THE FAT CONTENT OF ANIMAL FEED AND THE RELATIONSHIP WITH THE STUDY OF THE POSSIBILITY OF TRANSFER OF ORGANIC POLLUTANTS IN COW'S MILK

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Abstract

In the animal body, fats facilitate the absorption and accumulation of highly lipophilic organic pollutants. Considering that the presence of organic pollutants in the animal body is a result of the contamination of the administered feed, and considering that milk is a product with a high predisposition to the accumulation of organic pollutants, in order to evaluate the possibility of transfer and the incidence of organic pollutants, the purpose of this paper refers to the determination of the fat content of feed and milk as a preliminary step in the assessment of the possibility of identifying organic pollutants.

By means of the Soxhlet method procedures, the crude fat content was extracted from 21 feed samples and 4 cow's milk samples taken from three farms to be comparatively evaluated according to the incidence of organic pollutants found in the feed and milk samples within each.

The results obtained for the analyzed samples revealed an average crude fat content relative to DM between 0.79–4.64 % for feed and between 35.3–37.3 % for milk, on the F1 farm; 0.94–4.61 % for feed and 29.6 % for milk, on farm F2; 1.22–8.97 % for feed and 29.65 % for milk, on farm F3. Depending on the determined crude fat content, the possibility of identifying organic pollutants in the analyzed matrices from each farm was evaluated: F1–low (L); F2–medium (M); F3–high (H).

Keywords: *organic pollutants, feed, milk, fats*

INTRODUCTION

To achieve growth and productivity performances, but also to maintain and improve biological functions, the animal body needs energy (Kerr et al. 2015). Among the nutrients, lipids represent the most important energy-concentrated component, beneficial for meeting the increased nutritional requirements of different categories of animals.

In the animal body, according to Cherian (2020), the role of lipids from feed includes the provision of essential fatty acids for the body, the positive effects brought by energy intake being focused especially on growth performance (Cetingul et al. 2008; Cherian, 2020), on improving productivity by increasing the digestibility of feed and increasing the efficiency of feed conversion and utilization (Kerr et al. 2015), supporting reproduction, reducing the amount of methane formed during ruminal fermentation (Zubieta et al. 2021) and role in transportation and absorption of different compounds in the body (Pop et al. 2006; Erickson et al. 2020).

In fodder, lipids are found in the form of simple substances, compounds or as lipid derivatives, with the exception of concentrated fodder from oilseeds, whose fat proportion can vary between 30–50 % (Pop et al. 2006; Harvatine, 2017), most other animal feed sources being low in lipids (Pop et al. 2006; Cetingul et al. 2008).

Despite many positive roles, in the animal body, the intake of lipids can also have negattive effects because lipids can represent "accumulation deposits" for polluting substances with high lipophilicity (EFSA, 2005; Tao et al. 2009), the main way of pollutants go through the animal or human

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The manuscript was received: 07.10.2022
Accepted for publication: 02.11.2022

body, being the consumption of feeds and foods in which they can be found.

In recent years, related to the contamination of the human body with organic pollutants, the consumption of food of animal origin has been considered one of the main way of contamination (Kim et al. 2013; Rodriguez-Hernandez et al. 2015; Bedi et al. 2018; Vasconcelos Rego et al. 2019), in practice, from all productions of animal origin, milk being considered one of the most highly prone to the accumulation of polluting residues, as a result of the fact that most organic polluting compounds prefer substrates rich in fat (Jahed Khaniki, 2007). Given that during the study of persistent organic pollutants, authors such as Lake et al. (2013); Tremolada et al. (2014) highlighted in their work that any detection of persistent organic pollutants in the animal body or in their productions indicates a massive contamination of the administered feeds, thus it was considered that animal feed is the main source of milk contamination.

For these reasons, in order to be able to evaluate the possibility of transfer of organic pollutants from feed in cow's milk and to determine their incidence, the purpose of this paper refers to the characterization of feed and milk by fat content as an important preliminary step in the evaluation the possibility of identifying organic pollutants in the analyzed matrices.

On this point, for the future determination of organic pollutants from feed and milk samples, in this paper, was determinated the crude fat content of feed and milk samples taken from three dairy cow farms with home-grown feed and with different potential levels of pollution. Depending on the determined crude fat content was evaluated the possibility of identifying organic pollutants in the analyzed matrices from each farm.

MATERIAL AND METHOD

Sample collection

The determination of the fat content was carried out on a total of 25 feed and milk samples collected during the years 2021–2022 from three dairy cow farms located in the NE area of Romania, selected according to the expected level of pollution in their geographical area.

After collecting three partial samples for each matrix type, a total of 21 feed samples (wet, dry, pickled, concentrated and combined) and 4 milk samples were analyzed. Regarding these, the characterization of farms and samples and the coding used were described in Table 1.

In order to obtain representative samples, the collection and preparation of samples for analysis was carried out by referring to the general rules provided in the standards, but also by referring to different methods adapted according to various authors (Piskorska–Pliszczynska et al. 2017; Bedi et al. 2018 ; Miclean et al. 2019). In order to ensure the quality and reproducibility of the analyses, the sampling and preparation procedures of the samples were executed with the necessary precautions so that the applied methods and techniques do not influence the characteristics of the samples and prevent their potential contamination.

The collection and preparation of feed samples was carried out in accordance with the rules provided in the SR EN ISO 6497:2005 standards; SR EN ISO 6498:2012 and with the provisions of Regulation (EC) 152/2009–Annexes I–II. Fodder sampled from the three units was taken from covered storage areas, silos, warehouses or directly from the field (in varying quantities, depending on the type of fodder) and packed in plastic bags or paper bags, as appropriate, labeled and transported to the laboratory in preparation for analysis. Depending on the type of fodder, the samples were prepared for analysis by different procedures: drying at 60 ^{o}C in an electric oven (model ESAC-100), shredding (1–2 cm) or grinding in an electric mill (model Grindomix GM 200); the samples brought to fine powder were stored in aluminum bags, until the determinations.

Table 1 Origin and description of samples for analysis

Farm	Animal feed				Milk	
	Sample			Quantity *(kg)*	Sample	
F1 Farm ***Sadova, Suceava county*** - herd: 40 - feeding: seasonal ration - no sources of pollution	F1NH	Natural hay		1,5 – 2	F1M1	winter season
	F1CS	Corn silage		2 – 3		
	F1P	Pasture		2 – 3	F1M2	summer season
F2 Farm ***Rediu, Iași county*** - herd: 55 - feeding: single ration - rural area, location on the dominant direction of the wind from urban area	F2AF1	Alfalfa fresh–plot 1		2 – 3	F2M	-
	F2AF2	Alfalfa fresh–plot 2		2 – 3		
	F2AFH	Alfalfa hay		1,5 – 2		
	F2CFr	Corn fresh		2 – 3		
	F2CS	Corn silage		2 – 3		
	F2C	Corn		0,5 – 1		
	F2S	Soya meal		0,5 – 1		
	F2Mix	Mixed feed		1 – 2		
F3 Farm ***Dancu, Iași county*** - herd: 400; - feeding: single ration; - urban areal, location in the vicinity of industrial activities, airline, car traffic, waste incinerator	F3AF	Alfalfa fresh		2 – 3	F3M	-
	F3AFH	Alfalfa hay		1,5 – 2		
	F3AFS	Alfalfa silage		2 – 3		
	F3CFr	Corn fresh		2 – 3		
	F3CS	Corn silage		2 – 3		
	F3C	Corn		0,5 – 1		
	F3T	Triticale		0,5 – 1		
	F3BrG	Brewers grains		1 – 2		
	F3S	Șoya meal		0,5 – 1		
	F3Mix	Mixed feed		1 – 2		

Milk samples were collected according to the methods described by Rațu and Usturoi (2019), by taking average samples of 500–1000 ml of milk, directly from storage containers, milking being done mechanized in all three farms. Using a thermal bag, the average samples packed in labeled bottles were transported at temperatures of 4-6 ºC to the laboratory for analysis.

The preparation of the samples for the analysis was carried out according to STAS 6343-81 by homogenization of the milk and bringing the samples to 20±2ºC, immediately before the analysis.

Laboratory analysis

The determination of crude fat in feed samples was carried out according to SR ISO 6492:2001/Ac: 2016, respectively according to the procedures of the Soxhlet method, by extracting the crude fat from the samples with ethyl ether, using in this sense a Soxhlet extraction device attached to a thermo-adjustable electric battery.

Approximately 2–3 g of the sample, previously brought to constant temperature, by maintaining for 5 h at 103±2 ºC, was introduced into the extraction cartridges. The cartridges with samples were inserted into the extraction tube and drops of ether condensed on the refrigerant fell continuously over the samples. The extraction has a total duration between 8–10 h and at the end of the stage, the cartridges are removed and maintained for 1 h in the oven at 103±2 ºC.

The calculation of the crude fat content of the analyzed samples was made by the difference between the initial mass of the cartridges with samples and their post-degreasing mass, corresponding to relation (1). Relative to the dry matter of the analyzed samples, the expression of the results was given by the relation (2).

Fat (%)
$= (m_1 - m_2) / (m \times 100)$ *(1)*

Fat (% DM)
$= [(m_1 + m_2) \times 100 / m (100 - U_a)] \times 100$ *(2)*

m_1 = the initial mass of the cartridge +sample (g);
m_2 = mass of the cartridge + sample after degreasing (g);
m = sample mass (g).

The determination of crude fat in milk samples was carried out following the procedures of the Gerber acid-butyrometric method. 10 mL of H_2SO_4 (ρ=1.817 g/cm^3), 11 mL of well-homogenized milk and 1 mL of isoamyl alcohol (ρ=0.810 g/cm^3) were introduced into a butyrometer, after which the contents were vigorously stirred until the mixture formed was brown and homogeneous. The butyrometer with the mixture thus formed was inserted into the centrifuge (Nova Funke Gerber model) for 4 minutes, at approx. 1000–1200 rpm. After the centrifugation was completed, the butyrometer was kept at +65 ºC for 5 minutes and then the fat content was read on the graduated scale of the butyrometer.

Interpretation

For the values obtained from the laboratory determinations, the primary statistical estimators of position and variation were calculated: the mean values (x), the variance (S^2), the standard deviation (s), the standard deviation of the mean (±SD) and the coefficient of variation V %.

RESULTS AND DISCUSSIONS

Animal feed

The average results regarding crude fat content as an important parameter for the pollutant accumulation and transfer process for the feed samples collected from the three farms were presented in Table 2.

Values between 0.79–8.97 % DM crude fat were highlighted, with particularities for each farm, depending on the complexity of the administered rations.

Table 2 Mean ± SD values (% DM) of crude fat content of analyzed feeds samples

Farm	Sample (n=5)	Mean ± SD	Range	V (%)	
F1	F1NH	0.79 ± 0.05	0.66 – 0.94	16.13	*
	F1CS	3.52 ± 0.12	3.15 – 3.68	8.11	ns.
	F1P	2.64 ± 0.08	2.36 – 2.79	2.23	ns.
F2	F2AF1	3.39 ± 0.16	3.83 – 3.76	3.95	ns.
	F2AF2	3.67 ± 0.10	3.40 – 4.01	6.65	ns.
	F2AFH	1.69 ± 0.19	1.27 – 2.21	26.09	**
	F2CFr	2.79 ± 0.17	2.33 – 3.20	13.71	*
	F2CS	2.72 ± 0.12	2.41 – 3.13	10.26	*
	F2C	4.61 ± 0.04	4.45 – 4.69	2.03	ns.
	F2S	0.94 ± 0.06	0.81 – 1.13	14.84	*
	F2Mix	2.41 ± 0.02	2.36 – 2.46	1.85	ns.
F3	F3AF	2.35 ± 0.13	2.10 – 2.84	12.77	*
	F3AFH	1.81 ± 0.07	1.62 – 2.05	8.78	ns.
	F3AFS	2.04 ± 0.06	1.83 – 2.25	7.38	ns.
	F3CFr	2.54 ± 0.16	1.89 – 2.78	14.66	*
	F3CS	3.63 ± 0.09	3.45 – 3.90	5.71	ns.
	F3C	4.30 ± 0.07	4.14 – 4.56	3.91	ns.
	F3T	1.63 ± 0.12	1.32 – 2.04	17.46	*
	F3BrG	8.97 ± 0.17	8.67 – 9.62	4.27	ns.
	F3S	1.22 ± 0.07	1.10 – 1.50	13.90	*
	F3Mix	2.92 ± 0.07	2.73 – 3.15	5.96	ns.

ns.= no differences; * average differences; ** semnificative differences; V coefficient of variation; ± SD standard deviation

With the exception of brewers grains (F3B), for which was obtained a DM crude fat content of 8.97±0.17 %, the average values for the crude fat content did not exceed 2–4 % for the other feeds.

In general, for the green fresh fodder (F1P; F2AF1; F2AF2; F2CFr; F3AF; F3CFr) and for the pickled fodders (F2CS; F3CS; F3AFS) were highlighted average values between 2.35±0.13–3.67±0.10 % crude fat DM, respectively 2.04±0.06–3.63±0.09 % crude fat DM, these types of feed being especially associated with the rations from F2 farms and F3.

Lower values of crude fat content were reported for the forage samples, especially for the natural hay (F1NH) samples from the F1 farm, for which were obtained values between 0.66–0.94 % DM crude fat (0.79±0.05 % mean±SD), as well as for the concentrate feed samples, respectively the F2S soybean meal samples from the F2 farm, for which were obtained values between 0.81–1.13% crude fat DM (0.94±0.06 % mean±SD) and the F3S soybean meal samples from the F3 farm, for which were obtained values between 1.10–1.50 % crude fat DM (1.22±0.07 % mean±SD).

Compared to the literature, the average values regarding the crude fat content of the feed samples from the three farms (F1, F2, F3) were consistent with the average values obtained in similar research by Pop et al. (2006); Stanton et al. (2010); Donosă (2011); Van Saun (2013); Coșman et al. (2018); Simeanu et al. (2019) for the same parameter and for the same sample type.

The analysis of the relative variability of the results compared to the mean has indicated generally a homogeneity of the results obtained (ns.; V % <10) for an important proportion of the samples analyzed, for which were not identified differences. For another major proportion of the analyzed samples, the variability relative to the mean has indicated an average homogeneity of the results obtained (*; 10< V % <20) and only for a single sample (F2AFH) were identified differences between the results obtained for the same sample (**; V% >20; V=26.09).

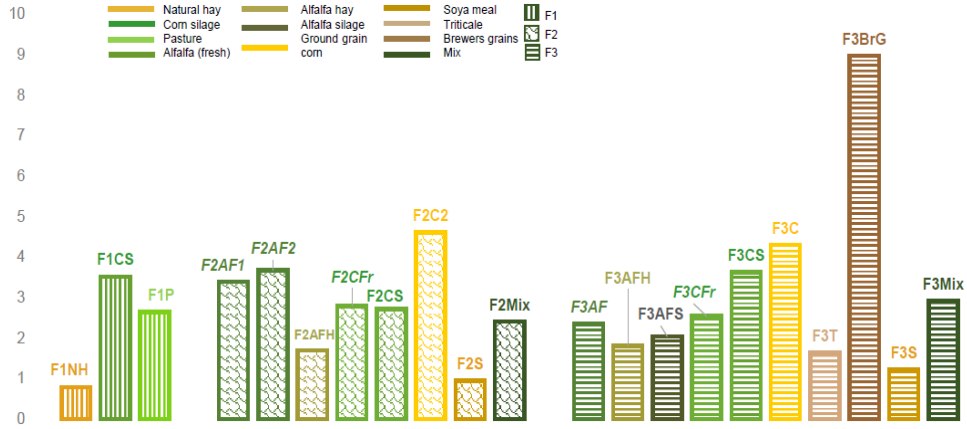

Fig. 1 Comparative values of the crude fat content in analyzed feeds (% DM)

The comparative analysis of the crude fat content of the feed samples was made (Figure 1) highlighting the value differences between the three analyzed farms (F1; F2; F3). For the assessment of the bioaccumulation potential of the pollutants in feed and their transfer into the animal body, the average results obtained were related to the percentage composition of the sample compared to the administered ration, presented in Figure 2.

The analysis of the crude fat content of the feed mix (F2Mix; F3Mix) shows that the fodder that compose the ration of the animals from the F3 farm were highlighted as having a richer crude fat content (2.92±0.07 % mean ±SD) than the fodder that compose the ration

of the animals from the F2 farm (2.41±0.02% mean±SD), an aspect due both to the complexity of the F3 ration compared to the F2 ration, but also highlighted by the fact that between the fodder common of both rations (F2–F3), such as alfalfa hay (AFH), corn silage (CS) or soybean meal (S), the crude fat content values obtained from the fodder analysis in F3 were higher than those obtained from the fodder analysis from F2 (AFH: 1.81% compared to 1.69%; CS: 3.63% compared to 2.72%; S: 1.22% compared to 0.94%).

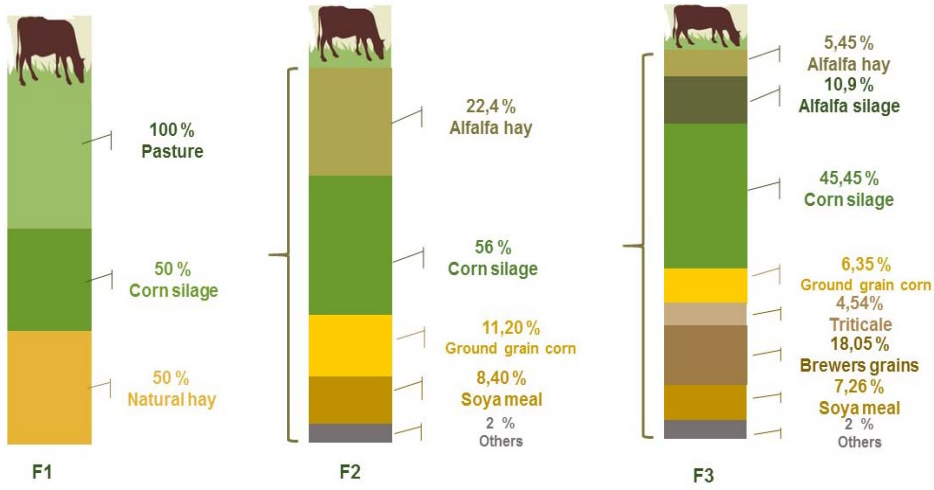

Fig. 2 Feed percentage composition from the administered rations from the analyzed farms

The lowest values of crude fat content at a comparative level between farms, were identified for the feeds administered to the animals of the F1 farm. Related to the particularities of the pollutants, respectively to their predilection for substrates rich in fats, depending on the average contents of fats found in the analyzed fodder, it can be appreciated that for the F3 farm the potential level of pollution could be the highest (H=High), followed by farm F2 (M=Medium), with a medium potential level of pollution and finally farm F1, where the potential level of pollution can be considered minimal (L=Low).

Milk

The analyzed milk samples (F1M1, F1M2; F2M; F3M) revealed an average crude fat content between 29.61±0.53–37.30±0.25 % DM, the results being presented in Table 3.

Table 3 Mean ± SD values (% DM) of crude fat content of analyzed milk samples

Farm	Sample (n=5)		Mean ± SD	Range	V (%)	
F1	F1M1		37.30 ± 0.25	36.49 – 38.11	1.53	ns.
	F1M2		35.38 ± 0.59	33.70 – 37.07	3.76	ns.
F2	F2M		29.61 ± 0.53	28.10 – 31.14	4.05	ns.
F3	F3M		29.65 ± 0.43	28.85 – 31.25	3.31	ns.

ns.= no differences; V coefficient of variation; ± SD standard deviation

For the F1 farm, the crude fat content of the two milk samples was between 36.49 – 38.11 % (37.30±0.25 % mean±SD) for the milk samples from the winter ration (F1M1) and between 33.70–37.07 % (35.38±0.59 % mean±SD) for milk samples from summer ration (F1M2).

The milk samples from the F2 farm were characterized following the determinations as having an average crude fat content between 28.10–31.14 % (29.61±0.53 % mean±SD, while the milk samples from the F3 farm showed an average crude fat content between 28.85–31.25 % (29.65±0.43 % mean ±SD), the analytical results obtained being in accordance with the value range established by the literature, which thus allows the characterization of the analyzed parameter as being within normal limits. Regarding variability, the analyzed milk samples were characterized as homogeneous, for all samples the coefficient of variability V % being less than 10 %.

The comparative analysis shown in Figure 3 regarding the average crude fat content in relation to total dry matter for the four milk samples revealed that the F1M1 milk samples from the F1 farm reported the highest crude fat content, while, the lowest low crude fat content was reported in the case of F2M2 samples.

The analytical values obtained when determining the crude fat content could indicate a potential level of quantitative accumulation of organic pollutants especially in the case of samples with higher fat content, such as samples F1M1 and F1M2, representative of the F1 farm and less in the case samples with lower fat content, such as F2M2 samples and F3M3 samples.

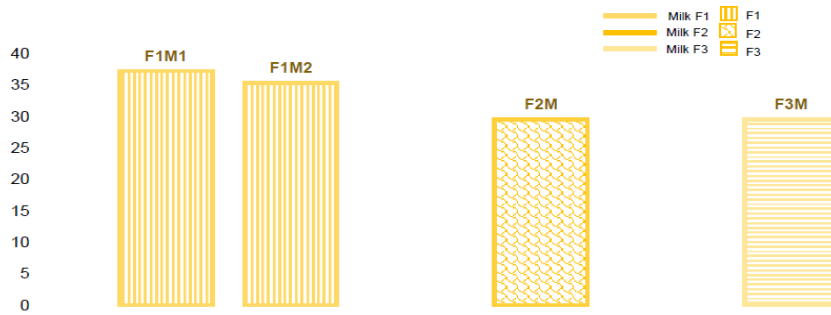

Fig. 3 Comparative values of the crude fat content in analyzed milk samples (% DM)

CONCLUSIONS

On the evaluation of the average crude fat content of feed and milk samples as a preliminary step for the evaluation of the potential for bioaccumulation of pollutants in feed and their transfer to the animal body and to animal productions, the analysis of the relative variability of the results with respect to the mean generally indicated a very good homogeneity for the results obtained.

Related to the preference of organic pollutants for substrates rich in fats, depending on the average fat contents found in the analyzed feeds, it was estimated that at the level of the F3 farm the potential level of pollution could be the highest (H=High), followed by the F2 farm (M=Medium), with a medium potential level of pollution and finally farm F1, where the potential level of pollution could be considered minimal (L=Low).

However, the differences regarding the content between the analyzed samples are too small so that the samples can be classified by risk categories. These things can only be clarified through a quantitative monitoring and quantification of the organic pollutants in the samples through advanced working methods and techniques , this research being only a preliminary evaluation stage of a potential contamination.

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