distribution of TU concentrations enabled to calculate, that 95% and 99% of the bovine urine samples has TU concentration below 4.50 and 14.85 μg L^{-1}, respectively. These values are very similar to those proposed by Le Bizec *et al.* (11) (6.2 and 12.1 μg L^{-1}), which were determined on the basis of results of the analysis of 1,098 samples, and showed that differences in animal origin and diet have no significant influence on the concentration level of TU in urine of fattening animals. Compared to cattle, percentiles of TU concentration in swine were about two times lower (2.3; 6.8 μg L^{-1}), which may be connected with a diet low in cruciferous plants.

The content of the feed with processed cruciferous plants, such as rapeseed meal in the amount of about 15% did not induce the formation of endogenous TU in urine of cows above CCα value. However, a long-term administration of feed with a high content of rapeseed meal may be the cause of an increase in TU in urine above 10 μg L^{-1}.

An additional peak with retention time shorter by 1 min and the same ratio of ions was found on the chromatograms of the non-compliant urine samples, next to the peak of TU. The observations may indicate that the unidentified peak is the tautomeric form of TU, and two forms of this compound, of several known (10), are present in urine samples. Occurrence of endogenous TU in urine in two tautomeric forms has not been described in the literature yet. Our observations may be an important indication to look for a marker that allows distinguishing exogenous and endogenous nature of this compound. At present no data are available, as concerns the possibility of the occurrence of two forms of the compound in urine after administration of TU to animals. The problem will be investigated in the future. Up till now, the exact origin of natural thiouracil is not fully explained and unambiguous criterions to discriminate endogenous and exogenous TU are still missing.

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