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## Toward the definition of a criterion to suspect thiouracil administration in animal husbandry

Bruno Le Bizec, Emmanuelle Bichon, Yoann Deceuninck, Stéphanie Prévost, Fabrice Monteau, Jean-Philippe Antignac, Gaud Dervilly-Pinel

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**Toward the definition of a criterion to suspect thiouracil administration in animal husbandry**

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**ABSTRACT**

Thyreostats are growth promoters banned in Europe since 1981. The identification of thiouracil (TU) in animal biological matrices is however not anymore systematically interpreted as the consequence of an illegal administration. Indeed, some experimental results pointed out a causality link between cruciferous-based diet and the presence of TU in urine of bovines. The present study aims at investigating, on a large scale ( $n > 1300$ ), the natural occurrence of thiouracil in urine samples collected in different animal species. TU was identified in main breeding animal species: bovine, porcine and ovine. The natural distribution of TU allowed proposing threshold values to differentiate compliant from suspect urine samples. Suggested values are  $5.7 \mu\text{g.L}^{-1}$  and  $9.1 \mu\text{g.L}^{-1}$  in male adult bovine (6-24 months),  $3.1 \mu\text{g.L}^{-1}$  and  $8.1 \mu\text{g.L}^{-1}$  in female adult bovine (6-24 months),  $7.3 \mu\text{g.L}^{-1}$  and  $17.7 \mu\text{g.L}^{-1}$  in calves ( $< 6$  months),  $3.9 \mu\text{g.L}^{-1}$  and  $8.8 \mu\text{g.L}^{-1}$  in female bovine ( $> 24$  months),  $2.9 \mu\text{g.L}^{-1}$  and  $4.1 \mu\text{g.L}^{-1}$  in porcine, at 95% and 99% confidence level respectively.

**Key words:** thiouracil, urine, mass spectrometry, ESI-, breeding animals, endogenous distribution

## 1 INTRODUCTION

2 Thyrostatic compounds are orally active drugs which may be used to increase the weight of  
3 cattle prior to slaughter. This weight gain is mainly due to increased water absorption and  
4 retention within edible tissue as well as filling of the gastro-intestinal tract by inhibiting thyroid  
5 hormones production [Kotter *et al.*, 1959; Derblom *et al.*, 1963]. In particular, 2-thiouracil (TU)  
6 is a very strong thyroid-inhibiting compound. The consequence of such abuse is not only the  
7 production of inferior meat quality, but overall the potential risk of drug residues to human  
8 health, since these compounds have indeed been classified by IARC as belonging to group  
9 2B that represents carcinogenic and teratogenic compounds. For these particular reasons,  
10 thyrostats are banned within the EU since 1981 (E.C. Directive 81/602) for animal  
11 production. In consequence, reliable, sensitive and specific methods, mandatory based on  
12 mass spectrometry, have been set up to ensure efficient control of their illegal use [Le Bizec  
13 *et al.*, 1997; De Wasch *et al.*, 2001; Pinel *et al.*, 2005; Zhang *et al.*, 2005]. The latest  
14 developed protocols [Pinel *et al.*, 2005; Pinel *et al.*, 2006a; Le Bizec *et al.*, 2009; Vanden  
15 Bussche *et al.*, 2010] exhibit performances allowing detection and identification of the  
16 thyrostat compounds in biological fluids and edible tissues in the  $\mu\text{g.kg}^{-1}$  or  $\mu\text{g.L}^{-1}$  range  
17 which is in accordance with the requirements of the European Union regarding the  
18 provisional Minimum Required Performance Limit (MRPL) provisionally fixed at  $10 \mu\text{g.L}^{-1}$ .  
19 These methods allow unambiguous detection of thyrostats abuse since the concentrations  
20 that have to be administrated (5 g / day) in order to observe the expected animal weight gain  
21 generate high urine concentrations ( $\gg 100 \mu\text{g.L}^{-1}$ ).

22  
23 Occasionally however, the occurrence of thiouracil in bovine urines from the national  
24 monitoring plan with levels in the range  $1\text{-}10 \mu\text{g.L}^{-1}$  raised the question of the origin of the  
25 substance [Vanden Bussche *et al.*, 2009]. The hypothesis of a contribution from animal feed  
26 was recently considered [Pinel *et al.*, 2006b] since some vegetables from the Cruciferous  
27 (Brassicaceous) family [Kennedy & Purves, 1941] are known to contain substances, called  
28 goitrogens, which impair iodine uptake by the thyroid and subsequently the conversion of T4

1 (thyroxine) to T3 (triiodothyronine). The experimental study of Pinel et al. (2006b) allowed  
2 drawing a correlation between a cruciferous-based animal feeding and the identification of  
3 TU in urine of animals; concentrations of TU eliminated in urine during the cruciferous diet  
4 were rather low and never exceeded  $10 \mu\text{g}\cdot\text{L}^{-1}$ . Even though the relation between feeding  
5 and thiouracil occurrence in urine has been established, this observation was the result of a  
6 single animal experiment which did not correspond to usual good farming practices, *i.e.* it  
7 was performed under *ad libitum* feeding on rapeseed cakes. To complete the global risk  
8 analysis, an epidemiological study was necessary to establish the distribution of TU in urine  
9 collected in breeding animal. A national monitoring plan has been used to provide urine  
10 samples and therefore integrate the potential variability associated to meat animals breeding  
11 conditions (species, age, gender, feed...). In this context, more than 1300 urine samples  
12 collected in different species (bovine, ovine, porcine) have been analysed for thiouracil  
13 according to Pinel et al. (2005). The objective was to investigate the natural distribution of TU  
14 and finally, when possible, to propose threshold values authorizing robust classification of  
15 samples toward compliant or suspect.

## 17 **EXPERIMENTAL**

### 18 *Urine samples*

19 The urinary sampling gathered 1322 specimens, involving bovine ( $n = 1098$ , including 362  
20 calves), porcine ( $n = 201$ ) and ovine ( $n = 26$ ) species. Samples have been collected all over  
21 the year 2006 and equally distributed, both over this period of time and on the French  
22 territory. Details of the sampling are summarized in Table 1.

### 24 *Data collection and analysis*

25 Three field laboratories, accredited for thyrostats according to ISO 17025:2005 and running  
26 the official French reference method (derivatisation with 3-iodobenzylbromide, SPE C18,  
27 detection and identification by LC-ESI(-)-MS/MS) (adapted from Pinel et al., 2005), have  
28 been involved in the data production. These laboratories have been submitted to proficiency

1 tests organised by the French National Reference Laboratory (NRL) which shows  $CC_{\alpha} < 0.7$   
2  $\mu\text{g.L}^{-1}$  for TU: they proved similar performances and homogeneous quantitative data (Z-score  
3 in the range +1). Data produced in the laboratories, which consisted in TU urinary  
4 concentrations, have been analysed in the present study using descriptive statistics.

## 6 RESULTS

7 It shall first be noted that the observed concentrations through the study did not follow a  
8 normal distribution whatever the species considered. Therefore, emphasis has been given to  
9 the determination of 95 and 99 centiles of the distributions. Nevertheless and owing to the  
10 high number of animals included in the study which improves the associated statistical  
11 confidence, mean values as well as p-values have also been investigated.

### 13 *Bovines*

14 Thiouracil concentrations levels determined in the 1098 analysed bovine urine samples (all  
15 gender and age considered together) are represented in Figure 1. While TU was not  
16 detected in 32% of the samples (observations included in the  $[0 - CC_{\alpha}]$  range), it could be  
17 identified and quantified in the majority of urines with concentrations ranging from decision  
18 limit ( $CC_{\alpha}$ ) to  $22.5 \mu\text{g.L}^{-1}$ ; mean and median values were equal to  $1.4 \mu\text{g.L}^{-1}$  and  $0.3 \mu\text{g.L}^{-1}$ ,  
19 respectively (Table 1). Several atypically elevated values were noticed (12 samples over the  
20 1098 were found between  $11.9$  and  $22.5 \mu\text{g.L}^{-1}$ ), explaining this significant difference  
21 between the mean and median values. From this urinary distribution, thresholds at 95% and  
22 99% could be established for bovines at  $6.2 \mu\text{g.L}^{-1}$  and  $12.1 \mu\text{g.L}^{-1}$ , respectively. Bovine  
23 samples have then been further investigated by age group in order to refine this preliminary  
24 result.

25 The particular case of calves which represented 362 animals out of the 1098 bovine samples  
26 was thus studied. The corresponding TU concentration levels are shown in Figure 2 and  
27 Table 1. TU could be identified and quantified in more than 80% of the urine samples and



1 concentrations ranged from decision limit ( $CC\alpha$ ) to  $22.5 \mu\text{g.L}^{-1}$ . Mean and median  
2 concentrations, which were equal to  $2.2 \mu\text{g.L}^{-1}$  and  $1.1 \mu\text{g.L}^{-1}$ , respectively, were significantly  
3 ( $p < 0.0001$ ) higher than the corresponding values for the bovines older than 6 months-old  
4 ( $n = 736$ ) reported at  $1.0 \mu\text{g.L}^{-1}$  and  $0.1 \mu\text{g.L}^{-1}$ , respectively (Table 1). The observed  
5 distribution of TU concentrations within the calves' population allowed classifying 95% and  
6 99% of the samples below  $7.3 \mu\text{g.L}^{-1}$  and  $17.7 \mu\text{g.L}^{-1}$ , respectively. These thresholds were  
7 found significantly higher than those proposed for bovines  $> 6$  months-old, reported at  
8  $5.0 \mu\text{g.L}^{-1}$  and  $8.8 \mu\text{g.L}^{-1}$ , respectively (Table 1). These significant differences between calves  
9 and older bovines therefore support the fact that these two populations have to be  
10 considered apart in the context of the present issue. The reasons for such differences  
11 remains however still unknown and may lie in different metabolisms, breeding or feeding  
12 conditions between calves and adult animals. The hereby study has furthermore evidenced  
13 that calves exhibit different TU patterns according to their age: it is obvious from Figure 3 that  
14 mean TU concentrations increase from very young animals (less than 1 month) to older ones  
15 (aged between 5 and 6 months) in the range  $1.5 - 4.6 \mu\text{g.L}^{-1}$ . Once again, it can be  
16 hypothesised that feeding conditions may contribute to this observation, and in particular for  
17 calves, the increase in the diet of the vegetable proportion may be considered. Any inter-sex  
18 difference was investigated for calves (Figure 4 and Table 1) but did not lead to any  
19 significant statistical difference ( $p\text{-value} = 0.4035$ ) between mean TU concentrations for  
20 males ( $2.1 \mu\text{g.L}^{-1}$ ) and females ( $2.4 \mu\text{g.L}^{-1}$ ), which does not support to define any gender-  
21 dependant thresholds for calves.

22  
23 Adult bovines ( $> 6$  months) were further investigated and the corresponding group therefore  
24 divided into two groups, i.e. animals aged between 6 and 24 months and animals older than  
25 24 months (Table 1). The reason for this additional categorisation lies in the fact that animals  
26 aged more than 24 months only consist in females, which therefore support their study apart.  
27 In this context, a close study of the animals aged between 6 and 24 months was performed.  
28 Thiouracil concentrations levels determined in the 540 analysed bovine urine samples are

1 represented in Figure 5. Thiouracil was identified and quantified in the majority of urines with  
2 concentrations ranging from decision limit ( $CC\alpha$ ) to  $20.8 \mu\text{g.L}^{-1}$ . Mean and median values  
3 were equal to  $1.1 \mu\text{g.L}^{-1}$  and  $0.1 \mu\text{g.L}^{-1}$ , respectively (Table 1); several atypically elevated  
4 values were noticed explaining this significant difference between the mean and median  
5 values. From this urinary distribution, thresholds at 95% and 99% could be established for  
6 bovines aged between 6 and 24 months at  $5.2 \mu\text{g.L}^{-1}$  and  $8.8 \mu\text{g.L}^{-1}$ , respectively. The inter-  
7 sex difference was also assessed as presented in Figure 6. Mean TU concentrations were  
8 found equal to  $1.4$  and  $0.8 \mu\text{g.L}^{-1}$  in male (M) and female (F) urine samples, respectively. On  
9 average, female animals present lower TU concentrations in their urine compared to males,  
10 even if the highest values have been observed in female urines. The difference between  
11 males and females was found significant, as attested by the low corresponding p-value ( $p =$   
12  $0.00095$ ), which justifies the proposition of sex-dependant thresholds as follows:  $5.7 \mu\text{g.L}^{-1}$   
13 (M) and  $3.1 \mu\text{g.L}^{-1}$  (F) at 95% and  $9.1 \mu\text{g.L}^{-1}$  (M) and  $8.1 \mu\text{g.L}^{-1}$  (F) at 99%. One hypothesis  
14 supporting this finding would lie in feeding conditions which are known to be different  
15 between female animals, mainly consisting in lactating cows, and male animals which  
16 represent meat producing animals.

17  
18 Finally, and as presented in Table 1, it could be demonstrated that 95% and 99% of the urine  
19 samples collected on cows older than 24 months present thiouracil concentrations,  
20 respectively below  $3.9$  and  $8.8 \mu\text{g.L}^{-1}$ .

21  
22 From these results it appears obvious that when considering bovine animals, three  
23 categories have to be defined, namely the calves (aged  $< 6$  months), the females aged more  
24 than 24 months and animals with an age range comprised between 6 and 24 months.

## 25 26 *Porcines*

27 Two hundred twenty one porcine urine samples have also been investigated for TU  
28 concentrations, which are reported in Figure 7 and Table 1. TU could be identified in more

1  
2  
3 1 than 80% of the samples. Concentrations ranging from decision limit ( $CC\alpha$ ) to  $7.0 \mu\text{g.L}^{-1}$   
4  
5 2 have been obtained while mean and median values were equal to  $0.9 \mu\text{g.L}^{-1}$  and  $0.5 \mu\text{g.L}^{-1}$ ,  
6  
7 3 respectively (Table 1). Thresholds at 95% and 99% could be proposed at  $2.9 \mu\text{g.L}^{-1}$  and  $4.1$   
8  
9 4  $\mu\text{g.L}^{-1}$ , respectively. A very low standard deviation ( $1.0 \mu\text{g.L}^{-1}$ ) was obtained for porcine which  
10  
11 5 certainly reflects the homogeneous sampling within this specie: all animals involved in the  
12  
13 6 study were indeed from a narrow age group in the range 1-8 months, and furthermore, much  
14  
15 7 standardised breeding practices are observed for porcine. No significant differences between  
16  
17 8 males and females could be observed ( $p = 0.2527$ ), which does not support the proposition  
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19 9 of gender-dependant thresholds. As compared to the bovine specie, the urinary TU  
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21 10 concentrations in porcine were found significantly lower ( $p = 0.0020$ , \*\*), this may be linked to  
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23 11 the feeding conditions which in this case consist in an omnivore diet including probably not  
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25 12 that much cruciferous.  
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#### 31 14 *Ovines*

32  
33 15 Twenty six urine samples collected on ovines have also been investigated in the present  
34  
35 16 study. The TU urinary concentrations were in the range  $CC\alpha - 14.3 \mu\text{g.L}^{-1}$ ; mean and median  
36  
37 17 values were  $3.3 \mu\text{g.L}^{-1}$  and  $2.2 \mu\text{g.L}^{-1}$ , respectively. No significant differences between males  
38  
39 18 and females could be observed ( $p = 0.2189$ ) and thresholds at 95% and 99% could be  
40  
41 19 proposed for this specie at  $12.9 \mu\text{g.L}^{-1}$  and  $14.3 \mu\text{g.L}^{-1}$ , respectively (Table 1). As compared  
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43 20 with other species, these values can be considered as rather high, however, because of the  
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45 21 reduced number of samples involved, the statistical confidence associated to the resulting  
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47 22 values should only be considered as indicative trends for the specie and would need further  
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49 23 confirmation through an extended study on a large number of animals.  
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#### 55 25 **CONCLUSION**

56  
57 26 The present study gives a comprehensive overview of the natural occurrence of thiouracil in  
58  
59 27 a large number ( $n > 1300$ ) of urine samples collected in different animal species over a year  
60  
28 on the French territory. TU was identified in main breeding animal species (bovine, porcine

1 and ovine) and in a large proportion of the samples involved (>70%). The natural distribution  
2 of TU allowed proposing threshold values to differentiate compliant from suspect urine  
3 samples. Suggested values are 5.7  $\mu\text{g.L}^{-1}$  and 9.1  $\mu\text{g.L}^{-1}$  in male adult bovine (6-24 months),  
4 3.1  $\mu\text{g.L}^{-1}$  and 8.1  $\mu\text{g.L}^{-1}$  in female adult bovine (6-24 months), 3.9  $\mu\text{g.L}^{-1}$  and 8.8  $\mu\text{g.L}^{-1}$  in  
5 female bovine (> 24 months), 7.3  $\mu\text{g.L}^{-1}$  and 17.7  $\mu\text{g.L}^{-1}$  in calves (< 6 months), 3.9  $\mu\text{g.L}^{-1}$  and  
6 8.8  $\mu\text{g.L}^{-1}$  in female bovine (> 24 months), 2.9  $\mu\text{g.L}^{-1}$  and 4.1  $\mu\text{g.L}^{-1}$  in porcine, at 95% and  
7 99% confidence level respectively.

8  
9 According to the CRL guidance paper (2007), a provisional MRPL at 10  $\mu\text{g.L}^{-1}$  has been  
10 proposed for thyrostatic compounds. This level of performance is undoubtedly too high if a  
11 Reference Point for Action (RPA) is decided around 10  $\mu\text{g.L}^{-1}$ . It should also be emphasised  
12 that robustness of the method regarding TU quantitation and control of the instability of such  
13 residue are two issues of prime interest which are genuine pre-requisite before efficient and  
14 non ambiguous implementation of the RPA.

15  
16 This work overall highlights the urgent need for a confirmatory criterion, should it be based  
17 on the discovery of a discriminant biomarker or isotope ratio measurement. Two strategies  
18 should be considered regarding the biomarker discovery which would either sign for the  
19 feeding or the abuse origin of the TU signal; in these cases, biomarkers of interest would  
20 either, and respectively, consist in vegetable precursors of the target molecule or direct  
21 metabolites (phase I or II) of the exogenous administered thiouracil.

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3 **1 ACKNOWLEDGEMENTS**  
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6 for Food, DGA) for financial support as well as Laboratoire Départemental des Landes,  
7 Laboratoire Départemental d'Analyse d'Ille-et-Vilaine, and Laboratoire Départemental de la  
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3 1 Kennedy T.H, Purves H.D. 1941. Studies on experimental goitre I: the effect of brassica  
4 2 seed diets on rats. The British Journal of Experimental Pathology 22:241.  
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8 4 Vanden Bussche J, Noppe H, Verheyden K, Wille K, Pinel G, Le Bizec B, De Brabander H.  
9 5 2009. Analysis of thyreostats : a history of 35 years. Anal. Chim. Acta 637:2-12.  
10 6  
11  
12 7 Vanden Bussche J, Vanhaecke L, Deceuninck Y, Verheyden K, Wille K, Bekaert K, Le Bizec  
13 8 B and De Brabander HF. 2010. Development and validation of an ultra-high performance  
14 9 liquid chromatography tandem mass spectrometry method for quantifying thyreostats in  
15 10 urine without derivatisation. J.Chromatogr. A 1217:4285-4293.  
16 11  
17  
18 12 Zhang L, Liu Y, Xie M.-X, Qiu Y-M. 2005. Simultaneous determination of thyrostatic residues  
19 13 in animal tissues by matrix solid-phase dispersion and gas chromatography-mass  
20 14 spectrometry. J. Chromatogr. A 1074:1.  
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**Table 1:** Detailed results of the data analysis for thiouracil urinary concentrations in the different animal species considered

Specie Sex	Total Bovine			Bovine (> 6 months)			Calves (< 6 months)			Porcine			Ovine		
	Male+Female	Male	Female	Male+Female	Male	Female	Male+Female	Male	Female	Male+Female	Male	Female	Male+Female	Male	Female
n	1098	474	553	736	279	409	362	195	144	201	56	19	26	13	6
mean	1,4	1,7	1,2	1,0	1,4	0,8	2,2	2,1	2,4	0,9	0,7	0,9	3,3	3,5	1,3
median	0,3	0,6	0,1	0,1	0,2	0,0	1,1	1,0	1,1	0,5	0,5	0,6	2,2	1,7	1,0
min	CC $\alpha$	CC $\alpha$	CC $\alpha$	CC $\alpha$	CC $\alpha$	CC $\alpha$	CC $\alpha$	CC $\alpha$	CC $\alpha$	CC $\alpha$	CC $\alpha$	CC $\alpha$	CC $\alpha$	CC $\alpha$	CC $\alpha$
max	22,5	22,5	20,8	20,8	11,9	20,8	22,5	22,5	18,7	7,0	3,4	5,3	14,3	14,3	3,0
95% Centile	6,2	6,1	5,9	5,0	5,7	3,3	7,3	6,7	9,0	2,9	1,8	5,3	12,9	14,3	3,0
99% Centile	12,1	11,2	13,3	8,8	9,1	8,4	17,7	21,4	14,1	4,1	3,4	5,3	14,3	14,3	3,0
s.d.	2,5	2,6	2,5	2,0	2,1	1,9	3,2	3,1	3,3	1,0	0,7	1,2	3,7	4,1	1,1
p-value (M vs F)						0,00010			0,40357			0,25277			0,21899

Bovine Sex	6-24 months			> 24 months
	Male+Female	Male	Female	Female
n	540	279	213	196
mean	1,1	1,4	0,8	0,8
median	0,1	0,2	0,0	0,1
min	CC $\alpha$	CC $\alpha$	CC $\alpha$	CC $\alpha$
max	20,8	11,9	20,8	15,7
95% Centile	5,2	5,7	3,1	3,9
99% Centile	8,8	9,1	8,1	8,8
s.d.	2,1	2,1	2,0	1,9
p-value (M vs F)			0,00095	



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6 2 **Figure Captions**  
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10 4 **Figure 1:** Distribution frequency observed for thiouracil concentration levels measured in  
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12 5 bovine urine samples (n = 1098)  
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16 7 **Figure 2:** Distribution frequency observed for thiouracil concentration levels measured in  
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18 8 calve urine samples (n = 362)  
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23 10 **Figure 3:** Thiouracil concentration levels measured in calves urine samples according to  
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25 11 their age when known (n = 296)  
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29 13 **Figure 4:** Distribution observed for thiouracil concentration levels measured in female (F,  
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31 n=144) versus male calve urine samples (M, n = 195)  
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36 16 **Figure 5:** Distribution frequency observed for thiouracil concentration levels measured in  
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38 17 bovine (6-24 months old) urine samples (n = 540)  
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43 19 **Figure 6:** Distribution observed for thiouracil concentration levels measured in bovine (6-24  
44  
45 20 months) female (F, n=213) and male urine samples (M, n = 279)  
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49 22 **Figure 7:** Distribution frequency observed for thiouracil concentration levels measured in  
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51 23 porcine urine samples (n = 201)  
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Figure 1

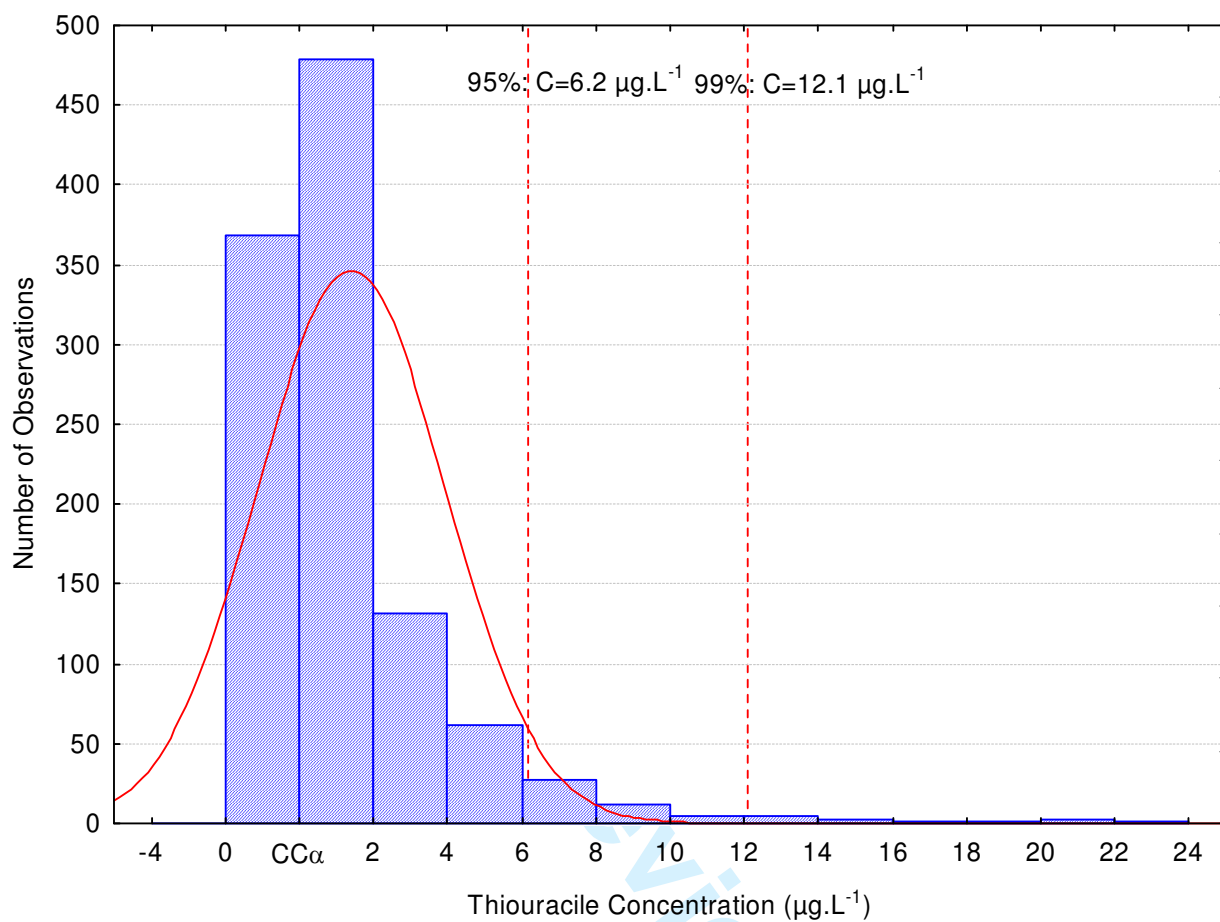


Figure 2

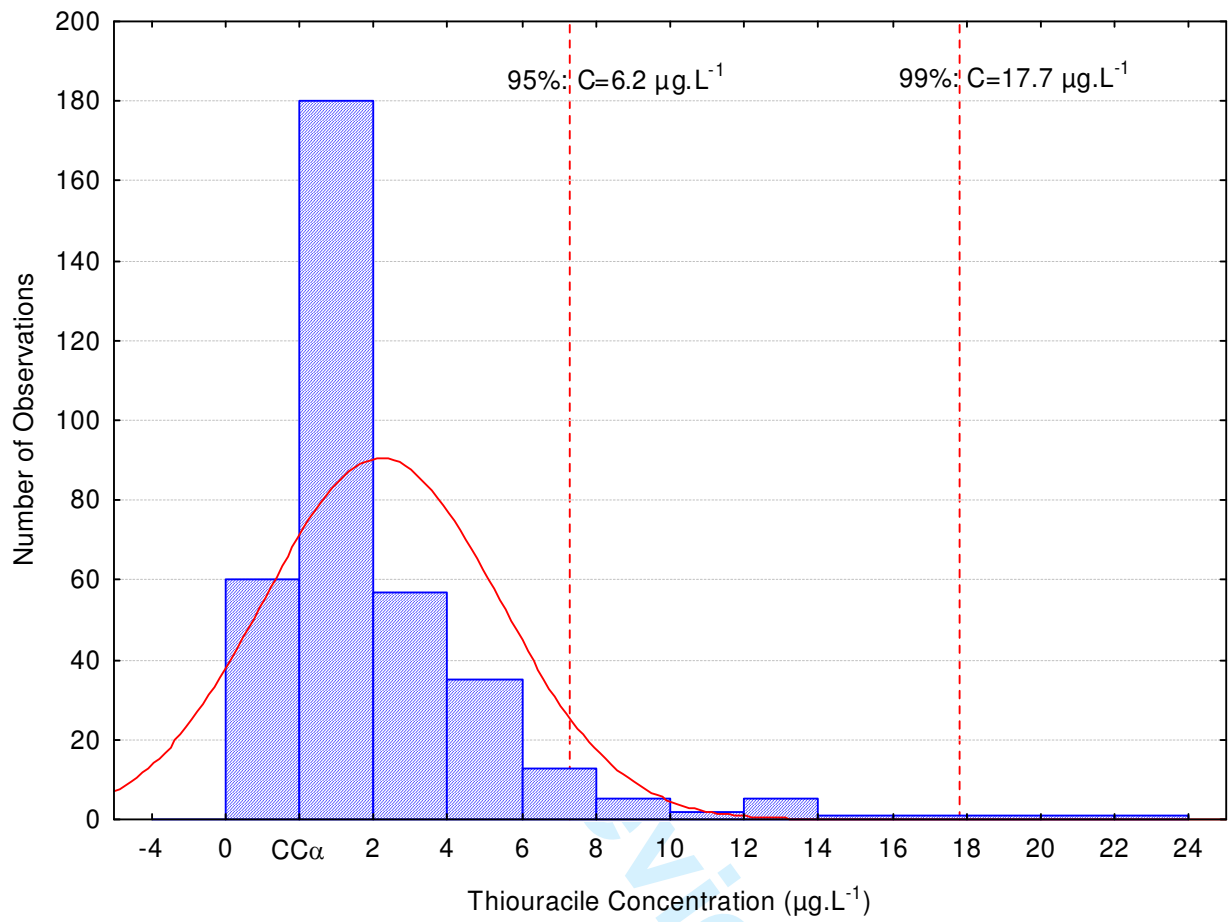
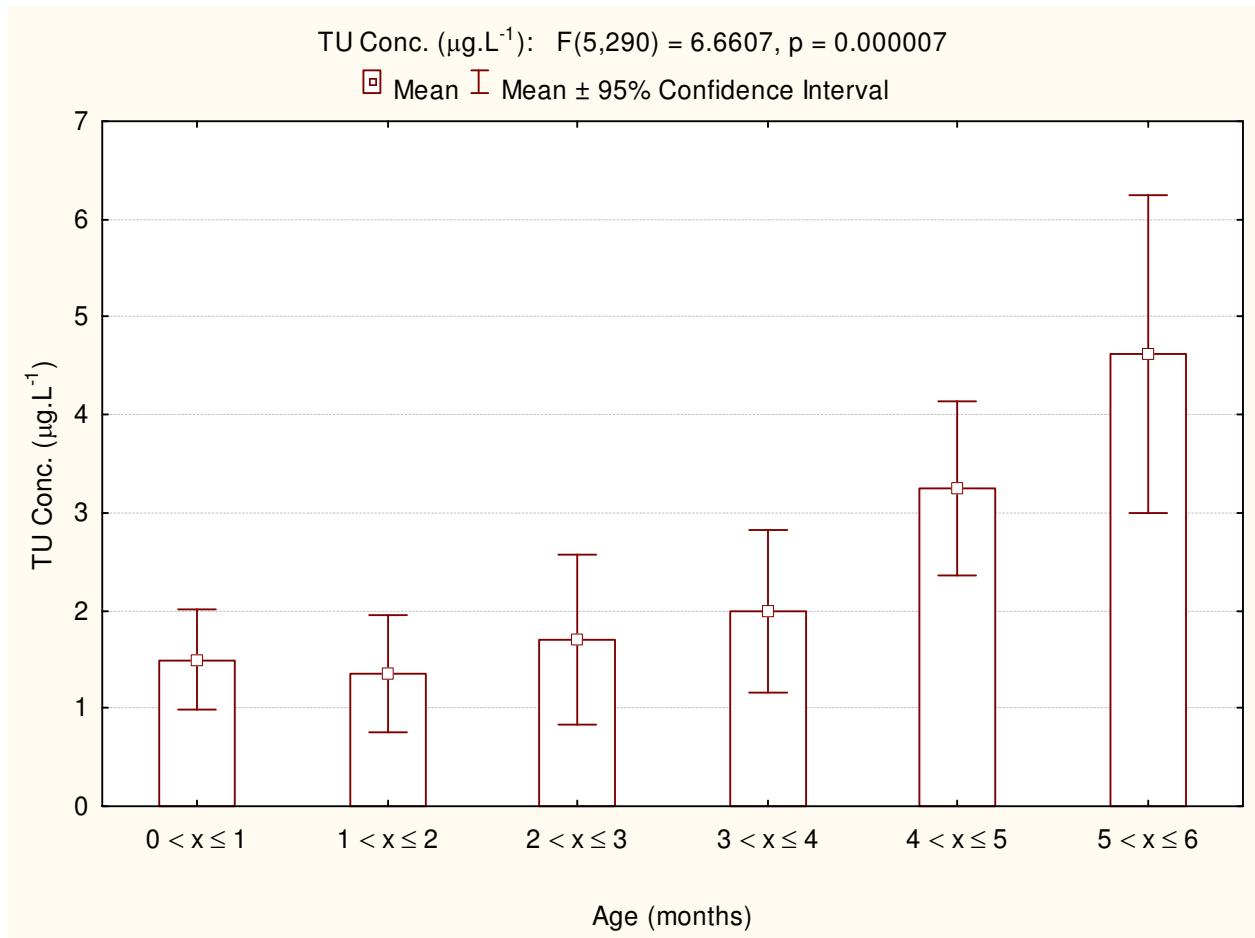


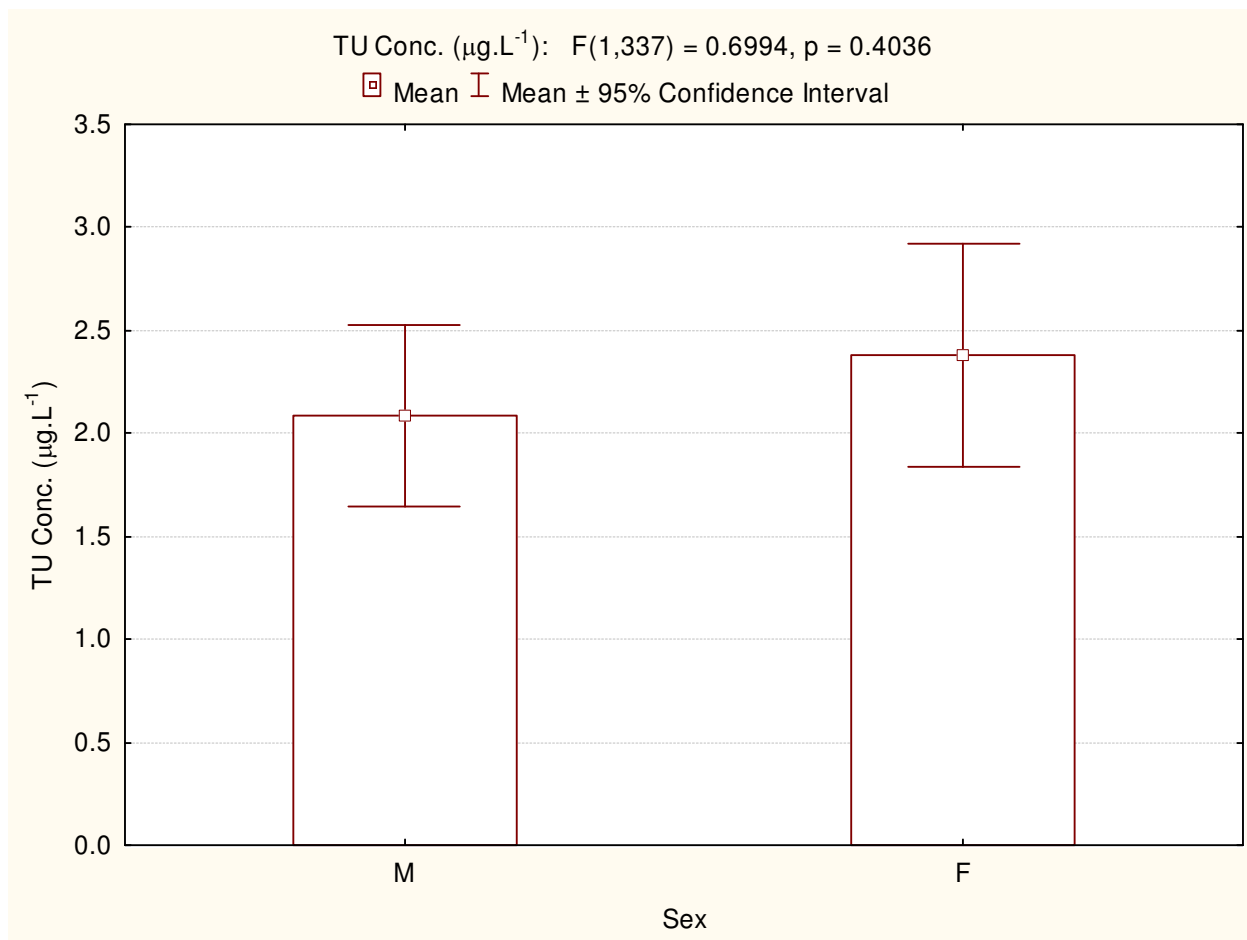
Figure 3



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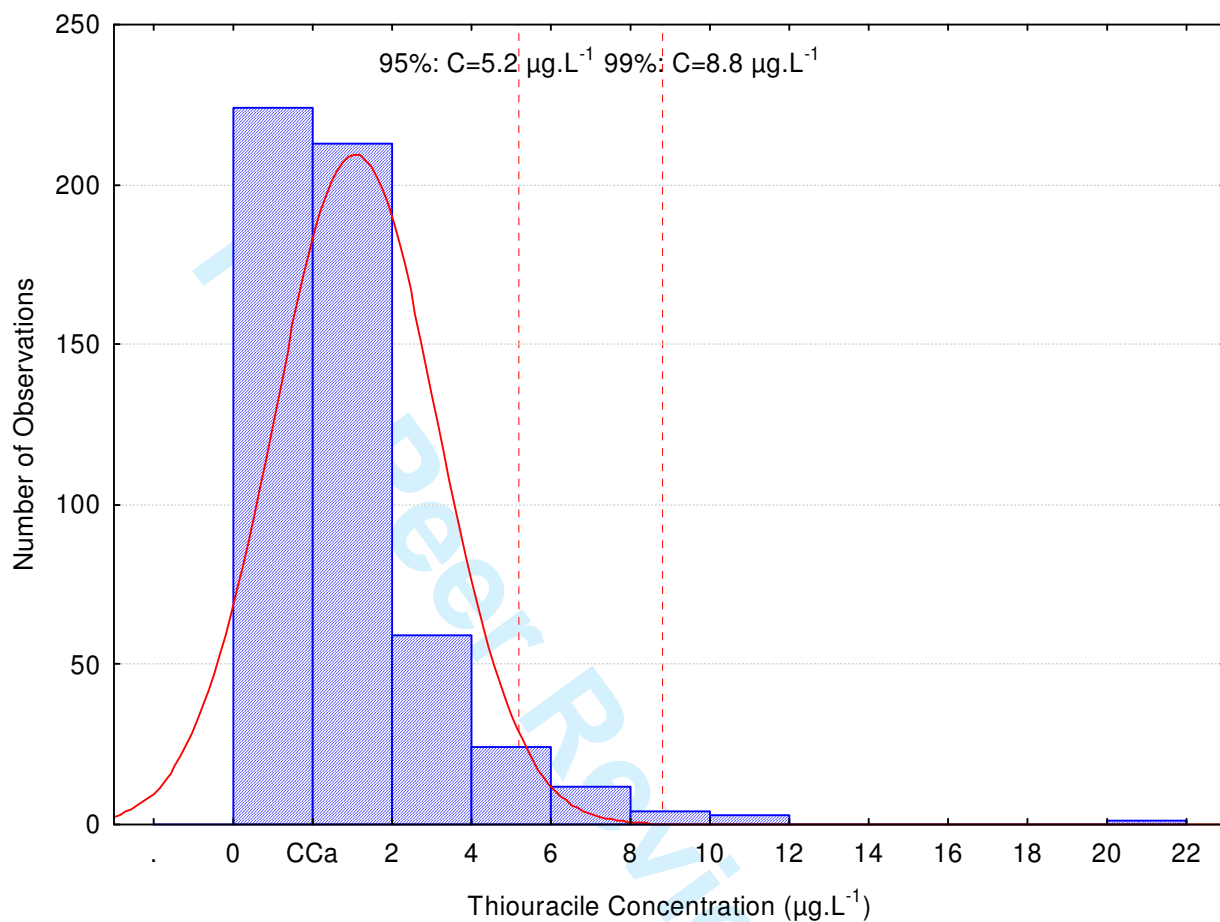
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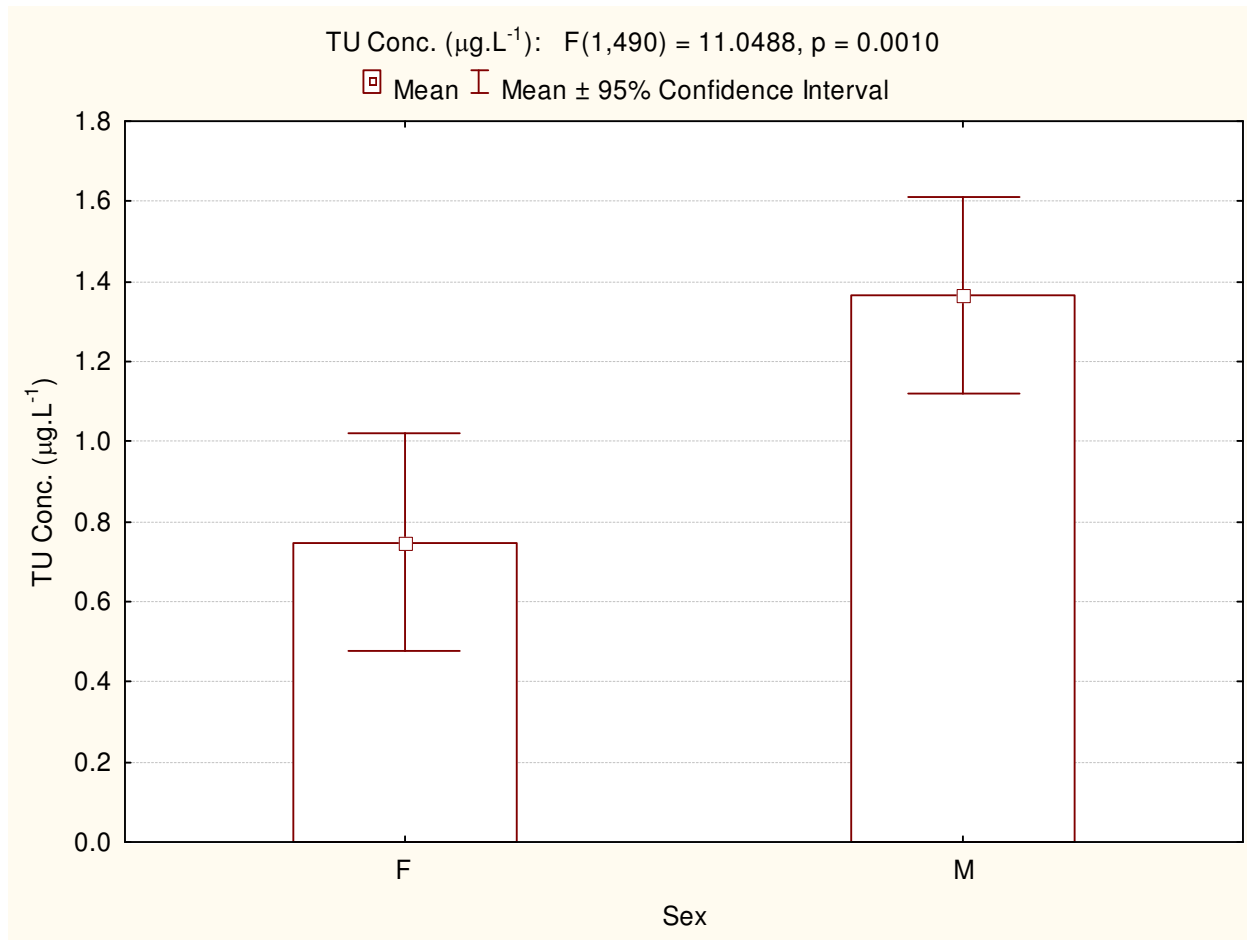
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Figure 5



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Figure 6



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Figure 7

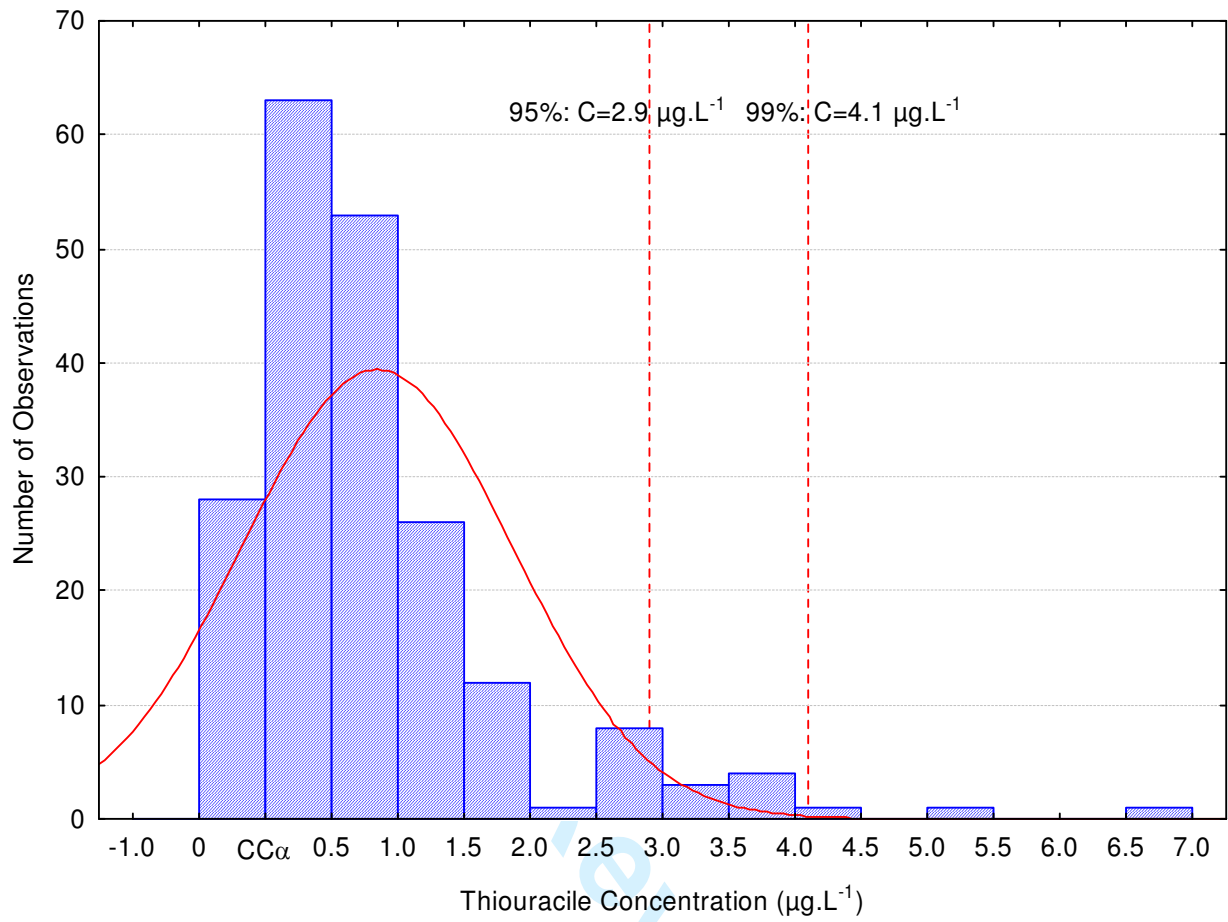




Table 1: Detailed results of the data analysis for thiouracil urinary concentrations in the different animal species considered

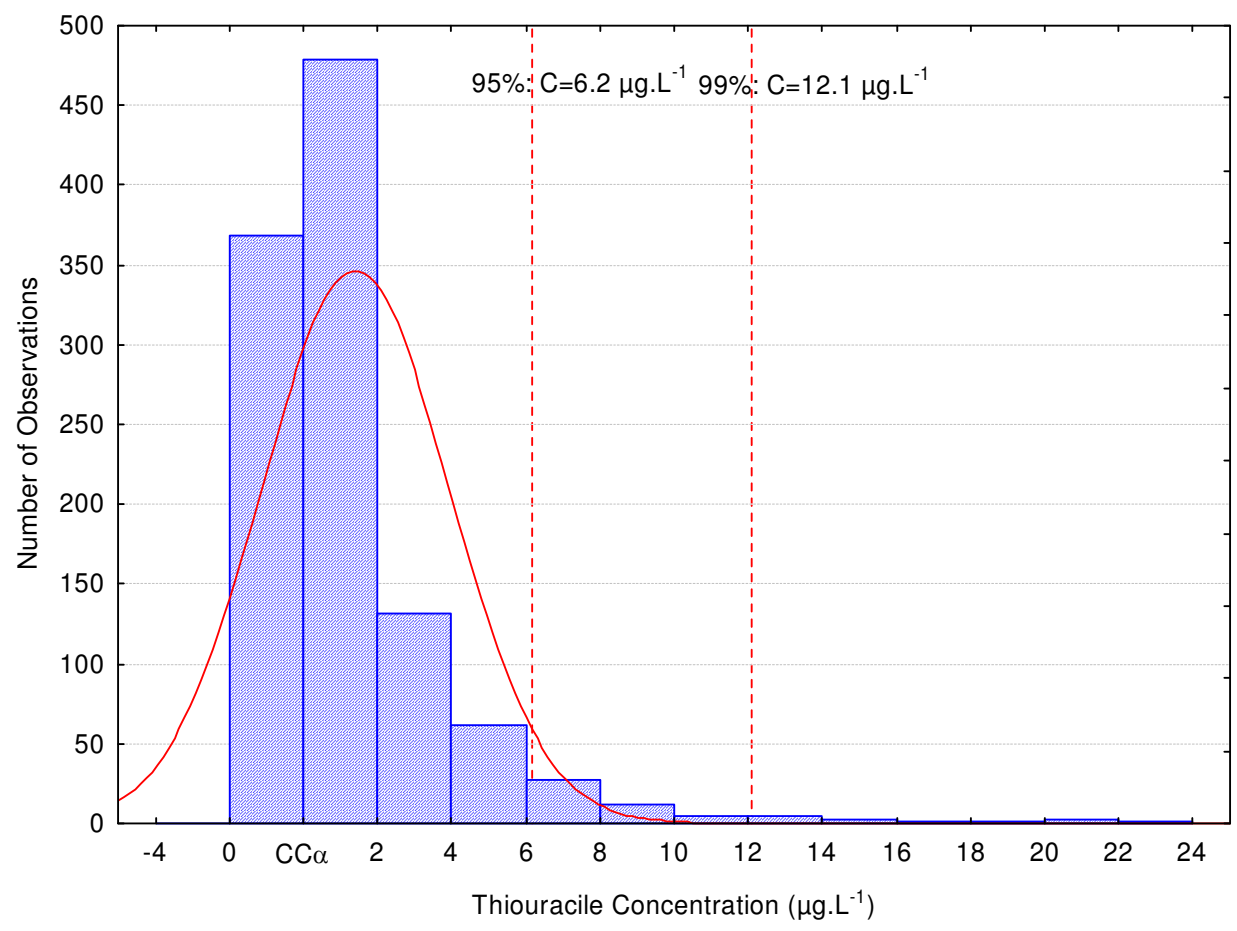
Specie Sex	Total Bovine			Bovine (> 6 months)			Calves (< 6 months)			Porcine			Ovine		
	Male+Female	Male	Female	Male+Female	Male	Female	Male+Female	Male	Female	Male+Female	Male	Female	Male+Female	Male	Female
n	1098	474	553	736	279	409	362	195	144	201	56	19	26	13	6
mean	1,4	1,7	1,2	1,0	1,4	0,8	2,2	2,1	2,4	0,9	0,7	0,9	3,3	3,5	1,3
median	0,3	0,6	0,1	0,1	0,2	0,0	1,1	1,0	1,1	0,5	0,5	0,6	2,2	1,7	1,0
min	CC $\alpha$	CC $\alpha$	CC $\alpha$	CC $\alpha$	CC $\alpha$	CC $\alpha$	CC $\alpha$	CC $\alpha$	CC $\alpha$	CC $\alpha$	CC $\alpha$	CC $\alpha$	CC $\alpha$	CC $\alpha$	CC $\alpha$
max	22,5	22,5	20,8	20,8	11,9	20,8	22,5	22,5	18,7	7,0	3,4	5,3	14,3	14,3	3,0
95% Centile	6,2	6,1	5,9	5,0	5,7	3,3	7,3	6,7	9,0	2,9	1,8	5,3	12,9	14,3	3,0
99% Centile	12,1	11,2	13,3	8,8	9,1	8,4	17,7	21,4	14,1	4,1	3,4	5,3	14,3	14,3	3,0
s.d.	2,5	2,6	2,5	2,0	2,1	1,9	3,2	3,1	3,3	1,0	0,7	1,2	3,7	4,1	1,1
p-value (M vs F)						0,00010			0,40357			0,25277			0,21899

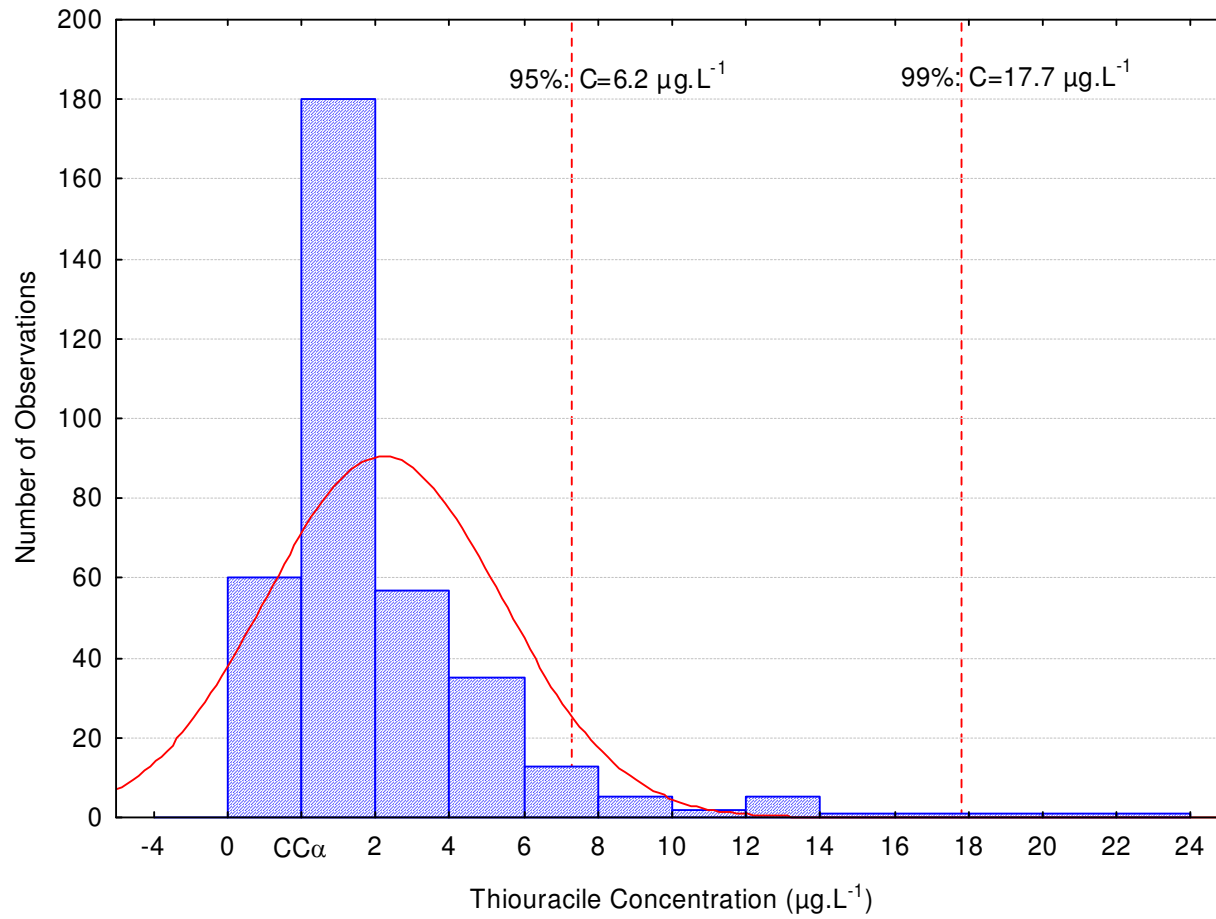
Bovine Sex	6-24 months			> 24 months
	Male+Female	Male	Female	Female
n	540	279	213	196
mean	1,1	1,4	0,8	0,8
median	0,1	0,2	0,0	0,1
min	CC $\alpha$	CC $\alpha$	CC $\alpha$	CC $\alpha$
max	20,8	11,9	20,8	15,7
95% Centile	5,2	5,7	3,1	3,9
99% Centile	8,8	9,1	8,1	8,8
s.d.	2,1	2,1	2,0	1,9
p-value (M vs F)			0,00095	

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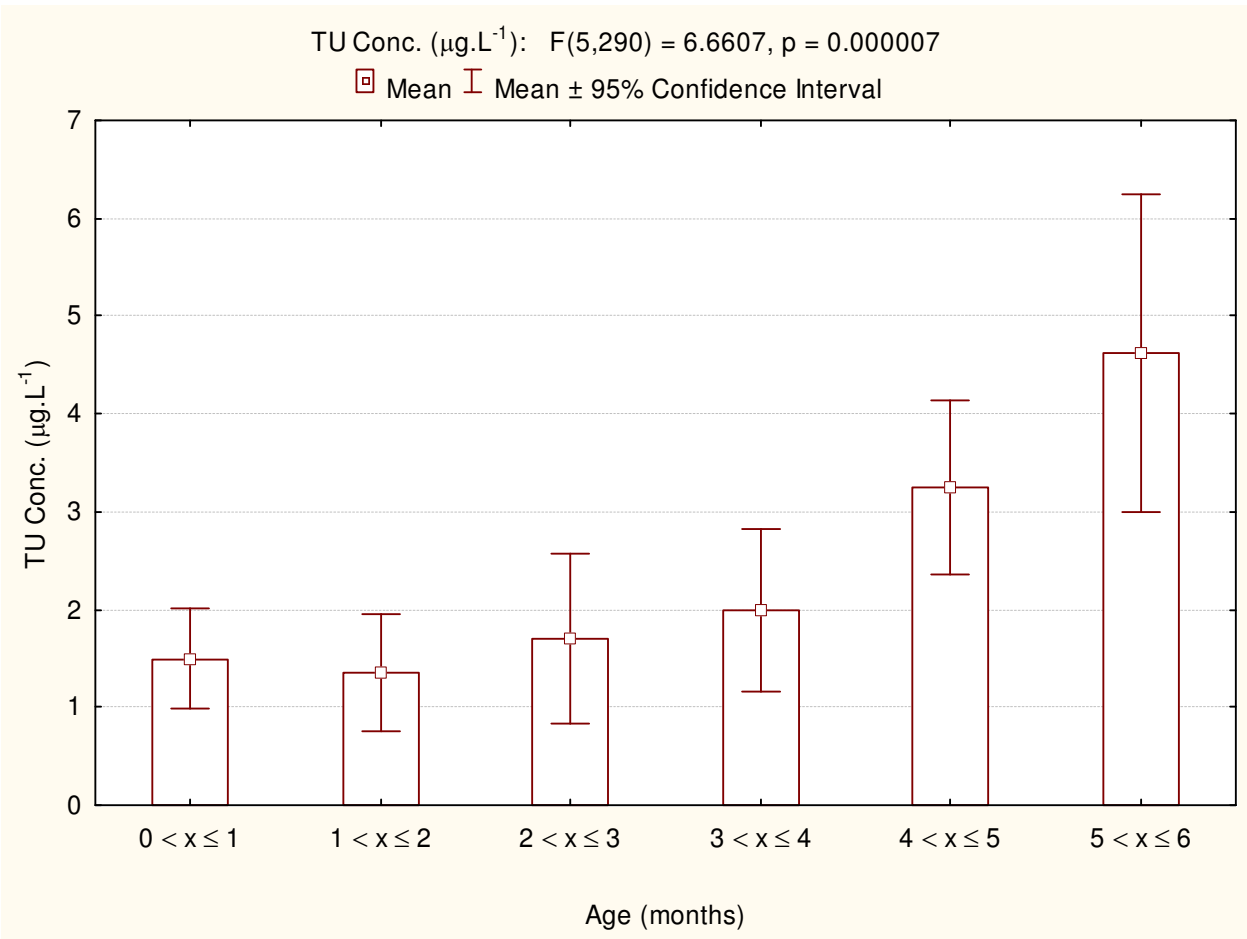
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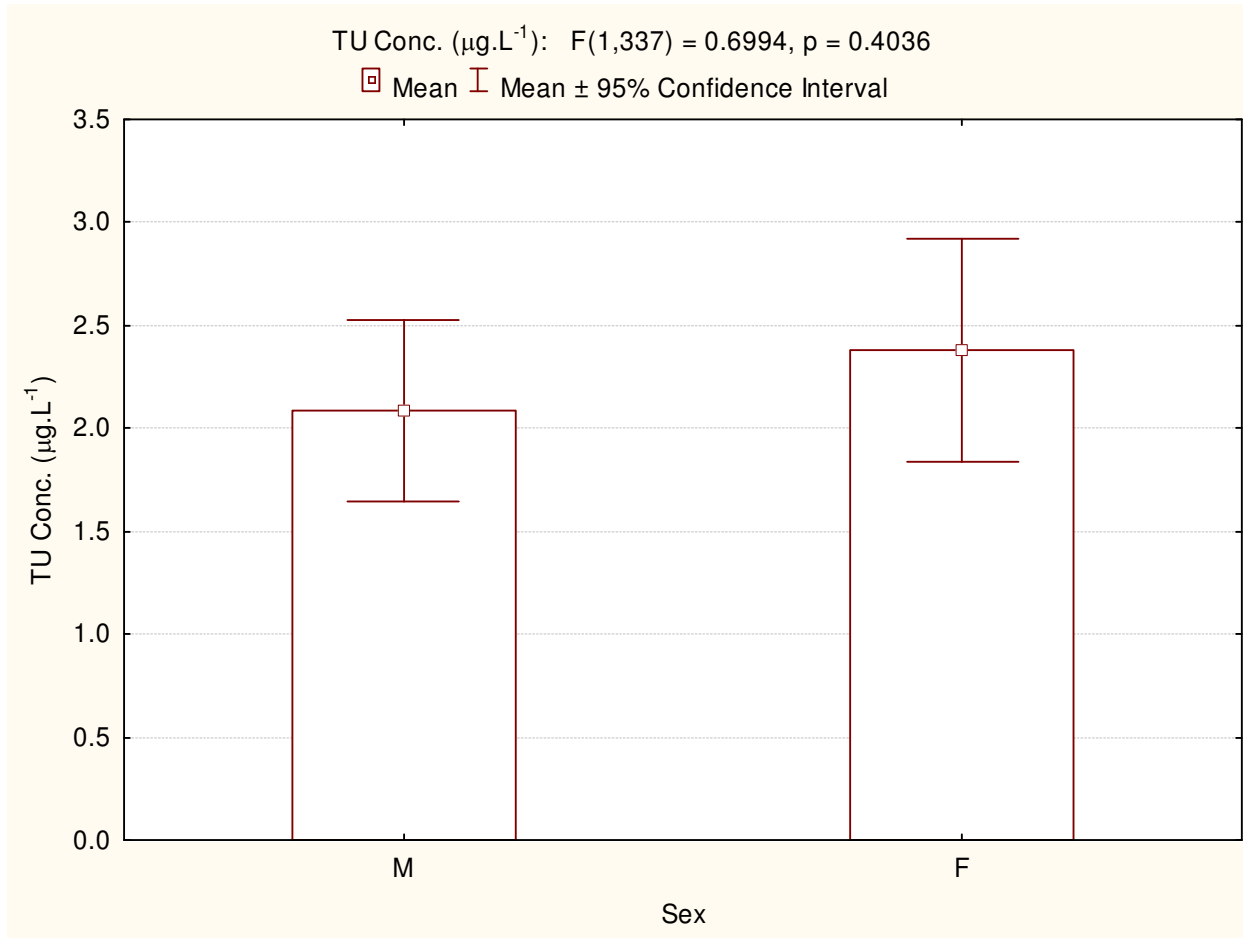




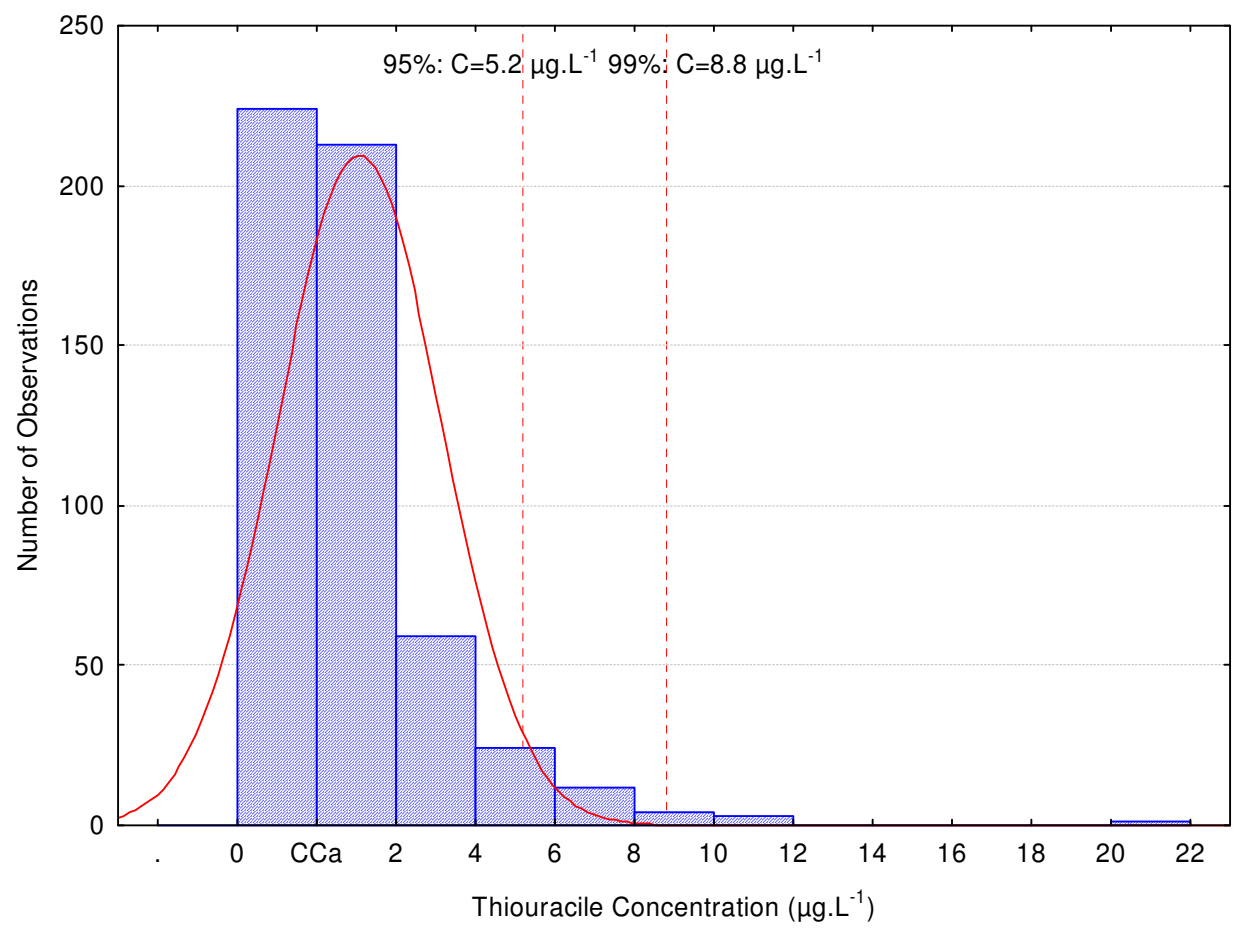
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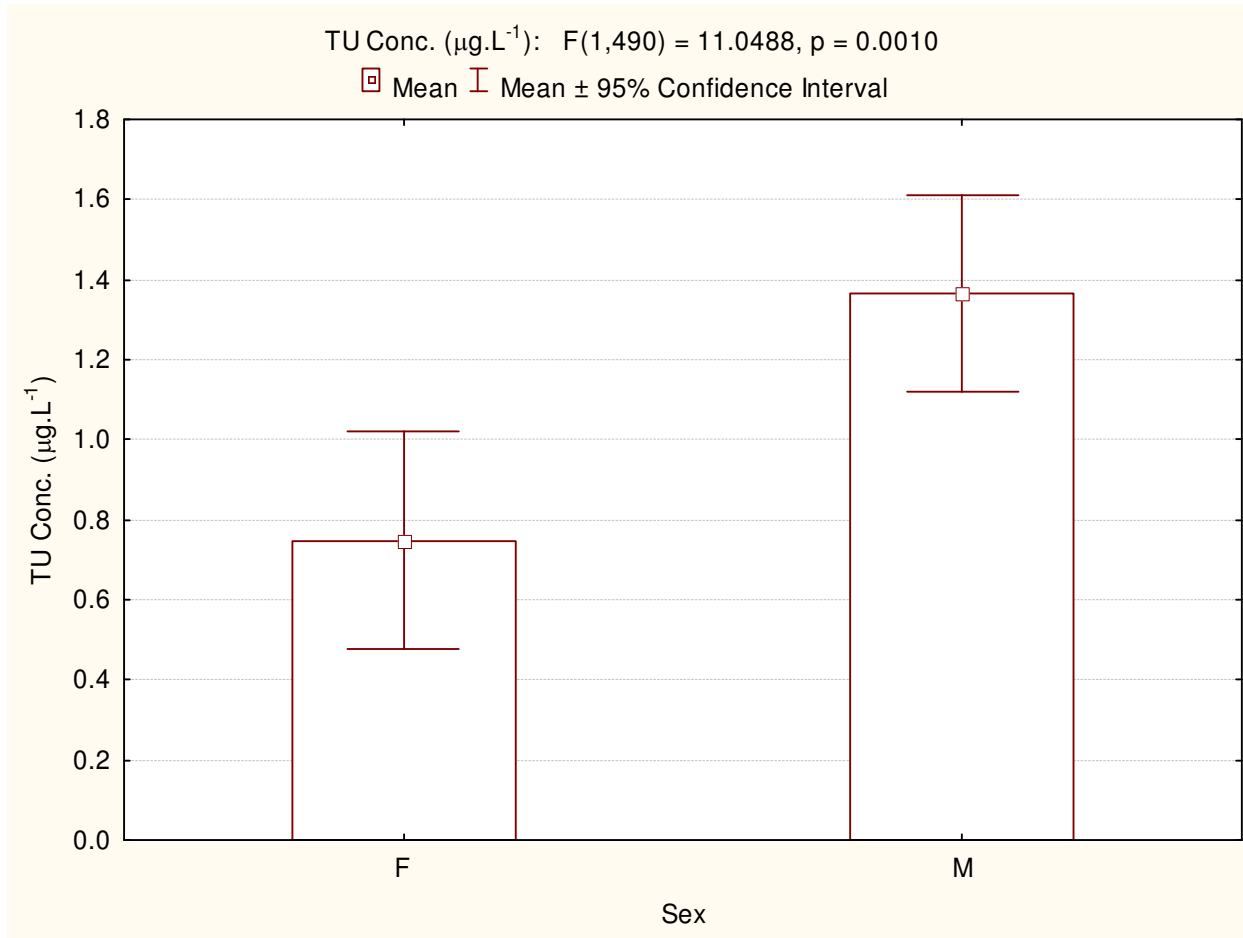
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