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South African Journal of Animal Science 2018, 48 (No. 6)

Determination of perfluorooctanoic acid (PFOA) and perfluorooctane sulfonic acid (PFOS) in livestock feeds

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(Received 6 April 2018; Accepted 22 July 2018; First published online 6 December 2018)

Abstract

The objective of this study was to examine the levels of perfluorooctanoic acid (PFOA) and perfluorooctane sulfonic acid (PFOS) in livestock feeds (calf starter, dairy cattle, fattening cattle, calf growth, lamb starter, lamb growth, dairy sheep, fattening sheep, chick, broiler and layer hen feeds) and feed raw materials (wheat, cottonseed pulp, wheat bran, maize, barley, lentil, wheat straw, bean straw, pea straw and meadow grass). PFOA and PFOS concentrations of 30 livestock feeds, 24 raw feed materials, 9 poultry feeds and 10 water samples were determined by LC-MS/MS. The findings indicated that all the evaluated livestock feeds and feed materials contain perfluorinated compounds. Poultry, cattle, feed raw materials and sheep feed samples were examined for PFOA concentrations and for PFOS concentrations, cattle, poultry, sheep and feed raw materials samples were examined. The highest PFOA concentrations were found in layer hen feed (7.55 µg/kg), dairy cattle feed (6.75 µg/kg) and fattening cattle feed (6.53 µg/kg) respectively. The highest PFOS levels were found in layer hen feed (0.882 µg/kg), calf feed (0.833 µg/kg) and dairy sheep feed (0.830 µg/kg).

Keywords: Feed, feed raw materials, LC-MS/MS, perfluorinated compound
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Introduction

Perfluorinated compounds (PFCs) are a group of synthetic chemicals characterized by their unique properties such as amphiphilicity and high resistance to degradation. Because of their unique features, PFCs are included in a wide range of products and materials such as protective coatings for cloths and carpets, paper coatings, insecticides, paints, cosmetics and fire-fighting foams, among many others (Buck *et al*., 2011). This widespread existence causes inevitable exposure of humans, and life in general, to them.

Dietary intake is a major route of exposure to perfluorinated compounds. PFCs are currently considered as emerging contaminants in the food chain. For this reason, the European Food Safety Authority (EFSA) has set the tolerable daily intakes (TDI) of PFOS and PFOA at 150 ng/kg/day and 1500 ng/kg/day, respectively (EFSA, 2008). Toxicology studies show that PFOS and PFOA are readily absorbed after oral exposure and accumulate primarily in the serum, kidney and liver. No further metabolism is expected (EFSA, 2008). PFOS and PFOA have a long half-life of about 4 years in humans. This continued exposure could increase body burdens to levels that could result in adverse outcomes (ATSDR, 2009).

Since nutrition is the main source of exposure to perfluorinated compounds, many studies have been conducted in recent years to identify perfluorinated compounds in various food products such as fish and beverages (Paiano *et al*., 2012; Squadrone *et al*., 2014; Vassiliadou *et al*., 2015), seafood (Domingo *et al*., 2012; Carlsson *et al*., 2014; Munschy *et al*., 2015), meat (Zhang *et al*., 2010; Hlouskova *et al*., 2013; Perez *et al*., 2014), cereals (Vestergren *et al*., 2012; D'Hollander *et al*., 2015; Ciccotelli *et al*., 2016), eggs (D'Hollander *et al*., 2011; Liu *et al*., 2016; Zafeiraki *et al*., 2016), vegetables and fruits (Herzke *et al*., 2013; Blaine *et al*., 2014; Heo *et al*., 2014), drinking water (Castiglioni *et al*., 2015; Zafeiraki *et al*., 2015; Schwanz *et al*., 2016), milk (Young *et al*., 2012; Eriksson *et al*., 2013; Barbarossa *et al*., 2014) and tea (Haug *et al*., 2010; Zheng *et al*., 2014). In these studies, the highest concentrations for PFOA and PFOS were found in

URL: http://www.sasas.co.za
ISSN 0375-1589 (print), ISSN 2221-4062 (online)
Publisher: South African Society for Animal Science
http://dx.doi.org/10.4314/sajas.v48i6.6

meat (Zhang *et al*., 2010; Vestergren *et al*., 2012; Heo *et al*., 2014), offal (Clarke *et al*., 2010) and other foods of animal origin (Wang *et al*., 2008; D'Hollander *et al*., 2011; Young *et al*., 2012; Barbarossa *et al*., 2014).

There are few studies on the determination of perfluorinated compounds in feeds (Vestergren *et al*., 2013; Kowalczyl *et al*., 2012; Stahl *et al*., 2009). However, in the literature, there is no previous research related to determination of perfluorinated compounds in commercially available livestock feeds. The objective of this study was to examine the levels of PFOA and PFOS in various feeds consumed by cattle, sheep and poultry.

Materials and Methods

Certified standards of PFOS (96%) and PFOA (98%) were purchased from Sigma Aldrich (UK). All chemicals were obtained from Merck (Darmstadt, Germany). All chemicals used were of analytical-reagent grade and were at least 99.5 % pure.

The feed and water samples used in the study were obtained from three different local breeding farms in the Hatay province which is located on the eastern Mediterranean coast of Turkey, at 36° 12' 0.0036'' North and 36° 10' 0.0048'' East geographic coordinates.

About 1 g of sample was homogenized with 5 mL of highly pure Milli-Q water. One mL of 0.5 M tetrabutylammonium (TBA) hydrogen sulphate solution and 2 mL of sodium carbonate buffer (0.25 M, pH 10) were added to 1 mL of the homogenate samples in a polypropylene tube and thoroughly mixed for extraction. Five mL of methyl tert-butyl ether (MTBE) were added to the above mixture and shaken for 20 min. The organic and aqueous layers were separated by centrifugation, and an exact volume of MTBE (4 mL) was removed from the solution. The aqueous mixture was rinsed with MTBE and separated twice; both the rinses were combined in a second polypropylene tube. The solvent was evaporated under nitrogen and replaced with 0.5 mL of methanol. This extract was filtered by a nylon mesh filter (0.2 µm) into an HPLC vial. Extraction blanks were prepared using Milli-Q water (Guerranti *et al*., 2013).

LC-MS/MS analysis was performed on an AB SCIEX 3200 QTRAP system. Betasil C18 column (50 x 2.1 mm i.d. 5 µm) was used. Ten microliters of each extract were injected in the LC – MS / MS with 2 mM ammonium acetate/methanol as the mobile phase starting at 10% methanol. At a flow rate of 300 µL/min the gradient increased to 95% methanol at 10 min before reverting to original conditions at 15 min. Column temperature was maintained at 25 ºC (Guerranti *et al*., 2013).

PFOA and PFOS concentrations were determined by comparing their peak areas with those of standards in LC-MS/MS. All analyses were repeated three times for each sample. All blank values were averaged, and the average value was subtracted from the detected PFOA and PFOS values. The limit of detection (LOD) was determined to be three times the standard deviation of the blank test values. The limit of quantification (LOQ) was taken as three times the LOD (Table 1).

Table 1 The values of retention time, coefficient of determination, limit of detection (LOD); limit of quantification (LOQ), perfluorooctanoic acid (PFOA) and perfluorooctane sulfonic acid (PFOS)

Compound name	Retention time min	Coefficient of determination R^2	LOD ng/mL	LOQ ng/mL
PFOA	2.57	1.000	0.042	0.139
PROS	2.63	1.000	0.042	0.007

As data sets were mostly not normally distributed, average values were calculated by using the Microsoft Office Excel 2007 programme. Values below the LOD were considered equal to zero.

Results

The average PFOA concentrations were found as ranging from 1.19 - 2.63 µg/kg, while PFOS levels were in the range of 0.097 - 0.396 µg/kg in the sheep (ruminant) feed samples. The highest concentrations of PFOA (3.87 µg/kg) and PFOS (0.830 µg/kg) were detected in dairy feed samples and the lowest concentrations n the starter feed samples (PFOA 0.96 µg/kg; PFOS 0.086 µg/kg). Median PFOA and PFOS concentrations in sequence was determined as; dairy sheep, fattening sheep, lamb growth, lamb starter and sheep (ruminant) feeds. Mean concentrations of PFOA and PFOS in cattle feeds were in the range of 2.20 - 4.55 µg/kg and 0.060 - 0.686 µg/kg, respectively. The high concentrations of PFOA were on average found

Table 2A The perfluorooctanoic acid (PFOA) and perfluorooctane sulfonic acid (PFOS) contents of the examined feed raw materials

Feed raw material	Wheat			Cotton seed meal			Wheat bran			Maize			Barley		
	Sample 1	Sample 2	Sample 3	Sample 1	Sample 2	Sample 3	Sample 1	Sample 2	Sample 3	Sample 1	Sample 2	Sample 3	Sample 1	Sample 2	Sample 3
PFOA µg/kg	4.48 ± 0.01	3.36 ± 0.01	3.31 ± 0.01	1.86 ± 0.01	1.85 ± 0.01	1.82 ± 0.01	1.28 ± 0.01	4.15 ± 0.01	2.26 ± 0.01	2.71 ± 0.01	4.37 ± 0.01	3.30 ± 0.01	3.51 ± 0.01	3.50 ± 0.01	2.48 ± 0.01
PFOS µg/kg	0.291 ± 0.001	0.048 ± 0.001	0.112 ± 0.001	0.078 ± 0.001	0.126 ± 0.001	0.094 ± 0.001	0.097 ± 0.001	0.096 ± 0.001	0.102 ± 0.001	0.118 ± 0.001	0.013 ± 0.001	0.092 ± 0.001	< 0	0.067 ± 0.001	0.052 ± 0.001

Feed raw material	Lentil			Wheat straw			Bean straw			Pea straw			Meadow grass		
	Sample 1	Sample 2	Sample 3	Sample 1	Sample 2	Sample 3	Sample 1	Sample 2	Sample 3	Sample 1	Sample 2	Sample 3	Sample 1	Sample 2	Sample 3
PFOA µg/kg	4.17 ± 0.01	3.42 ± 0.01	3.18 ± 0.01	3.63 ± 0.01	3.49 ± 0.01	3.17 ± 0.01	0.98 ± 0.01	0.68 ± 0.01	0.89 ± 0.01	1.16 ± 0.01	1.08 ± 0.01	1.23 ± 0.01	2.70 ± 0.01	2.98 ± 0.01	3.12 ± 0.01
PFOS µg/kg	0.081 ± 0.001	0.044 ± 0.001	0.039 ± 0.001	0.421 ± 0.001	0.214 ± 0.001	0.155 ± 0.001	< 0	< 0	< 0	0.142 ± 0.001	0.110 ± 0.001	0.125 ± 0.001	0.195 ± 0.001	0.188 ± 0.001	0.210 ± 0.001

Table 2B The perfluorooctanoic acid (PFOA) and perfluorooctane sulfonic acid (PFOS) contents of the examined feeds

Mixed Feed	Calf starter			Calf growth meal			Dairy cattle ration			Fattening cattle		
	Sample 1	Sample 2	Sample 3	Sample 1	Sample 2	Sample 3	Sample 1	Sample 2	Sample 3	Sample 1	Sample 2	Sample 3
PFOA µg/kg	1.04 ± 0.01	3.39 ± 0.01	2.18 ± 0.01	2.72 ± 0.01	5.04 ± 0.01	5.88 ± 0.01	2.25 ± 0.01	6.75 ± 0.01	1.28 ± 0.01	6.53 ± 0.01	0.94 ± 0.01	1.57 ± 0.01
PFOS µg/kg	0.578 ± 0.001	0.557 ± 0.001	0.397 ± 0.001	0.833 ± 0.001	0.588 ± 0.001	0.638 ± 0.001	0.042 ± 0.001	0.056 ± 0.001	0.699 ± 0.001	< 0	0.153 ± 0.001	0.026 ± 0.001

Mixed Feed	Lamb starter ration			Lamb growth			Dairy sheep			Fattening sheep		
	Sample 1	Sample 2	Sample 3	Sample 1	Sample 2	Sample 3	Sample 1	Sample 2	Sample 3	Sample 1	Sample 2	Sample 3
PFOA µg/kg	0.96 ± 0.01	1.44 ± 0.01	1.18 ± 0.01	1.45 ± 0.01	1.78 ± 0.01	1.60 ± 0.01	1.72 ± 0.01	2.29 ± 0.01	3.87 ± 0.01	2.52 ± 0.01	1.48 ± 0.01	0.98 ± 0.01
PFOS µg/kg	0.086 ± 0.001	0.108 ± 0.001	0.097 ± 0.001	0.088 ± 0.001	0.123 ± 0.001	0.102 ± 0.001	0.233 ± 0.001	0.126 ± 0.001	0.830 ± 0.001	0.097 ± 0.001	0.420 ± 0.001	0.112 ± 0.001

PFOA: perfluorooctanoic acid; PFOS: perfluorooctane sulfonic acid in dairy cattle rations (6.75 µg/kg), fattening cattle (6.53 µg/kg) and calf growth feed (5.88 µg/kg). While the mean concentrations of PFOA were ranked in sequence; calf growth feed, dairy cattle, fattening cattle and calf starter feeds, the mean concentrations of PFOS were ranked: calf growth feed, calf starter, dairy, cattle fattening and cattle feeds. PFOA concentrations were higher than PFOS concentrations in both sheep and cattle feeds (Table 2A and 2B).

The highest average PFOA concentrations were found in wheat (3.72 µg/kg), lentil (3.59 µg/kg), maize (3.46 µg/kg) and wheat straw (3.43 µg/kg). The highest amounts of PFOS were also identified to be 0.263 µg/kg in wheat straw; 0.150 µg/kg in wheat; 0.126 µg/kg in pea straw samples. PFOS was not detected in any of the bean straw samples.

The PFOA and PFOS concentrations in poultry feeds were identified in concentrations from 0.31 to 7.55 µg/kg and < 0 to 0.882 µg/kg, respectively (Table 3). The mean PFOA concentrations were 1.91 µg/kg in chick feed, 5.30 µg/kg in boiler feed and 6.35 µg/kg in layer hen feed. The average PFOS concentrations for poultry feeds were determined to be between 0.198 and 0.398 µg/kg. For poultry feeds the mean concentrations of PFOA were ranked as the highest in the layer hen feed, followed by broiler feed and chick feed, and the mean PFOS concentrations of were the highest in the chick feed, then layer hen feed and broiler pullet feed (Table 3).

Table 3 The perfluorooctanoic acid (PFOA) and perfluorooctane sulfonic acid (PFOS) contents of the examined poultry feeds

Mixed feed	Chick feed			Broiler feed			Layer chicken		
	Sample 1	Sample 2	Sample 3	Sample 1	Sample 2	Sample 3	Sample 1	Sample 2	Sample 3
PFOA µg/kg	1.55 ± 0.01	3.88 ± 0.01	0.31 ± 0.01	5.25 ± 0.01	5.72 ± 0.01	4.93 ± 0.01	7.55 ± 0.01	6.23 ± 0.01	5.26 ± 0.01
PFOS µg/kg	0.682 ± 0.001	0.405 ± 0.001	0.108 ± 0.001	0.010 ± 0.001	0.351 ± 0.001	0.234 ± 0.001	0.882 ± 0.001	< 0	0.134 ± 0.001

In water samples taken from local fattening farms, the mean PFOA and PFOS concentrations were found to be 1.72 µg/L and 0.063 µg/L, respectively (Table 4).

Table 4 The perfluorooctanoic acid (PFOA) and perfluorooctane sulfonic acid (PFOS) contents examined in water samples

Sample	PFOA (µg/kg)	PFOS (µg/kg)
$Water_1$	1.94 ± 0.01	0.117 ± 0.001
$Water_2$	1.29 ± 0.01	0.0.97 ± 0.001
$Water_3$	1.73 ± 0.01	0.0.45 ± 0.001
$Water_4$	2.71 ± 0.01	< 0
$Water_5$	1.28 ± 0.01	0.058 ± 0.001
$Water_6$	2.05 ± 0.01	0.052 ± 0.001
$Water_7$	1.89 ± 0.01	0.046 ± 0.001
$Water_8$	1.32 ± 0.01	0.108 ± 0.001
$Water_9$	1.30 ± 0.01	0.062 ± 0.001
$Water_{10}$	1.64 ± 0.01	0.041 ± 0.001

Discussion

In the literature not many studies had focused on the concentration of perfluorinated compounds in animal feeds. PFOA concentrations of 29 to 537 µg/kg and PFOS concentrations of 12 to 240 µg/kg were found in the grass samples by Kowalczyk and his colleagues (2012). Furthermore, they detected 857 – 2845 µg/kg of PFOA and 123 – 597 µg/kg of PFOS in hay samples. These values are notably high in comparison to our study. The authors noted that such high results could be due to extensive environmental pollution experienced in Germany in 2006. Vestergren *et al*. (2013) reported that the average PFOS levels in silage were 6.3 ± 2.1 ng/kg and in barley 3.9 ± 1.7 ng/kg. They also identified that the average PFOA levels were 13 ± 4.4 ng/kg in silage and 8.3 ± 2.8 ng/kg in barley. Our average values were higher than the values reported by Vestergren *et al* (2013). Stahl et al. (2009) studied the uptake of PFOA and PFOS from soil into maize, rye grass and wheat, and showed transfer of PFOA and PFOS into the stalks, stems and produce of the plant. The uptake of PFOA and PFOS into the plants was directly proportional to the PFOA and PFOS concentrations in the irrigated soil.

Since there are no studies related to the PFOA and PFOS contents of commercial feeds in the literature we are unable to compare these results to other data.

Several factors play a role in the accumulation of perfluorinated compounds in feeds. Many of the PFCs are soluble in water, and a number of studies have indicated that PFCs are dispersed in the environment through normal hydrological processes (Delinsky *et al*., 2009). To lessen the amount of sludge that is put in landfills, biosolids are often used as fertilizers and applied to pastures where animals graze or where feed crops grown for animals. The transfer of PFCs from biosolids to the soil in amended fields has been observed. Studies have shown that perfluorinated compounds can pass from biosolids to the soil in the range of 0.17-317 ng/g (Washington *et al*., 2010). There are also studies showing that these compounds pass from soil to plants such as maize, spring wheat, oats, barley and perennial ryegrass (Stahl *et al*., 2009; Yoo *et al*., 2011; Renner, 2009). Since the perfluorinated compounds are transported through the food chain, they migrate to animals that eat these plants and then to people who eat these animals or their products.

Plants growing in soils contaminated with highly harmful perfluorinated compounds as a result of environmental pollution may absorb and transfer these harmful compounds to the human food-chain. Although these compounds are not normally an ingredient of commercial feeds, the plants exposed to perfluorinated compounds may be included into livestock feed.

Conclusions

We suggest the reducing of the concentration of perfluorinated compounds in the soil because perfluorinated compounds may be transported into the food chain via herbivorous animals which feed on plants grown in contaminated soils, and can eventually end up in animal products consumed by people.

Acknowledgements

This research (16486) was supported by the Scientific Research Projects Coordination Unit of Mustafa Kemal University. The authors would like to thank the Scientific Research Projects Coordination Unit of Mustafa Kemal University for financial support.

Authors' Contributions

SEÖ participated in designing the study, laboratory analysis, manuscript writing and constructive revision of the manuscript. ŞS was involved in the design of the study data analysis, manuscript writing and interpretation of the data analysis. MK participated in laboratory analysis and manuscript writing.

Conflict of Interest Declaration

The authors declare that they have no competing interests.

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