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COMPREHENSIVE EVALUATION OF LIPIDIC CONTENT IN DRY PET FOOD RAW MATERIALS: COMPARISON BETWEEN FRESH MEATS AND MEAT MEALS

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Abstract

In a balanced diet for companion animals, the lipid component represents an important nutrient and source of energy, moreover it increases the palatability of the food. Dogs and cats are unable to synthesize essential fatty acids (EFAs) needed for their metabolism, therefore, they must be taken with the diet. The majority of dry pet food nowadays on the market are produced starting from fresh meats and meat meals which have a different lipid composition. This study was conducted to analyse the lipid component of the raw materials used for the production of dry pet food, paying particular attention to the polyunsaturated fatty acids (PUFAs), the ω-3 and ω-6, whose presence is fundamental for pet health. The crude fats of both fresh meats and meat meals were analysed by a gravimetric method while the lipid profile was determined by LC/MS-QTOF (Liquid Chromatography/Mass Spectrometry-Quadrupole Time Of Flight) in order to evaluate the lipid component, in terms of saturated, monounsaturated and polyunsaturated fatty acids of the different raw materials used for dry pet food production. The results demonstrated that fresh meats have a better lipid profile, having a higher concentration of PUFAs compared to meat meals, thus making fresh meats the best choice as raw materials for dry pet food production from the lipid point of view.

Key words*: Dry Pet Food, Lipid Content, Saturated Fatty Acids (SFAs), Monounsaturated Fatty Acids (MUFAs), Polyunsaturated Fatty Acids (PUFAs).*

INTRODUCTION

The market of dry pet food is constantly expanding and new formulations are proposed, making it necessary to have a more accurate assessment of the raw materials used in the production process (Zicker, 2008). Pet food should ensure the right supply of nutrients so that the animal can enjoy a good state of health. In this respect, the fats present in dry pet food play a very important role, since many of these are not naturally produced by the body; therefore they must necessarily be included in the regular diet (Bauer, 2008; Lenox, 2016). Some of the main categories of fats that should be present in dry pet food are represented by monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs), ω-3 and ω-6. Their intake ensures normal lipid metabolism and brings numerous beneficial effects on pet health (Ahlstrøm et al., 2004; Granato et al., 2000; Hilton, 1989; Moussa et al., 2000; Watson, 1998; Yaqoob, 2002). A few studies have also disclosed how some fatty acids can have antioxidant effects (Giordano & Visioli, 2014; Richard et al., 2008). It has been shown that the intake of PUFAs brings numerous benefits to both human and animal health (Alessandri et al., 1998; Calder & Yaqoob, 2009; Kouba & Mourot, 2011; Lara et al., 2007; Newton, 1996; Simopoulus, 2001; Sioen et al., 2008). Some of these PUFAs, such as ω-6 linoleic acid, arachidonic acid, ω-3 α-linolenic acid, eicosapentaenoic acid and

docosahexaenoic acid are essential fatty acids (EFAs) for the health of pets (Lenox, 2016; MacDonald et al., 1983; Rivers et al., 1975; Wander et al., 1997; Watson, 1998). PUFAs play a structural role in cell membranes and act as precursors to eicosanoids such as prostaglandins and leukotrienes (Lenox, 2016; Watson, 1998). It has also been shown how nutritional deficiencies of PUFAs are at the basis of pathologies affecting the skin, such as dermatitis, dry and rough coat and dry and itchy skin (Ahlstrøm et al., 2004; Bauer, 1994; Lenox, 2016; Palmquist, 2009; Watson, 1998). PUFAs are also fundamental for the reproductive efficiency of the animal, for renal function and the regulation of the immune system (Alonge et al., 2019; Brown et al., 1998; Brown et al., 2000; Filburn & Griffin, 2005; Hall et al., 2003; Lenox, 2016; Pawlosky & Salem, 1996; Wander et al., 1997). Based on all these findings, it is then clear how a healthy dry pet food should contain a suitable concentration of MUFAs and PUFAs in order to allow pets to enjoy a good state of health.

The majority of dry pet foods nowadays found on the market are produced starting from two different kinds of raw materials: fresh meats and meat meals (Thompson, 2008) (Figure 1).

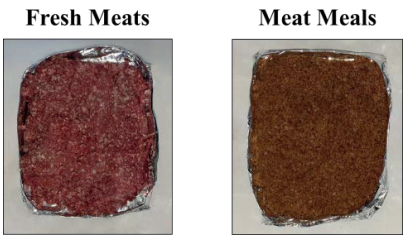

Figure 1. Representation of a type of raw material used in this study

Fresh meats are obtained from the waste of meat intended for human consumption, while meat meals derive from meat by-product processing according to the Regulation (EC) No 1069/2009 of the European Parliament and of the Council of 21/10/2009. These meals are mainly produced by pet food manufacturers to supply protein sources in order to make pet kibbles; however, the intensive industrial process they undergo may cause the onset of oxidation processes and a partial degradation of the raw materials (Camire et al., 1990; Lankhorst et al., 2007; Rokey et al., 2010; Singh et al., 2007; Tran et al., 2008; Williams et al., 2006). In fact, one of the main cause of alteration of pet food is due to the pro-oxidant action of oxygen and light (Piergiovanni & Limbo, 2010), capable of inducing the formation of radical species and Reactive Oxygen Species (ROS) that damage different molecules, lipids included (Lin et al., 1998).

An appropriate storage of raw materials, therefore, seems to be fundamental for the preservation of the nutritional qualities of the food, not least a good selection of the raw materials used for dry pet food production is an essential step for the manufacture companies to obtain better quality products.

The aim of this study is to analyse the lipidic component of the raw materials usually used for dry pet food production. The crude fats of both fresh meats and meat meals were analysed by a gravimetric method. At the same time, a lipidomic analysis was carried out using LC/MS–QTOF (Liquid Chromatography/Mass Spectrometry-Quadrupole Time Of Flight) in order to evaluate the presence and concentration of saturated, monounsaturated and polyunsaturated fatty acids in the different raw materials.

MATERIALS AND METHODS

Raw Materials

Raw materials used in this study are listed in Table 1 and they consist of: chicken fresh meat for companion animal food, 10 batches from pet food manufacturers (Italy), chicken meat meal for companion animal food, 10 batches from pet food manufacturers (Italy); pork fresh meat for companion animal food, 10 batches from pet food manufacturers (Italy), pork meat meal for companion animal food, 10 batches from pet food manufacturers (Italy); salmon fresh meat for companion animal food, 10 batches from pet food manufacturers (Italy), salmon meat meal for companion animal food, 10 batches from pet food manufacturers (Italy).

Table 1. List of raw materials used in this study.

Raw Materials		
Chicken	Fresh meat for companion animal food	10 batches from pet food manufacturers
	Meat meal for companion animal food	10 batches from pet food manufacturers
Pork	Fresh meat for companion animal food	10 batches from pet food manufacturers
	Meat meal for companion animal food	10 batches from pet food manufacturers
Salmon	Fresh meat for companion animal food	10 batches from pet food manufacturers
	Meat meal for companion animal food	10 batches from pet food manufacturers

Drying Procedure

Samples of fresh meats and meat meals were dried according to the method described by da Silva et al. (2018). Briefly, an exact amount of raw material (40 g) was dried in oven (Termaks TS 8136) at 90°C for 6 hours, then it was cooled down at room temperature in an desiccator containing silica gel. Samples were then weighed using OHAUS™ Analytical Balance (Pioneer™) until a stable weight was reached.

Determination of Crude Fat content

An amount corresponding to 1 gram of each dry sample was finely weighed through the use of OHAUS™ Analytical Balance (Pioneer™), and placed in previously weighed glass vials. Diethyl ether was then added to the vials in order to solubilize the lipid component. The samples were then shaken for 15 minutes (Multi Reax, Heidolph) to facilitate the lipid solubilization process. Subsequently, the samples were centrifuged at 6000 × g in order to precipitate the insoluble component. The supernatant was then discarded and the samples were weighed with the vials. The process was repeated until a stable weight was reached. The crude fats, corresponding to the part removed with the solvent, were calculated as the difference between the initial weight and the sample residual weight.

Sample preparation

For the lipid extraction, a quantity corresponding to 100 mg of each dry sample was carefully weighed in an Eppendorf tube and 1 mL of 10 mM Butylated Hydroxytoluene in Methanol/Methyl *tert*-butyl ether/Chloroform (1:1:1) was added. The samples were then shaken 30 minutes at 1500 rpm at room temperature in a Thermomixer (T-Shaker Thermomixers, EuroClone). Subsequently, the samples were centrifuged at 1500 × g for 10 minutes at room temperature (Eppendorf™ 5415D Centrifuge). The supernatant containing lipid fraction of the sample was then recovered (Pellegrino et al., 2014).

To release the fatty acid components of glycerolipids and phospholipids, strong basic hydrolysis was performed. An aliquot of 100 µL of the supernatant, obtained as described above, was transferred into a new 2 mL Safe-Lock Eppendorf tube with 80 µL of a freshly prepared solution of 2% NaOH in Methanol/Water 8:2. The tube was shaken and heated in a Thermomixer at 60°C for 30 minutes. Afterwards, the solution was cooled at room temperature, acidified with 20 µL of 12 M HCl and 1 mL of n-Hexane was added. The tube was vortexed for 10 seconds and centrifuged at 1500 × g for 5 minutes. Finally, 250 µL of the supernatant, containing all fatty acids, was transferred in an autosampler vial for subsequent analysis.

Determination of Fatty Acid content

LC/MS analysis was conducted using an Agilent 6530 LC/MS-QTOF system. Fatty acids were separated using a Kinetex C18 column (4.6 mm × 100 mm, 2.6 µm, Phenomenex) with a 15 minutes linear gradient from 40% to 90% of Acetonitrile/Water 60:40 (Solvent A) and Isopropyl Alcohol (Solvent B), both containing 10 mM Ammonium Acetate. The column operated at 20°C with a flow of 0.8 mL/min.

Liquid Chromatography was interfaced to Mass Spectrometer with an Agilent JetStream source. Mass Spectrometer acquired negative ions in Full-Scan mode in the mass range of 100-1700 with mass accuracy of 1.5 ppm. This was achieved by continuous infusion in the source of a reference mass solution (Agilent G1969-85001). LC/MS raw files were aligned and processed using Batch Recursive Feature Extraction algorithm of MassHunter Profinder (Agilent B.08.00). Afterwards, data with a score > 90% were imported in Mass Profiler (Agilent B.08.01). Fatty Acid Database was downloaded

from LIPID MAPS® Structure Database (LMSD) (Sud et al., 2007) and adapted to work in Agilent Mass Profiler. Only fatty acids with a score > 90% were retained. At the end of the workflow, a matrix data reporting the abundance of the peaks of 40 Fatty Acids (9 saturated, 7 monounsaturated ad 24 polyunsaturated) was created and used to determine lipid content.

Statistical analysis

Data shown in this study, regarding the analysis of the crude fat content and the lipid profile of the raw materials used for the production of dry pet food, are reported as mean values of the ten analysed batches (Table 1) ± standard error of the mean (SEM). The t-Student test was used to investigate the significance of the different lipid content in fresh meats and meat meals. The level of significance for the data was set at $p < 0.05$. All statistical tests were done using GraphPad Prism 6.00 for Windows (GraphPad Software, Inc., San Diego, CA).

RESULTS AND DISCUSSIONS

In this study the crude fat content was initially evaluated for each raw material.

The results shown in Figure 2 represent the average of the crude fat values obtained for each batch of fresh meats and meat meals analysed. The analysis reveals how fresh meats exhibit a significantly higher crude fat content compared to meat meals.

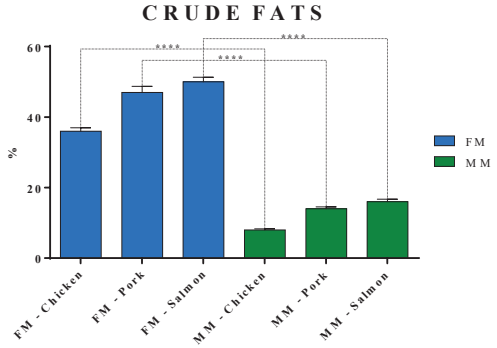

Figure 2. Crude fat content of fresh meat (FM) and meat meal (MM) for companion animal food determined by gravimetric analysis (expressed as percentage values on 100 g of dry sample). Data are reported as mean ± SEM, n = 10, ****$p < 0.0001$

The crude fat level in fresh meats, reported as weight percentage with respect to dry sample weight, ranges from about 36% in the case of chicken to 50% in the case of salmon, whereas a crude fat content lower than 20% is peculiar to all meat meals, reaching a minimum value of 8% in chicken meat meals.

As a rule, the amount of raw fats found in meat meals was always reduced by a factor of at least three compared to the amount found in the corresponding fresh meats. This feature may result from the fact that fresh meats, unlike meat meals, do not undergo treatments and manipulations that can cause loss of the crude fat content.

The crude fat content recommended for dry pet foods is no less than 15% (Case et al., 2010; Rolinec et al., 2016); thus, a deficit of fat concentration in dry pet food is dangerous, because fats are one of the main sources of energy in food and also represent the source of fatty acids (Rolinec et al., 2016). In addition, crude fats also play a key role in contributing to the palatability and the texture of food (Bauer, 2006). In this respect, the results obtained suggest that fresh meats, as well as being healthier due to their adequate lipid content, could also result more palatable for pets.

Subsequently, the fatty acid content in each raw material was evaluated through LC/MS-QTOF. The results reported in Figure 3 show the average of saturated, monounsaturated and polyunsaturated fatty acids present in the different batches of fresh meats and meat meals analysed.

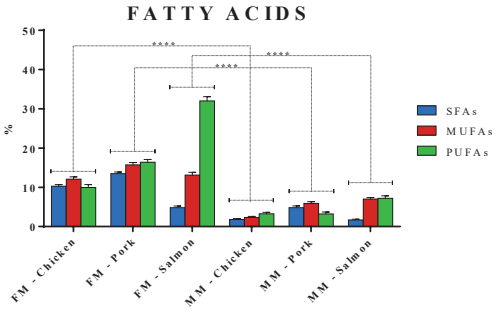

Figure 3. Fatty acid content of fresh meat (FM) and meat meal (MM) for companion animal food determined by LC/MS-QTOF (expressed as percentage values on 100 g of dry sample). Data are reported as mean ± SEM, n = 10, ****$p < 0.0001$

The results are reported as percentage values of each lipid class compared to dry samples. Broadly speaking, the content of saturated, monounsaturated and polyunsaturated fatty acids is significantly higher ($p < 0.0001$) in fresh meats for all the raw materials analysed. Saturated fatty acids (SFAs) are on average about three times more abundant in fresh meats than meat meals, with this ratio being the highest in the case of chicken samples. However, the largest concentrations are recorded for pork fresh meats, where SFAs reach the value of 13.5% relative to the dry sample.

Although fresh meats have been found to have a higher quantity of SFAs compared to meat meals, these fatty acids are mainly long-chain SFAs (LC-SFAs) (Figure 4), *i.e.* between 11 and 20 carbon atoms (C), which are less likely to increase the serum concentrations of cholesterol than very- and ultra-long-chain SFAs (VLC-SFAs and ULC-SFAs), respectively $20 < C \leq 25$ and $C \geq 26$ (Grundy, 1994; Sassa & Kihara, 2014). In fact, all the samples analysed have a concentration of LC-SFAs higher than 90% of total SFAs. In general, all the fresh meats analysed show a statistically significant higher content of LC-SFAs, VLC-SFAs and ULC-SFAs compared to meat meals.

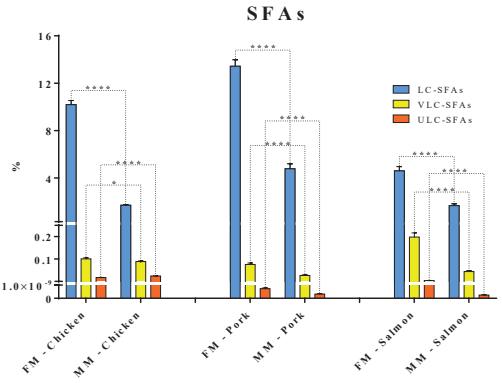

Figure 4. LC-SFA, VLC-SFA and ULC-SFA content of fresh meat (FM) and meat meal (MM) for companion animal food determined by LC/MS-QTOF (expressed as percentage values on 100 g of dry sample). Data are reported as mean ± SEM, n = 10, $*p < 0.05$, $****p < 0.0001$

Similarly, to what described above, even MUFAs are more abundant in fresh meats, with an average abundance about three times higher than the corresponding meat meals (Figure 3). As in the case of SFAs previously analysed, chicken again shows the greatest increase, about five times, compared to meat meals; while the highest MUFA concentration (15.7%) is found for pork fresh meats.

The MUFA content is mostly composed of long-chain fatty acids in all the samples analysed (Figure 5). However, fresh meats, also in this case, show a significantly higher content of LC-, VLC- and ULC-MUFAs compared to meat meals, except for ULC-MUFAs in salmon fresh meats and meat meals whose difference is not statistically significant.

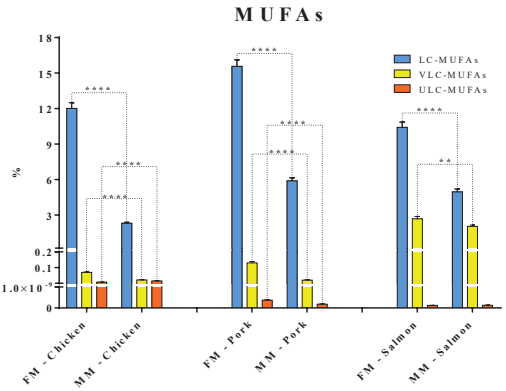

Figure 5. LC-MUFA, VLC-MUFA and ULC-MUFA content of fresh meat (FM) and meat meal (MM) for companion animal food determined by LC/MS–QTOF (expressed as percentage values on 100 g of dry sample). Data are reported as mean ± SEM, n = 10, $**p < 0.01$, $****p < 0.0001$

The LC-MUFA increase is more evident in both fresh meats and meat meals of chicken and pork, where LC-MUFAs are always significantly higher than the 98% of the total MUFA composition. This is beneficial inasmuch as some of them may play a role in lowering serum concentrations of cholesterol and modulating immune functions (Grundy, 1994; Yaqoob, 2002). Moreover, other studies showed that LC-MUFAs have positive effects on cardiovascular health, while VLC-MUFAs seem to have adverse effects (Li et al., 2014).

As far as the total fatty acid composition is concerned, particularly evident is instead the greater content of PUFAs in fresh meats, which is on average more than four times higher than the corresponding meat meals (Figure 3). The

highest concentration of PUFAs is found in salmon fresh meats, reaching 32% of the dry sample weight. This is coherent with the data found in literature according to which fish meats are richer in PUFAs than the other meats, particularly ω-3 fatty acids, which are known for their stimulatory action of anti-inflammatory responses in the case of skin diseases pet (Ahlstrøm et al., 2004; Ricci et al., 2009; Scott & Miller, 1993).

As for chicken and pork fresh meats, higher concentrations of PUFAs are again found compared to the related meat meals. In chicken fresh meats particularly rich in ω-6, the concentration of PUFAs is about 10% of the total weight, while in pork fresh meats it is 16.4%. These findings demonstrate that also for these two raw materials the fresh meat lipid profile is better than that of meat meals, in the light of the innumerable health benefits of PUFAs and for their antioxidant power as well (Alessandri et al., 1998; Giordano & Visioli, 2014; Kouba & Mourot, 2011; Richard et al., 2008; Simopoulus, 2001).

The PUFA carbon backbone length analysis shows a statistically significant higher content of LC-, VLC- and ULC-PUFAs in fresh meats than meat meals in all raw materials analysed; moreover, the LC-PUFA content results to be higher than VLC-PUFA and ULC-PUFA content in all the samples (Figure 6).

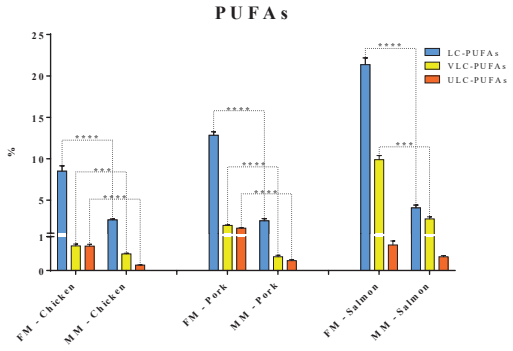

Figure 6. LC-PUFA, VLC-PUFA and ULC-PUFA content of fresh meat (FM) and meat meal (MM) for companion animal food determined by LC/MS–QTOF (expressed as percentage values on 100 g of dry sample). Data are reported as mean ± SEM, n = 10, ***$p < 0.001$, ****$p < 0.0001$

Salmon shows the highest content of LC-PUFAs and VLC-PUFAs in both fresh meats and meat meals, and the highest concentrations of these between health and promoting nutrients are found in salmon fresh meats (Calder & Yaqoob, 2009; Lara et al., 2007; Newton, 1996; Sioen et al., 2008).

These findings are consistent with the literature data that highlight how salmon is particularly rich in LC-PUFAs (Henriques et al., 2014; Sprague et al., 2016; Tocher et al., 2019). PUFAs bring numerous benefits to animal health (Lenox, 2016; MacDonald et al., 1983; Rivers et al., 1975; Wander et al., 1997; Watson, 1998), in fact, LC-PUFAs have cardioprotective, immunoprotective and anti-inflammatory effects (Palmquist, 2009).

The results obtained then show that meat meals have a significantly lower content of all the categories of fatty acids analysed. This could be justified by the treatments and processes employed for meat meal production, which could cause the degradation of the raw material with the loss of important nutrients such as EFAs (Camire et al., 1990; Lankhorst et al., 2007; Piergiovanni & Limbo, 2010; Rokey, 2010; Singh et al., 2007; Tran et al., 2008; Williams et al., 2006).

In this preliminary study, it has been shown how there are significant differences in the lipid content of the diverse raw material used for dry pet food production, which have important consequences in the quality of the final products. These results could help the manufacturing companies to shed light on which raw materials are the best choice for the production of healthier dry foods for dogs and cats.

CONCLUSIONS

Fatty acids, especially PUFAs, are essential in the diet of dogs and cats as they provide energy, modulate inflammation, act as precursors of eicosanoids and prostaglandins, play a structural role in the composition of biological membranes, affect the health of the skin and coat and more generally promote a healthy development of pets. In particular, ω-3 and ω-6 fatty acids are fundamental in animal diet, as pets are unable to synthesize them on their own. This study has shown how the different raw materials used in the production of dry pet food,

fresh meats and meat meals, have a quantitatively and qualitatively different lipid composition.

As opposed to meat meals, fresh meats appear to be the best raw materials that can be used for the production of dry food for pets, both in terms of crude fat content and in terms of MUFA and PUFA content. These results may therefore provide a new approach for the production of better-quality dry pet food, allowing manufacturer companies to better understand how to proceed in the formulation of new products with improved qualities.

In conclusion, this study has clearly shown how fresh meats, from the lipid point of view, appear to be the best choice as raw material for the production of dry food for companion animals.

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