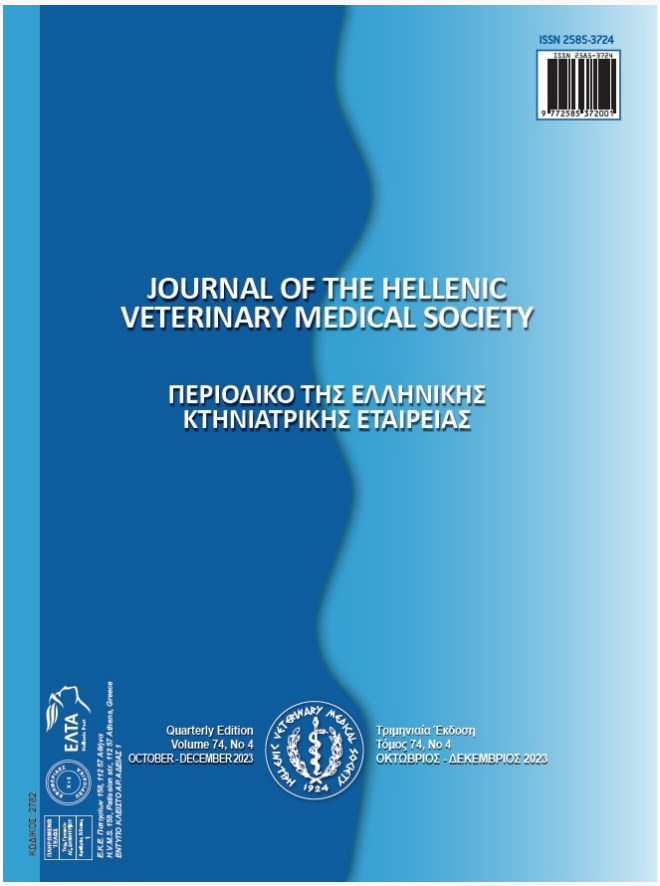
Journal of the Hellenic Veterinary Medical Society

Vol 75, No 1 (2024)



Determination of Zearalenone and their metabolites in sheep urine by ELISA Method.

S Teqja, J Mavromati

doi: 10.12681/jhvms.34009

To cite this article:

Teqja, S., & Mavromati, J. (2024). Determination of Zearalenone and their metabolites in sheep urine by ELISA Method. *Journal of the Hellenic Veterinary Medical Society*, *75*(1), 7019–7026. https://doi.org/10.12681/jhvms.34009

J HELLENIC VET MED SOC 2024, 75 (1): 7019-7026
ΠΕΚΕ 2024, 75 (1): 7019-7026

Research article
Ερευνητικό άρθρο

Determination of Zearalenone and their metabolites in sheep urine by ELISA Method

S. Teqja[1*], J. Mavromati[2]

[1]*Laboratory of Toxicology and Veterinary Drug Residues, Department of Toxicology, Food Safety and Veterinary Institute Tirana, Albania*

[2]*Department of Veterinary Public Health, Faculty of Veterinary Medicine, Agricultural University of Tirana, Albania*

ABSTRACT: The Fusarium metabolite mycotoxin zearalenone (ZEA) is of concern because of its lifelong estrogenic effects in animals. Zeranol as its metabolite was a widely used in animals as growth promoters to increase body weight. But, the use of zeranol as a growth promoter in animals that produce products for human consumption has been banned in the European Union (EU). As no data exist on the occurrence of zearalenone and zeranol in sheep urine in Albania we have analyzed samples of them by ELISA method. The method is based on a competitive enzyme immunoassay, ELISA for the quantitative analysis of zearalenone and its metabolites in urine. The overall recoveries of the analysis were in range of 80%-103%. Eighty nine (n=89) sheep urine samples were taken in the study during the last 2 years until now. Eighty (n= 80) samples were found to be compliant below the Screening Target Concentration (Cut-Off) value (0.404 - 0.839 ng/ml). Nine (n=9) samples were found to be suspect with the ELISA method above the Cut-Off value. After analyzing the suspect samples with the UPLC-MS/MS confirmatory method, two (n=2) sheep urine samples were positive above decision limit for confirmation (CCα). In the first sample concentration found is 1 and 1.5 in the second sample 3 and 4.5 μg/kg for α-Zearalanol and β-Zearalanol respectively. The high levels of α-Zearalanol and β-Zearalanol concentrations found in urine indicate a zearalenone contamination of animal feed. This comes as a consequence of poor animal feed storage and leads to the accumulation of ZEA before harvest time or abuse has occurred. Further studies should be conducted to contribute to the knowledge disposition of zearalenone and α-Zearalanol in animals to give an accurate answer about possible contamination or illegal use.

***Keywords*:** mycotoxins, zearalenone, zeranol, sheep urine, ELISA

Corresponding Author:
Teqja Suela, Laboratory of Toxicology and Veterinary Drug Residues, Department of Toxicology, Food Safety and Veterinary Institute Tirana, Albania. Rruga Aleksander Moisiu NR.82 Tirana, Albania.
E-mail address: teqja.suela@yahoo.com

Date of initial submission: 19-03-2023
Date of acceptance: 01-06-2023

INTRODUCTION

Mycotoxins, which are produced by fungi, are secondary metabolites found in food and feed at all stages of the food chain. Mycotoxin-contaminated cereal grain and animal feed are frequently found throughout the world (Changwon et al., 2020).

Zearalenone it is mainly formed pre harvest but its synthesis might continue under poor storage conditions. The climatic conditions during plant development prior to harvest are the major determinants for the ZEA contamination level of feed (Dänicke et al., 2015).

ZEA is typically detected in high levels in samples of natural animal feed, because of their improper storage and lead to zearalenone accumulation before the harvest time (Zhang et al., 2018; N. D. Krout-Greenberg, 2013).

The Fusarium toxin zearalenone (ZEA) is of concern due to its pronounced estrogenic effects in mammalian species. ZEA contaminates various grain-based food and cereal along with modified forms which contribute to overall mycoestrogen exposure (Nurshad et al., 2019).

Resorcylic acid lactones (RALs) and their structural are shown in the picture below:

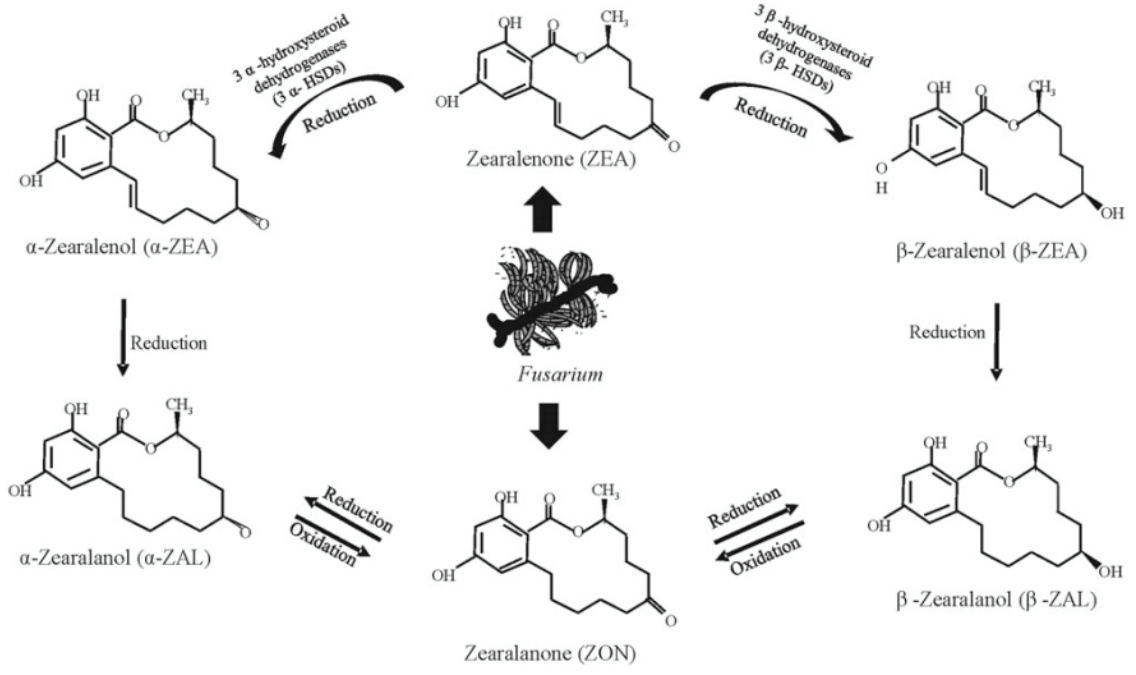


Figure 1. Zearalenone (ZEA) and it's metabolites (ZON, α-ZEA, β-ZEA, α-ZAL, β –ZAL) (Rai, et al., 2019)

These compounds are heat resistant and are difficult to inactivate and remove during cooking or processing (Yin et al., 2020).

Zearalenone was known to be a toxic substance of extensive concern to livestock. Decades later it was found that zearalenone is an oestrogen agonist (Jana Nandan, 2018).

In the literature, a wide variety of clinical effects attributed to zearalenone have been described. Abnormal estrus cycles, swollen vulvas, reduced milk production, decreased fertility, vaginitis and mammary gland enlargement are the most common findings reported in mammalian species. Single or multiple effects have been observed from the aforementioned changes. A change in the estrus cycle can manifest itself in various forms. Irregular, prolonged or skipped heats are commonly associated with zearalenone effects. While these abnormal estrus changes are not exclusively specific to zearalenone toxicity, one should investigate feed related causes when increases in abnormal estrus cycles are observed on farm (Lafayette et al., 2023).

The Fusarium mycotoxin ZEA is of concern because of its lifelong estrogenic effects in animals (Liu et al., 2007; Wang et al., 2007). α-zearalanol is a non-steroidal oestrogenic growth promoter that in-

creases live-weight gain in food animals following implantation. But, the use of α-zearalanol for growth promotion in animals that produce products for human consumption has been banned in the European Union (EU) (Cooper et al., 2002; Launay et al., 2004).

The determination of the banned anabolic substance α-zearalanol and the metabolites taleranol and zearalanone in urine is complicated because the occurrence of the structurally-related mycotoxin zearalenone. The corresponding α- and β-zearalenol metabolites which possess similar estrogenic properties (Bennekom et al., 2002; Bagnati et al., 1991).

In order to contribute to the knowledge disposition of α-zearalanol and other RALs in animals, and to give an accurate method for their detection after possible illegal use or contamination, we describe in this paper a procedure for the analysis of ZEA and its metabolite in urine and report the levels of these substances in sheep urine.

The objective of this study was to assess the risk of mycotoxin ZEA exposure posed to Albania livestock. Random samples (n=89) of sheep urine were collected and analyzed for zearalenone and its metabolites.

Immunoassay methods are considered cost-efficient and easy-to-use with sufficient specificity and sensitivity. This method can satisfy the requirements for rapid detection. ELISA is the most commonly developed immunoassay method for detecting mycotoxins and veterinary drugs (Yin et al., 2020).

As no data exist on the occurrence of zearalenone in urine in Albania we have analyzed zearalenone and its metabolites by ELISA method, as biomarkers of exposure in urines from sheep. The method is based on a competitive enzyme immunoassay ELISA for the quantitative analysis of zearalenone and its metabolites in urine. The overall recoveries of the analysis were in rage of 80%-103%.

MATERIALS AND METHODS

Study area

During the year 2021 seventeen samples were collected, while during the year 2022 seventy two urine samples from sheep were collected. All samples were collected randomly, using official sampling methods.

The samples were taken from different regions of Albania as Berat, Diber, Durres, Elbasan, Fier, Gjirokaster, Korça, Kukes, Lezhe, Shkoder, Tirana, Vlora.

Figure 2. The map of coordinates' from which the samples were taken

Table 1. The coordinates' sampling

Coordinates from the sample collections	
Berat	40.7086° N, 19.9437° E
Tirana	41.3275° N, 19.8187° E
Korçe	40.6141° N, 20.7778° E
Gjirokaster	40.0673° N, 20.1045° E
Elbasan	41.1102° N, 20.0867° E
Durres	41.3246° N, 19.4565° E
Diber	41.5888° N, 20.2356° E
Kukes	42.0756° N, 20.4259° E
Fier	40.7275° N, 19.5628° E
Vlore	40.4661° N, 19.4914° E
Lezhë	41.7861° N, 19.6461° E
Shkoder	42.0693° N, 19.5033° E

The distribution of the samples is presented in the Table 2:

Table 2. Sampling by location

Country	2021	2022
Diber		4
Durres		2
Elbasan	2	4
Fier	2	8
Gjirokastra	1	3
Korça	5	16
Lezhe	1	4
Shkoder	1	3
Tirana	1	5
Vlora	2	14
Berat	2	7
Kukes		2
Total	17	72

Sampling

The urine samples were packed in sterile plastic containers, transported in refrigerated boxes and were labeled with a specific code, then a form was filled out, which contains all the data about the animal from which the sample was taken, such as age, registration number, sampling date, sampling time, sampling method, reason for sampling, transport conditions and farm address. The samples were stored at -20°C until the analysis was performed.

Reagents and Standards

Standards and reagents are provided by the kit (I'screen Zeranol).

Reagents not provided by the kit:

alpha-Zearalanol CAS Nr.26538-44-3(Sigma Aldrich)

alpha-Zearalenol CAS Nr.36455-72-8(Sigma Aldrich)

beta-Zearalenol CAS Nr.71030-11-0(Sigma Aldrich)

beta-Zearalanol CAS Nr.42422-68-4(Sigma Aldrich)

Zearalanone CAS Nr.5975-78-0(Sigma Aldrich)

Zearalenone CAS Nr.17924-92-4(Sigma Aldrich)

Helix Pomatia β-glucuronidase (Sigma Aldrich)

Methanol (Sigma Aldrich)

Sample Extraction

0.5 ml of urine sample was dilute with 2.5 ml of sodium acetate buffer 50 mM pH 4.8. Helix Pomatia β-glucuronidase 10 μl was added. Incubation for 2 h at 37°C was performed. Solid Phase Extraction (SPE) procedure was done for purification of urine samples as follow. C18 columns were equilibrate with 3 ml methanol, 2 ml Tris HCl / methanol. After that samples of urine were applied through the column. Columns were washed 2 ml Tris HCl / methanol and 2 ml of methanol 40%. Columns were completely dry for 2 minutes, then elute with 1 ml of 80% methanol. Eluate was evaporating to dryness under a stream of nitrogen/air. Residues were dissolved in 0.5 ml of Dilution Buffer.

Zearalenone and its metabolites analysis

Urine samples were analyzed using an Enzyme immunoassay ELISA kit (I'screen Zeranol), following exactly the instructions of the manual included in the kit.

Calibration curve in solvent in the range 0 - 3 ng/ml was used to calculate the concentration in ng/ml of ZEA and its metabolites as shown in the figure below.

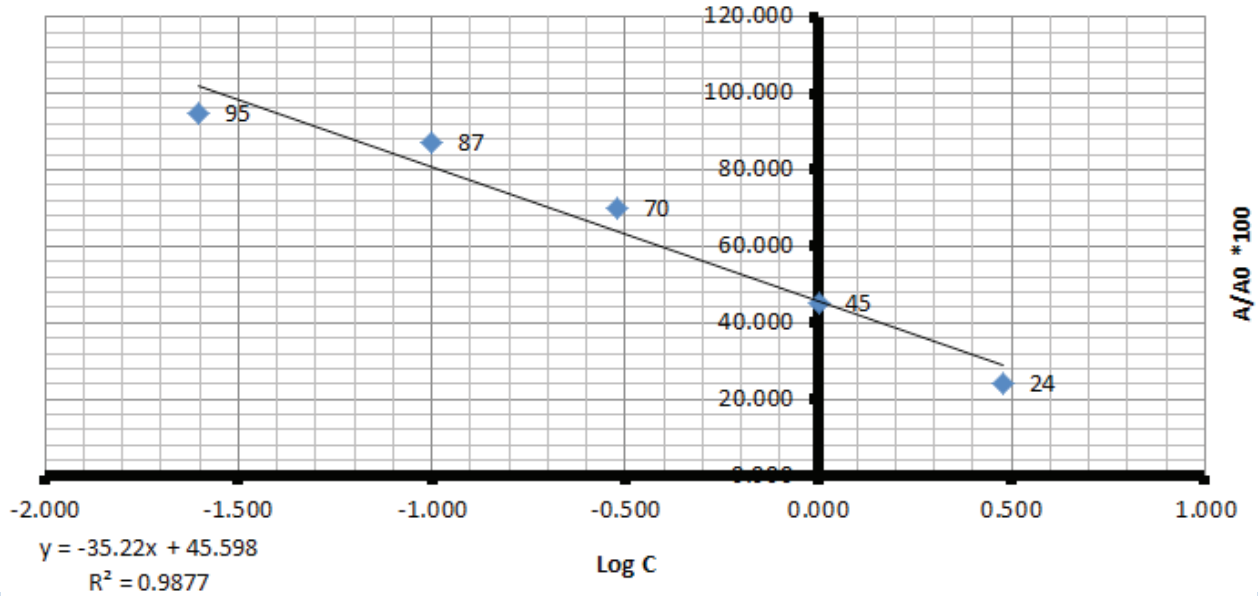


Figure 3. The standard curve in a semi-logarithmic system.

Statistical analyses

The data were statistically analyzed using Excel 2013, the T-test was performed to compare values, and Paired Two Sample for comparison of variances was utilized. Analysis of variance, and simple regression analysis were also applied.

Validation procedure

The acceptance criteria of the method are as follows:

Table 3. Assay specifications

Criterion	Range
Mean A_0 absorbance (per manufacturer)	≥ 0.7 OD 450_{nm}
A/A_0 50% (per manufacturer)	0.22-0.64 ng/ml
Detection limit (per manufacturer)	0.25 ppb

After performing the analysis with the ELISA kit, the results were processed only if the acceptance criteria presented in the table above were met.

To calculate threshold value T and the Cut-Off Fm, were analyzed 20 negative quality control samples (QC) and 20 positive quality control samples (QC) spiked at ½ minimum method performance requirements (MMPRs).

T= mean concentration negative QC+1.64*SD

Cut Off FM= mean concentration positive QC-1.64*SD

Cut-Off Fm (Screening Target Concentration)

QC (quality control)

SD (Standard deviation)

The urine sample were spiked at the level 0.5 ng/ml for α-zearalanol, β-zearalanol and 1 ng/ml

Zearalenone, Zearalanone, α-zearalenol, β-zearalenol, following the (EURL_MMPR_guidance, 2020).

All the samples concentration found above the Screening Target Concentration Cut-Off FM value were declared suspected samples. All the suspected samples were transported to CER Group to be analyzed with RALs confirmatory method using UP-LC-MS/MS (Ultra-high performance liquid chromatography-tandem mass spectrometry).

RESULTS

During 2021 seventeen sheep urine samples were analyzed and their concentrations are presented in the figure below.

During 2022 seventy two sheep urine samples were analyzed and their concentrations are reflected in the figure below.

Table 4. Validation parameters for zearalenone and its metabolites

Item	Zearalenone	Zearalanone	α-zearalenol	β-zearalenol	α-zearalanol	β-zearalanol
Spike level	1 ng/ml	1 ng/ml	1 ng/ml	1 ng/ml	0.5 ng/ml	0.5 ng/ml
Mean calculated	1.077 ng/ml	1.025 ng/ml	1.137 ng/ml	1.050 ng/ml	0.492 ng/ml	0.535 ng/ml
Recovery	82%	87%	88%	80%	95%	103%
SD	0.243	0.113	0.249	0.237	0.054	0.046
Cut offFm (ng/ml)	**0.679**	**0.839**	**0.728**	**0.662**	**0.404**	**0.460**

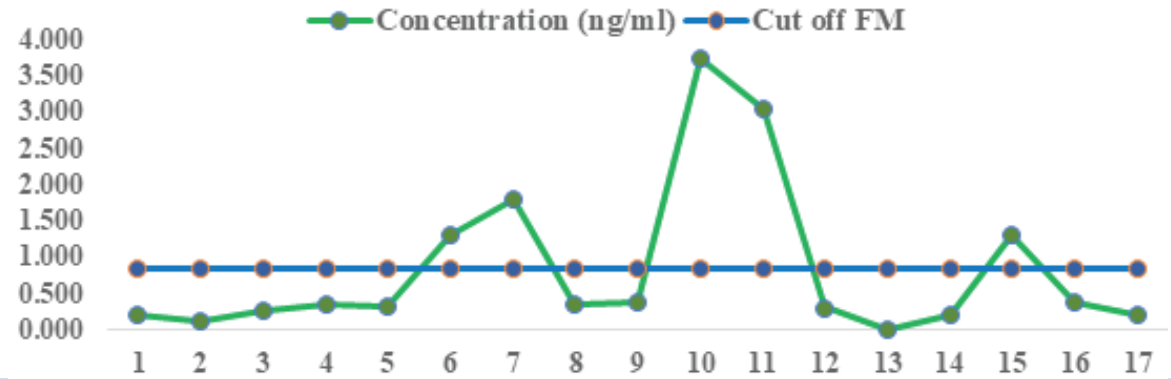


Figure4. Zearalenone and its metabolites concentration in sheep urine during 2021

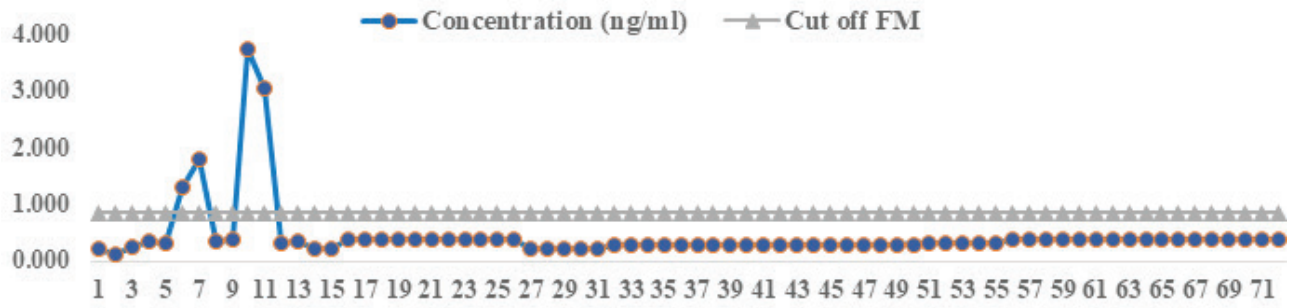


Figure5. Zearalenone and its metabolites concentration in sheep urine during 2022

Table 5, reveals the concentration results of zearalenone and its metabolites evaluated in sheep urine during 2021.

Results (table 5) show that the zearalenone and its metabolites concentration for seventeen samples analyzed in 2021 have turned out to be smaller than the Cut off FM value for twelve samples which are considered as compliant samples.

Zearalenone and its metabolites concentration for five samples have turned out to be higher than Cut off FM value which are considered as suspected samples and were transported to CER Group to be analyzed with RALs confirmatory method using UPLC-MS/MS (Ultra-high performance liquid chromatography-tandem mass spectrometry).

Results (table 6) show the zearalenone and its metabolites concentration for seventy two samples analyzed in 2022, have turned out to be smaller than the Cut-Off FM value for sixty eight samples which are considered as compliant samples.

Zearalenone and its metabolites concentration for four samples have turned out to be higher than Cut-Off FM value which are considered as suspected samples and were transported to CER Group to be analyzed with RALs confirmatory method using UPLC-MS/MS.

Table 5. Reveals the results of zearalenone and its metabolites concentration in sheep urine during 2021

Analytes	Cut off FM (ng/ml)	Tested samples	Compliant samples (≤ Cut off FM)	Suspected Samples (≥ Cut off FM)
Zearalenone	0.679	17	12	5
Zearalanone	0.839		12	5
α-zearalenol	0.728		12	5
β-zearalenol	0.662		12	5
α-zearalanol	0.404		12	5
β-zearalanol	0.460		12	5

Table 6. Zearalenone and its metabolites concentration in sheep urine during 2022.

Analytes	Cut_off(ng/ml)	Tested samples	Compliant samples (≤ Cut_off FM)	Suspected Samples (≥ Cut_off FM)
Zearalenone	0.679	72	68	4
Zearalanone	0.839		68	4
α-zearalenol	0.728		68	4
β-zearalenol	0.662		68	4
α-zearalanol	0.404		68	4
β-zearalanol	0.460		68	4

Table7. The CC alpha values for 6 parameters analyzed.

Analytes	CCα (μg/kg)
Zearalenone	0.91
Zearalanone	0.92
α-zearalenol	0.91
β-zearalenol	0.87
α-zearalanol	0.38
β-zearalanol	0.38

After analyzing the suspected samples with the confirmatory method, two samples were positive for α-Zearanol and β-Zearanol the concentration found and their location are shown in the table below:

Table8. Positive samples concentration.

Number	Region	Sampling Period	Analytes	Concentration (μg/kg)
1	Korca	March 2022	α-zearalanol	1
			β-zearalanol	1.5
2	Korca	March 2022	α-zearalanol	3
			β-zearalanol	4.5

The results found above the CCα were reported to the Albanian authorities.

DISCUSION

Eighty nine sheep urine samples were taken in the study during the period 2021-2022. Two sheep urine samples were positive, in the first sample concentration found are 1 and 1.5 and in the second sample 3 and 4.5 μg/kg for α-Zearalanol and β-Zearalanol respectively.

To protect consumers from these contaminants, most international organizations and countries have set regulations for permissible levels in cattle origin foods while zeranol is banned for use in livestock and must not be detected in cattle origin foods in the EU.

α-zearalanol (zeranol) is a synthetic oestrogenic derivative of the mycotoxin zearalenone, which is produced by Fusarium moulds and a resorcylic acid lactone. Since 1996 its application has been banned in the European Union.

The use of α-zearalanol in animals as a growth promoter is another alternative that causes α-zearalanol residues in animal products. Furthermore, results of examinations of endocrine-disrupting potentials of zeranol were incongruous (Directive of The European Parliament and of the Council 96/22/EC 1996).

The main oestrogenic anabolic compounds that might be used (illegally) as growth promoters in meat-producing animals are α-zearalanol, 17b-oestradiol, ethynyloestradiol (EE2) and diethylstilbestrol (DES) (Arts et al., 1998; Bagnati et al., 1991; Lafayette, 2023; Launay et al., 2004).

The use of α-zearalanol as an anabolic agent in animals has only an insignificant effect on the overall potential human exposure to estrogenic compounds naturally present in our food supply. Due to the very low hormonal activity of α-zearalanol, total dietary exposure does not produce adverse effects on human health (Wang et al., 2007).

ZEA contamination in cereal based food and cereals, pose a significant health risk to animals worldwide and humans, possibly contribute to considerable economic losses (Rai et al., 2019).

Considering the consumer health risk, specific regulations for zearalenone in food stuffs have been establish by European Union (EU). EU legislations defines that maximum permitted limits for ZEA should be 100-200 μg/kg in unprocessed cereals, 20 μg/kg in processed cereal foods, 75 μg/kg for processed cereals and 50 μg/kg in cereal snacks (EFSA 2011).

A tolerable daily intake (TDI) of 0.25 ZEA mg /kg body weight per day was assumed for the consumer (EFSA2011; Rose, 2011).

Guidance values for ZEA, for animal feed intended for sheep are 0.5 mg/kg, it has been recommended under Commission Recommendation 2016/1319/EC (EFSA 2017).

Whereby the frequency of positively detected in urine samples appeared to be higher in spring. Based on the current knowledge on carryover of zearalenone from feed to urine, it can be concluded that foodstuffs of animal origin do not pose a significant risk for the consumer (Dänicke et al., 2015).

CONCLUSIONS

It is concluded that ELISA method is relatively economical and rapid for screening a-zearalanol residues in urine, so it could be used as an alternative for the UPLC-MS/MS method. To investigate the α-zearalanol and its metabolite in routine, screening of a large number of sheep urine samples is necessary.

The presence of both α-Zearalanol and zearalenone is considered as the result of mycotoxin contamination.

The high levels of α-Zearalanol and β-Zearalanol concentrations found in urine indicate a zearalenone contamination of animal feed. This comes as a consequence of poor animal feed storage and leads to the accumulation of ZEA before harvest time.

Animal feed need to be stored in a better way to avoid zearalenone contamination in animal feed and foodstuffs of animal origin.

Whereby the frequency of positively detected in urine samples appeared to be higher in spring. Based on the current knowledge on carryover of zearalenone from feed to urine, it can be concluded that foodstuffs of animal origin do not pose a significant risk for the consumer.

The concentrations of α-Zearalanol found in sheep urine are not sufficient to prove that abuse has occurred. Further studies should be conducted to contribute to the knowledge disposition of α-Zearalanol and other RALs in animals to give an accurate method for their detection after possible contamination or illegal use.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this article.

REFERENCES

Ali N, Degen G H, (2019). Biomonitoring of zearalenone and its main metabolites in urines of Bangladeshi adults. Food and Chemical Toxicology.130: 276–283.

Arts CJM, van Baak MJ, van de Velde-Fase K, Witkamp RF, Elliot CJ, Armstrong HS, Cooper J (1998). Comparison of conventional immunoassays and the oestrogen radioreceptor assay for screening for the presence of oestrogenic anabolic compounds in urine samples. The Analyst. 123(12): 2579-2583.

Bagnati R, Oriundi M P, Russo V, Danese M, Berti F, Fanelli R (1991). Determination of zeranol and β-zearalanol in calf urine by immunoaffinity extraction and gas chromatography—mass spectrometry after repeated administration of zeranol. Journal of ChromatographyB: Biomedical Sciences and Applications. 564(2): 493–502.

Bennekom EO, Brouwer L, Laurant EHM, Hooijerink H, Nielen MWF (2002). Confirmatory analysis method for zeranol, its metabolites and related mycotoxins in urine by liquid chromatography-negative ion electrospray tandem mass spectrometry.AnalyticaChimicaActa. 473(1-2): 151–160.

"COMMUNITY REFERENCE LABORATORIES RESIDUES (CRLs)."https://food.ec.europa.eu/system/files/2016-10/cs_vet-med-residues_guideline_validation_screening_en.pdf[accessed 20 January 2010].

Commission Decision 2002/657/EC - BVLhttps://eurl-residues.eu/wp-content/uploads/2020/12/EURL_MMPR_guidance-paper_final.pdf[accessed September 2020].

Commission Implementing Regulation (EU) 2021/808 of 22 https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32021R0808&-from=EN[accessed 22 March 2021].

Council Directive 96/22/EChttps://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:31996L0022&from=EN[accessed 29 April 1996].

Cooper KM, Ribeiro L, Alves P, Vozikis V, Tsitsamis S, Alfredsson G, Lövgren T, Tuomola M, Takalo H, Iitia A, Sterk SS, Blokland M, Kennedy DG. (2003). Interlaboratory ring test of time-resolved fluoroimmunoassays for zeranol and α-zearalenol and comparison with zeranol test kits. Food Additives and Contaminants. 20(9):804-812.

Cooper K M, Tuomola M, Lahdenperä S, Lövgren T, Elliott C T, Kennedy DG. (2002). Development and validation of dry reagent time-resolved fluoroimmunoassays for zeranol and α -zearalenol to assist in distinguishing zeranol abuse from Fusarium spp. toxin contamination in bovine urine. Food Additives and Contaminants. 19(12):1130-1137.

Dänicke S, Winkler J, (2015). Invited review: Diagnosis of zearalenone (ZEN) exposure of farm animals and transfer of its residues into edible tissues (carry over). Food and Chemical Toxicology. 84: 225-249.

EURL GUIDANCE ON MINIMUM METHOD PERFORMANCE REQUIREMENTS (MMPRs) FOR SPECIFIC PHARMACOLOGICALLY ACTIVE SUBSTANCES IN SPECIFIC ANIMAL MATRICESHttps://eurl-residues.eu/wp-content/uploads/2020/12/EURL_MMPR_guidance-paper_final.pdf[accessed September 2020].

Jana N, Nanda S, (2018). Resorcylic acid lactones (RALs) and their structural congeners: recent advances in their biosynthesis, chemical synthesis and biology. New Journal of Chemistry.

Krout-Greenberg ND, Puschner B, Davidson MG, DePeters EJ. (2013). Preliminary study to assess mycotoxin concentrations in whole corn in the California feed supply. Journal of Dairy Science. 96(4):2705-2712.

Knutsen H-K, Alexander J, Barregård L,Bignami M, Brüschweiler B, Ceccatelli S, Cottrill B, Dinovi M, Edler L, Grasl-Kraupp B, Hogstrand C, Hoogenboom LR, Nebbia CS, Petersen A, Rose M, Roudot AC, Schwerdtle T, Vleminckx C, Vollmer G, Wallace H, Dall'AstaC, Dänicke S, Eriksen GS, Altieri A, Roldán-Torres R, Oswald IP (2017). Risks for animal health related to the presence of zearalenone and its modified forms in feed. EFSA Journal, 15(7).

Lafayette, The Presence, Effect, and Diagnosis of Zearalenone in Dairy Cattle West. "Winter 2003 Newsletter."West Lafayette:406 S. University West Lafayette, IN 47907.

Launay FM, Ribeiro L, Alves P, Vozikis V, Tsitsamis S, Alfredsson G, Sterk SS, Blokland M, Iitia A, Lövgren T, Tuomola M, Gordo, Kennedy DG (2004). Prevalence of zeranol, taleranol and Fusarium spp. toxins in urine: implications for the control of zeranol abuse in the European Union. Food Additives and Contaminants. 21(9): 833-839.

Launay FM, Ribeiro L, Alves P, Vozikis V, Tsitsamis S, Alfredsson G, Sterk SS, Blokland M, Iitia A, Lövgren T, Tuomola M, Gordon A, Kennedy DG (2004). Prevalence of zeranol, taleranol and Fusarium spp. toxins in urine: implications for the control of zeranol abuse in the European Union. Food Additives and Contaminants. 21(9):833-839

Launay FM, Young PB, Sterk SS, Blokland MH, Kennedy DG (2004) Confirmatory assay for zeranol, taleranol and the Fusarium spp. toxins in bovine urine using liquid chromatography-tandem mass spectrometry. Food Additives and Contaminants. 21(1):52-62.

Liu Y, Zhang C, Yu X, Zhang Zh, Zhang X, Liu R, Liu X, Gong Zh (2007) Development and evaluation of immunoassay for zeranol in bovine urine. Journal of Zhejiang University SCIENCE B. 8(12): 900-905.

Miles CO, Erasmuson, A F, Wilkins AL, Towers NR, Smith B L, Garthwaite I, Scahill G, Bryan G, Hansen RP (1996). Ovine Metabolism of Zearalenone to α-Zearalanol (Zeranol). Journal of Agricultural and Food Chemistry. 44(10): 3244-3250.

Rai A, Das M, Tripathi A (2019). Occurrence and toxicity of a fusarium mycotoxin, zearalenone. Critical Reviews in Food Science and Nutrition. 1-20.

Scientific Opinion on the risks for public health related to the presence of zearalenone in food. (2011) EFSA Journal, 9(6): 2197.

Takagi M, Uno S, Kokushi E, Shiga S, Mukai S, Kuriyagawa T, Takagaki K, Hasunuma H, Matsumoto D, Okamoto K, Shahada F, Chenga T, Deguchi E, Fink-Gremmels J (2011). Measurement of urinary zearalenone concentrations for monitoring natural feed contamination in cattle herds: On-farm trials. Journal of Animal Science. 89(1): 287-296.

Wang S, Wang XH (2007). Analytical methods for the determination of zeranol residues in animal products. Food Additives and Contaminants. 24(6): 573–582.

Yang Ch, Song G, Lim W (2020). Effects of Mycotoxin-Contaminated Feed on Farm Animals. Journal of Hazardous Materials. 122087.

Yin M, Hu Xi, Sun Y, Xing Y, Chai Sh, Xing G, Yang Y, Teng M, Li Q, Wang Y, Deng R, Zhang G (2020). The broad-spectrum and ultra-sensitive detection of zeranol and its analogues by an enzyme-linked immunosorbent assay in cattle origin samples. RSC Advances. 10(35):20809-20816.

Liu Y, Zhang C, Yu X, Zhang Z, Zhang X, Liu R, Liu X, Gong Zh (2007). Development and evaluation of immunoassay for zeranol in bovine urine. Journal of Zhejiang University SCIENCE B. 8(12):900-905.

Zhang G-L, Feng Y-L, Song J-L, Zhou X-Sh (2018). Zearalenone: A Mycotoxin With Different Toxic Effect in Domestic and Laboratory Animals' Granulosa Cells. Frontiers in Genetics. Volume 9