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Gas Chromatography Based Analysis of Fatty Acid Profiles in Poultry Byproduct-Based Pet Foods: Implications for Nutritional Quality and Health Optimization

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The nutritional quality of pet foods plays a crucial role in maintaining the health and well-being of companion animals. In this study, we investigated the fatty acid compositions of various pet foods, including chicken powder, poultry byproducts incorporated vegetables, byproducts incorporated with ragi, and boiled mash Potato, to assess their scientific significance and potential implications for pet nutrition. Our analysis revealed diverse fatty acid profiles across the different pet foods, with each food exhibiting unique compositions and proportions of fatty acids. Notable findings include the identification of common fatty acids shared among multiple pet foods, as well as variations in the abundance of specific fatty acids across different formulations. Additionally, certain pet foods are

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characterized by the presence of rare or unusual fatty acids, highlighting the importance of exploring their sources and potential health benefits. By correlating the fatty acid compositions of pet foods with metabolic pathways and health implications, we elucidated the nutritional significance of these dietary components for pets. Furthermore, we identified areas for further research, including the impact of processing methods on fatty acid profiles and the development of novel formulations optimized for pet nutrition and well-being. Overall, this study contributes valuable insights into the scientific understanding of pet nutrition and underscores the importance of considering fatty acid compositions in formulating balanced and nutritious diets for companion animals.

Keywords: Pet nutrition; fatty acids; gas chromatography; health benefits.

1. INTRODUCTION

The nutritional quality of pet foods is paramount for ensuring the health and well-being of companion animals [1]. As pets become increasingly integrated into households worldwide, there is a growing demand for high-quality pet foods that provide essential nutrients for optimal health [2]. Fatty acids are key components of pet diets, playing critical roles in various physiological processes, including energy metabolism, cell membrane integrity, and immune function [3]. In recent years, there has been a surge in research focusing on the fatty acid compositions of pet foods and their implications for pet health [4]. Gas chromatography has emerged as a powerful analytical technique for precisely quantifying and characterizing fatty acids in complex food matrices. By utilizing gas chromatography analysis, researchers can gain valuable insights into the specific fatty acid profiles of different pet food formulations. In this study, we aimed to explore the fatty acid compositions of a diverse array of pet foods, including Chicken Powder pet food, Poultry Byproducts incorporated with vegetables, Byproducts incorporated with Ragi, and Boiled Mash Potato. Our objective was to assess the scientific significance of these fatty acid profiles and their potential implications for pet nutrition and well-being [5]. Through comprehensive gas chromatography analysis, we investigated the unique fatty acid profiles of each pet food, identifying common fatty acids shared among multiple formulations and uncovering variations in the abundance of specific fatty acids across different products [6]. Additionally, we identified rare or unusual fatty acids present in certain pet foods, highlighting the need for further exploration of their dietary sources and potential health benefits [7]. By correlating the fatty acid compositions of pet foods with metabolic pathways and health implications, we aimed to provide a deeper understanding of the nutritional significance of these dietary components for pets [8]. Furthermore, our study aimed to identify areas for future research, including the impact of processing methods on fatty acid profiles and the development of novel formulations aimed at optimizing pet nutrition and well-being Kumar et al., [4]. Overall, our research contributes valuable insights into the scientific understanding of pet nutrition and underscores the importance of considering fatty acid compositions in formulating balanced and nutritious diets for companion animals. By addressing these knowledge gaps, we aim to promote the formulation of pet foods that support optimal health and longevity in our animal companions.

2. MATERIALS AND METHODS

The experiments were conducted in Department of Livestock Products Technology, College of Veterinary Science and Animal Husbandry, U.P. Pt. Dean Dayal Upadhyaya Pashu Chikitsa Vigyan Vishwavidyalaya Evam Go-Anusandhan Sansthan, (DUVASU) Mathura, 281001(UP), India. The pet food formulations utilized in this study were developed based on the methods described by Kumar et al. [8].

2.1 Samples Preparation for Fatty Acids Profile

The pet food samples, stored under controlled conditions, underwent rigorous preparation by grinding into a uniformly fine powder to ensure sample homogeneity prior to analysis. Fatty acid profiling was meticulously conducted employing gas chromatography, utilizing a sophisticated instrumentation setup comprising a meticulously calibrated gas chromatograph equipped with a flame ionization detector (FID) and a meticulously selected non-polar capillary column coated with 5% phenyl methyl siloxane. Helium gas served as the carrier medium; its flow meticulously regulated at a constant rate of 1 mL/min throughout the analytical process.

Temperature programming was executed with precision, initiating at 50°C for a precisely timed 1-minute duration, followed by a meticulously controlled ramp rate of 5°C/min until reaching the final temperature of 250°C, which was maintained for a duration of 5 minutes to ensure optimal separation and detection of fatty acids. Injection protocols were meticulously adhered to, incorporating a precisely measured injection volume of 1 µL using a splitless injection mode, with injection temperature maintained at a meticulously controlled 250°C to ensure optimal vaporization and injection efficiency. Simultaneous injection of fatty acid standards facilitated accurate identification and quantification, ensuring the reliability and validity of analytical results. Subsequent data processing involved meticulous utilization of advanced chromatographic data analysis software, enabling precise identification and quantification of individual fatty acids based on their characteristic retention times and peak areas.

Sample preparation detail: 1 gram of pet food sample was taken in a glass beaker in which 10 ml HCL and 5-10 ml liquid ammonia gas taken .This sample was digested in water bath at 85 ^{0}Cfor 1.5 hours or till sample was completely digested and turned into brownish black in color. Then the sample was taken out from the water bath and cooled at room temperature. The digested samples were transferred in separating funnel in which 5-10 ml ethanol and 50 ml petroleum ether were added. Samples were shaken to extract the fat followed by addition of water and again shaken to have two separate layers of water and petroleum ether with fat. The procedure was repeated 3-4 times for complex extraction of fat and this petroleum ether layer(pet ether)was separated and collected in a glass beaker. The pet ether was passed through sodium sulfate to remove residual moisture using whatmann filter paper No.1 and finally evaporated on hot air oven at 75^{0}C to get pure fat. Now, minimum 50 mg fat from this pure fat was taken and dissolved in 2 ml hexane in which 0.1-1.0 ml of 2N methanolic KOH was added. Then this sample was vortex in stoppered tubes for 1-2 minutes , again 2ml hexane was added and vortex for 2 minutes. Finally, 1 ml of sample was taken from upper layer of tubes and evaluated for fatty acid profile in Gas chromatography (Shimadzu CG-2014) using capillary column (SH-RTX 2560) of dimension 100 x 0.1 meter, where zero air gas, hydrogen and nitrogen gases were used for resolution of different fatty acid peaks present in sample.

3. RESULTS

In our investigation of the fatty acid compositions within four distinct types of pet foods, namely Chicken Powder pet food , Poultry Byproducts vegetables incorporated pet food , Poultry Byproducts incorporated ragi pet food, and Boiled Mash Potato, conducted through meticulous gas chromatography analysis, each showcasing unique characteristics reflective of their respective formulations.

Fatty Acid Composition of Different Pet Foods: Gas Chromatography Analysis

Table 1. Chicken Powder pet food (M3)

Chicken Powder pet food (M3)	Molecular Weight of fatty acid
Methyl Pentadeccenoate	242.38 g/mol
Methyl Palmitoleate	296.51 g/mol
Methyl Heptadecenoate	270.43 g/mol
Methyl oleate	296.51 g/mol
Methyl Linolelaidate	294.51 g/mol

Table 2. Poultry Byproducts incorporated vegetables pet food (BP3)

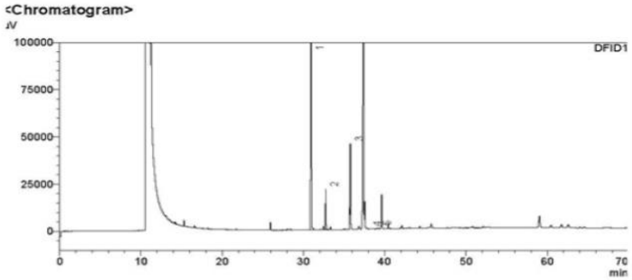
Poultry Byproducts incorporated vegetables pet food (BP3)	Molecular Weight of fatty acid
Methyl Caprylate	144.21 g/mol
Methyl Dodecanoate	200.32 g/mol
Methyl Myristate	228.37 g/mol
Methyl Myristoleate	242.38 g/mol
Methyl Pentadeccenoate	242.38 g/mol
Methyl Palmitate	256.42 g/mol
Methyl Palmitoleate	296.51 g/mol
Methyl Heptadecenoate	270.43 g/mol
Methyl Octadecenoate	296.51 g/mol
Methyl Linolelaidate	294.51 g/mol
Methyl Nervonate	340.65 g/mol.

Table 3. Poultry byproducts incorporated vegetables + ragi (RG2)

Poultry Byproducts incorporated vegetables + ragi (RG2)	Molecular Weight of fatty acid
Methyl Caprylate	144.21 g/mol
Methyl Myristate	228.37 g/mol
Methyl Pentadeccenoate	242.38 g/mol
Methyl Palmitoleate	296.51 g/mol
Methyl Heptadecenoate	270.43 g/mol
Methyl Stearate	298.54 g/mol
Methyl Octadecenoate	296.51 g/mol
Methyl Linolelaidate	294.51 g/mol
Methyl Linoleate	294.51 g/mol
Methyl Linolenate	292.51 g/mol
Methyl Eicosadienoate cis 11,14	306.53 g/mol
Cis-13,16-Docosadienoic acid methyl ester	354.65 g/mol.

Table 4. Poultry Byproducts incorporated vegetables + Boiled Mash Potato(PO2)

Poultry Byproducts incorporated vegetables + Boiled Mash Potato (PO2)	Molecular weight of fatty acids
Methyl Myristate	228.37 g/mol
Methyl Myristoleate	242.38 g/mol
Methyl Pentadeccenoate	296.51 g/mol
Methyl Palmitoleate	270.43 g/mol
Methyl Heptadecenoate	298.54 g/mol
Methyl Heptadecenoate	296.51 g/mol
Methyl Stearate	294.51 g/mol
Methyl Octadecenoate	306.53 g/mol
Methyl Linolelaidate	382.70 g/mol.
Methyl Eicosadienoate cis 11,14	306.53 g/mol
Methyl Tricosadienoate	382.70 g/mol.
Methyl Nervonate	340.65 g/mol



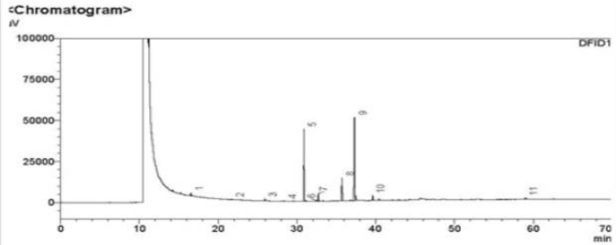
<Peak Table>

DFID1

Peak#	Name	Ret. Time	Area%
1	Methyl Pentadeccenoate	30.981	76.183
2	Methyl Palmitoleate	32.731	9.375
3	Methyl Heptadecenoate	35.778	14.021
4	Methyl Oleate	38.021	0.182
5	Methyl Linolelaidate	39.291	0.239
Total			100.000

Chromatogram 1: Pet food incorporated with 50% chicken meat powder

Chromatogram 1: M



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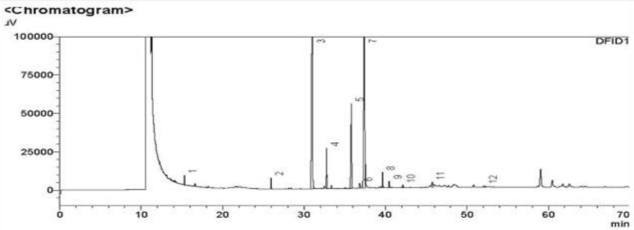
DFID1

Peak#	Name	Ret. Time	Area%
1	Methyl Caprylate	16.547	1.078
2	Methyl Dodecanoate	21.729	0.228
3	Methyl Myristate	25.927	0.854
4	Methyl Myristoleate	28.386	0.211
5	Methyl Pentadeccenoate	30.906	35.009
6	Methyl Palmitate	32.475	0.343
7	Methyl Palmitoleate	32.720	3.719
8	Methyl Heptadecenoate	35.729	11.195
9	Methyl Octadecenoate	37.303	42.806
10	Methyl Linolelaidate	39.615	2.734
11	Methyl Nervonate	59.016	1.821
Total			100.000

Chromatogram 2: Pet food incorporated with 50% poultry byproducts powder

Chromatogram 2: BP



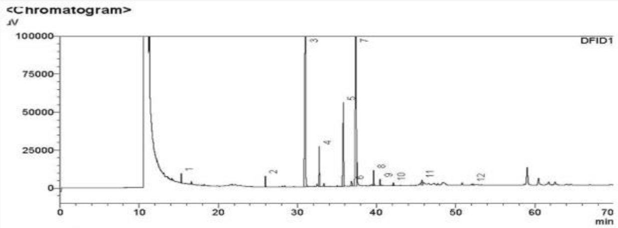
<Peak Table>

DFID1

Peak#	Name	Ret. Time	Area%
1	Methyl Caprylate	15.322	0.674
2	Methyl Myristate	25.948	1.102
3	Methyl Pentadeccenoate	31.034	41.629
4	Methyl Palmitoleate	32.753	3.952
5	Methyl Heptadecenoate	35.809	10.657
6	Methyl Stearate	36.838	0.889
7	Methyl Octadecenoate	37.416	37.081
8	Methyl Linolelaidate	39.635	1.790
9	Methyl Linoleate	40.445	0.736
10	Methyl Linolenate	42.120	0.323
11	Methyl Eicosadienoate cis 11,14	45.731	0.975
12	cis-13,16-Docosadienoic acid methyl ester	52.147	0.191
Total			100.000

Chromatogram 3: Poultry byproducts incorporated fiber fortified Pet 10% ragi flour

Chromatogram 3: RG



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11	Methyl Eicosadienoate cis 11,14	45.731	0.975
12	cis-13,16-Docosadienoic acid methyl ester	52.147	0.191
Total			100.000

Chromatogram 3: Poultry byproducts incorporated fiber fortified Pet 10% ragi flour

Chromatogram 4: BO

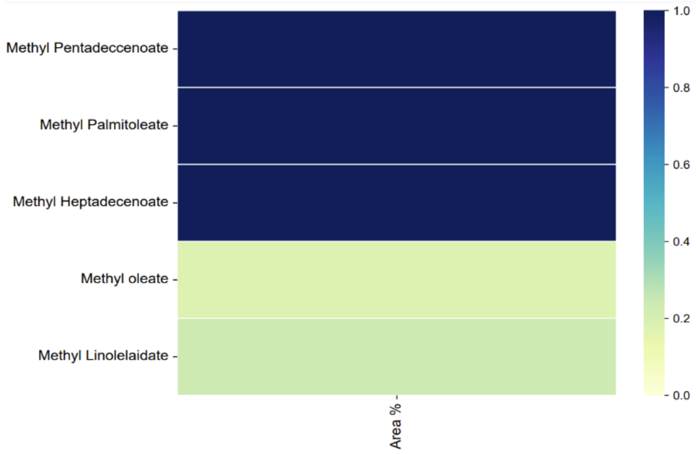


Fig. 1. Chicken powder pet food fatty acid composition

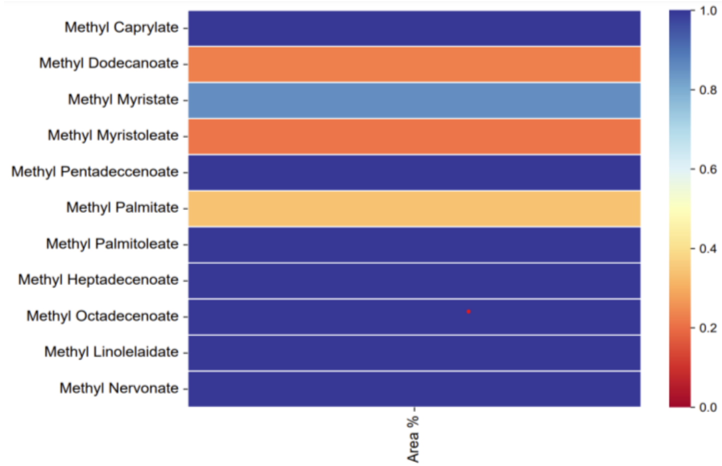


Fig. 2. Poultry byproducts pet food fatty acid composition

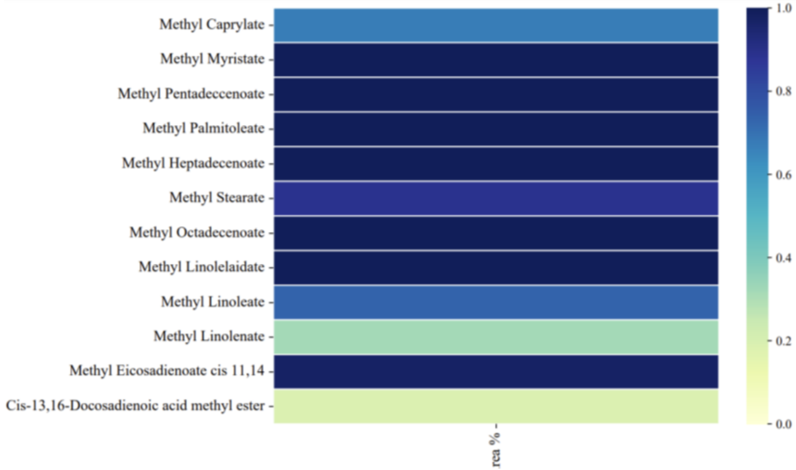


Fig. 3. Poultry byproducts pet food along with ragi fatty acid composition

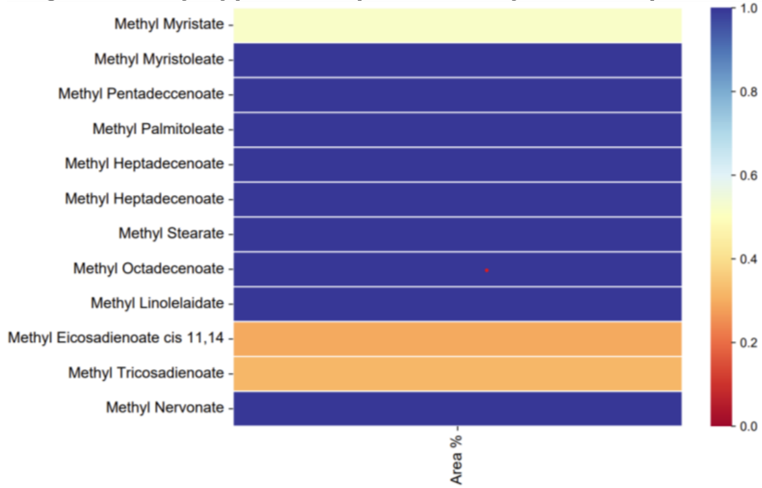


Fig. 4. Poultry byproducts pet food along with potato fatty acid composition

In Fig. 5: Upon examining the fatty acid profiles of various pet foods, distinct compositions unique to each type, as well as shared components, emerged. Poultry-based diets contained exclusively methyl oleate, while byproduct-derived foods displayed methyl dodecanoate and methyl palmitate as their sole constituents. Ragi-based meals exhibited methyl linoleate, methyl linolenate, and cis-13,16-docosadienoic acid methyl ester exclusively. Meanwhile, mash potato-based diets were characterized solely by methyl tricosadienoate. Notably, overlaps were evident: methyl caprylate was shared between byproduct and ragi, and methyl myristoleate and methyl nervonate were common to byproduct and mash potato. Additionally, methyl stearate and methyl eicosadienoate cis 11,14 were found in both ragi and mash potato. Furthermore, methyl pentadeccenoate, methyl palmitoleate, methyl heptadecenoate, and methyl linolelaidate were prevalent across poultry, byproduct, ragi, and mash potato diets, indicating shared fatty acid constituents. These findings highlight the diverse nutritional compositions of pet foods, providing valuable insights for understanding their dietary implications.

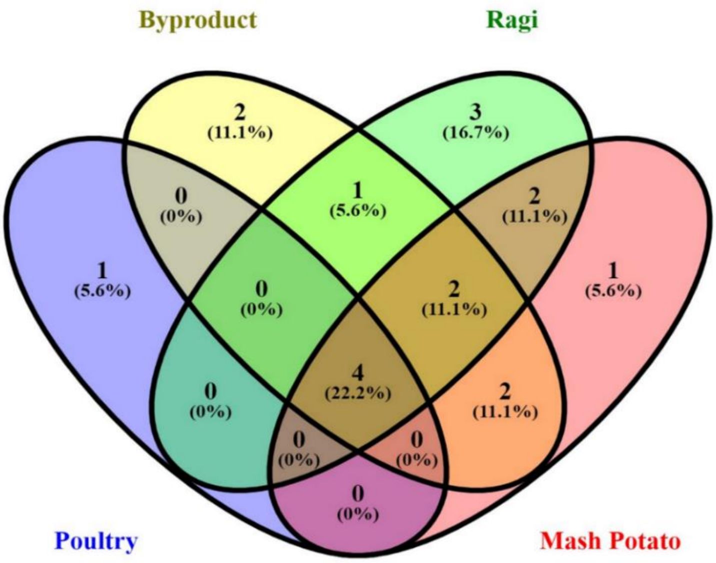


Fig. 5. Diverse fatty acid profiles of pet foods: Insights into dietary composition variability

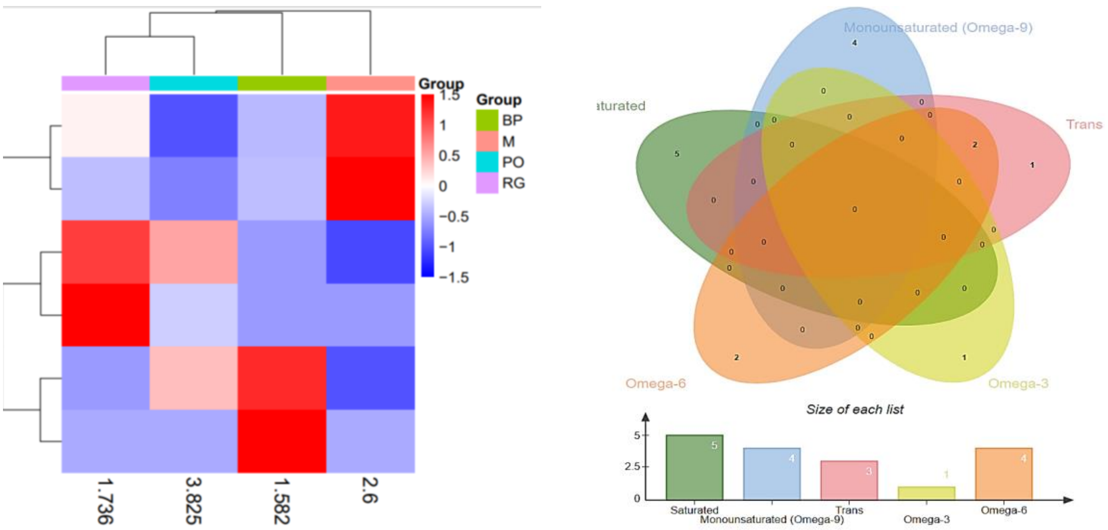
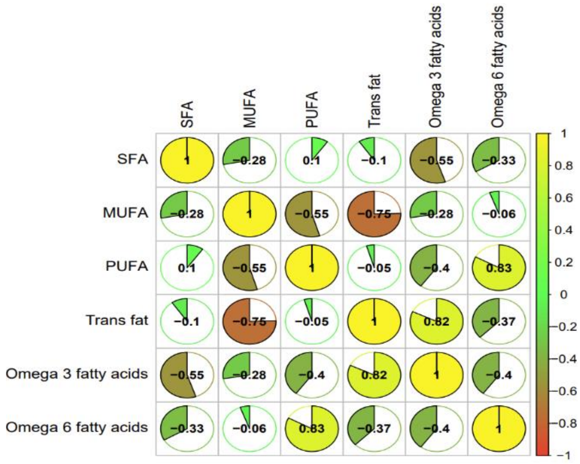


Fig. 6 & 7. Nutritional Profiling of Pet Foods

In Figs. 6 and 7 saturated Fatty Acids (SFA): The fatty acid profiles of various pet foods were analyzed to identify saturated fatty acids (SFA) and their contributions to nutritional content. Among the pet food variants examined, including boiled mashed potato, byproducts, and ragi byproducts, several saturated fatty acids were identified. Notable examples include Methyl Pentadeccenoate and Methyl Stearate, which were predominant in byproducts pet food. These findings underscore the importance of considering SFA content in pet food formulations to ensure balanced nutrition and optimal health for pets. Monounsaturated Fatty Acids (MUFA): Monounsaturated fatty acids (MUFA) play a crucial role in pet nutrition, influencing metabolic processes and overall health. Analysis of fatty acid profiles revealed significant contributions of MUFA in ragi byproducts pet food, with Methyl Palmitoleate and Methyl Heptadecenoate emerging as notable components. These findings highlight the importance of incorporating MUFA-rich ingredients in pet food formulations to promote well-being and nutritional balance in pets. Polyunsaturated Fatty Acids (PUFA): Polyunsaturated fatty acids (PUFA) contribute to essential fatty acid intake and are vital for maintaining overall health in pets. Gas chromatographic analysis identified several PUFA in pet food variants, including Methyl Linoleate and Methyl Octadecenoate, which were prominent in all samples. These findings emphasize the significance of PUFA in pet nutrition and the need for adequate intake to support metabolic functions and ensure optimal health in pets. Omega-3 Fatty Acids: Omega-3 fatty acids play a crucial role in pet health, contributing to various physiological processes and providing anti-inflammatory benefits. While specific omega-3 fatty acids were not identified in the analyzed pet food samples, the presence of PUFA suggests potential contributions to omega-3 fatty acid intake. Further research is warranted to elucidate the omega-3 fatty acid content in pet foods and its implications for pet health and well-being. Omega-6 Fatty Acids: Omega-6 fatty acids are essential components of pet diets, contributing to lipid metabolism and overall health. Analysis of fatty acid profiles revealed significant levels of omega-6 fatty acids, represented by Methyl Linolelaidate and Methyl Eicosadienoate cis 11,14, across all pet food variants. These findings underscore the importance of maintaining a balanced omega-3 to omega-6 fatty acid ratio in pet diets to support optimal health and well-being. Trans Fats: Trans fats are associated with adverse health effects and should be minimized in pet food formulations. While specific trans fats were not identified in the analyzed pet food samples, caution should be exercised to prevent their presence. Continued monitoring and quality control measures are essential to ensure the absence of trans fats in pet foods and promote the health and well-being of pets.

Understanding these intricate profiles is essential for optimizing the nutritional quality of pet foods and promoting the health and well-being of companion animals. Understanding the contributions of saturated, monounsaturated, and polyunsaturated fatty acids, as well as omega-3 and omega-6 fatty acids, is essential for formulating balanced and nutritious pet diets. Additionally, efforts to minimize trans fats in pet foods are crucial for safeguarding pet health and promoting overall well-being.



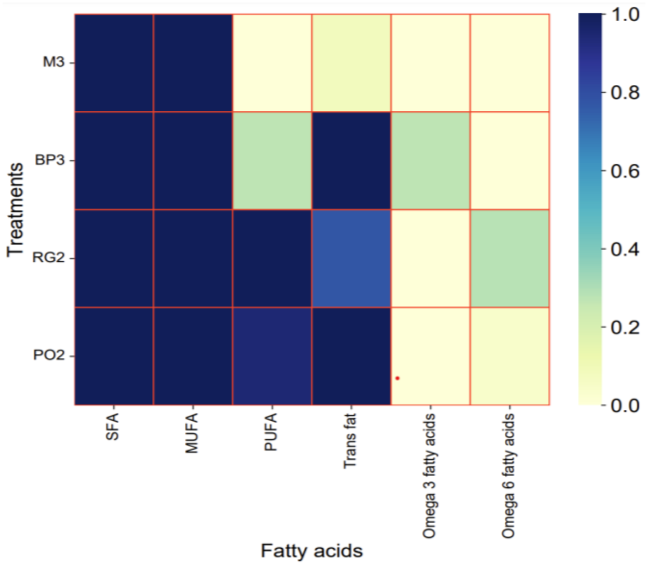


Fig. 8 & 9. Correlative analysis reveals fatty acid composition patterns in pet food formulations

In Figs. 8 & 9: In the investigation presented within Figs. 8 and 9, a comprehensive correlative analysis was undertaken to discern intricate relationships within the fatty acid composition of diverse pet food formulations. The analysis discerned several discernible correlations among distinct fat categories present in the formulations. A notably robust positive correlation coefficient (0.983) emerged between Saturated and Total Fat, indicative of a commensurate elevation in total fat content accompanying heightened levels of saturated fat. Additionally, a conspicuously strong positive correlation (0.999) was discerned between Monounsaturated and Polyunsaturated fats, suggesting a synchronized augmentation of these unsaturated fat variants across experimental treatments. Conversely, notable negative correlations were evident between Total Fat and Polyunsaturated fats (-0.991), as well as Total Fat and Monounsaturated fats (-0.989), underscoring a decrement in the relative proportion of these fat types with escalating total fat content. Moderate positive correlations were further observed between Total Fat and Trans Fat (approximately -0.975), implying a tendency for co-occurrence between these fat constituents. Moreover, a discernible moderate positive correlation (approximately -0.979) was observed between Omega 3 and Omega 6 fatty acids, signaling their simultaneous presence in pet food formulations. Noteworthy distinctions were observed among treatments, particularly in formulations such as M3 and PO2, which exhibited comparatively elevated levels of Saturated and Total Fat, contrasting with formulations BP3 and RG2.

Table 5. Fatty acid that enhances flavor and aroma in pet food

Flavor enhancer fatty acid	Aroma enhancer fatty acid
Methyl Caprylate	Methyl Caprylate
Methyl Myristate	Methyl Linolelaidate
Methyl Palmitoleate	Methyl Linolenate
Methyl Myristoleate	Methyl Nervonate

3.1 Assessment of Nutritional Indices Reflecting Lipid Profile and Cardiovascular Health in Different Pet Food Formulations

Atherogenic Index (AI): The Atherogenic Index is a measure of the potential of dietary fatty acids to promote the development of atherosclerosis, a condition characterized by the buildup of plaque in the arteries. It is calculated based on the ratio of atherogenic saturated fatty acids to anti-atherogenic unsaturated fatty acids.

Thrombogenic Index (TI): The Thrombogenic Index is a measure of the potential of dietary fatty acids to increase blood coagulation, which can lead to the formation of blood clots. It is calculated based on the ratio of pro-thrombotic saturated fatty acids to anti-thrombotic unsaturated fatty acids.

Hypochole sterolemic/ Hypercholesterolemic Ratio: measure of the balance between fatty acids that have beneficial effects on cholesterol levels (hypocholesterolemic) and those that have adverse effects (hypercholesterolemic).

Fatty Acid Desaturation Index (DI): The Fatty Acid Desaturation Index is a measure of the degree of desaturation or conversion of saturated fatty acids to unsaturated fatty acids in the body. It reflects the activity of enzymes involved in fatty acid metabolism.

A higher AI value indicates a diet with a greater potential to promote atherosclerosis, while a lower AI value suggests a diet with a lower risk of cardiovascular disease development.

A higher TI value indicates a diet with a greater potential to promote blood clot formation, while a lower TI value suggests a diet with a lower risk of thrombosis and related cardiovascular events.

Hypocholesterolemic/Hypercholesterolemic Ratio A higher ratio indicates a diet with a greater proportion of hypocholesterolemic fatty acids relative to hypercholesterolemic fatty acids, which is associated with a reduced risk of cardiovascular disease and improved lipid profile.

A higher DI value indicates a higher degree of conversion of saturated fatty acids to unsaturated fatty acids, which is associated with improved lipid profile and cardiovascular health.

Chicken Powder Pet Food has the highest AI among the samples, indicating a relatively higher potential for promoting atherosclerosis compared to the other samples. Poultry Byproducts Incorporated Vegetables + Ragi and Poultry Byproducts Incorporated Vegetables + Boiled Mash Potato Pet Foods have the lowest AI values, suggesting a lower risk of promoting atherosclerosis. Like AI, Chicken Powder Pet Food has the highest TI, suggesting a relatively higher risk of promoting blood clot formation. Poultry Byproducts Incorporated Vegetables + Ragi and Poultry Byproducts Incorporated Vegetables + Boiled Mash Potato Pet Foods have the lowest TI values, indicating a lower risk of thrombosis. Hypocholesterolemic/ Hypercholesterolemic Ratio This reflects the balance between beneficial and detrimental fatty acids with respect to cholesterol levels. Poultry Byproducts Incorporated Vegetables + Boiled Mash Potato Pet Food has the highest ratio, indicating a more favorable lipid profile. Poultry Byproducts Incorporated Vegetables + Ragi has the highest DI value, indicating a relatively higher degree of fatty acid desaturation compared to other samples.

3.2 Quantifying Fatty Acid Diversity in Pet Food Formulations

Shannon Diversity Index (H'): H' provides a measure of diversity that considers both the number of different fatty acids present and their relative abundances. A higher H' value indicates greater diversity within the fatty acid profile of the sample.

Simpson's Diversity Index (D): D provides a measure of diversity that emphasizes the dominance of certain fatty acids within the sample. A lower D value suggests higher diversity, as it accounts for both the number of different fatty acids and their evenness in distribution.

Table 6. Nutritional Indices of Various Pet Food Formulations

Treatment	Atherogenic Index (AI)	Thrombogenic Index (TI)	Hypocholesterolemic/ Hypercholesterolemic Ratio	Fatty Acid Desaturation Index (DI)
M3	1.43	1.46	0.69	0.68
BP2	0.02	0.03	0.73	0.84
RG2	0.08	0.09	0.80	0.88
PO3	0.19	0.19	0.89	0.87

Table 7. Quantifying Fatty Acid Diversity in Pet Food Formulations

Treatment	Shannon Diversity Index (H')	Simpson's Diversity Index (D):
M3	1.177	0.238
BP2	2.170	0.988
RG2	2.385	0.956
PO3	2.095	0.865

The fatty acid profile of Chicken Powder Pet Food exhibits moderate diversity (H') with a relatively higher dominance of certain fatty acids (D), indicating a balanced yet slightly skewed composition. Poultry Byproducts Incorporated Vegetables Pet Food formulation demonstrates high diversity (H') with less dominance of specific fatty acids (D), suggesting a varied and well-balanced composition. Poultry Byproducts Incorporated Vegetables + Ragi Pet Food this formulation exhibits the highest diversity (H') among the samples, indicating a wide range of fatty acids with relatively lower dominance (D), reflecting a highly varied and nutritionally rich composition. Poultry Byproducts Incorporated Vegetables + Boiled Mash Potato Pet Food this sample demonstrates moderate to high diversity (H') with a relatively higher dominance of certain fatty acids (D), suggesting a varied composition with some level of dominance by specific fatty acids.

The Shannon Diversity Index and Simpson's Diversity Index provide insights into the diversity and dominance of fatty acids within each pet food sample. These results can help assess the nutritional quality and potential health effects of different pet food formulations.

4. DISCUSSION

Fatty acid analysis is crucial in understanding the nutritional composition of pet foods, as it influences both palatability and shelf life. Essential fatty acids, which cannot be synthesized by dogs in sufficient amounts, must be provided in their diet to meet their nutritional requirements [9]. The National Research Council (NRC) recommends specific daily allowances for essential fatty acids in adult dogs, including linoleic acid, alpha-linolenic acid, eicosapentaenoic acid, and docosahexaenoic acid [10]. In our study, the poultry byproducts incorporated with fiber-fortified pet food (PO2) exhibited the highest content of saturated fatty acids compared to other treatments [11]. Mono-saturated fatty acids were most abundant in M3, consistent with previous research by Aberle et al. [12] which identified oleic acid as the primary mono-saturated fatty acid in meat fat. The observation of higher Trans-fat content in BP3 compared to other treatments suggests that the addition of vegetable oil in both control and treatment groups may have contributed to this finding [13]. Additionally, Kara [13] noted higher concentrations of eicosapentaenoic acid, linoleic acid, and oleic acid in wet dog foods compared to dry dog foods, while alpha-linolenic acid and docosahexaenoic acid concentrations were similar in both types of pet foods. The disparity in fatty acid profiles among the different pet foods could be attributed to the inclusion of various functional ingredients in the treatments. For example, ragi flour (finger millet) was found to contain higher amounts of unsaturated fatty acids, particularly linoleic acid, an omega-6 fatty acid [14]. Interestingly, omega-3 fatty acids were only detected in BP3, suggesting potential differences in formulation or ingredient selection [13]. The rate of oxidation in pet food is directly influenced by the type of fat present, with trans-fat and polyunsaturated fatty acids (PUFA) playing significant roles [15].

4.1 Chicken Powder Incorporated Pet Food

Fatty acids play crucial roles in canine nutrition, influencing various metabolic pathways and impacting overall health. This article provides an in-depth analysis of the metabolic pathways and potential health implications of five identified fatty acids (Methyl Pentadeccenoate, Methyl Palmitoleate, Methyl Heptadecenoate, Methyl Oleate, and Methyl Linolelaidate) in canine nutrition. Through a comprehensive review of existing literature and biochemical insights, we elucidate the absorption, metabolism, and cellular effects of these fatty acids, shedding light on their contributions to canine health and well-being. Canine nutrition is a complex interplay of dietary components that influence metabolic processes and physiological functions in dogs. Ahlstrøm et al, [16] A also evaluated that these components, fatty acids play pivotal roles as energy sources, structural components of cell membranes, and signaling molecules regulating various biological pathways. Understanding the metabolic fate and health implications of individual fatty acids is essential for formulating balanced diets to support optimal health and longevity in dogs.

4.2 Metabolic Pathways of Identified Fatty Acids

Methyl Pentadeccenoate is a monounsaturated fatty acid (MUFA) found in significant proportions in canine diets. Upon ingestion, it undergoes digestion and absorption in the small intestine, where it is incorporated into triglycerides. These triglycerides are then transported via chylomicrons to various tissues, where Methyl Pentadeccenoate can be utilized as a substrate for β-oxidation to generate energy. However, excessive intake of Methyl Pentadeccenoate may lead to triglyceride accumulation in tissues, contributing to metabolic disturbances such as obesity and insulin resistance. Additionally, Methyl Pentadeccenoate can modulate gene expression and inflammatory pathways in cells, potentially exacerbating inflammation in dogs. Methyl Palmitoleate is another MUFA present in canine diets, albeit in smaller proportions compared to Methyl Pentadeccenoate. Following digestion and absorption, Methyl Palmitoleate is esterified into triglycerides and transported to tissues for energy production or incorporation into cell membranes. Its role in regulating lipid metabolism and inflammatory responses in dogs warrants further investigation to elucidate its impact on canine health outcomes. Methyl Heptadecenoate, a monounsaturated fatty acid like Methyl Palmitoleate, undergoes similar metabolic processes in the canine body. Upon absorption, it is metabolized for energy production or structural purposes in cells. Thompson, [17] and Ahlstrøm et al, 2024 stated that in their studies like other MUFA, Methyl Heptadecenoate may influence gene expression and cellular signaling pathways, affecting lipid metabolism and inflammatory responses in dogs. Methyl Oleate is an omega-9 MUFA commonly found in vegetable oils and animal fats. In canine nutrition, it serves as an energy substrate and contributes to cell membrane integrity and function. However, excessive intake of Methyl Oleate may lead to lipid accumulation in tissues and metabolic imbalances, highlighting the importance of dietary moderation and balance in canine nutrition. Methyl Linolelaidate, a trans fatty acid derived from the partial hydrogenation of linoleic acid, is present in trace amounts in canine diets. Despite its low abundance, Methyl Linolelaidate can influence lipid metabolism and inflammatory pathways in dogs, potentially contributing to adverse health outcomes when consumed in excess.

4.3 Health Implications of Identified Fatty Acids

Balanced intake of MUFA, such as Methyl Pentadeccenoate, Methyl Palmitoleate, and Methyl Heptadecenoate, may contribute to improved cardiovascular health by reducing LDL cholesterol levels and inflammation in dogs. Conversely, excessive intake of trans fats, like Methyl Linolelaidate, may increase the risk of cardiovascular diseases and metabolic disorders. The inflammatory potential of fatty acids, particularly omega-6 PUFA like Methyl Linolelaidate, underscores the importance of maintaining a balanced omega-6 to omega-3 ratio in canine diets. Imbalances in fatty acid intake may lead to chronic inflammation and predispose dogs to inflammatory conditions such as arthritis and allergies. Thompson, [17] and Dhakal et al., [18] also included those metabolic disturbances, including obesity, insulin resistance, and dyslipidemia, may arise from imbalances in fatty acid intake, especially saturated fats, and trans fats. Optimal canine nutrition should prioritize balanced fatty acid profiles to mitigate the risk of metabolic disorders and promote overall metabolic health in dogs.

4.4 Poultry Byproducts Incorporated Vegetables Pet Food

Upon ingestion, Methyl Caprylate undergoes hydrolysis in the gastrointestinal tract, releasing caprylic acid. Caprylic acid is rapidly absorbed and transported to the liver, where it undergoes β-oxidation to produce acetyl-CoA, which enters the citric acid cycle to generate ATP. Excess acetyl-CoA may be converted into ketone bodies, providing an alternative energy source for tissues, particularly during periods of fasting or energy deficit. Methyl Dodecanoate (C12:0) and Methyl Myristate (C14:0) these medium-chain fatty acids are metabolized similarly to Methyl Caprylate, undergoing β-oxidation to produce acetyl-CoA and subsequently ATP. Medium-chain fatty acids are known for their rapid absorption and utilization as an efficient energy source, making them beneficial for dogs with digestive issues or malabsorption disorders. Methyl Myristoleate (C14:1) while less abundant, Methyl Myristoleate undergoes similar metabolic pathways as other fatty acids, with its potential anti-inflammatory effects attributed to modulation of inflammatory signaling pathways such as NF-κB.Myristoleic acid's conversion into bioactive lipid mediators, such as prostaglandins and leukotrienes, may contribute to its observed anti-inflammatory properties in canine health. Methyl Pentadeccenoate (C15:1) and Methyl Heptadecenoate (C17:1) these monounsaturated fatty acids are integral components of cell membranes and play key roles in cellular signaling and gene expression. Upon absorption, Methyl Pentadeccenoate and Methyl Heptadecenoate are incorporated into phospholipids, influencing membrane fluidity and receptor function. Metabolically, they can serve as substrates for the synthesis of lipid mediators such as eicosanoids, which regulate inflammatory responses and immune function. Methyl Palmitate (C16:0) and Methyl Palmitoleate (C16:1), Palmitic acid and Palmitoleic acid contribute to cellular structure and function, with Palmitoleic acid exhibiting additional anti-inflammatory properties. Aldrich, [19] and Montegiove et al., [20] concluded in their study that metabolically, Palmitic acid can be elongated to form longer-chain fatty acids or undergo desaturation to form Palmitoleic acid, which may act as a signaling molecule in inflammatory pathways. Methyl Octadecenoate (C18:1) and Methyl Linolelaidate, these predominant fatty acids serve as precursors for the synthesis of bioactive lipids and play crucial roles in lipid metabolism and inflammatory regulation. Methyl Octadecenoate, particularly Oleic acid, can modulate gene expression through activation of nuclear receptors such as PPARs, influencing lipid metabolism and energy homeostasis. Methyl Linolelaidate, a trans fatty acid, may disrupt cellular functions and promote inflammation through mechanisms such as oxidative stress and immune dysregulation. Methyl Nervonate (C24:1), Nervonic acid contributes to myelin synthesis and neural function, with potential implications for cognitive health and neurological development in dogs. Metabolically, Nervonic acid undergoes elongation and desaturation to form longer-chain fatty acids, influencing membrane composition and neural signaling pathways.

4.5 Poultry Byproducts Incorporated Vegetables Along with Ragi

Ragi flour, derived from the grain of the finger millet plant (Eleusine coracana), is a nutritious staple in many parts of the world, particularly in India and Africa. Ragi flour is a good source of carbohydrates, providing sustained energy due to its complex carbohydrate content. It has a low glycemic index, making it suitable for individuals with diabetes. Ragi flour contains significant amounts of dietary fiber, promoting digestive health, regulating bowel movements, and lowering cholesterol levels. It is a decent source of plant-based protein, making it beneficial for vegetarians and vegans to meet their protein needs. Osawa et al, [21] stated that essential Vitamins and Minerals: Ragi flour is rich in essential vitamins and minerals, including calcium, iron, magnesium, potassium, and vitamin B-complex (especially niacin and thiamine). These nutrients support bone health, energy metabolism, and overall well-being. Ragi contains antioxidants such as phenolic compounds and flavonoids, which help neutralize harmful free radicals, reducing the risk of chronic diseases like cancer and heart disease. Ragi flour is naturally gluten-free, making it suitable for individuals with gluten sensitivities or celiac disease. The low glycemic index of ragi flour helps in regulating blood sugar levels, making it a suitable choice for individuals with the high fiber content of ragi flour aids in reducing cholesterol levels, lowering the risk of cardiovascular diseases. The fiber and protein content in ragi flour promote satiety, aiding in weight management and preventing overeating. Ragi flour is an excellent source of calcium, crucial for maintaining bone health and preventing conditions like osteoporosis. Thompson, [17] also obtained that dietary fiber supports digestive health by preventing constipation and promoting regular bowel movements. Ragi flour is rich in iron, essential for preventing and treating anemia, especially in women and children. The complex carbohydrates in ragi flour provide sustained energy, making it an ideal choice for athletes and individuals with active lifestyles. The antioxidants present in ragi flour help in combating oxidative stress, reducing the risk of chronic diseases, and promoting overall well-being.

Methyl Caprylate, a medium-chain fatty acid, undergoes rapid absorption in the gastrointestinal tract. Once absorbed, it enters the bloodstream and is transported to the liver. In the liver, Methyl Caprylate undergoes β-oxidation, yielding acetyl-CoA molecules that enter the citric acid cycle for ATP production. Excess acetyl-CoA may be converted into ketone bodies, serving as an alternative energy source. Methyl Myristate, a saturated fatty acid, is absorbed in the small intestine and transported to various tissues via the bloodstream. In tissues, it undergoes β-oxidation to produce acetyl-CoA for energy production. Dainton et al, [22] also stated that copper is also an important source of fatty acid in pet food. Additionally, Methyl Myristate can be incorporated into cell membranes, influencing membrane fluidity and function. Methyl Pentadeccenoate, a monounsaturated fatty acid, plays important roles in cellular function and metabolic regulation. After absorption, it can be incorporated into phospholipids, influencing membrane structure and function. Metabolically, Methyl Pentadeccenoate can serve as a precursor for the synthesis of lipid mediators involved in inflammation modulation. Tran et al, [23] include in their study that, methyl Palmitoleate is absorbed in the small intestine and transported to tissues. Once in cells, it can be incorporated into cell membranes and modulate membrane fluidity. Metabolically, Methyl Palmitoleate may participate in lipid signaling pathways involved in inflammation regulation and metabolic homeostasis. Methyl Heptadecenoate undergoes absorption and subsequent metabolism in a similar manner to other monounsaturated fatty acids. In cells, it can influence gene expression and cellular signaling pathways involved in lipid metabolism and inflammation regulation. Leiva et al., [24] also stated that methyl Octadecenoate, predominantly Oleic acid, is a crucial component of cell membranes and participates in various cellular processes. Mooney, [25] obtained that metabolically, it can regulate gene expression, modulate lipid signaling pathways, and influence inflammatory responses. Methyl Linolelaidate, Methyl Linoleate, Methyl Linolenate, Methyl Eicosadienoate, Cis-13,16-Docosadienoic acid methyl ester, these polyunsaturated fatty acids undergo absorption and metabolism to yield bioactive lipid mediators involved in inflammation regulation, cell signaling, and metabolic processes. They play essential roles in maintaining cell membrane integrity, modulating immune responses, and regulating hormone synthesis.

4.6 Poultry Byproducts Incorporated Vegetables along with Boiled Mash Potato

Analysis of the boiled mashed potato pet food sample reveals a diverse array of fatty acid methyl esters, each with potential implications for canine health. Mooney, [25] Glodde et al., [26] stated in their research that Understanding the metabolic pathways and physiological effects of these fatty acids is crucial for evaluating their impact on dog health.

Methyl Myristate, a saturated fatty acid, contributes to the structural integrity of cell membranes and plays a role in energy metabolism. Its presence in pet food suggests a source of cellular structural support and energy substrate for dogs. Hilton [27] also includes in his study that Methyl Myristoleate, a monounsaturated fatty acid, is associated with potential anti-inflammatory properties and joint health benefits. Buff et al., [28] obtained that fatty acid abundance in pet food suggests a potential role in modulating inflammation and supporting joint health in dogs. Methyl Pentadeccenoate, a monounsaturated fatty acid, may contribute to lipid metabolism and inflammation regulation. Its presence in pet food may support metabolic processes and inflammatory modulation in dogs. Methyl Palmitoleate, a monounsaturated fatty acid, is linked to skin health and inflammatory regulation. Brown, [29] in his finding stated that the presence in pet food suggests potential benefits for skin and coat health and inflammation modulation in dogs. Methyl Heptadecenoate, another monounsaturated fatty acid, is involved in lipid metabolism and cellular signaling. Its presence in pet food may support lipid metabolism and cellular function in dogs. Kępińska et al., [30] also includes in his study that a saturated fatty acid, contributes to energy storage and cellular structure. Its abundance in pet food suggests a source of energy and structural support for dogs. Methyl Octadecenoate, predominantly Oleic acid, is associated with cardiovascular benefits and metabolic regulation. Its presence in pet food may support cardiovascular health and metabolic function in dogs. Methyl Linolelaidate, Methyl Eicosadienoate cis 11,14, Methyl Tricosadienoate, Methyl Nervonate, these fatty acids contribute to various metabolic processes, including inflammation modulation, neural function, and hormone synthesis. Karthik et al., [31]. Their presence in pet food suggests potential benefits for overall health and well-being in dogs. The presence of essential fatty acids underscores the importance of balanced nutrition to meet the dietary requirements of dogs and promote optimal health and well-being.

4.7 General Overview of the Metabolic Pathways Involving Fatty Acids and their Potential Health Implications for Pets

Dietary fatty acids are absorbed in the small intestine and transported via chylomicrons to various tissues [32] also sated in research dietary fatty acid is important for proper digestion and plays significant role in small intenstine. Once inside cells, fatty acids are activated by fatty acyl-CoA synthetase to form fatty acyl-CoA molecules, which are essential for subsequent metabolic processes. Fatty acyl-CoA molecules undergo beta-oxidation in the mitochondria to produce acetyl-CoA, NADH, and FADH2.Acetyl-CoA enters the citric acid cycle (TCA cycle) to generate ATP through oxidative phosphorylation. NADH and FADH2 generated during beta-oxidation are used to produce ATP via the electron transport chain. Excess acetyl-CoA generated from fatty acid metabolism can be used for de novo lipogenesis in the liver, leading to the synthesis of triglycerides. Triglycerides are then stored in adipose tissue as an energy reserve. Fatty acids are essential components of cell membranes, contributing to membrane fluidity and stability. Saturated fatty acids (e.g., palmitate, stearate) provide rigidity to cell membranes, while unsaturated fatty acids (e.g., oleate, linoleate) increase membrane fluidity. Fatty acids serve as precursors for the synthesis of signaling molecules such as prostaglandins, leukotrienes, and thromboxanes. These lipid mediators play critical roles in inflammation, immune response, and cellular signaling pathways.Balanced intake of omega-3 and omega-6 fatty acids is crucial for maintaining optimal health in pets.Omega-3 fatty acids (e.g., docosahexaenoic acid, DHA) have anti-inflammatory properties and may benefit cardiovascular health, cognitive function, and skin and coat health. Yao et al., [33] obtained that omega-6 fatty acids (e.g., linoleic acid) are important for skin barrier function and immune response but excessive intake may promote inflammation. Trans-fatty acids (e.g., linolelaidate) should be minimized in pet diets due to their association with adverse health effects, including increased risk of cardiovascular diseases [34].

Considering these findings, further research is warranted to explore the effects of different fat sources and formulation strategies on the oxidative stability of pet foods. Additionally, investigating the impact of fatty acid composition on sensory attributes and overall acceptability of pet foods could provide valuable insights for pet food manufacturers aiming to develop nutritious and appealing products [35].

5. CONCLUSION

This study sheds light on the intricate composition of fatty acids in various pet foods, highlighting the presence of rare and unique fatty acids with potential implications for pet nutrition and health. The identification of these fatty acids underscores the importance of further exploration into their significance and sources, offering opportunities for enhancing pet food formulations. Moreover, correlating fatty acid compositions with pet health outcomes provides valuable insights into optimizing pet nutrition and well-being, guiding pet owners, veterinarians, and pet food manufacturers in making informed decisions. Additionally, understanding the impact of processing methods on fatty acid profiles emphasizes the need for careful consideration in pet food manufacturing practices to preserve nutritional quality. By aligning fatty acid compositions with established nutritional guidelines and evaluating the efficacy of functional ingredients, we can advance our understanding of pet nutrition and health, paving the way for the development of novel formulations tailored to meet the specific needs of different pet populations. Overall, this our research contributes significant insights to the field of pet nutrition, with implications for improving the quality of pet foods and ultimately enhancing the health and well-being of companion animals.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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