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LIFE-F4F (Food for Feed)

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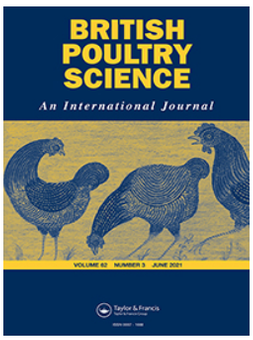

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The food for feed concept. Performance of broilers fed hotel food residues

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ABSTRACT

1. In recent times the use of food waste in animal diets has gained considerable attention because of the increasing demand to cover the needs of human population and the high prices of conventional, arable based, animal feeds.
2. The aim of the present study was to investigate the effect of adding dried human food waste to the diet of meat-type chickens (broilers). Two hundred, one-day-old broilers were divided into two treatment groups, with 10 replicate pens containing 10 birds per pen. The duration of the study was 42 days. In the control (C), the diet did not contain any food waste, whereas in the second treatment (T) food waste residues from hotels made up 15% of the diet. Diets had similar crude protein and metabolisable energy content.
3. Feed intake and body weight were recorded in order to calculate weight gain and feed conversion ratio (FCR). Carcase and breast muscle yield, the weight of selected internal organs and the level of selected biochemical and haematological parameters were determined. Quality of breast muscle meat was assessed.
4. Broilers fed the control treatment consumed more feed and gained more weight compared to broilers fed waste; however, the FCR was similar. No major differences were seen for internal organ weights and haematological parameters, although some differences were observed in colour traits and shear force of meat. It was concluded that there is a potential for use of food waste in broiler diets.

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Broilers; carcase yield; food residues; feed; growth performance; meat quality; waste

Introduction

The large amount of human food that gets wasted annually around the world has increased the concern regarding environmental impact and has raised the importance of reusing and handling food waste. The global human population is continuously increasing and, as a result, more food waste is expected to be available. Hence, it is necessary to find alternative sources of human food and a way to utilise waste, which could be a valuable source of protein (Kamlesh and Saraswat 2000). Every year, around 1.3 billion tons of edible food for human consumption gets lost or wasted in a global scale, which accounts for one third of the food that is annually produced (Gustavson et al. 2011). Food loss occurs in early processing stages, during production, post-harvest and at retail (FAO 2019; Gustavson et al. 2011; Parfitt et al. 2010). Food waste is not included in losses and needs to reflect the actual levels of the supply chain, in particular retail and consumption level (FAO 2019; Gustavson et al. 2011; Parfitt et al. 2010).

The generation of food waste can lead to a degradation of the environment (Lee et al. 2007). Anaerobic digestion and composting go some way to handling food waste but they have a high environmental impact due to eutrophication, potential toxicity and acidification (Salemdeeb et al. 2016). Using food waste in animal feed may be a more effective method, as less feed needs to be produced when human food waste is used as a substitute for other raw materials and emissions are avoided (Salemdeeb et al. 2016). Moreover, food waste may contain useful bioactive compounds (Georganas et al. 2020), such as long-chain polyunsaturated fatty acids (PUFA) (Astley and Finglas 2016; Donati 2018), carotenoids, vitamins, polyphenols (Astley and Finglas 2016) and peptides. These substances are found in small amounts in human food (Astley and Finglas 2016; Santos et al. 2019) and may be of benefit to animals (Martín Ortega and Segura Campos 2019). It is worth noting that the transformation of food waste in feed has already been used in some parts of the world, including Texas, Florida and New Jersey in the USA (Westendorf et al. 1996). As already observed by Cho et al. (2004a), (2004b), growth of broilers and egg production from laying hens, when fed with 10% inclusion of dried food leftovers, were not significantly different. It has been indicated that the integration of food waste with grains, cereal by-products or commercial corn-soy diets improved pig performance (Chae et al. 2000; Heitman et al. 1956; Myer et al. 1999; Westendorf 2000a).

An important limiting factor regarding incorporating food waste into animal feed is the variability in the nutrient content (Westendorf 2000a), which can unbalance diet formulations. Another factor that influences the food composition can be the period of the year that residues had been

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gathered (Esteban et al. 2005). In addition, the high level of moisture affects the final product (Westendorf 2000b). Other problems are the potential pathogenic content or rapid oxidation and biological instability of human food waste (Jayathilakan et al. 2012). According to previous studies, food waste may transmit diseases in some cases (for example, the foot and mouth disease outbreak in the UK in 2001 from airline food leftovers; BBC 2016) and this might be an obstacle for its reutilisation (EU 2009; Westendorf, Zirkle Pas, and Gordon 1996). In 2002, the EU banned the use of catering waste in animal feed with the exception of fur animals (EU 2002, 2009, 2011). Processes are required in order to avoid the transmission of disease amongst animals and from animal-to-human (Westendorf et al. 1996) and eliminate any pathogens (Sancho et al. 2004). Biosecurity can be achieved with heating treatments, boiling, addition of chemical substances, ensiling and composting (EU 2009; Kelley and Walker 2000; Westendorf et al. 1999). Despite the safety instructions, consumers worry about the suitability of food waste, nutrient content and the potential transmission of diseases (Chung 2001). A change of policy, support from industries and consumers and the improvement in the infrastructures of food waste collection are necessary in order to re-legislate the use of food waste in animal feed (Salemdeeb et al. 2016).

The present study was part of a LIFE project on the transformation of food waste from hotels to pig and poultry feed. The project involved solar drying of food waste prior to use (an environment-friendly procedure). In the project, several types of food waste were examined as potential feedstuffs for monogastrics including, but not limited to, leftovers that were solely from plant origin, those that contained meat residues and others that underwent sterilisation. The aim of the present study was to assess the performance of broilers fed dried hotel food waste.

Materials and methods

Food waste collection, animals, diets and experimental procedures

The food waste originated from hotels in Crete that were participating in the project. Trained personnel working at the hotels placed the leftovers/food waste into plastic bags and used specific bins for collection, and all waste was refrigerated. Following collection, a refrigerated truck transported the waste from the hotels to the experimental unit. In an air-cooled pre-treatment unit, the collected food waste was hand-sorted, ground and pulverised. Immediately after pulverisation, a high-powered pump introduced the pulp into the solar drying unit. Food waste was not stored at any point during the transformation procedure.

Several analyses were carried out to determine the nutrient levels, including physicochemical and compositional analysis. Such analyses were of high importance since they provide feedback about the characteristics of the input material and the quality of the dried end-product. Results from the analyses determined the principal food waste categories; fresh fruit and vegetables, as well as salad waste, represented the greatest portions (58.29%), while meat and fish represented 4.90%, cooked meals 25.42%, bread and bakery products 5.7%, dairy (excluding milk) and eggs 0.79% (Table 1).

Table 1. Compositional analysis (% w/w) for sorted food residues.

Component category (%)	
Fresh vegetables and salads	13.92
Bread and bakery	5.70
Fresh fruit	44.37
Meat and fish	4.90
Cooked meals and snacks	25.42
Dairy (excluding milk) and eggs	0.79
Condiments, sauces, herbs and spices	0.34
Desserts	0.22
Confectionery and snacks	0.09
Processed fruit	0.03
Other	3.48
Impurities	0.74

Food waste was analysed for several known pathogens, such as *Escherichia coli, Salmonella spp., Clostridium perfringens, Staphylococcus spp., Listeria spp.* and, specifically, *L. monocytogenes*. Similarly, amino acid content was determined in order to be able to formulate diets and the metabolisable energy value was 15.4 MJ/kg calculated using the formula:

ME (Mj/kg) = 0.1551 x % crude protein + 0.3431 x % ether extract + 0.1669 x % starch + 0.1301 x % total sugars*

(*expressed as sucrose).

Proximate analysis of food waste showed dry matter (DM), crude protein (CP), ether extract (EE) and crude fibre (CF) of 92.74%, 23.76%, 20% and 6.26%, respectively (Table 2).

Two hundred male, 1-day-old, Aviagen Ross 308 broilers were obtained from a commercial hatchery for the feeding experiment. The duration of the experiment was 42 days, and the housing and care of broilers was approved by the Ethical Committee of the Agricultural University of Athens and complied with directive 2010/63/EC (EU 2010) on the protection of animals used for scientific purposes.

Pen was denoted as the experimental unit. There were 10 replicate pens of each of two dietary treatments, namely, control (C) and treatment (T) with 10 broilers per pen and 100 per treatment. Birds were assigned to a pen (measuring

Table 2. Determined composition (%) and calculated analysis of the food waste material.

Determined composition (%)	
Dry matter	92.74
Ash	6.27
Crude protein	23.76
Ether extract	20.00
Crude fibre	6.26
Starch	26.80
Total sugars	3.50
Calculated analysis	
Metabolisable energy (MJ/kg)	15.48
Ca (g/kg)	8.6
Mg (g/kg)	0.9
P (g/kg)	3.2
Available P (g/kg)	1.1
Na (g/kg)	7.9
K (g/kg)	9.2
Lys (g/kg)	8.9
Meth (g/kg)	3.7
Cyst (g/kg)	2.7
M + C (g/kg)	6.5
Threo (g/kg)	6.7
Arg (g/kg)	7.9
Iso (g/kg)	8.4
Hist (g/kg)	4.2
Val (g/kg)	9.9
Tyr (g/kg)	5.0
Glyc (g/kg)	9.4

Table 3. Composition (%) of the starter (0–10 d), grower (11–24 d) and finisher (25–42 d) diets.

	Starter		Grower		Finisher	
Ingredients	Control	Treatment	Control	Treatment	Control	Treatment
Food waste	-	15	-	15	-	15
Maize	48.50	45.14	52.12	47.97	57.62	53.47
Soybean meal	42.83	34.21	38.98	31.19	33.43	25.64
Vitamin and Mineral Premix[1]	0.20	0.20	0.20	0.20	0.20	0.20
Limestone	0.84	0.55	0.78	0.48	0.74	0.45
NaCl	0.37	0.07	0.37	0.07	0.37	0.07
Methionine	0.36	0.39	0.31	0.33	0.27	0.28
Soybean oil	4.46	1.64	5.17	2.45	5.59	2.86
Lysine	0.24	0.37	0.17	0.28	0.16	0.27
Threonine	0.10	0.24	0.07	0.11	0.04	0.09
Monocalcium Phosphate	2.02	2.06	1.76	1.80	1.50	1.54
Choline	0.08	0.13	0.07	0.12	0.08	0.13

[1]Premix supplied per kg of diet: 13,000 IU vitamin A (retinyl acetate), 3,500 IU vitamin D_3 (cholecalciferol), 70 mg vitamin E (DL-α-tocopheryl acetate), 7 mg vitamin K_3, 8.5 mg thiamin, 8 mg riboflavin, 5 mg pyridoxine, 0.020 mg vitamin B_{12}, 50 mg nicotinic acid, 15 mg pantothenic acid, 1.5 mg folic acid, 0.15 mg biotin, 1 mg iodine, 50 mg iron, 75 mg manganese, 15 mg copper, 0.3 mg selenium, 75 mg zinc

Table 4. Analysed composition (%) of the starter (0–10 d), grower (11–24 d) and finisher (25–42 d) diets.

	Starter		Grower		Finisher	
Ingredients	Control	Treatment	Control	Treatment	Control	Treatment
Dry matter	88.74	88.55	89.23	88.45	89.30	89.25
Ash	5.87	5.60	5.52	5.25	4.95	4.47
Crude protein	22.82	22.69	21.98	21.02	18.88	18.67
Ether extract	5.88	6.02	6.32	7.09	7.25	7.71
Crude fibre	4.00	4.01	3.82	3.66	3.29	3.02

Table 5. Calculated analyses of the starter (0–10 d), grower (11–24 d) and finisher (25–42 d) diets.

	Starter		Grower		Finisher	
Ingredients	Control	Treatment	Control	Treatment	Control	Treatment
ME (MJ/kg)	12.55	12.55	12.97	12.97	13.39	13.39
Sodium (g/kg)	1.6	1.6	1.6	1.6	1.6	1.6
Ca (g/kg)	9.6	9.6	8.7	8.7	7.8	7.8
Available P (g/kg)	4.8	4.8	4.4	4.4	3.9	3.9
Lysine (g/kg)	14.4	14.4	12.9	12.9	11.5	11.5
Methionine +cysteine (g/kg)	10.8	10.8	9.9	9.9	9.0	9.0
Threonine (g/kg)	9.7	10.5	8.8	8.8	7.8	7.8

2 m^2) bedded with chopped wheat straw litter. The maximum stocking density in the pens did not exceed 33 kg/m^2at any time, following directive 2007/43/EC (EU 2007). The house environmental conditions (light and ventilation) were controlled according to commercial recommendations, and heat was provided with a heating infrared lamp per pen.

Broilers were fed three different diets depending on growing phase; starter (0–10 d), grower (11–24 d) and finisher (25–42 d). In the control (C) group, broilers were fed a basal diet based on corn and soybean meal with no food waste product added. In the treatment (T) group, food waste product was added to starter, grower and finisher diet at a level of 15%. The inclusion rate was selected in order to achieve similar dietary metabolisable energy and crude protein content between treatments and to meet *Ross 308 Broiler Nutrition Specifications* (Aviagen 2014). The composition, determined and calculated analysis of the diets is presented in Tables 3, 4 and 5 respectively. Feed and water were provided *ad libitum*.

Experimental diets from the three growing phases were milled through a 1-mm screen before analysis. The DM was determined after drying in an oven at 100 for 24 h (Method 930.15; AOAC 1995). Ash was measured after combusting for 5 h at 550 and fat (as EE) was measured with Soxhlet apparatus (Soxtec Avanti 2050; Foss Tecator AB, Hoganas, Sweden). The Kjeldahl nitrogen (N) analysis was performed in an autoanalyser unit (Kjeltec 2300; Foss Tecator AB, Hoganas, Sweden) and CP was calculated as N × 6.25 (Method 954.01; AOAC 1995). The CF was measured using the filter bag system (ANKOM 220 FiberAnalyzer; ANKOM Technology, NY, US).

Determination of performance parameters

Body weight (BW) was recorded on the first day of the experimental period and at the end of each feeding phase. Feed intake was recorded and feed conversion ratio (FCR) was calculated. Total mortality was calculated as the number of broilers that died throughout the study compared to the initial number of broilers placed.

Carcase analysis

On day 42 of age, 60 broilers (30 per treatment and three per replicate pen) were randomly selected and sacrificed to investigate treatment effects on carcase yield. After chilling at 4 for 24 h, carcases were weighed to estimate the percentage of carcase yield. The breast muscle was removed from carcases, weighed and expressed as a percentage of final body weight. The right part of the breast muscle was removed from the cold carcase and used for the determination of meat quality indices (pH_{24}, colour, shear force and cooking loss).

Measurement of pH_{24} and colour

The pH was measured via the insertion of an electrode attached to a pH meter (Sentron 1001 pH System, Roden, Netherlands) 24 h *post-mortem* into the right section of the breast muscle. Two buffers and pH 4.0 and 7.0 at room temperature were used for the calibration of the (Merck, Darmstadt, Germany). Colour traits were determined in the breast muscle after 30 min at air room temperature, and for every sample, there were three measurements taken. A Miniscan XE (HunterLab, Reston, USA) was used to determine colour using the Hunter

Table 6. Performance of broilers for the whole experimental period (0–42 d).

	Treatment [a]			
Parameter	C (n = 10)	T (n = 10)	SEM	P-value
Initial BW (g/broiler)	40.05	39.75	0.25	0.997
Final BW 42d (g/broiler)	3098.2	2794.1	49.99	<0.001
ABWG (g/broiler)	3058.1	2754.3	50.03	<0.001
AFI (g/broiler)	4586.9	4289.6	73.94	0.011
FCR(g feed/g gain)	1.50	1.56	0.021	0.129
Mortality %	7	2	1.78	0.072
Carcase yield (%)	75.78	75.60	0.250	0.597
Breast yield (%)	29.48	30.28	0.376	0.138

[a]C, control group with no food waste; T, treatment group with 15% of food waste added.

BW: body weight; AFI: Average feed intake of the total experimental period (0–42 days); ABWG: Average body weight gain of the total experimental period (0–42 days); FCR: Feed conversion ratio (g feed/g gain) of the total experimental period (0–42 days); SEM: pooled standard error of means.

Lab L^* (lightness), a^* (redness), b^* (yellowness) system (CIE (Commission Internationale de l'Eclairage) 1976), which was standardised using white and black tiles.

Measurement of cooking loss and shear force

For the determination of cooking losses, the right section of breast muscle was weighed, placed in plastic bags, cooked for 30 min at 85°C in a temperature-controlled water bath. Subsequently, samples were left under running tap water for 15 min, dried and weighed to measure the percentage of cooking loss. For the evaluation of shear force, the method published by ***Cason et al. (1997)*** was used. In detail, shear force was measured using the Zwick Testing Machine (Model Z2.5/TN1S; Zwick GmbH & Co, Ulm, Germany) equipped with a shear blade (Warner-Bratzler G146; Instron, Grove City, PA, US) and three strips from the breast muscle of 1 cm^2 were cut, parallel to muscle fibres and peak force values were marked in N/mm^2.

Internal organs, haematological and biochemical parameters

Blood samples from 20 broilers at 42 d of age in total (10 per treatment, one per replicate pen) were collected by exsanguination after slaughter in order to examine selected haematological and biochemical parameters. In detail, haematocrit (%), asparate aminotransferase (SGOT-AST) (IU/l), alanine aminotransferase (SGPT-ALT) (IU/l), blood urea nitrogen (BUN) (mg/dl), γ-glutamyltransferase (γ-GT) (IU/l), alkaline phosphatase (IU/l), cholesterol (mg/dl), total proteins (g/dl) and fractions of albumins (g/dl) and globulins (g/dl) were assessed using an automatic ABX Pentra 400 analyser (Horiba-ABX, Montpellier, France). Moreover, from the same 20 broilers, the heart, spleen, liver, kidney, bursa of Fabricius and gizzard were removed and their weight was expressed as a percentage of final body weight (g/100 g body weight).

Table 7. Treatment effects on internal organ weight and selected biochemical and haematological parameters.

	Treatment [a]			
Parameter	C (n = 10)	T (n = 10)	SEM	P-value
Heart (%)	0.507	0.505	0.017	0.935
Spleen (%)	0.097	0.096	0.008	0.903
Liver (%)	1.60	1.59	0.045	0.916
Kidney (%)	0.159	0.157	0.010	0.886
Bursa of Fabricius (%)	0.199	0.194	0.018	0.597
Gizzard (%)	1.25	1.22	0.066	0.730
SGOT AST(IU/l)	522.3	519.3	85.74	0.385
SGPT ALT (IU/l)	5.50	4.70	0.858	0.518
BUN (IU/l)	1.41	0.98	0.176	0.120
γ-GT (IU/l)	22.30	23.10	2.520	0.825
Phosphatase (IU/l)	3207.0	2260.8	400.2	0.112
Cholesterol (mg/dl)	143.3	157.5	4.758	0.049
Total proteins (g/dl)	2.80	2.71	0.087	0.475
Albuomin (g/dl)	1.19	1.22	0.038	0.587
Globulin (g/dl)	1.61	1.49	0.060	0.178
Haematocrit (%)	29.56	29.50	1.470	0.485

[a]C, control group with no food waste; T, treatment group with 15% of food waste added.

Statistical analysis

Experimental data are presented as means and standard errors. Treatment effects on performance (body weight, feed consumption, carcase yield), internal organ weight, selected biochemical and haematological parameters and carcase quality were calculated using t-tests for independent samples and Mann–Whitney U tests for normally and non-normally distributed variables, respectively. Normality of data distribution was assessed using the Shapiro–Wilks test and graphical methods (Q–Q plots). For all tests, significance was set at $P < 0.05$. Statistical analyses were performed with SPSS software (version 20.0, IBM, USA).

Results

Broiler performance

In Table 6, the effects of feeding dried food waste on broilers' body weight, feed consumption, FCR and carcase yield are presented. Final BW at d 42 of age for the T group was significantly lower than that of the control. The ABWG was statistically significantly lower for the broilers in the T group compared to those of the C group. Similarly, lower AFI was noted for broilers of the T group compared to those of the C group; however, when calculated, the FCR did not differ between the two groups. As far as broilers carcase characteristics are concerned, they were not affected by the inclusion of food waste at 15%.

Biochemical, haematological parameters and internal organ weight

Several biochemical and haematological parameters, as well as internal organ weights, expressed as a percentage of final body weight, were examined in order to investigate potential treatment effects on broiler health. Data are presented in Table 7. The weight of internal organs indicated that the consumption of the diet containing 15% of food waste did not have any negative impact on the broilers. The haematological parameters were not affected by feeding food waste. However, cholesterol was statistically different and was lower for birds in the C group.

Meat quality traits

The parameters for carcase and meat quality are summarised in Table 8. Feeding a diet containing 15% of food waste affected some colour traits, especially lightness (L*) and yellowness (b*). In the breast meat of birds in group T, yellowness (b*) values decreased significantly compared to

Table 8. Treatment effects on selected parameters of carcase quality.

Parameter	Treatment [a]			
Colour traits	C (n = 10)	T (n = 10)	SEM	P-value
L*	56.22	54.18	0.840	0.094
a*	6.06	5.70	0.270	0.123
b*	17.43	15.84	0.474	0.023
Physical traits				
pH_{24}	6.22	6.21	0.026	0.829
Cooking loss (%)	13.62	12.98	0.644	0.485
Shear force (100 N/mm^2)	11.81	10.85	0.504	0.081

[a]C, control group with no food waste; T, treatment group with 15% of food waste added.
L* = lightness, a* = redness, b* = yellowness

those in the control group. Similarly, a tendency for lower L* values in the breast meat was observed for the T group broilers. The addition of food waste had no effect on pH_{24} and cooking loss, whereas shear force values tended to be lower in T, when compared to C broilers.

Discussion

In the current study, broilers fed a diet containing 15% of food waste (CP = 23.76%, EE = 20%) gained less weight and had lower feed intake in comparison with the control group. The FCR did not differ between C and T treatments, indicating a similar utilisation of dietary nutrients. Cho et al. (2004b) reported that, when feeding dried leftover food (DLF) (CP = 20.62%, EE = 9.99%) at different inclusion levels, the final body weight of broilers was slightly greater for the control group and that the FCR deteriorated with increasing dried food waste inclusion. However, Navidshad et al. (2009) stated that feeding a modified meat meal (60% meat meal, 15% wheat bran, 10% feather meal and 15% zeolite) had no negative effects on final weight gain, feed intake or FCR when 20, 35, 50, 65 and 80 g/kg were added to broiler diets. These differences may be attributed to the different types of food that were present in the waste. In another trial on monogastrics, weight gain and feed intake of growing and finishing pigs were reduced following the addition of 40% dried food waste (CP = 25%, EE = 17.3%) (Chae et al. 2000). Growth performance of finishing pigs was not affected by diets containing 25% or 50% food waste mixture (CP = 22%, EE = 23.9%; Kwak and Kang 2006). Although not directly comparable, many studies support the use of bakery waste. Al-Tulaihan et al. (2004) did not find any differences in final BW, feed intake and FCR when broilers diets were supplemented with 30% of dried bakery waste (CP = 12.22%, EE = 1.32%). In the present study, carcase and breast yield were similar between treatments and did not appear to be affected by the addition of 15% of food waste. These results were in agreement with Kwak and Kang (2006), who observed that carcase weight was not affected, even for food waste (CP = 22%, EE = 23.9%) inclusion of up to 50%.

Some physical and colour traits of broilers meat were affected when food waste was added to the diets. The pH_{24} of meat in T treatment was similar to those in C treatment. The evaluation of pH_{24} is important as it affects the meat colour and cooking loss percentages (Apple et al. 2005). Moreover, the lower the muscle pH is the poorer the quality of meat (Ryu and Kim 2005). Colour traits in the present study showed differences between the two treatments. The L* and b* factors were numerically higher for C group as well as shear force. The lower value for L* colour trait in T treatment indicated a darker meat and which may have been associated with the concentration of myoglobin. Meat quality parameters, such as colour traits, pH, cooking loss and shear force, were similar to reports using different levels of dehydrated food waste product (CP = 15.79%, EE = 15.98%) in chicken diets (Chen et al. 2007). In other studies, meat pH was not affected by adding food waste in diets for growing-finishing pigs; the L* factor increased in the group with 100% food waste inclusion (Kjos et al. 2000). In another study, Choe et al. (2017) found higher pH in their control group while higher values of lightness and yellowness were noted in the pigs fed a diet containing food waste for the growing-finishing period and until 4 weeks prior slaughtering (CP = 26.59%). In the current trial, food waste inclusion in the diet had no negative impact on cooking loss of the breast meat.

The weights of heart, spleen, liver, kidney, bursa of Fabricius and gizzard of the T group birds were not different compared to those given the C dietary treatment. These results were in partial agreement with Chen et al. (2007) who reported no significant variations in heart and liver weights from broilers fed diets containing food waste product; however, when the inclusion was increased to 5%, 10% or 20%, the gizzard weights followed a linear increasing pattern. In another report (Cho et al. 2004b), no differences in the weight of heart, liver and gizzard were seen for broilers fed diets with increasing levels of dried food waste (10%, 20% and 30%) as compared to a control group.

In the current study, the serum AST and serum γ-GT showed similar values among the treatments, which was in contrast to the work of Chen et al. (2007), where same serum markers increased linearly with higher dehydrated food waste level. Liver enzyme levels were measured in order to investigate a potential disease. The concentration of enzyme γ-GT mainly relates to the function of chicken kidneys, while AST is generally found in liver, skeletal muscle, heart muscle, brain and kidney. These parameters were within the normal range in the present study and this indicated undamaged kidney tissue. Serum cholesterol concentrations in broilers fed diets containing 15% of food waste were higher compared to broilers fed the C diet, which might be due to the composition of fat in the food waste. This was in agreement with the findings of Cho et al. (2004b), who reported elevated serum cholesterol levels in broilers fed dried leftover food waste compared to a control group

In conclusion, the results of the present study showed that feeding broilers a diet containing 15% dried food waste from hotels had no negative effects on mortality, carcase or breast yield. Final broiler weight was affected, but FCR was similar between treatments. Biochemical parameters did not indicate any physiological malfunctions due to the dietary inclusion of dried food waste. For meat quality parameters, some impact was detected in colour traits, i.e., decreased yellowness in broilers fed the food waste supplemented diet. Further investigation

is required to optimise the inclusion level of food waste in conventional broiler diets.

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Disclosure statement

Authors declare no conflict of interest.

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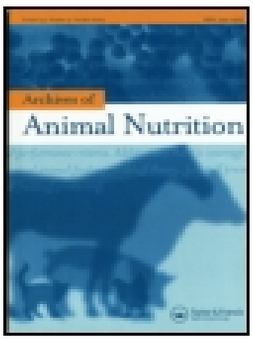

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Impact of the dietary inclusion of dried food residues on the apparent nutrient digestibility and the intestinal microbiota of dogs

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ABSTRACT

The use of food residues for animal nutrition might imply ecological and economic advantages; however, their effects as a potential ingredient have not yet been evaluated in dogs. In the present study, four diets with 0, 5, 10 and 15% dried food residues (DFR), derived from hotel catering, were fed to 10 healthy adult dogs. At the end of each three-week feeding period, faeces and blood were collected. The apparent nutrient digestibility was calculated by the dietary inclusion of titanium dioxide as an inert marker. The results demonstrated that the apparent crude protein digestibility and ether extract digestibility decreased with increasing amounts of DFR in the diets ($p < 0.05$). In addition, an increase of the faecal concentrations of acetic acid, propionic acid, *n*-butyric acid and total short-chain fatty acids (SCFA) was observed ($p < 0.05$). Faecal ammonium and lactate concentrations, as well as plasma phenol and indole concentrations, were not linearly affected by the dietary inclusion of DFR. The relative abundance of *Fusobacteria* in the faeces of the dogs decreased, and the relative abundance of *Actinobacteria* and *Bacteroidetes* increased with increasing amounts of DFR in the diets ($p < 0.05$). In conclusion, the DFR seemed to be intensively fermented by the intestinal microbiota of the dogs, as indicated by the increased faecal SCFA concentrations and the shifts in the composition of the faecal microbiota. Dietary inclusion levels of up to 5% can be recommended based on our results, as the observed lower apparent crude protein and ether extract digestibility might limit the use of food residues for dogs at higher amounts.

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1. Introduction

It has been estimated that about 88 million tons of food waste are produced in the EU every year (Stenmarck et al. 2016). Worldwide, about 1.3 billion tons of food are lost or wasted yearly (FAO 2011). Given its economic and ecological impact, different strategies

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have been initiated to reduce the generation of food waste. The EU funded project "Food for Feed" (F4F) (LIFE15 ENV/GR/000257), for instance, aims to evaluate the potential use of hotel catering waste in animal nutrition. Food waste has been fed to farm animals for a long time (Westendorf 2000), but this has either declined for efficiency reasons in the course of intensive animal feeding (Doua et al. 2018), or is part of legal restrictions in several world regions. In the European Union, feeding catering waste to farm animals other than fur animals has been prohibited by the Regulation (EC) No 1774/2002, as catering waste can be a vector for infectious diseases. In particular, foot-and-mouth disease and classic swine fever might be transmitted by raw food waste (Doua et al. 2018). The Regulation (EC) No 1774/2002 has been repealed by the Regulation (EC) No 1069/2009, retaining the ban of catering waste for the nutrition of farmed animals other than fur animals. For processed petfood, it is also currently prohibited to use catering waste as an ingredient (Commission Regulation (EU) No 142/2011). Although legal restrictions are therefore a barrier for the potential use of food waste for animal nutrition in the European Union, the global need for a reduction of food waste might be a driver of policy changes. However, this would only be possible if the food residues can be considered safe, both with regard to hygiene standards and animal health.

In the present study, dried food residues (DFR) were evaluated as a dietary ingredient for dogs. In particular, the effects on the apparent nutrient digestibility and the intestinal microbiota were investigated.

2. Methods

The study was approved by the Landesamt für Gesundheit und Soziales (LAGeSo), Berlin, Germany (G 0233/18).

2.1. Dried food residues

The food residues were separately collected from the kitchen of the four participating hotels in Crete, Greece, for the purposes of the F4F project. On average, about 850 kg food residues were collected daily, where at least 100 kg were used for the determination of the composition of the food residues.

The food residues´ characterisation of the hotel's catering was based on ASTM D5231-92 standard (2008). This test method describes procedures for measuring the composition of unprocessed solid waste by employing manual sorting, and it was adapted herein for the analysis of the hotels' food residues. After sorting the food residues into predetermined categories, the net weight of the material was measured. Each weighing was performed twice.

The food residues used for the purposes of the current study consisted of: fresh fruits (44.4%), cooked meals and snacks (25.4%), fresh vegetables and salads (13.9%), bread and bakery (5.71%), meat and fish (4.90%), dairy products (excluding milk) and eggs (0.79%), impurities (0.74%), sauces, herbs and spices (0.34%), desserts (0.22%), confectionary and snacks (0.09%), processed fruits (0.03%), and others (3.48%). Percentages are reported per wet weight of the non-dried food residues.

The impurities were manually removed from the food residues. The material was consequently ground and dried using solar greenhouse drying. This was performed in the pilot plant developed by the F4F project in Heraklion, Crete. The particle sizes of the final dried product used for the present study was less than 5 mm.

2.2. Study design

Ten healthy adult beagle dogs (five females, five males, aged 44.1 ± 11.7 months) were fed a complete diet based on poultry meal and rice flour (Table 1) with or without DFR (0, 5, 10 or 15%). The analysed dry matter and nutrient concentrations of the DFR and the experimental diets are presented in Tables 2, 3 and 4.

The diets were fed to the dogs for 3 weeks each. As the acceptance and tolerance of DFR were unknown, the diets were fed in increasing order regarding their amount of DFR, allowing an early detection of potential intolerances. At the end of the feeding periods, urine and faecal samples were collected for 4 d each. In addition, fasting blood samples were collected at the end of the feeding periods. The urine samples were stored at −20°C, and the faecal samples at −80°C until further analysis. After storage for 1 h at room temperature, the blood samples were centrifuged for ten min at 1811 *g* and 4°C (Heraeus Megafuge 1.0 R, Thermo Scientific), and the plasma was stored at −20°C until further analysis.

Table 1. Diet composition.

	Experimental diets			
Ingredients [%]	0% DFR[†]	5% DFR	10% DFR	15% DFR
Poultry meal (low ash)	22.7	21.2	19.8	17.9
Rice flour	65.8	63.7	61.6	59.2
Rapeseed oil	5.66	4.68	3.72	2.90
Cellulose	1.36	1.16	0.97	0.79
Vitamin and mineral premix[‡]	1.16	1.16	1.15	1.16
Potassium hydrogen carbonate	0.66	0.57	0.40	0.35
Sodium chloride	0.53	0.44	0.34	0.24
Blood meal[§]	1.12	1.10	1.10	1.11
Magnesium supplement[#]	0.21	0.21	0.21	0.21
Vitamin B supplement[$]	0.07	0.07	0.07	0.08
Dicalcium phosphate	-	0.03	0.05	0.12
Calcium carbonate	-	-	-	0.01
Cod liver oil	0.53	0.53	0.52	0.53
Dried food residues	-	4.95	9.87	15.2
Titanium oxide	0.20	0.20	0.20	0.20

[†]DFR, dried food residues; [‡]provided per kg premix (according to the manufacturer): crude protein, 249 g; ether extract, 34 g; crude fibre, 13 g; crude ash, 342 g; magnesium, 55 g; sodium, 27 g; iodine, 200 mg; iron, 1.5 g; copper, 900 mg; zinc, 8 g, manganese, 1 g; vitamin E, 5 g; vitamin B_1, 500 mg; vitamin B_2, 1 g; vitamin B_6, 200 mg; vitamin B_{12} 5 mg; pantothenic acid 3 g (napfcheck BARF Ergänzung, Napfcheck, Planegg, Germany); [§]provided per kg blood meal (according to the manufacturer): crude protein, 920 g; iron, 2.8 g (Fortain®, Fortan GmbH & Co. KG, Wuppertal, Germany); [#]provided per kg supplement (according to the manufacturer): magnesium, 310 g (Futtermedicus Optisolo Magnesium Pulver, Futtermedicus, Fürstenfeldbruck, Germany); [$]provided per kg supplement (according to the manufacturer): crude protein 73 g, ether extract, 11 g; crude fibre, 245 g; crude ash, 87 g; sodium, 1 g; vitamin B_1, 3.5 g; vitamin B_2, 3.5 g; vitamin B_6, 3 g; vitamin B_{12}, 0.625 g; calcium D-pantothenate, 12 g; niacin, 30 g; folic acid, 1.25 g; biotin, 0.125 g (Futtermedicus Optisolo B-Komplex-Pulver, Futtermedicus, Fürstenfeldbruck, Germany).

Table 2. Analysed dry matter (DM) and nutrient concentrations of the experimental diets.

	Experimental diets			
	0% DFR[†]	5% DFR	10% DFR	15% DFR
DM [g/100 g fresh matter]	92.9	92.8	92.6	92.5
Crude ash [g/100 g DM]	4.40	4.78	4.71	4.64
Crude protein [g/100 g DM]	24.9	24.9	25.1	24.8
Ether extract [g/100 g DM]	9.94	9.94	9.49	9.87
Crude fibre [g/100 g DM]	0.95	0.92	0.90	0.92
Total dietary fibre [g/100 g DM]	3.46	3.15	3.52	3.91
Insoluble dietary fibre [g/100 g DM]	3.24	2.89	2.79	3.55
Soluble dietary fibre [g/100 g DM]	0.22	0.26	0.73	0.36
Calcium [g/100 g DM]	0.72	0.72	0.70	0.70
Phosphorus [g/100 g DM]	0.55	0.57	0.58	0.54
Potassium [g/100 g DM]	0.38	0.42	0.45	0.48
Magnesium [g/100 g DM]	0.19	0.18	0.19	0.20
Sodium [g/100 g DM]	0.27	0.30	0.29	0.31
Titanium oxide [g/100 g DM]	0.23	0.21	0.21	0.21
Copper [mg/100 g DM]	1.79	1.60	1.52	1.44
Zinc [mg/100 g DM]	13.7	14.3	12.9	12.7
Iron [mg/100 g DM]	9.94	9.92	9.99	9.79
Manganese [mg/100 g DM]	2.63	2.66	2.26	2.55

[†]DFR, dried food residues; analysed composition: DM 91.2 g/100 g; per 100 g DM: crude ash 5.97 g, crude protein 25.9 g, ether extract 24.7 g, crude fibre 3.46 g, calcium 0.61 g, phosphorus 0.42 g, potassium 0.87 g, magnesium 0.09 g, sodium 0.82 g.

Table 3. Analysed amino acid concentrations of the experimental diets [g/100 g dry matter].

	Experimental diets			
	0% DFR[†]	5% DFR	10% DFR	15% DFR
Alanine	1.34	1.60	1.40	1.15
Arginine	1.33	1.50	1.35	1.14
Aspartic acid	0.59	0.67	0.57	0.55
Cysteine	0.32	0.35	0.31	0.33
Glutamic acid	2.55	3.12	2.83	2.35
Glycine	1.57	1.83	1.58	1.35
Isoleucine	0.67	0.87	0.73	0.66
Leucine	1.40	1.66	1.49	1.32
Lysine	0.48	0.57	0.49	0.45
Methionine	0.60	0.56	0.56	0.62
Phenylalanine	0.04	0.04	0.04	0.74
Proline	1.07	1.23	1.10	0.94
Serine	0.78	0.97	0.88	0.72
Threonine	0.66	0.80	0.76	0.63
Tyrosine	0.53	0.56	0.50	0.42
Valine	1.88	2.01	1.97	1.79

[†]DFR, dried food residues.

Table 4. Analysed fatty acid concentrations of the dried food residues used for the experimental diets.

Fatty acids	Contents [% of TFA†]	Fatty acids	Contents [% of TFA]
C6:0	0.06	C18:0	8.25
C8:0	0.30	C18:1 trans isomers	0.27
C10:0	0.76	C18:1 n-9 c	42.3
C11:0	0.00	C18:2 n-6 t	0.00
C12:0	2.22	C18:2 n-6 c	10.4
C14:0	2.93	C18:2 n-9 c, n-11 t	0.41
C14:1	0.09	C18:3 n-3	0.68
C15:0	0.26	C20:0	0.00
C16:0	24.3	C20:2	4.29
C16:1	1.35	C20:3 n-3	0.10
C17:0	0.26	C20:5	0.39
C17:1	0.13	C22:0	0.21
		C22:6	0.07
		C23:0	0.04
		C24:0	0.00

†TFA, total fatty acids.

The daily amount of feed was calculated based on the recommendations of the NRC (2006), and weekly adjusted to maintain body weight of the dogs. The feed intake of the dogs was recorded daily and the body weight of the dogs weekly.

2.3. Analyses

The nutrient concentrations of the DFR and experimental diets, as well as the faecal mineral concentrations were analysed as described previously (Passlack and Zentek 2013). Faecal crude protein and ether extract concentrations were determined using the same methods as for the feed analyses. Total dietary fibre and insoluble dietary fibre were measured using a commercial assay kit (Megazyme Ltd., Ireland). The concentration of titanium dioxide (TiO_2) in the diets and faeces was measured photometrically (Ultrospec 2100 pro photometer, Amersham Pharmacia Biotech Inc., Piscataway, NJ, USA). To determine the apparent nutrient digestibility, the following formula was used:

Apparent digestibility [%] = 100 – {(TiO_2 in diet [%]/TiO_2 in faeces [%]) · (Nutrient in faeces [%]/Nutrient in diet [%]) · 100}.

The fatty acids in the DFR were extracted and methylated according to the method of O'Fallon et al. (2007). For the determination of the fatty acid profile in the DFR, an Agilent 6890 N gas chromatograph, equipped with an HP-88 capillary column (60 m × 0.25 mm i.d. with 0.20 µm film thickness, Agilent) and a flame ionis ation detector, was used. Tridecanoic acid was used as an internal standard for the chromatographic analysis (Fluka, Sigma Aldrich).

The faecal pH was measured after thawing of the samples (Seven Multi pH metre, Mettler-Toledo GmbH). The microbial metabolites in the faeces of the dogs were determined as described by Paßlack et al. (2015, 2018). The faecal microbiota was

analysed using 16S rDNA sequencing (Illumina NextSeq500 sequencer, LGC, Berlin, Germany) as described by Paßlack et al. (2021). In brief, the DNA was extracted from the faeces (0.2 g per sample) with a commercial extraction kit (QIAamp Fast DNA stool mini kit, Qiagen). The instructions of the manufacturer were only modified by considering a lysis step at 90°C. For the analysis of the DNA extracts, amplicon sequencing was used, where the V3-V4 region of the 16S rDNA gene was targeted, with two 150-base pair reads. After the combination of forward and reverse reads (BBMerge tool; Bushnell et al. 2017) and demultiplexing, the 16S rDNA sequences could be analysed using the QIIME2 pipeline (Bolyen et al. 2019) and the SILVA SSU database (Yilmaz et al. 2014). Tables were constructed for genera with more than five sequences per sample according to Huse et al. (2007). Thus, only dominant bacterial sequences are reported.

The concentrations of phenols and indoles in the plasma of the dogs were measured as described by Paßlack et al. (2021). Phenol and indole concentrations in the urine were determined according to Eisenhauer et al. (2019).

2.4. Statistics

For the statistical analysis, IBM SPSS Statistics 27 (SPSS Inc., Chicago, IL, USA, 2013) was used. Data in the Tables are presented as mean ± standard deviation. For group comparisons, polynomial linear and quadratic contrasts were calculated (General Linear Model repeated measures, within-subject factor: dried food residues; number of levels: four). In addition, a one-factorial analysis of variance (fixed factor: diet) was performed to compare the dietary treatment groups, using the Scheffé (variance equality) or Tamhane 2 (variance inequality) post hoc tests. Both for the polynomial contrasts and the post hoc tests, $\alpha = 0.05$ was considered to be statistically significant. Visualisation of the sequencing data was done via principal component analysis with singular value decomposition (SVD) that decomposes the variability of the data into the between and within groups variability and performing SVD on both parts, as laid out in the webtool clustVis (Metsalu and Vilo 2015).

Concerning the 16S rDNA sequencing data, values < 0.5% in all groups are only presented if a group difference was detected by the polynomial contrast calculation or post hoc tests. The full sequencing data set is available in the NCBI BioProject database (PRJNA731312).

3. Results

3.1. Animal health, feed intake, body weight and apparent nutrient digestibility

All dogs were healthy throughout the study. With increasing amounts of DFR in the diets (0, 5, 10 and 15%), the daily amount of feed had to be increased in order to maintain body weight of the dogs (Table 5). In addition, increasing amounts of dietary DFR reduced the apparent crude protein digestibility and ether extract digestibility (Table 5).

Table 5. Body weight of dogs, feed intake, apparent nutrient digestibility, faecal dry matter (DM), pH and concentrations of ammonium, lactate and short-chain fatty acids (SCFA) in the faeces of dogs fed a diet with varying amounts of dried food residues (DFR).

	Experimental diets				Polynomial contrasts (*p*-values)	
	0% DFR	5% DFR	10% DFR	15% DFR	Linear	Quadratic
Body weight [kg]	12.4 ± 1.24	12.7 ± 1.20	12.4 ± 1.13	12.5 ± 1.22	0.542	0.304
Feed intake [g DM/d]	228 ± 18.9^{ab}	212 ± 9.55^{a}	224 ± 27.8^{ab}	241 ± 20.8^{b}	0.004	< 0.001
Apparent digestibility [%]						
Crude protein	82.0 ± 4.44^{ab}	83.4 ± 5.14^{a}	78.1 ± 10.2^{ab}	74.0 ± 6.71^{b}	0.003	0.220
Ether extract	94.1 ± 2.67^{a}	93.8 ± 3.30^{a}	89.7 ± 3.70^{b}	92.8 ± 1.62^{ab}	0.013	0.128
Faecal DM [%]	28.5 ± 3.07	32.4 ± 2.11	30.2 ± 3.05	28.5 ± 4.93	0.754	0.052
Faecal pH	7.25 ± 0.20^{ab}	7.27 ± 0.18^{a}	6.93 ± 0.27^{bc}	6.85 ± 0.31 ^{c}	0.001	0.606
Faecal contents [µmol/g]						
Ammonium	31.5 ± 8.73	36.5 ± 10.4	36.2 ± 8.47	34.6 ± 5.13	0.501	0.242
L-lactate	0.01 ± 0.01	0.29 ± 0.54	0.01 ± 0.02	0.01 ± 0.01	0.126	0.131
D-lactate	0.02 ± 0.04	0.13 ± 0.18	0.06 ± 0.07	0.04 ± 0.06	0.911	0.045
Acetic acid	70.5 ± 14.2	81.9 ± 15.3	93.5 ± 20.2	87.7 ± 24.1	0.025	0.094
Propionic acid	34.0 ± 8.79	38.2 ± 11.4	46.3 ± 14.0	40.4 ± 10.5	0.039	0.072
i-butyric acid	3.79 ± 1.01	4.07 ± 1.02	4.70 ± 1.19	4.01 ± 0.93	0.261	0.223
n-butyric acid	12.3 ± 3.83^{a}	17.1 ± 5.28^{ab}	21.3 ± 6.45^{bc}	25.1 ± 7.80 ^{c}	0.002	0.830
i-valeric acid	4.42 ± 1.48	5.14 ± 1.50	5.72 ± 1.87	4.23 ± 1.08	0.993	0.029
n-valeric acid	2.49 ± 2.09	4.81 ± 3.88	2.38 ± 1.91	4.60 ± 2.99	0.396	0.958
Total SCFA	128 ± 25.8^{a}	151 ± 29.5^{ab}	174 ± 38.1^{b}	166 ± 40.2^{ab}	0.009	0.079
Faecal SCFA [%]						
Acetic acid	55.4 ± 3.39	54.3 ± 3.84	53.8 ± 2.99	52.7 ± 3.46	0.062	0.990
Propionic acid	26.5 ± 3.28	24.9 ± 3.09	26.3 ± 3.50	24.3 ± 2.33	0.045	0.862
i-butyric acid	2.97 ± 0.49^{a}	2.68 ± 0.31^{ab}	2.71 ± 0.30^{ab}	2.45 ± 0.38^{b}	0.033	0.912
n-butyric acid	9.69 ± 2.13^{a}	11.5 ± 3.54^{ab}	12.5 ± 3.78^{ab}	15.2 ± 3.30^{b}	0.001	0.642
i-valeric acid	3.43 ± 0.74^{a}	3.36 ± 0.37^{a}	3.25 ± 0.48^{ab}	2.57 ± 0.41^{b}	0.009	0.112
n-valeric acid	2.06 ± 1.63	3.35 ± 2.75	1.52 ± 1.41	2.81 ± 1.67	0.891	0.998

Different superscript letters in the same row indicate significant group differences ($p < 0.05$).

3.2. Faecal dry matter, pH and bacterial metabolites

The faecal dry matter concentrations were not affected by the dietary treatments (Table 5). Concentrations of acetic acid, propionic acid, *n*-butyric acid and total short-chain fatty acids (SCFA) increased with increasing dietary DFR (linear contrasts; $p < 0.05$) (Table 5). Accompanied by the increase of faecal SCFA, the faecal pH decreased with increasing dietary inclusion levels of DFR (linear contrast: $p = 0.001$). When calculated as percentage of total SCFA, a decrease of propionic acid, *i*-butyric acid and *i*-valeric acid, and an increase of *n*-butyric acid was observed with increasing amounts of DFR in the diets ($p < 0.05$). Faecal ammonium and lactate concentrations were not linearly affected by increasing amounts of DFR in the experimental diets (Table 5).

3.3. Phenol and indole concentrations in the urine and plasma

No unidirectional effect of the diets on the phenol and indole concentrations in the urine and plasma of the dogs could be detected (Table 6). However, a quadratic effect was observed for the 7-methylindole concentrations in the urine ($p = 0.037$) and the indoxyl sulphate concentrations in the plasma ($p = 0.035$) of the dogs, with the highest concentrations at 5% dietary DFR and 10% dietary DFR, respectively.

Table 6. Phenol and indole concentrations in the urine[†] and plasma[§] of dogs ($n = 10$) fed a diet with varying amounts of dried food residues (DFR).

	Experimental diets				Polynomial contrasts (p-values)	
	0% DFR	5% DFR	10% DFR	15% DFR	Linear	Quadratic
Urine [µg/ml]						
Phenol	4.93 ± 1.92	6.09 ± 1.83	5.23 ± 2.23	5.71 ± 1.85	0.557	0.647
Indole	2.01 ± 0.76	4.79 ± 5.21	2.27 ± 1.53	2.51 ± 0.89	0.649	0.181
3-methylindole	2.01 ± 0.71	3.85 ± 4.56	2.28 ± 1.15	1.66 ± 0.52	0.194	0.141
7-methylindole	0.34 ± 0.59[a]	4.49 ± 5.67[ab]	2.30 ± 1.79[b]	1.46 ± 0.49[b]	0.563	0.037
Indoxyl sulphate	291 ± 177	307 ± 100	331 ± 226	272 ± 136	0.845	0.345
Plasma [µg/ml]						
Indoxyl sulphate	3.09 ± 3.31	4.39 ± 2.81	5.27 ± 4.21	3.28 ± 2.01	0.618	0.035
Phenol	0.62 ± 0.22	0.61 ± 0.05	0.64 ± 0.09	0.66 ± 0.22	0.104	0.799

[†]All values for p-cresol, 4-ethylindole, 2-methylindole and 2,3-dimethylindole were below the detection limit; only in the group 0% DFR three values of 7-methylindole were above the detection limit; [§]all values of p-cresol, indole and 3-methylindole were below the detection limit.
Different superscript letters in the same row indicate significant group differences ($p < 0.05$).

3.4. Faecal microbiota

Sequencing of the 16S rRNA gene yielded a mean number of combined reads (OTU) of 33,821 (± 14,605.7) sequences per sample.

Figure 1 depicts the faecal bacterial composition of the dogs by principal component analysis. The control group (0% DFR) showed a tighter cluster formation compared to the other treatment groups. However, differences between treatments seemed to be not dependent on the DFR inclusion level, as the faecal bacterial composition of the dogs fed 5% and 10% DFR showed a higher variability compared to the 15% DFR treatment.

In accordance with the principal component analysis, alpha diversity of the faecal microbiota of the dogs was not unidirectionally affected by the experimental diets (Table 7). However, marked changes in the relative abundance of bacterial phyla (Table 8), orders (Table 9) and genera (Table 10) in the faeces of the dogs were observed.

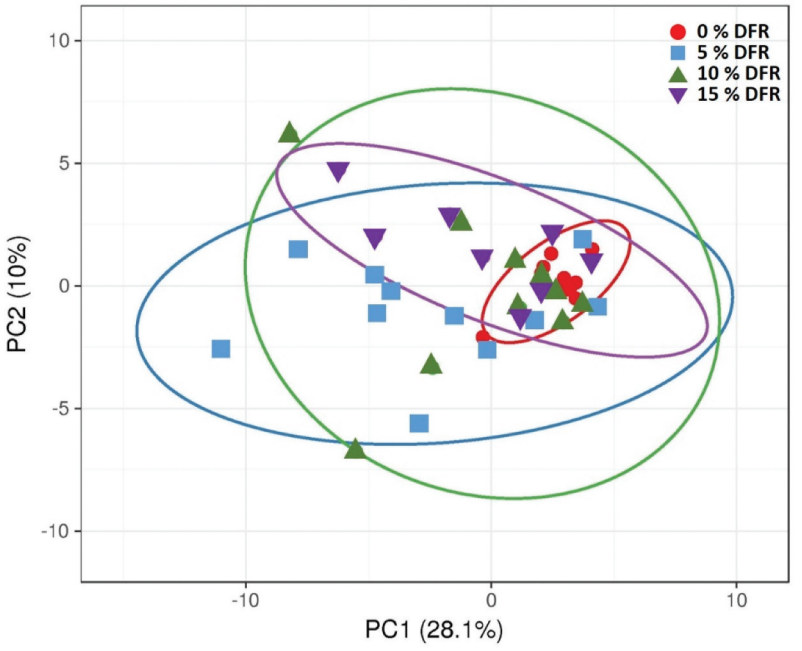

Figure 1. Principal component analysis of 16S rDNA sequencing of the faecal microbiota of dogs fed a diet with varying amounts of dried food residues (DFR). Prediction ellipses depict a 95% probability for new observations to fall inside the ellipse.

Table 7. Alpha diversity indices of the faecal microbiota of dogs (n = 10) fed a diet with varying amounts of dried food residues (DFR).

	Experimental diets				Polynomial contrasts (p-values)	
	0% DFR	5% DFR	10% DFR	15% DFR	Linear	Quadratic
Richness	31.9 ± 5.55^{a}	48.9 ± 9.33^{b}	44.7 ± 9.08^{b}	42.5 ± 10.4^{ab}	0.086	0.001
Shannon	1.82 ± 0.37	2.31 ± 0.43	2.09 ± 0.54	2.27 ± 0.41	0.080	0.279
Evenness	0.53 ± 0.11	0.60 ± 0.12	0.55 ± 0.14	0.62 ± 0.12	0.261	0.898
Simpson	0.27 ± 0.13	0.18 ± 0.10	0.23 ± 0.19	0.20 ± 0.12	0.374	0.469

Different superscript letters in the same row indicate significant group differences ($p < 0.05$).

Table 8. Relative abundance [%] of dominant bacterial phyla in the faeces of dogs fed a diet with varying amounts of dried food residues (DFR).

	Experimental diets				Polynomial contrasts (p-values)	
	0% DFR	5% DFR	10% DFR	15% DFR	Linear	Quadratic
Actinobacteria	1.21 ± 1.09 (9)†	3.63 ± 4.58 (10)	3.04 ± 2.19 (10)	2.30 ± 1.49 (9)	0.044	0.063
Bacteroidetes	9.34 ± 6.93^{a} (10)	8.92 ± 4.83^{a} (10)	12.0 ± 10.8^{ab} (10)	21.8 ± 8.70^{b} (10)	0.006	0.052
Firmicutes	69.7 ± 19.2 (10)	73.6 ± 11.6 (10)	72.2 ± 19.2 (10)	64.8 ± 12.7 (10)	0.381	0.282
Fusobacteria	19.2 ± 16.2 (10)	13.2 ± 7.33 (10)	11.9 ± 8.64 (10)	10.2 ± 7.72 (10)	0.043	0.562
Proteobacteria	0.67 ± 0.37 (9)	0.66 ± 0.53 (10)	0.88 ± 1.11 (10)	1.18 ± 0.66 (10)	0.059	0.638

†Number of positive samples.
Different superscript letters in the same row indicate significant group differences ($p < 0.05$).

Table 9. Relative abundance [%] of dominant bacterial orders in the faeces of dogs fed a diet with varying amounts of dried food residues (DFR).

	Experimental diets				Polynomial contrasts (*p*-values)	
	0% DFR	5% DFR	10% DFR	15% DFR	Linear	Quadratic
Bacteroidales	9.34 ± 6.93[a] (10)[†]	8.92 ± 4.83[a] (10)	12.0 ± 10.8[ab] (10)	21.8 ± 8.70[b] (10)	0.006	0.052
Betaproteo-bacteriales	0.51 ± 0.22 (9)	0.57 ± 0.49 (9)	0.61 ± 0.59 (9)	1.08 ± 0.64 (10)	0.124	0.517
Bifidobacteriales	1.08 ± 0.95 (6)	0.55 ± 0.58 (7)	0.79 ± 0.62 (10)	0.59 ± 0.36 (7)	0.513	0.666
Clostridiales	22.7 ± 16.5 (10)	26.1 ± 14.4 (10)	21.3 ± 9.22 (10)	16.2 ± 8.74 (10)	0.067	0.403
Coriobacteriales	0.49 ± 0.48 (9)	3.24 ± 4.49 (10)	2.25 ± 1.99 (10)	1.84 ± 1.26 (9)	0.111	0.037
Erysipelotrichales	39.1 ± 34.5 (10)	23.6 ± 26.9 (10)	24.1 ± 25.0 (10)	38.0 ± 25.2 (10)	0.915	0.094
Fusobacteriales	19.2 ± 16.2 (10)	13.2 ± 7.33 (10)	11.9 ± 8.64 (10)	10.2 ± 7.72 (10)	0.043	0.562
Lactobacillales	13.1 ± 19.2 (6)	23.7 ± 24.8 (10)	26.4 ± 26.5 (10)	10.2 ± 12.0 (10)	0.999	0.188

[†]Number of positive samples.
Different superscript letters in the same row indicate significant group differences ($p < 0.05$).

Table 10. Relative abundance [%] of dominant bacterial genera in the faeces of dogs fed a diet with varying amounts of dried food residues (DFR).

	Experimental diets				Polynomial contrasts (*p*-values)	
	0% DFR	5% DFR	10% DFR	15% DFR	Linear	Quadratic
Allobaculum	12.4 ± 10.9 (8)	6.81 ± 5.87 (10)	6.34 ± 5.44 (10)	6.74 ± 5.59 (10)	0.357	0.032
Alloprevotella	1.32 ± 0.94[a] (10)	2.60 ± 1.21[ab] (9)	4.88 ± 4.96[ab] (10)	4.61 ± 2.92[b] (10)	0.018	0.473
Asaccharospora	0.59 ± 0.53 (7)	0.16 ± 0.13 (8)	0.24 ± 0.20 (8)	0.15 ± 0.11 (8)	0.140	0.243
Bacteroides	5.88 ± 4.78 (10)	5.13 ± 3.35 (9)	4.38 ± 6.06 (10)	8.38 ± 6.81 (10)	0.510	0.219
Bifidobacterium	1.05 ± 0.93 (6)	0.52 ± 0.56 (7)	0.77 ± 0.61 (10)	0.57 ± 0.35 (7)	0.502	0.645
Blautia	3.41 ± 2.82 (10)	5.09 ± 3.24 (10)	4.27 ± 2.30 (10)	3.61 ± 2.61 (10)	0.927	0.264
Clostridium sensu stricto 1	0.63 ± 0.65 (6)	0.54 ± 0.44 (9)	0.52 ± 0.68 (6)	0.28 ± 0.29 (4)	-*	-
Collinsella	0.18 ± 0.16 (5)	0.83 ± 0.61 (9)	0.74 ± 0.37 (9)	0.57 ± 0.38 (8)	0.176	0.145
Dubosiella	1.71 ± 1.11 (7)	2.37 ± 1.87 (9)	1.27 ± 1.16 (10)	1.97 ± 1.02 (9)	0.921	0.676
Enterococcus	0.84 (2)	7.09 ± 16.7 (7)	0.17 ± 0.19 (7)	0.26 ± 0.35 (4)	-	-
Faecalibacterium	0.17 ± 0.12 (7)	0.45 ± 0.46 (9)	0.81 ± 0.78 (9)	0.55 ± 0.59 (8)	0.048	0.562
Faecalitalea	1.70 (1)	0.05 (2)	0.05 (1)	(0)	-	-

(*Continued*)

Table 10. (Continued).

	Experimental diets				Polynomial contrasts (*p*-values)	
	0% DFR	5% DFR	10% DFR	15% DFR	Linear	Quadratic
Fusobacterium	14.7 ± 11.1 (10)	12.7 ± 7.40 (10)	11.6 ± 8.56 (10)	9.85 ± 7.68 (10)	0.123	0.965
Holdemanella	0.02 (1)	0.10 ± 0.11 (7)	0.23 ± 0.21 (9)	0.71 ± 0.96 (4)	-	-
Lachno-clostridium	0.96 ± 1.45 (4)	0.83 ± 0.88 (9)	0.55 ± 0.71 (10)	0.62 ± 0.72 (7)	0.955	0.444
Lactobacillus	3.81 ± 3.63 (3)	1.95 ± 3.06 (9)	25.0 ± 26.2 (10)	9.84 ± 11.9 (10)	0.305	0.811
Parabacteroides	0.05 ± 0.03 (6)	0.09 ± 0.04 (8)	0.15 ± 0.16 (7)	0.11 ± 0.10 (9)	0.004	0.336
Parasutterella	0.44 ± 0.24 (7)	0.53 ± 0.52 (8)	0.40 ± 0.48 (9)	0.92 ± 0.69 (10)	0.244	0.718
Peptoclostridium	11.2 ± 11.1^{ab} (10)	9.72 ± 5.68^{a} (10)	6.15 ± 3.44^{ab} (10)	3.47 ± 2.56^{b} (10)	0.013	0.776
Peptostrepto-coccus	1.51 ± 1.53 (8)	0.77 ± 0.92 (5)	1.24 ± 1.25 (9)	1.69 ± 1.82 (9)	0.671	0.447
Prevotella_9	0.36 ± 0.36 (7)	0.35 ± 0.31 (9)	0.35 ± 0.31 (9)	4.45 ± 10.6 (9)	0.354	0.355
Prevotellaceae Ga6A1 group	1.41 ± 2.75 (9)	0.23 ± 0.32 (10)	0.80 ± 1.04 (6)	1.27 ± 1.27 (7)	0.801	0.211
Romboutsia	1.06 ± 0.59 (9)	1.43 ± 1.06 (10)	1.43 ± 1.65 (10)	1.60 ± 2.58 (10)	0.522	0.799
Streptococcus	10.7 ± 15.4 (6)	20.8 ± 13.8 (8)	1.39 ± 1.89 (6)	0.12 (1)	-	-
Terrisporobacter	0.36 ± 0.39 (4)	0.36 ± 0.29 (10)	0.50 ± 0.36 (7)	0.23 ± 0.15 (7)	-	-
Unknown *Atopobiaceae*	1.03 ± 0.97 (6)	6.69 ± 9.28 (6)	3.90 ± 3.40 (7)	2.45 ± 2.16 (8)	0.047	0.449
Unknown *Clostridiales*	0.40 ± 0.37 (4)	0.12 (1)	0.10 (2)	0.80 (1)	-	-
Unknown *Clostridiales* Family XIII	1.22 ± 0.90 (10)	1.41 ± 0.78 (10)	1.25 ± 0.59 (10)	0.65 ± 0.54 (10)	0.072	0.087
Unknown *Erysipelo-trichaceae*	30.4 ± 30.6 (9)	15.4 ± 21.9 (9)	17.7 ± 21.7 (9)	31.5 ± 21.6 (9)	0.960	0.113
Unknown *Fusobacteriaceae*	0.48 ± 0.22^{a} (5)	0.20^{ab} (2)	0.10 ± 0.13^{b} (5)	0.10 ± 0.18^{b} (7)	-	-
Unknown *Lachnospiraceae*	2.06 ± 1.87 (10)	3.21 ± 2.34 (10)	3.27 ± 1.61 (9)	2.13 ± 1.72 (10)	0.923	0.168
Unknown *Muribaculaceae*	0.49 ± 0.41 (8)	0.94 ± 0.90 (10)	1.33 ± 1.20 (10)	3.23 ± 3.68 (10)	0.037	0.388
Unknown *Peptostrept-ococcaceae*	0.55 (2)	0.16 ± 0.09 (8)	0.27 ± 0.24 (5)	0.21 ± 0.21 (4)	-	-

†Number of positive samples; *polynomial contrasts could not be calculated.
Different superscript letters in the same row indicate significant group differences ($p < 0.05$).

The relative abundance of *Actinobacteria* and *Bacteroidetes* increased and the relative abundance of *Fusobacteria* decreased with increasing amounts of DFR in the diets ($p < 0.05$).

On the order level, an increase of the relative abundance of *Bacteroidales* and a decrease of *Fusobacteriales* was observed with increasing dietary DFR ($p < 0.05$).

Increasing amounts of DFR in the diets further increased the relative abundance of the genera *Alloprevotella, Faecalibacterium, Parabacteroides*, unknown *Atopobiaceae* and unknown *Muribaculaceae* and decreased the relative abundance of *Peptoclostridium* ($p < 0.05$).

4. Discussion

In the present study, four experimental diets with varying amounts of DFR were fed to healthy adult dogs. Although comparable nutrient concentrations were achieved in all diets, it is important to notice that the origin of the nutrients differed, as the ingredients of the basal diet were partly replaced by the DFR. The food residues used for the experimental diets were mainly based on fresh fruits (~ 44%), cooked meals and snacks (~ 25%), and fresh vegetables and salads (~14%). Additionally, meat and fish, as well as bread and bakery were included in smaller amounts (~5 and 6%, respectively). Thus, different nutrients were provided by the DFR, including relatively high amounts of protein (~26%), fat (~25%) and nitrogen-free extracts (~37%).

The observed lower apparent crude protein and ether extract digestibility of the diets containing DFR indicates that the DFR were less digestible compared to the main protein and fat sources in the basal diet (poultry meal and rapeseed oil, respectively). A variety of factors can affect the digestibility of diets, including nutrient quality and composition (e.g. amino acid and fatty acid pattern, fibre content), heat treatment or antinutritional factors (McNab 1975; Gilani et al. 2012). The food residues collected for the present study were solar-dried, implicating a gentle heat treatment. However, about 25% of the DFR used consisted of cooked meals and snacks. It cannot be excluded that the heat treatment of the food residues, both during food preparation in the hotel kitchen and during solar-drying of the material, might have affected the apparent nutrient digestibility of the diets. With regard to the fibre content of the experimental diets, variations were noted for total, insoluble and soluble dietary fibre. These variations, however, were relatively small and not unidirectional. The amino acid pattern was comparable among the diets, whereas the fatty acid pattern of the diets was not determined in this study. Overall, the impact of the heat treatment and nutritional composition of the DFR on the apparent nutrient digestibility of the experimental diets cannot be clarified at this point. Future studies are necessary to identify potential strategies to improve the digestibility of diets containing DFR.

The results on the apparent crude protein und ether extract digestibility are in line with the observation that the daily amount of feed had to be increased when the diets with the DFR were fed in order to maintain body weight of the dogs. Thus, a lower energy utilisation can be assumed, probably resulting from the reduced protein and fat digestibility of the diets containing DFR. The undesired effects of the DFR on the apparent nutrient digestibility might limit their future use in petfood. Thus, lower inclusion levels in complete diets might be preferable in order to avoid an impaired nutrient and energy utilisation for the animals. In addition, alternative applications with lower demands on nutrient digestibility (e.g. inclusion of DFR in snacks) might be interesting for the potential use of DFR in canine nutrition, and should therefore be further investigated in future studies.

Given the lower apparent crude protein digestibility when feeding the diets with DFR, it can also be assumed that the undigested protein may have served as a substrate for the intestinal microbiota. However, neither faecal ammonium and branched-chain fatty acid concentrations, nor phenols in the urine and plasma of the dogs, all products of bacterial protein fermentation (Nyangale et al. 2012), were affected by the different experimental diets. In addition, only a trend ($p < 0.1$) for an increased relative abundance of *Proteobacteria* in the faeces of the dogs was observed with increasing amounts of DFR in the diets. Overall, the lower apparent protein digestibility when feeding the diets with DFR seemed to have no effect on the metabolic activity and composition of the intestinal microbiota of the dogs. Nevertheless, limitations in the determination of apparent protein digestibility due to endogenous nitrogen secretion and microbial protein degradation (Darragh and Hodgkinson 2000) and also limitations in the use of faecal samples for the investigation of the intestinal microbiota (Tang et al. 2020) require a careful data interpretation.

Although no specific effect of the lower apparent protein digestibility when feeding the diets with DFR was associated with changes in the metabolic activity and composition of the faecal microbiota of the dogs, a notable effect of the inclusion of DFR in the experimental diets on the faecal microbiota could still be observed. Both the concentrations of SCFA and the composition of the microbiota in the faeces of the dogs were affected, indicating that the food residues were intensively fermented by the intestinal microbiota. The faecal concentrations of acetic acid, propionic acid, *n*-butyric acid and total SCFA were increased with increasing amounts of DFR in the diets. SCFA are particularly produced by microbial fermentation of undigestible carbohydrates (Verbeke et al. 2015). In addition, branched-chain fatty acids can also be formed by microbial protein degradation in the intestine (Macfarlane et al. 1992). It can be assumed that especially fibre-rich ingredients of the DFR (e.g. fruits and vegetables) were fermented by the intestinal microbiota of the dogs, resulting in an increase of faecal SCFA and a decrease of the faecal pH. When the percentage of the single SCFA related to the total amount of SCFA was calculated, a decrease of propionic acid, *i*-butyric acid and *i*–valeric acid was observed, while the concentration of *n*-butyric acid increased with increasing amounts of DFR in the diets. The total and percentage increase of butyric acid could be discussed as a potential beneficial effect of the dietary inclusion of DFR, as butyric acid acts as a main energy source for epithelial cells in the colon and may contribute to gut health (Markowiak-Kopec and Slizewska 2020).

A variety of bacteria can produce butyric acid, for instance *Faecalibacterium* spp. (Oliphant and Allen-Vercoe 2019; Markowiak-Kopec and Slizewska 2020). An increase of the relative abundance of the genus *Faecalibacterium* in the faeces of the dogs was noted, when increasing amounts of DFR were included in the diets. Although the relative abundance of *Faecalibacterium* was low in the present study, the observed increase when feeding the diets with DFR may have contributed to the increase of butyric acid in the faeces of the dogs. In this context, it has been reported that the dietary inclusion of potato fibre also increased the relative abundance of *Faecalibacterium* (Panasevich et al. 2015) and the concentrations of butyrate and further SCFA in the faeces of dogs (Panasevich et al. 2013). In addition, the dietary inclusion of beet pulp (Middelbos et al. 2010) and potato fibre (Panasevich et al. 2015) decreased *Fusobacteria* in the faeces of dogs. The present study also demonstrated a decrease of the relative abundance of this phylum in

the faeces of the dogs with increasing amounts of dietary DFR, supporting the hypothesis that especially fibre-rich ingredients of the DFR might have been fermented by the intestinal microbiota of the dogs in the present study.

The dietary inclusion of DFR increased the relative abundance of the phyla *Actinobacteria* and *Bacteroidetes* in the faeces of the dogs. Within the phylum *Actinobacteria*, the relative abundance of the genus unknown *Atopobiaceae* was increased, and within the phylum *Bacteroidetes*, the relative abundance of the order *Bacteroidales* and the genera *Alloprevotella, Parabacteroides* and unknown *Muribaculaceae* was increased when feeding the diets with DFR. While carbohydrates are the main substrate for *Actinobacteria* (Oliphant and Allen-Vercoe 2019), both carbohydrates and protein can serve as a substrate for *Bacteroidetes* (Oliphant and Allen-Vercoe 2019). As the DFR used in the present study were composed of different ingredients, the effect on the faecal microbiota might not be solely attributed to the provision of a single substrate, although the main effect seems to arise from the provision of undigestible carbohydrates. In this context, it can also be speculated that the bacterial fermentation of undigested carbohydrates might have led to unfavourable conditions for bacterial protein fermentation, as evidenced by the reduced relative abundance of the proteolytic *Fusobacteria* in the faeces of the dogs when feeding the diets with DFR. In addition, this might also explain, why the observed lower apparent protein digestibility in these groups did not affect the composition of the faecal microbiota in this study.

Interestingly, the relative abundance of the genus *Peptoclostridium* in the faeces of the dogs decreased with increasing amounts of DFR in the diets. *Peptoclostridium* has been found to be enriched in the faeces of dogs suffering from canine parvovirus infection (Zheng et al. 2018). The observed decrease in the relative abundance of *Peptoclostridium* when feeding the diets with DFR might therefore be considered to be a beneficial effect, although it should be noted that all dogs were healthy throughout the study, revealing no gastrointestinal disorders.

Apparently, the dietary inclusion of DFR diversified the bacterial composition in the faeces of the dogs. However, individual animal differences in response to the dietary treatment may have played an additional role in the present study. This is evident by high standard deviations observed for some bacterial groups, but also by the principal component analysis performed. While a very tight cluster of the faecal microbiota was formed when the dogs received the control diet (0% DFR), feeding the DFR led to a larger variability of the faecal microbiota, although without a clear dependence on the dietary inclusion level. Individual variations of the canine intestinal microbiota have also been demonstrated in other studies (Suchodolski et al. 2005; Forster et al. 2018), and should be considered for data interpretation, especially at small sample sizes (Pilla and Suchodolski 2020). Thus, based on our results, it can be concluded that the dietary inclusion of DFR enriched the diversification of the individual faecal microbiota of dogs.

With regard to the use of DFR as a potential ingredient for dog food, it should finally be underlined that the composition of food residues might be variable, requiring a careful chemical analysis or standardised collection and processing procedures. Depending on the composition of food residues, the results obtained in the present study might not be necessarily transferable in general, but can give an insight into the effects of this potential "new" feed ingredient in dogs.

5. Conclusions

The inclusion of 5–15% DFR in a diet for dogs was accompanied with shifts in the metabolic activity and the composition of the faecal microbiota compared to the control diet (0% DFR). Lower inclusion levels of DFR might be preferable in complete diets, as the observed decreased apparent crude protein and ether extract digestibility when feeding the diets with DFR might limit their future use as a dietary ingredient for dogs.

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Data availability statement

The data that support the findings of this study are available from the corresponding author, upon reasonable request.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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Investigations on the Use of Dried Food Residues as a Potential Dietary Ingredient for Cats

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Abstract: The potential use of food residues for pet food could significantly contribute to food waste reduction. In the present study, the effects of the inclusion of dried food residues (DFR) (0, 5, 10 and 15%) in a complete diet were evaluated in seven healthy adult cats. At the end of each three-week feeding period, feces were collected. The analysis of the fecal microbiota by 16S rDNA sequencing demonstrated a marked increase of the bacterial alpha-diversity with increasing dietary inclusion levels of DFR. In addition, an increase in the relative abundance of *Coriobacteriales*, *Collinsella* and *Lachnoclostridium*, as well as of propionate and n-valerate in the feces of the cats, was detected. The dietary inclusion of DFR decreased the apparent crude protein digestibility and tended to decrease the apparent crude fat digestibility. Overall, the DFR seemed to be highly fermentable in the intestine of cats, which markedly affected the diversity of the fecal microbiota. As this effect might be critical for a balanced gut microbiota, but also along with the observed depressing effects of DFR on the apparent crude protein and crude fat digestibility, lower inclusion levels are recommended if used as a potential ingredient for cat food in the future.

Keywords: cats; hotel catering; diet; microbiota; feces

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1. Introduction

Human food residues were reused as a dietary component for farm animals for a long time [1]. However, regardless of their nutritional value and the contribution to food waste reduction by the recycling, concerns on the hygienic safety of food residues lastly caused legal restrictions. In the European Union, for instance, catering waste is currently prohibited as a feed component for livestock [2], but also for pet animals [3].

Nevertheless, there is a global need to reduce the food waste production [4]. The European Union is currently funding the project "Food for Feed (F4F)" (LIFE15 ENV/GR/000257), which evaluates the recycling of hotel catering into animal nutrition. The project is aware of the legal restrictions on the use of food residues, but aims to promote more research in this field, which could potentially also contribute to policy changes in the future. As a part of this project, we recently investigated the impact of the inclusion of dried food residues (DFR) in a complete diet for dogs [5]. The results demonstrated a clear and dose-dependent effect on the fecal microbiota of the animals and indicated that especially fiber-rich ingredients of the DFR were microbially fermented. In addition, a decrease in the

apparent crude protein and crude fat digestibility was observed with increasing amounts of DFR in the diets [5].

Whether comparable effects of the dietary inclusion of food residues might also occur in cats, however, cannot be directly concluded from the related study in dogs. Dogs are considered to be more omnivorous, whereas cats are strictly carnivorous [6]. The cat's ability to digest starch is lower than that of dogs, whereas the protein requirement is higher [7]. These dietary variations between dogs and cats might also affect the composition of the intestinal microbiota in general, and it can be assumed that potential differences in the gut microbiota might also result in divergent responses to dietary interventions.

It was therefore the aim of the present study to evaluate the effects of increasing concentrations of DFR in a complete diet for cats, mainly focusing on the impact on the composition and metabolic activity of the fecal microbiota, but also on the apparent nutrient digestibility.

2. Materials and Methods

The study received approval by the Landesamt für Gesundheit und Soziales (LAGeSo) in Berlin, Germany (approval number G 0233/18).

2.1. Diets

A complete basic diet was composed based on raw ground beef, rice flour, rapeseed oil, cellulose and mineral and vitamin supplements (Table 1). Titanium dioxide was included as an inert marker to determine the apparent nutrient digestibility. In order to improve the feed acceptance, a supplement based on chicken liver (Fresshilfe, anibio, SPECHT BIO-PHARMA, Reinbek, Germany) was added to the daily amount of feed of each cat (1/2 teaspoon/cat/day).

Table 1. Composition of the experimental diets with or without dried food residues (DFR).

Ingredient (%)	0% DFR	5% DFR	10% DFR	15% DFR
Ground beef	81.0	78.9	76.5	75.1
Rice flour	12.4	10.52	8.51	5.59
Rapeseed oil	3.18	2.56	2.09	1.68
Cellulose	0.80	0.56	0.39	0.17
Vitamin and Mineral premix	0.44	0.45	0.46	0.48
Potassium hydrogen carbonate	0.20	0.14	0.08	-
Sodium chloride	0.30	0.17	0.08	-
Blood meal	0.51	0.60	0.70	0.80
Magnesium supplement	0.02	0.02	0.01	-
B Vitamin Supplement	0.07	0.08	0.10	0.08
Dicalcium phosphate	0.12	0.09	0.08	-
Calcium carbonate	0.58	0.56	0.50	0.50
Cod liver oil	0.18	0.19	0.20	0.20
DFR [1]	-	4.96	10.1	15.2
Titanium dioxide	0.20	0.20	0.20	0.20

[1] Composition of the DFR, per wet weight of the non-dried food residues [5]: fresh fruits (44.4%), cooked meals and snacks (25.4%), fresh vegetables and salads (13.9%), bread and bakery (5.71%), meat and fish (4.90%), dairy products (excluding milk) and eggs (0.79%), impurities (0.74%; manually removed before the further processing of the food residues), sauces, herbs and spices (0.34%), desserts (0.22%), confectionary and snacks (0.09%), processed fruits (0.03%), and others (3.48%).

Dried food residues were included in the basic diet at 0, 5, 10 and 15%. The food residues were collected in hotel kitchen in Heraklion, Greece, as part of the project "Food for Feed (F4F)". The food residues were ground and solar dried, resulting in a final particle size of less than 5 mm. A compositional analysis of the collected hotel catering was performed based on the ASTM D5231-92 standard [8]. More details can be found elsewhere [5]. A microbial analysis of the DFR regarding hygiene safety was performed by the Laboratory of Microbiology of the Harokopio University in Athens by taking into consideration the Scientific Opinion of the Panel on Biological Hazards [9], the European

Feed Manufacturers' Guide [10] and the Commission Regulation (EC) No 2073/2005 [11]. The presence of microorganisms was determined using plate count techniques on selective substrates. The DFR have been found to be free of *Salmonella* spp., *Listeria monocytogenes*, *Escherichia coli*/Total Coliforms, *Staphylococcus aureus*, *Clostridium perfringens* and yeasts. The number of total mesophilic bacteria was $<10^2$ colony forming units (CFU)/g DFR.

The analyzed dry matter and nutrient concentrations of the experimental diets and DFR are presented in Table 2. The analyses were performed as described previously [5,12].

Table 2. Analyzed dry matter and nutrient concentrations of the experimental diets.

	0% DFR [1]	5% DFR	10% DFR	15% DFR
		g/100 g		
Dry matter	42.0	43.2	44.6	45.3
		g/100 g dry matter		
Crude ash	5.07	5.02	5.23	5.15
Crude protein	41.8	41.8	41.5	42.3
Crude fat	24.8	25.0	25.5	26.4
Crude fiber	1.22	1.36	1.08	1.57
Calcium	0.67	0.66	0.61	0.65
Phosphorus	0.52	0.53	0.54	0.53
Potassium	0.93	0.88	0.92	0.92
Magnesium	0.12	0.12	0.12	0.13
Sodium	0.45	0.43	0.48	0.49
Titanium dioxide	0.43	0.45	0.44	0.44
		mg/100 g dry matter		
Copper	1.02	1.15	1.21	1.18
Zinc	20.6	20.5	18.6	19.3
Iron	13.3	14.2	14.3	15.3
Manganese	1.75	1.77	1.63	1.71

[1] Analysis of the dried food residues (DFR): dry matter 91.2 g/100 g; per 100 g dry matter: crude ash 5.97 g, crude protein 25.9 g, crude fat 24.7 g, crude fiber 3.46 g, calcium 0.61 g, phosphorus 0.42 g, potassium 0.87 g, magnesium 0.09 g, sodium 0.82 g [5].

2.2. Animals and Study Design

Seven healthy adult cats (European Shorthair; 4 neutered males, 1 intact male, 2 intact females; 72.9 ± 42.0 months old) received the experimental diets in ascending order of the DFR inclusion level. The daily amount of feed was calculated according to the NRC [7] and was weekly adjusted to maintain the body weight of the cats. The feed intake of the animals was recorded daily, and the body weight weekly throughout the study.

Each feeding period consisted of 3 weeks. On the last four days of the feeding periods, the cats were individually housed for urine and feces collection. Fasting blood was collected at the end of each feeding period.

2.3. Calculation of the Apparent Nutrient Digestibility

The fecal crude protein, crude fat and titanium dioxide concentrations were analyzed as described elsewhere [5,12]. The apparent nutrient digestibility was calculated using the following formula:

Apparent nutrient digestibility (%) = 100 − [(% titanium dioxide in the diet/% titanium dioxide in the feces) × (% nutrient in the feces/% nutrient in the diet) × 100].

2.4. Fecal Microbiota and Microbial Metabolites

The microbiota in the feces of the cats was analyzed by 16S rDNA sequencing, using the llumina NextSeq500 sequencer (LGC, Berlin, Germany). DNA extraction from the feces was performed with a commercial kit (QIAamp Fast DNA stool mini kit, QIAGEN GmbH, Hilden, Germany). The instructions of the manufacturer were followed, with the exception of a lysis step at 90 °C. For amplicon sequencing, the V3–V4 region of the 16S

rDNA gene was targeted, and the 16S rDNA sequences were finally analyzed with the QIIME2 pipeline [13] and the SILVA SSU database [14]. More details on the method used are provided by Paßlack et al. [15]. The full data set of the sequencing is available at the NCBI BioProject database under ID PRJNA755187.

The concentrations of microbial metabolites in the feces of the cats were measured as described in detail by Paßlack et al. [16,17]. In short, short-chain fatty acid (SCFA) concentrations were analyzed by gas chromatography (Model 19095 N-123, Agilent Technologies, CA, USA), lactate with high-performance liquid chromatography (HPLC Agilent 1100, Agilent Technologies, CA, USA), and ammonium colorimetrically (Tecan Sunrise™ microplate reader, Tecan Austria GmbH, Grödig, Austria) by the Berthelot reaction.

2.5. Plasma and Urine Analysis

After collection, the blood was stored at room temperature for 1 h before centrifugation (10 min, 4 °C, 1811× *g*; Heraeus Megafuge 1.0R, Thermo Scientific, Karlsruhe, Germany). The plasma was frozen at −20 °C until the further analyses. The indoxyl sulfate concentrations were measured in the plasma using the method of Chen et al. [18] and high-performance liquid chromatography (HPLC Agilent 1100, Agilent Technologies, CA, USA). The phenol and indole concentrations in the plasma were also measured with the HPLC Agilent 1100 (Agilent Technologies, CA, USA), more details are provided by Paßlack et al. [15]. The urinary phenol and indole concentrations were determined by gas chromatography (GC 6890 N, Agilent Technologies), as specified by Eisenhauer et al. [19]. The urine pH was measured with a pH meter (Seven Multi pH meter, Mettler-Toledo GmbH, Schwerzenbach, Switzerland), directly after the urine collection at 6.30 h and 12.30 h. The urinary anions and cations were analyzed as described by Passlack and Zentek [12].

2.6. Statistical Data Analysis

The statistical data analysis was performed with IBM SPSS Statistics 27 (SPSS Inc, Chicago, IL, USA; 2020). Data are presented as mean ± standard deviation. Polynomial contrasts were calculated for group comparisons (General Linear Model repeated measures, within-subject factor: DFR, number of levels: four), with $\alpha = 0.05$ being statistically significant.

For the sequencing data, values < 0.5% in all groups are only presented in the Tables in the case of a significant group difference.

In addition, a principal component analysis was performed to visualize the sequencing data. For this, the web tool ClustVis was used [20].

3. Results

3.1. Animal Health, Body Weight, Fecal Dry Matter, Feed Intake

The cats were healthy throughout the study, and the dietary inclusion of DFR did not affect their body weight or fecal dry matter concentrations (Table 3). As one cat refused the diet after the first feeding period, it was replaced by another cat with a comparable age. Another cat refused the diet in the second, but not in the other feeding periods. The feed intake of the cats that completed the study was not influenced by the inclusion of DFR in the diets (Table 3).

3.2. Apparent Nutrient Digestibility

The apparent crude protein and crude fat digestibility was high in general (Table 3). However, with increasing dietary concentrations of DFR, the apparent crude protein digestibility decreased from 93.9 ± 2.06 to 90.0 ± 1.91% (linear contrast: P = 0.007). In addition, a trend for a lower apparent crude fat digestibility with increasing dietary DFR inclusion levels was observed (linear contrast: P = 0.062).

Table 3. Feed intake, body weight, fecal dry matter, and apparent crude protein and crude fat digestibility of cats (n = 7) fed a diet with varying amounts of dried food residues (DFR).

	0% DFR	5% DFR	10% DFR	15% DFR	Polynomial Contrasts (P Value)	
					Lin.	Quadr.
Feed intake (g dry matter/kg body weight/day)	10.1 ± 1.51	10.1 ± 1.27	9.60 ± 1.16	9.33 ± 1.25	0.169	0.661
Body weight (kg)	5.36 ± 1.14	5.79 ± 1.19	5.99 ± 1.08	5.95 ± 1.17	0.135	0.092
Fecal dry matter (%)	44.4 ± 11.5	41.0 ± 11.9	43.6 ± 6.80	41.3 ± 6.10	0.974	0.335
Apparent crude protein digestibility (%)	93.9 ± 2.06	92.8 ± 1.90	91.4 ± 1.57	90.0 ± 1.91	0.007	0.870
Apparent crude fat digestibility (%)	97.3 ± 2.23	97.4 ± 0.56	96.5 ± 1.90	96.0 ± 1.31	0.062	0.196

Lin.: Linear; Quadr.: Quadratic.

3.3. Fecal Microbiota

A marked increase in the microbiota´s diversity in the feces of the cats was noted with increasing amounts of DFR in the diets (linear contrasts, $P < 0.05$) (Table 4). This effect was also demonstrated by the principal component analysis, where the cats receiving no DFR showed a tighter cluster formation of the fecal microbiota compared to the other groups, especially to the group receiving 15% dietary DFR (Figure 1).

Table 4. Alpha diversity indices of the fecal microbiota of cats (n = 7) fed a diet with varying amounts of dried food residues (DFR).

	0% DFR (n = 7)	5% DFR (n = 6)	10% DFR (n = 7)	15% DFR (n = 7)	Polynomial Contrasts (P Value)	
					Linear	Quadratic
Richness	33.0 ± 6.38	36.5 ± 4.04	46.9 ± 5.58	51.4 ± 5.77	0.001	0.075
Shannon	1.46 ± 0.45	1.91 ± 0.21	2.17 ± 0.16	2.37 ± 0.14	0.011	0.297
Evenness	0.42 ± 0.12	0.53 ± 0.05	0.56 ± 0.03	0.60 ± 0.04	0.043	0.194
Simpson	0.33 ± 0.14	0.24 ± 0.05	0.18 ± 0.04	0.15 ± 0.04	0.043	0.310

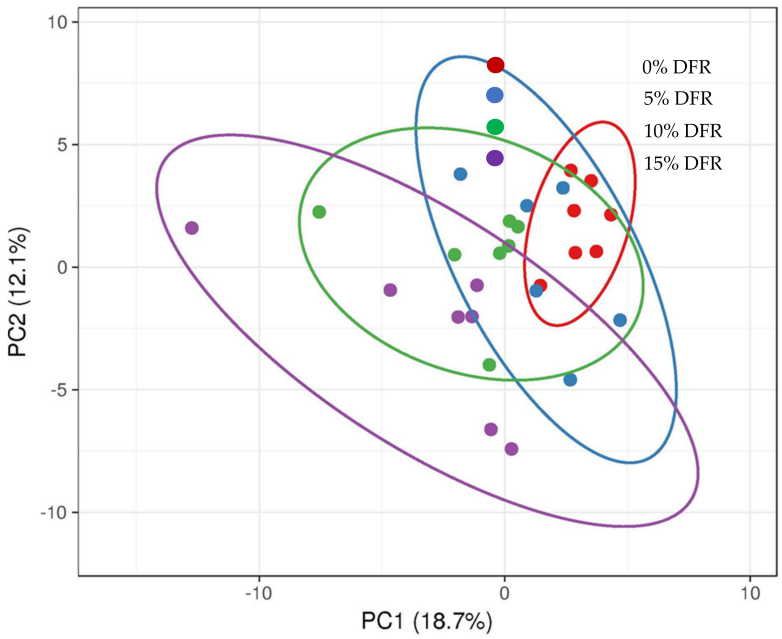

Figure 1. Principal component analysis of the fecal microbiota of cats (n = 7) fed a diet without or with 5, 10 and 15% dried food residues (DFR). The prediction ellipses show a 95% probability for new observations to fall inside these ellipses.

While only quadratic effects of the dietary DFR inclusion were detected on the phylum level (Table 5), a linear increase in the relative abundance of the order *Coriobacteriales* (Table 6) and the genera *Collinsella*, *Lachnoclostridium*, *Libanicoccus* and *Romboutsia* (Table 7) was observed with increasing dietary concentrations of DFR (linear contrasts, $P < 0.05$).

Table 5. Relative abundance (%) of dominant bacterial phyla in the feces of cats (n = 7) fed a diet with varying amounts of dried food residues (DFR).

	0% DFR	n [1]	5% DFR	n	10% DFR	n	15% DFR	n	Polynomial contrasts (P value)	
									Linear	Quadratic
Actinobacteria	42.3 ± 33.7	(7)	42.7 ± 31.8	(6)	52.8 ± 14.6	(7)	37.4 ± 8.19	(7)	0.674	0.008
Bacteroidetes	8.42 ± 16.3	(5)	8.42 ± 17.3	(6)	2.76 ± 2.03	(7)	9.07 ± 5.04	(7)	0.786	0.379
Firmicutes	51.5 ± 32.0	(7)	48.6 ± 28.8	(6)	44.3 ± 15.5	(7)	53.2 ± 7.36	(7)	0.686	0.031
Fusobacteria	0.08 ± 0.04	(3)	0.54 ± 0.45	(3)		(0)	0.34 ± 0.57	(4)	*	*

[1] Number of positive samples; * Polynomial contrasts could not be calculated.

Table 6. Relative abundance (%) of dominant bacterial orders in the feces of cats (n = 7) fed a diet with varying amounts of dried food residues (DFR).

	0% DFR	n [1]	5% DFR	n	10% DFR	n	15% DFR	n	Polynomial Contrasts (P Value)	
									Lin.	Quadr.
Bacteroidales	8.41 ± 16.3	(5)	8.41 ± 17.3	(6)	2.76 ± 2.03	(7)	9.07 ± 5.04	(7)	0.786	0.380
Bifidobacteriales	32.0 ± 31.4	(5)	27.6 ± 25.0	(5)	21.9 ± 17.4	(7)	2.37 ± 3.29	(7)	0.231	0.064
Clostridiales	43.8 ± 27.7	(7)	44.9 ± 30.4	(6)	39.5 ± 16.5	(7)	42.1 ± 10.3	(7)	0.404	0.688
Coriobacteriales	19.4 ± 19.8	(7)	19.6 ± 11.5	(6)	30.9 ± 7.77	(7)	35.0 ± 7.92	(7)	<0.001	0.113
Erysipelotrichales	3.22 ± 2.38	(7)	1.72 ± 1.17	(6)	2.93 ± 1.66	(7)	5.54 ± 3.59	(7)	0.051	0.013
Fusobacteriales	0.08 ± 0.04	(3)	0.54 ± 0.45	(3)		(0)	0.34 ± 0.57	(4)	*	*
Lactobacillales	6.87 ± 13.1	(4)	1.04	(1)	0.03	(2)	0.55 ± 1.02	(4)	*	*
Selenomonadales	0.56 ± 0.72	(5)	1.80 ± 2.50	(6)	2.23 ± 1.55	(6)	5.25 ± 6.54	(7)	0.493	0.809
Unknown Firmicutes	0.73	(2)		(0)		(0)	0.05	(2)	*	*

[1] Number of positive samples; * Polynomial contrasts could not be calculated; Lin.: Linear; Quadr.: Quadratic.

Table 7. Relative abundance (%) of dominant bacterial genera in the feces of cats (n = 7) fed a diet with varying amounts of dried food residues (DFR).

	0% DFR	n [1]	5% DFR	n	10% DFR	n	15% DFR	n	Polynomial Contrasts (P Value)	
									Lin.	Quadr.
Alloprevotella	0.74 ± 0.89	(4)	1.47 ± 2.51	(5)	0.15 ± 0.08	(6)	3.31 ± 4.79	(5)	0.538	0.467
Bacteroides	0.47 ± 0.46	(5)	0.58 ± 0.55	(6)	0.40 ± 0.21	(7)	0.73 ± 0.58	(7)	0.830	0.748
Bifidobacterium	32.0 ± 31.3	(5)	27.5 ± 24.9	(5)	21.8 ± 17.4	(7)	2.35 ± 3.27	(7)	0.230	0.064
Blautia	24.8 ± 20.4	(7)	20.8 ± 15.2	(6)	17.0 ± 7.61	(7)	16.4 ± 2.27	(7)	0.361	0.615
Clostridium sensu stricto 1	6.89 ± 11.6	(7)	4.54 ± 6.81	(6)	1.73 ± 2.44	(3)	0.05 ± 0.03	(3)	0.298	0.386
Collinsella	11.0 ± 10.8	(7)	13.8 ± 8.62	(6)	23.9 ± 5.82	(7)	28.7 ± 8.70	(7)	0.003	0.799
Faecalibacterium		(0)	0.64	(1)	0.17 ± 0.16	(6)	0.12 ± 0.14	(6)	*	*
Fusobacterium	0.08 ± 0.04	(3)	0.50 ± 0.42	(3)			0.34 ± 0.57	(4)	*	*
Holdemanella	1.18 ± 1.70	(4)	0.79 ± 1.04	(5)	1.00 ± 0.90	(7)	1.84 ± 1.39	(6)	0.175	0.348
Lachnoclostridium	1.68 ± 1.17	(7)	4.46 ± 3.38	(6)	3.68 ± 1.54	(7)	4.95 ± 1.87	(7)	0.027	0.234
Lachnospira		(0)	0.67	(2)	1.19 ± 1.16	(3)	0.34 ± 0.28	(3)	*	*
Lachnospiraceae NK4A136 group	0.12 ± 0.07	(5)	0.56 ± 0.75	(4)	0.22 ± 0.17	(7)	0.17 ± 0.09	(7)	0.149	0.401
Lactobacillus		(0)	1.03	(1)	0.02	(1)	0.01	(2)	*	*
Libanicoccus	0.12 ± 0.08	(5)	0.16 ± 0.14	(5)	0.46 ± 0.19	(7)	0.50 ± 0.15	(7)	0.003	0.945

Table 7. *Cont.*

	0% DFR	n [1]	5% DFR	n	10% DFR	n	15% DFR	n	Polynomial Contrasts (P Value)	
									Lin.	Quadr.
Marvinbryantia	0.64 ± 0.14	(4)	1.37 ± 0.70	(3)	0.58 ± 0.33	(5)	0.39 ± 0.21	(7)	0.131	0.035
Megamonas	0.11 ± 0.14	(4)	1.11 ± 1.61	(4)	0.22 ± 0.30	(5)	0.30 ± 0.39	(5)	0.881	0.520
Megasphaera	0.26 ± 0.33	(4)	1.67 ± 2.52	(3)	1.60 ± 1.10	(6)	6.39 ± 6.88	(5)	*	*
Olsenella	14.1 ± 27.3	(4)	10.7 ± 14.0	(3)	6.77 ± 4.59	(6)	4.62 ± 5.49	(7)	0.914	0.480
Paeniclostridium	0.71 ± 0.54	(6)	0.27 ± 0.13	(3)		(0)		(0)	*	*
Peptoclostridium	3.54 ± 1.85	(7)	4.76 ± 4.27	(6)	3.65 ± 2.94	(7)	5.01 ± 2.65	(7)	0.588	0.812
Prevotella 9	12.1 ± 19.3	(3)	6.50 ± 14.6	(6)	2.08 ± 1.75	(7)	5.68 ± 3.82	(7)	0.653	0.331
Romboutsia	0.21 ± 0.12	(4)	0.53 ± 0.72	(4)	0.22 ± 0.16	(5)	0.41 ± 0.23	(4)	0.027	0.396
Sellimonas	0.50 ± 0.42	(6)	0.77 ± 0.69	(4)	0.69 ± 0.54	(7)	0.42 ± 0.43	(6)	0.834	0.540
Solobacterium	2.28 ± 2.46	(7)	0.98 ± 0.77	(5)	1.68 ± 1.73	(7)	3.57 ± 2.67	(7)	0.384	0.164
Streptococcus	6.62 ± 12.6	(4)		(0)	0.04	(1)	1.06	(2)	*	*
Subdoligranulum	0.07	(2)	2.50 ± 3.78	(3)	4.01 ± 7.57	(7)	3.77 ± 6.54	(7)	*	*
Unknown Clostridiales Family XIII	0.52 ± 0.39	(6)	0.35 ± 0.24	(6)	0.48 ± 0.59	(7)	0.98 ± 1.17	(7)	0.905	0.540
Unknown Lachnospiraceae	3.74 ± 2.26	(7)	4.98 ± 3.04	(6)	6.00 ± 2.06	(7)	7.03 ± 1.76	(7)	0.069	0.865

[1] Number of positive samples; * Polynomial contrasts could not be calculated; Lin.: Linear; Quadr.: Quadratic.

3.4. Fecal Microbial Metabolites

The ammonium and lactate concentrations in the feces of the cats were not affected by the dietary inclusion of DFR; however, the concentrations of propionic and n-valeric acid markedly increased with increasing amounts of DFR in the diets (linear contrasts, $P < 0.05$) (Table 8). When calculated as % of total SCFA, propionic acid increased and n-butyric acid decreased with increasing dietary inclusion levels of DFR (linear contrasts, $P < 0.05$).

Table 8. Concentrations of bacterial metabolites in the feces of cats (n = 7) fed a diet with varying amounts of dried food residues (DFR).

	0% DFR	5% DFR	10% DFR	15% DFR	Polynomial Contrasts (P Value)	
					Linear	Quadratic
µmol/g						
Ammonium	25.0 ± 6.17	21.4 ± 4.43	20.7 ± 6.96	23.4 ± 7.66	0.147	0.491
L-lactate	0.04 ± 0.07	0.15 ± 0.27	0.00 ± 0.00	0.00 ± 0.00	0.130	0.338
D-lactate	0.08 ± 0.11	0.30 ± 0.49	0.02 ± 0.03	0.05 ± 0.09	0.201	0.340
Acetic acid	69.4 ± 42.1	61.2 ± 15.2	67.4 ± 31.2	93.8 ± 49.0	0.188	0.253
Propionic acid	27.9 ± 15.5	29.8 ± 6.40	33.3 ± 11.8	45.8 ± 19.8	0.025	0.485
i-butyric acid	3.62 ± 1.14	3.22 ± 0.74	3.87 ± 0.96	4.72 ± 1.95	0.099	0.245
n-butyric acid	15.6 ± 7.78	13.5 ± 5.97	14.7 ± 3.86	15.2 ± 7.52	0.225	0.808
i-valeric acid	4.41 ± 1.31	3.57 ± 1.38	4.68 ± 1.21	5.19 ± 2.36	0.416	0.273
n-valeric acid	6.42 ± 3.13	5.68 ± 4.46	8.29 ± 2.73	9.19 ± 4.54	0.016	0.709
Total SCFA	127 ± 66.0	117 ± 22.8	127 ± 57.8	174 ± 81.8	0.089	0.215
% SCFA						
Acetic acid	51.0 ± 9.28	52.1 ± 6.86	49.6 ± 4.72	52.9 ± 4.71	0.673	0.592
Propionic acid	21.4 ± 2.20	25.6 ± 3.32	25.3 ± 2.62	26.7 ± 2.35	0.003	0.158
i-butyric acid	3.44 ± 1.45	2.82 ± 0.79	3.08 ± 0.53	2.83 ± 0.62	0.325	0.463
n-butyric acid	13.8 ± 4.77	11.5 ± 3.88	11.6 ± 1.74	9.05 ± 2.25	0.032	0.883
i-valeric acid	4.58 ± 2.69	3.16 ± 1.38	3.77 ± 1.01	3.13 ± 0.88	0.243	0.571
n-valeric acid	5.77 ± 2.86	4.86 ± 3.48	6.60 ± 1.81	5.44 ± 1.40	0.401	0.685

Lin.: Linear; Quadr.: Quadratic; SCFA: Short-chain fatty acids.

3.5. Urine pH and Composition, Phenols and Indoles in the Urine and Plasma

The urine pH of the cats decreased with increasing dietary concentrations of DFR (Table 9). No significant effects of the diets on the urinary anion and cation concentrations were observed, however, there was a trend (P = 0.092) for increasing phosphate concentrations in the urine with increasing dietary inclusion levels of DFR (Supplementary Table S1). The p-cresol concentrations markedly increased in the urine, when the DFR were included in the diets (linear contrast, P = 0.008), whereas a slight, but significant decrease of urinary 7-methylphenol was noted (linear contrast, P = 0.007) (Table 9).

Table 9. Phenol and indole concentrations [1] in the urine of cats (n = 7) fed a diet with varying amounts of dried food residues (DFR).

	0% DFR	5% DFR	10% DFR	15% DFR	Polynomial Contrasts (P Value)	
					Lin.	Quadr.
pH	8.20 ± 0.22	7.94 ± 0.35	7.81 ± 0.30	7.67 ± 0.28	0.002	0.586
			µg/mL			
Phenol	0.14 ± 0.07	0.28 ± 0.16	0.17 ± 0.06	0.27 ± 0.18	0.386	0.452
4-ethylphenol	0.01 ± 0.01	0.01 ± 0.02	0.01 ± 0.02	0.05 ± 0.06	0.224	0.162
3-methylphenol	0.10 ± 0.02	0.08 ± 0.03	0.10 ± 0.03	0.12 ± 0.10	0.447	0.884
7-methylphenol	0.08 ± 0.04	0.03 ± 0.03	0.02 ± 0.01	0.01 ± 0.02	0.007	0.037
p-cresol	0.53 ± 0.47	0.62 ± 0.24	1.20 ± 0.35	1.78 ± 0.70	0.008	0.848
Indole	0.21 ± 0.48	0.08 ± 0.07	0.15 ± 0.10	0.19 ± 0.08	0.768	0.381
Indoxyl sulfate	2.43 ± 0.63	1.63 ± 1.17	2.02 ± 0.88	2.26 ± 1.17	0.315	0.069

[1] 2-methylphenol, 2,3-dimethylphenol: most values were below the detection limit; Lin: Linear; Quadr.: Quadratic.

The analysis of phenols and indoles in the plasma of the cats could only detect a quadratic effect of the DFR on the indoxyl sulfate concentrations, with the lowest concentrations, when the DFR were included in the diet at 5% (P = 0.037) (Table 10).

Table 10. Phenol and indole concentrations [1] in the plasma of cats (n = 7) fed a diet with varying amounts of dried food residues (DFR).

	0% DFR	5% DFR	10% DFR	15% DFR	Polynomial Contrasts (P Value)	
					Lin.	Quadr.
			µg/mL			
p-cresol	0.52 ± 0.51	0.67 ± 0.92	0.57 ± 0.39	0.75 ± 0.45	0.373	0.945
Phenol	0.59 ± 0.17	0.53 ± 0.01	0.53 ± 0.01	0.56 ± 0.02	0.570	0.292
			mg/mL			
Indoxyl sulfate	3.01 ± 1.04	1.56 ± 1.31	2.82 ± 1.66	3.03 ± 1.07	0.033	0.037

[1] indole, 3-methylindole: all values were below the detection limit.

4. Discussion

In the present study, DFR, derived from hotel catering, were evaluated as a dietary ingredient for cats. The results demonstrated that the diversity of the fecal microbiota markedly increased with higher amounts of DFR in the diet. Along with an increase in the relative abundance of some bacterial orders and genera, as well as of propionate and n-valerate in the feces, an intensive microbial fermentation of the DFR in the intestine of the cats can be assumed.

The richness of the fecal microbiota markedly increased when treatments without and with 15% DFR were compared. In general, a decreased microbial diversity has been observed in cats with chronic enteropathy [21], chronic kidney disease [22] or diabetes mellitus [23], indicating potential negative effects on the intestinal microbiota of different diseases (or vice versa). Based on these results, it can be speculated that a decreased diversity of the gut microbiota might be critical in cats. On the other hand, a marked increase in the bacterial diversity as observed in the present study by the dietary inclusion

of DFR could also be considered as a potentially undesired effect. As a stable and balanced intestinal microbiota is important for gut health in cats [24], major shifts in the bacterial community in the intestine could be harmful. Nevertheless, it should also be mentioned that the cats of the present study were healthy throughout the experimental period, and no negative impact of increasing dietary DFR inclusion levels could be detected on the fecal consistency as an indicator of intestinal function.

In particular, the relative abundance of *Coriobacteriales*, *Collinsella* and *Lachnoclostridium* was increased by the dietary inclusion of DFR. These bacterial groups can mainly metabolize different types of carbohydrates [25–27]. The results of the present study correspond with the data obtained when feeding DFR from the same batch to dogs, where carbohydrate fermenting bacteria were also promoted by the dietary inclusion of DFR [5]. It should be mentioned, however, that the composition of the DFR used in the present study cannot be postulated for food residues in general. Instead, the ingredients of food residues might vary depending on the collection procedure. Variations in the nutritional composition of food residues might also lead to varying responses of the intestinal microbiota. Nevertheless, the present results provide valuable insights into the use of DFR as an ingredient for a complete diet for cats. Given the marked effects on the fecal microbiota at higher dietary inclusion levels, which could also be detrimental for a balanced gut microbiota, lower amounts of DFR in a diet might be preferable if used as a potential ingredient in the future.

The apparent crude protein digestibility decreased with the increasing amounts of DFR in the diets. In addition, a trend for a decreased apparent crude fat digestibility was observed. The results confirm the data obtained in dogs when feeding DFR of the same composition [5]. The underlying mechanism of these depressing effects on the nutrient digestion, however, cannot be clarified at this stage. High dietary fiber concentrations, as well as different fiber fractions, can negatively affect the apparent nutrient digestibility in cats [28,29]. Although the dietary fiber fractions were not specified in the present study, the crude fiber concentrations were comparable between the experimental diets and were relatively low in general. It can therefore be assumed that fiber-rich ingredients of the DFR might not have been the main reason for the observed depressing effects on the apparent crude protein and crude fat digestibility. However, as was hypothesized also in our previous work [5], the heat treatment of the DFR could have potentially affected the nutrient digestibility. This might relate to the heating in the hotel kitchen, as about 25% of the food residues were cooked meals and snacks, and the solar drying of the collected leftovers. It should also be considered that the food residues used for the present study were not sterilized, but for the potential future use as a component for pet food, this might be a prerequisite to ensure the hygienic quality of this material. Steam-sterilizing temperatures might additionally affect the nutrient digestibility of DFR included in diets, which should be further investigated in future studies.

Interestingly, the urine pH of the cats decreased with increasing dietary inclusion levels of DFR. The dietary nutrient composition, which significantly affects the urine pH [30], was comparable for all treatments, and no significant effect of the diets on the anion and cation concentrations in the urine of the animals could be observed. However, there was a trend for increasing urinary phosphate concentrations at higher amounts of DFR in the diets, which could have affected the urine pH of the cats. In addition, it can be speculated that the increasing p-cresol concentrations in the urine might also have contributed to the decrease in the urine pH. P-cresol is a metabolite of bacterial protein fermentation and considered to be potentially harmful, e.g., for the integrity of the intestinal epithelial barrier or by a suppression of the immune function [31]. As the apparent protein digestibility decreased with increasing dietary DFR in the present study, it can be hypothesized that the undigested protein was partly microbially fermented, resulting in an increased p-cresol concentration in the urine of the cats. It should, however, be noted that neither the fecal ammonium concentrations, as another important metabolite of microbial protein fermentation [32], nor the plasma p-cresol concentrations were increased by the DFR in the diets, which is why an extensive bacterial protein degradation in the intestine of the cats might be

excluded. Nevertheless, the results indicate that not only carbohydrates, but also protein was microbially fermented when DFR were included in the diet.

As a limitation of the present study, the diets were fed in ascending order of their amount of DFR, and not as a cross-over design. This study design was used to early detect potential adverse reactions to the DFR, i.e., at the lowest inclusion level. Although we considered sufficiently long feeding periods to detect diet-related effects on the intestinal microbiota and apparent nutrient digestibility, a potential influence of the preceding on the subsequent feeding period cannot be fully excluded and should therefore be considered for data interpretation.

The diets of the present study were based on raw ground beef and only mixed with a small amount of rice flour and additional mineral and vitamin supplements. Despite the raw and not heat-treated meat, the diet composition can be considered to be comparable to a standard canned diet for cats. In contrast, dry extruded diets for cats often contain higher amount of cereals or other starch-rich ingredients, which usually also results in a lower protein and fat content. As the diet format can affect the composition of the fecal microbiota in cats [33], the results of the present study might not be fully transferable to pet food in general. However, they can provide an important basis for the potential commercial use of DFR as a dietary ingredient for cats.

Overall, the present study demonstrates that food residues could not only be used as a dietary component for dogs, as recently evaluated [5], but also for cats. Considering the demonstrated limitations of dietary DFR, particularly the effects on the apparent nutrient digestibility and the intestinal microbiota of cats, but also of dogs [5], lower inclusion levels ($\leq$5%) are recommended. Given the large amounts of pet food produced per year, e.g., 29.33 million tons in the year 2020 [34], even a small inclusion level of DFR in a diet for cats and dogs could significantly help to reduce the environmental footprint of the pet food industry in the future.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/su132111603/s1, Table S1: Concentrations of anions and cations in the urine of cats (n = 7) fed a diet with varying amounts of dried food residues (DFR).

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RESEARCH ARTICLE

In vitro digestion and microbial fermentation of dried food residues, a potential "new" component for pet food, and different non-digestible carbohydrate sources

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Abstract

Food residues are often fed to dogs in private households and might also be a potential "new" ingredient for pet food in the future. As food residues might contain not only digestible, but also fermentable substrates, an effect on the intestinal microbiota can be assumed. In the present study, two batches of dried food residues (DFR) collected from hotels in Crete were microbially fermented in an *in vitro* batch culture system with canine fecal inoculum: non-sterile DFR including meat (DFR_m), sterile DFR including meat (DFR_{ms}) and sterile DFR without meat (DFR_{wms}). Different non-digestible carbohydrate sources (beet pulp, wheat bran, inulin, carrot pomace, brewer´s spent grains, cellulose and lignocellulose) were included for comparison. Inulin, cellulose and lignocellulose were only used as raw materials, while the other test substrates were incubated as raw and enzymatically pre-digested substrates. After incubation for 24 hours, the raw food residues markedly increased the concentrations of bacterial metabolites in the fermenters, although smaller effects were observed for the DFR_{wms}. When the enzymatically pre-digested food residues were incubated, the effects were more pronounced for the DFR_{ms} and DFR_{wms}. In general, when compared with the other test substrates, the food residues were microbially fermented to a comparable or partly higher extent. Interestingly, high n-butyrate concentrations were measured in the inocula, both after incubation of the raw and pre-digested food residues. In conclusion, the food residues contained enzymatically digestible and microbially fermentable substrates. If considered as a potential future ingredient for pet food, a standardization of the collection and processing of food residues might be necessary in order to reduce compositional variability and varying effects on the intestinal microbiota.

Competing interests: The authors have declared that no competing interests exist.

Introduction

Given that 1.3 billion tons of food are lost or wasted every year [1], new strategies for food waste reduction are of increasing interest. For instance, the project "Food for Feed (F4F)" (LIFE15 ENV/GR/000257) aims to investigate the potential use of dried food residues (DFR) for animal nutrition. Although legal restrictions currently exist, food residues might be particularly interesting as a potential future ingredient for pet food. In practice, dogs often receive table scraps by their owners [2, 3], making the commercial use of food residues also conceivable.

One major concern of feeding catering waste to animals is its hygienic quality, as several pathogens that could be potentially present in recycled food leftovers may not only be harmful for the animals, but also for human consumers throughout the food chain [4]. A heat treatment of food residues designated for animal nutrition is therefore necessary to ensure the hygienic safety of this material [4].

Another issue of the use of food leftovers for feed is its nutritional composition. Food residues might contain both enzymatically digestible and microbially fermentable substrates. Thus, the dietary inclusion of food residues might also affect the fermentative activity and composition of the intestinal microbiota of animals.

In vitro fermentation systems are well established to simulate intestinal conditions and to evaluate the microbial fermentation of certain substrates [5]. As it has been demonstrated that feces are an adequate inoculum [6], these non-invasive models also contribute to the "3R" principle ("reduction, replacement and refinement") of animal experiments.

In the present study, a batch culture system was used to incubate raw and enzymatically pre-digested food residues with canine fecal inoculum. To compare the effects on the microbial fermentation, different non-digestible carbohydrate sources, varying in their fermentative capacity, were also included. The results of this study should contribute to a better understanding of the effects of food residues on the intestinal microbiota of dogs and might therefore also allow for an evaluation of the suitability of food residues as a potential future ingredient for pet food.

Material and methods

Animals and feces collection

Fresh fecal samples were collected from healthy adult dogs kept in the facilities of the Institute of Animal Nutrition, Freie Universität Berlin. All dogs were fed a standard complete dry extruded diet. The dogs were indoor housed with constant light and temperature conditions and had daily access to a clean outdoor area.

Test substrates

Ten test substrates were microbially fermented in the *in vitro* system: Two different batches of DFR (batch 1: non-sterilized and sterilized DFR including meat (DFR_m, DFR_{ms}); batch 2: sterilized DFR without meat (DFR_{wms})), beet pulp, wheat bran, carrot pomace, brewer´s spent grains, cellulose, lignocellulose and inulin. The composition of the test substrates is presented in Tables 1 and 2. Details on the chemical analyses are provided elsewhere [7, 8].

For the compositional analysis of the DFR_m/DFR_{ms}, the ASTM D5231-92 (reapproved 2008) standard [9] was adapted as described by Paßlack et al. [8]. For the production of the DFR_{wms}, meat was manually removed from the food residues. The composition of the DFR_{wms} was calculated by determining the relative amount of meat in food residues collected during the analysis period (autumn 2017—autumn 2018) and adjusting the average composition of

Table 1. Composition (% wet weight) of the food residues used for the present study, and compositional variation (minimum—maximum values) of the food residues collected during the project period[1].

	Present study		Project period		
	DFR_m/DFR_{ms}	DFR_{wms}	Minimum	-	Maximum
Fresh fruits	44.4	46.7	39.7	-	51.3
Cooked meals and snacks	25.4	26.73	19.3	-	32.4
Fresh vegetables and salads	13.9	14.6	9.58	-	17.5
Bread and bakery	5.71	6.00	3.36	-	11.1
Meat and fish	4.90	0.00	3.11	-	8.96
Dairy products (excluding milk) and eggs	0.79	0.83	0.11	-	1.72
Impurities	0.74	0.77	0.32	-	1.42
Sauces, herbs and spices	0.34	0.36	0.00	-	0.90
Desserts	0.22	0.23	0.00	-	0.48
Confectionary and snacks	0.09	0.09	0.00	-	0.35
Processed fruits	0.03	0.03	0.00	-	0.11
Others	3.48	3.66	1.38	-	6.64

[1] Collection of hotel catering leftovers from autumn 2017—autumn 2018 (n = 4 collection periods); DFR_m: non-sterile dried food residues with meat (composition already published elsewhere [8]); DFR_{ms}: sterile dried food residues with meat; DFR_{wms}: sterile dried food residues without meat.

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the collected food residues without meat accordingly. Table 1 also provides data on the compositional variation of the food residues collected during the F4F project period (autumn 2017—autumn 2018) (n = 4 sampling periods).

The food residues were collected from hotel catering in Crete, Greece, ground to a particle size of 10 mm and solar dried in a specific pilot unit in Heraklion, developed in the course of

Table 2. Analyzed dry matter (DM) and nutrient concentrations of the test substrates used in the present study.

	DFR_m	DFR_{ms}	DFR_{wms}	Beet pulp	Wheat bran	Carrot pomace	Brewer´s spent grains	Cellulose	Ligno-cellulose	Inulin
g/100 g										
DM	91.2	91.1	86.4	94.1	90.9	94.4	92.4	95.2	90.9	94.1
g/100 g DM										
Crude protein	25.9	28.0	31.1	8.62	13.2	10.5	27.9	0.45	0.77	0.14
Crude fat	24.7	23.9	21.5	0.01	1.58	2.33	9.77	0.00	0.37	0.00
Crude fiber	3.46	3.10	4.86	17.3	13.1	22.7	14.4	73.1	65.6	0.00
Crude ash	5.97	6.56	7.98	6.64	1.80	5.10	5.74	0.14	0.41	0.00
Acid detergent fiber	3.84	5.08	7.97	18.3	12.4	28.5	20.9	51.9	73.5	0.00
Neutral detergent fiber	20.3	19.6	20.6	41.2	45.6	44.0	69.8	94.6	94.7	0.00
Soluble dietary fiber	0.81	0.40	1.43	19.4	4.80	21.1	1.76	0.17	1.40	-[1]
Insoluble dietary fiber	10.8	14.0	12.4	47.1	57.4	46.2	53.7	96.6	93.5	-[1]
Total dietary fiber	11.6	14.4	13.8	66.5	62.2	67.3	55.5	96.8	94.9	-[1]
Calcium	0.61	0.58	1.08	1.34	0.04	0.77	0.74	0.02	0.10	0.00
Phosphorus	0.42	0.43	0.47	0.09	0.35	0.17	0.67	0.01	0.01	0.01
Potassium	0.87	1.01	1.36	0.71	0.49	0.89	0.19	0.01	0.04	0.00
Magnesium	0.09	0.09	0.12	0.19	0.12	0.14	0.32	0.01	0.01	0.00
Sodium	0.82	0.94	1.20	0.07	0.01	0.33	0.05	0.03	0.01	0.00

[1] Below the detection limit (insoluble dietary fiber: 0.380%, total dietary fiber: 0.678%); DFR_m: non-sterile dried food residues with meat; DFR_{ms}: sterile dried food residues with meat; DFR_{wms}: sterile dried food residues without meat.

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the project "Food for Feed (F4F)" (LIFE15ENV/GR/000257). For the sterilization (DFR_{ms} DFR_{wms}), the solar dried samples were treated for 20 minutes at 121˚C and 2 bars.

The DFR_m, DFR_{ms}, DFR_{wms}, beet pulp, wheat bran, carrot pomace and brewer´s spent grains were added to the *in vitro* system both as raw material and enzymatically pre-digested substrate. Cellulose, lignocellulose and inulin were added as raw material without enzymatic pre-digestion. All the raw substrates were ground at a particle size of 0.5 mm. Fecal suspension without a test substrate was incubated as a blank control.

Enzymatic pre-digestion of the test substrates

To simulate the microbial fermentation of the substrates in the large intestine, i.e., after digestion by mammalian enzymes, the test substrates were enzymatically pre-digested using a modified method based on the studies of Gauthier et al. [10], Savoie and Gauthier [11] and Minekus et al. [12]. For each test substrate, the enzymatic pre-digestion was performed with 4 replicates. Simulated Gastric Fluid (SGF) and Simulated Intestinal Fluid (SIF) were prepared as described by Minekus et al. [12]. The SIF was stored overnight at 37˚C before use.

As a first step, 0.5 g test substrate was mixed with 3 ml SGF. Then, 1 μl $CaCl_2$ (0.3 M) was mixed in, and a pH of 3 was adjusted by adding HCl (6 M). Subsequently, 400 μl porcine pepsin (100 mg/ml, dissolved in SGF; activity of porcine pepsin: at least 250 U/mg, according to the manufacturer, Sigma-Aldrich/Merck KGaA, Darmstadt, Germany) were added, mixed, and the solution was filled up to 5 ml with ultrapure water. This solution was mixed and incubated at 37˚C for 2 hours in an incubation shaker (Heidolph Inkubator 1000 and Heidolph Unimax 1010, Heidolph Instruments GmbH & CO. KG, Schwabach, Germany).

To stop the pepsin digestion, NaOH (1 M) was added to the solution to adjust a pH of 7. Afterwards, 2 ml of the SIF solution were added, mixed and 1.25 ml porcine bile extract (100 mg/ml, dissolved in SIF) were added. After mixing, 10 μl $CaCl_2$ (0.3 M) were added and mixed again. Subsequently, 1.25 ml pancreatin from porcine pancreas (160 mg/ml, dissolved in SIF; pancreatin from porcine pancreas 8 × USP specifications, Sigma-Aldrich/Merck KGaA, Darmstadt, Germany) were mixed to the solution. The pH of the solution was adjusted to 7 using NaOH, and the solution was finally filled up with ultrapure water to 10 ml. After incubation at 37˚C for 2 hours in an incubation shaker (Heidolph Inkubator 1000 and Heidolph Unimax 1010, Heidolph Instruments GmbH & CO. KG, Schwabach, Germany), the pancreatin digestion was stopped by incubating the samples on ice for 30 minutes.

In a last step, the samples were washed. For this, dialysis membranes (Spectra/Por® 7 MWCO 1000, 38 mm, Carl Roth, Karlsruhe, Germany) were soaked in water for 15 minutes first. The low ends of these membranes were sealed (Spectra/Por® Universal, 50 mm, Carl Roth, Karlsruhe, Germany), and the enzymatically pre-digested samples were pipetted into the membranes. Afterwards, the top ends of the membranes were also sealed (Spectra/Por® Universal, 50 mm, Carl Roth, Karlsruhe, Germany). The membranes were incubated in 5 l water at 4˚C for 24 hours using a magnetic stirrer (IKA RH-KT/C, Sigma-Aldrich/Merck KGaA, Darmstadt, Germany). During the incubation time, the water was changed once. After the incubation, the membranes were opened and the samples were quantitatively transferred into 50 ml tubes. The samples were deep frozen at -80˚C and freeze-dried afterwards (Alpha 1–4 LSC, Martin Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz, Germany).

To prove the efficiency of the pre-digestion, the crude protein amount in the inoculum was measured before and after the enzymatic treatment, and the crude protein digestibility (%) was calculated as follows: 100—((protein amount in the inoculum after the pre-digestion (g) / protein amount in the inoculum before the pre-digestion (g)) * 100).

Table 3. Calculated protein digestibility of the test substrates[1] after the enzymatic pre-digestion, but before the microbial fermentation. Means and pooled standard error of the means (SEM).

	Protein digestibility (%)
DFR_m	73.8
DFR_{ms}	76.8
DFR_{wms}	69.2
Beet pulp	37.6
Wheat bran	80.8
Carrot pomace	34.2
Brewer´s spent grains	84.4
Pooled SEM	3.77

[1] Not calculated for cellulose, lignocellulose and inulin, as these substrates contain only small amounts of protein (see Table 2). DFR_m: non-sterile dried food residues with meat; DFR_{ms}: sterile dried food residues with meat; DFR_{wms}: sterile dried food residues without meat.

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The results of the protein digestibility measurements are presented in Table 3. Due to the neglectable protein amounts present in cellulose, lignocellulose and inulin, the protein digestibility was not calculated for these substrates.

Given the small quantities of substrates used for the pre-digestion trials, and that the pre-digested material was mainly used for a microbial fermentation afterwards, only the protein digestion was calculated as main variable of the pre-digestion, but not the starch or fat digestibility additionally.

Microbial fermentation

For the microbial fermentation, the protocol of Vierbaum et al. [13] was slightly modified by using 0.5 g of each raw test substrate or the remaining substrate after enzymatic pre-digestion, respectively for the fermentation. The test substrates were weighed in filter bags (Ankom Fiber Filter Bags, F57, ANKOM Technology, Macedon NY, USA).

In a first step, 4 g fresh feces were weighed in 50 ml tubes each. The following steps were performed under anaerobic conditions. The feces were diluted (1:10) with PRAS medium (in g/l aqua bidestillata: 0.5 g KH_2PO_4, 0.5 g K_2HPO_4, 5.0 g $NaHCO_3$, 1.0 g NaCl, 0.1324 g $CaCl_2$ x 2 H_2O, 0.1 g $MgSO_4$ x 7 H_2O, 500 µl Resazurine (0.2%), 5.0 g cysteine hydrochloride; sterilized for 15 minutes at 121˚C [14]) and mixed for 2 minutes. After sedimentation for 10 minutes, the supernatant of all tubes was pipetted into one sterile bottle and mixed afterwards (fecal suspension).

In a next step, 90 ml PRAS medium were pipetted into 125 ml afnor bottles (Zscheile & Klinger GmbH, Hamburg, Germany). Afterwards, one welded filter bag with test substrate was placed into a bottle, and 10 ml of the fecal suspension were added. As a blank control, a filter bag without test substrate was placed into a bottle with PRAS medium and fecal suspension. The bottles were sealed and incubated for 24 hours in a waterbath (37˚C) and an incubation shaker (Heidolph Inkubator 1000 and Heidolph Unimax 1010, Heidolph Instruments GmbH & CO. KG, Schwabach, Germany).

For each test substrate and blank control, the microbial fermentation was performed in 4 replicates on different days.

Gas production

For the measurement of the gas volume in the bottles after incubation, a burette (50 ml) was connected with a separation funnel by a tube. The burette was filled with water up to the zero

graduation. A canula was connected with the burette by a tube. When the canula was perforating the cover of the incubation bottles, the gas volume in the bottles could be measured by the water displacement from the burette into the separation funnel.

pH measurement and sample collection

After the measurement of the gas production, the incubation bottles were placed on ice for 30 minutes. The bottles were then opened and the pH was measured in the fecal suspension using a pH meter (Seven Multi, Mettler-Toledo GmbH, Schwerzenbach, Switzerland). One ml aliquots of the fecal suspension were stored at -20˚C until further analysis of bacterial metabolites.

Dry matter loss of the test substrates after incubation

The filter bags were weighed before incubation (tare weight). In addition, the amount of test substrate filled into the filter bag was weighed (t_0). After the incubation, the welded filter bags, which included the fermented test substrates, were cleaned with distilled water. The filter bags were predried with a tissue and placed into acetone for 5 minutes to remove the remaining fluid. The bags were dried in a compartment dryer at 104˚C overnight (Heraeus T5042, Heraeus, Hanau, Germany). After cooling in a desiccator (Duran, DN 300 Novus Duran, Wertheim, Germany), the weight of the welded filter bags was determined. The dry matter loss of the test substrates was calculated as follows:

1. Correction factor for the tare weight of the filter bags after incubation: c = weight (g) of the blank control filter bag after incubation/weight (g) of the blank control filter bag before incubation
2. Weight of the test substrate after incubation (g): t_1 = weight (g) of the welded filter bag after incubation–(tare weight of the filter bag before incubation (g) * c)
3. Dry matter loss of the test substrate (%) = 100 –(t_1 (g)/ t_0 (g) * 100)

Bacterial metabolites in the fecal suspension after incubation

After thawing of the frozen aliquots, the fecal suspension was centrifuged at 14.000 x g and 20˚C for 10 minutes (Thermo Scientific Heraeus Fresco21, Thermo Fisher Scientific, Waltham, MA, USA). Afterwards, 200 μl of the supernatant were mixed with 100 μl hexanoic acid (5 mmol/l, internal standard). The mixture was filled up to 1 ml with oxalic acid (1% w/v), and the concentrations of short-chain fatty acids (SCFA) in the solution were subsequently measured using a gas chromatograph (Agilent Technologies 6890N, auto sampler G2614A, injection tower G2613A, Network GC Systems, Böblingen, Germany) and a polyethylene column (Agilent 19095N-123 HP-INNOWAX, Agilent Technologies, Böblingen, Germany).

For the measurement of D- and L-lactate, 500 μl of the fecal suspension were mixed with 500 μl $CuSO_4$ solution (0.5 mmol/l). Subsequently, 100 μl of Carrez I solution (17 g zinc chloride in 100 ml purified water) and 100 μl of Carrez II solution (15 g potassium ferrocyanide (II) in 100 ml purified water) were added. The samples were centrifuged at 14.000 x g and 4˚C for 10 minutes (Thermo Scientific Heraeus Fresco21, Thermo Fisher Scientific, Waltham, MA, USA), and the supernatant was filtered through a syringe filter (0.2 μm). The lactate concentrations in the solution were measured using high-performance liquid chromatography (HPLC Agilent 1100, Agilent Technologies, Böblingen, Germany; pre-column Phenomenex C 18, 4.0×2.0 mm, Phenomenex Ltd., Aschaffenburg, Germany; analytical column Phenomenex Chirex 3126 (D)-penicillamine, 150×4.6 mm, Phenomenex Ltd., Achaffenburg, Germany).

For the determination of ammonium, the fecal suspension was centrifuged at 14.800 x g and 20˚C for 10 minutes (Thermo Scientific Heraeus Fresco21, Thermo Fisher Scientific, Waltham, MA, USA), and the supernatant was diluted (1:90 and 1:100) with 100 mM 3-(N-morpholino)propanesulfonic acid (pH 6.8). Twenty μl of this mixture were pipetted into the wells of a microtiter plate. One hundred μl phenol nitroprusside and 100 μl alkaline hypochlorite were added into each well afterwards. Resulting from the Berthelot reaction, indophenol was formed, and the extinction was measured every 1.3 minute for 20 minutes at 420 nm (Tecan MPlex Microplate Reader, Tecan Austria GmbH, Grödig, Austria).

Statistical data analysis

The data were analyzed using SPSS 27 (SPSS Inc., Chicago, Illinois, USA), and are presented in tables as means and the pooled standard error of the means (SEM). For group comparisons, a one-factorial analysis of variance (fixed factor test substrate) and Scheffe´ (variance equality) or Tamhane 2 (variance inequality) post hoc tests were considered. Different letters in the same row indicate significant group differences ($P < 0.05$). For the comparison of the raw and enzymatically pre-digested substrates, normality of the data was tested (Kolmogorov-Smirnov and Shapiro Wilk tests), and groups were compared using the t test (parametric data) or Mann-Whitney U-test (nonparametric data).

Results

Microbial fermentation of the raw test substrates

The gas production was lowest, when no test substrate was incubated in the canine fecal suspension (blank control), and highest, when the DFR_{ms} were microbially fermented (Table 4). A low gas production was also observed, when cellulose and lignocellulose were incubated, while especially the incubation of DFR, beet pulp, wheat bran and carrot pomace resulted in a high gas production ($P < 0.05$, when these test substrates were compared with the blank control and cellulose incubation).

The microbial fermentation of the raw test substrates did not affect the pH in the inocula.

The highest ammonium concentrations were measured in the inocula, when the DFR_m and DFR_{ms} were incubated, with group differences compared to inulin, beet pulp and DFR_{wms}.

The incubation of the DFR_m, DFR_{ms} and DFR_{wms} also resulted in the highest L-lactate concentrations in the inoculum, and differed compared to the blank control, cellulose, lignocellulose, brewer´s spent grains and beet pulp. A comparable effect was observed for the D-lactate concentrations in the inoculum, with highest concentrations after incubation of the DFR_m, DFR_{ms} and DFR_{wms}, and lower concentrations after the blank control, cellulose, lignocellulose and wheat bran treatment. The D-lactate concentrations were also higher, when the DFR_m and DFR_{ms} were incubated when compared to the brewer´s spent grains, carrot pomace and beet pulp fermentation.

The acetate concentrations were low in the blank control (mean 1.32 μmol/ml) and differed after the microbial fermentation of carrot pomace, beet pulp, DFR_{ms} and DFR_{wms} (means 6.51–9.76 μmol/ml). The concentrations of propionate, i-butyrate, i-valerate and n-valerate in the inocula were not different among the groups. Higher n-butyrate concentrations were observed after incubation of the DFR_m and DFR_{ms} when compared to the blank control, cellulose, lignocellulose, brewer´s spent grains, inulin, carrot pomace and beet pulp treatment. The concentrations of total SCFA were low in the blank control (mean 1.67 μmol/ml), but higher, when the DRF_m, DFR_{ms}, DFR_{wms}, beet pulp and carrot pomace were microbially fermented (means 9.30–17.1 μmol/ml).

Table 4. Gas production, pH, and microbial metabolites in canine fecal suspension after incubation with different raw test substrates, as well as dry matter (DM) loss of the test substrates after incubation. Means and pooled standard error of means (SEM).

	Blank control[1]	DFR_m	DFR_{ms}	DFR_{wms}	Beet pulp	Wheat bran	Carrot pomace	Brewer´s spent grains	Cellulose	Ligno-cellulose	Inulin	SEM
Gas (ml)	6.56^{a}	38.3^{be}	47.1^{efg}	33.0^{bdg}	30.4^{bdg}	28.7^{bc}	28.8^{bc}	16.6^{acd}	9.64^{a}	11.2^{ac}	21.3^{abcd}	1.78
pH	7.41	6.59	6.61	6.67	6.60	6.82	6.73	7.00	7.29	7.32	6.81	0.06
DM loss of the test substrate (%)	-	58.6	55.6	56.9	44.1	33.8	33.7	15.6	4.99	10.2	67.1	3.11
µmol/ml												
Ammonium	10.5^{abc}	22.7^{b}	20.6^{bc}	14.3^{a}	12.5^{a}	18.6^{abc}	14.4^{ac}	16.0^{abc}	13.0^{abc}	12.8^{abc}	10.5^{a}	0.66
L-lactate	0.03^{a}	2.02^{b}	1.90^{bd}	2.33^{be}	0.73^{ac}	0.84^{acd}	0.96^{ce}	0.23^{a}	0.03^{a}	0.03^{a}	0.76^{acd}	0.11
D-lactate	0.03^{a}	1.43^{e}	1.63^{e}	1.76^{ec}	0.53^{ac}	0.44^{ad}	0.65^{bcd}	0.35^{ac}	0.02^{a}	0.04^{a}	1.10^{abe}	0.09
Acetate	1.32^{a}	10.2^{abc}	9.76^{bc}	6.86^{bc}	9.05^{b}	7.79^{abc}	6.51^{bc}	4.32^{abc}	1.49^{ac}	1.86^{ac}	3.21^{abc}	0.49
Propionate	0.16	1.98	1.90	1.52	1.91	1.44	1.46	0.54	0.20	0.26	1.03	0.11
i-butyrate	0.05	0.27	0.37	0.12	0.13	0.16	0.19	0.08	0.16	0.04	0.12	0.03
n-butyrate	0.12^{a}	4.50^{b}	3.77^{bc}	2.27^{abc}	1.34^{a}	1.81^{ac}	1.10^{a}	0.39^{a}	0.15^{a}	0.13^{a}	0.41^{a}	0.21
i-valerate	0.02	0.09	0.05	0.05	0.02	0.07	0.01	0.08	0.12	0.06	0.04	0.01
n-valerate	0.00	0.07	0.08	0.11	0.02	0.29	0.02	0.01	0.00	0.00	0.01	0.02
Total SCFA	1.67^{a}	17.1^{b}	15.9^{b}	10.9^{bcde}	12.5^{bd}	11.6^{abcde}	9.30^{bde}	5.43^{ae}	2.12^{ac}	2.37^{ac}	4.82^{ad}	0.78
Mol %												
Acetate	74.5	58.9	60.6	62.8	72.4	67.1	69.4	78.5	70.5	76.3	64.0	1.25
Propionate	10.9^{a}	11.5^{a}	11.8^{a}	14.1^{ab}	15.2^{ab}	12.6^{a}	15.9^{ab}	10.2^{a}	9.87^{a}	11.3^{a}	21.3^{b}	0.55
i-butyrate	4.41	1.95	2.75	1.33	1.13	1.79	2.37	1.87	6.49	2.34	4.33	0.55
n-butyrate	8.66^{a}	26.7^{b}	24.0^{bd}	20.4^{bc}	11.0^{ac}	15.3^{acd}	12.0^{ac}	7.52^{a}	7.83^{a}	6.08^{a}	8.99^{a}	1.01
i-valerate	1.18	0.58	0.32	0.52	0.14	0.74	0.05	1.72	5.17	3.67	1.09	0.33
n-valerate	0.32	0.45	0.46	0.90	0.18	2.55	0.26	0.24	0.17	0.27	0.24	0.21

[1]Incubation without test substrate; DFR_m: non-sterile dried food residues with meat; DFR_{ms}: sterile dried food residues with meat; DFR_{wms}: sterile dried food residues without meat; SCFA: short-chain fatty acids; Different letters in the same row indicate significant group differences ($P < 0.05$).

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When the relative amount of the single SCFA (% of total SCFA) in the inocula was calculated, no group differences could be detected for acetate, i-butyrate, i-valerate and n-valerate. Higher relative amounts of propionate were measured after the microbial fermentation of inulin (mean 21.3 mol %) when compared to the blank control, cellulose, lignocellulose, brewer´s spent grains, wheat bran, DFR_m and DFR_{ms} treatment (means 9.87–12.6 mol %). The microbial fermentation of the DFR_m, DFR_{ms} and DFR_{wms} resulted in the highest relative amounts of n-butyrate (means 20.4–26.7 mol %), while lower amounts of n-butyrate were measured after the blank control, cellulose, lignocellulose, inulin and brewer´s spent grains treatment (means 6.08–8.99 mol %). In addition, the relative amounts of n-butyrate were higher after the microbial fermentation of the DFR_m and DFR_{ms} when compared to the inoculation of carrot pomace and beet pulp.

Microbial fermentation of the enzymatically pre-digested test substrates

The microbial fermentation of the enzymatically pre-digested test substrates resulted in a higher gas and ammonium production compared to the blank control (Table 5). The pH in the inoculum was comparable among all groups.

The concentrations of L-lactate were higher after the microbial fermentation of enzymatically pre-digested wheat bran when compared to all other test substrates and the blank control, whereas the D-lactate concentrations in the inocula did not differ among the groups.

Table 5. Gas production, pH, and microbial metabolites in canine fecal suspension after incubation with different enzymatically pre-digested test substrates, as well as dry matter (DM) loss of the test substrates after incubation. Means and pooled standard error of means (SEM).

	Blank control[1]	DFR_m	DFR_{ms}	DFR_{wms}	Beet pulp	Wheat bran	Carrot pomace	Brewer´s spent grains	SEM
Gas (ml)	6.56^{a}	36.8^{b}	39.1^{b}	38.8^{b}	38.6^{b}	42.0^{b}	41.4^{b}	37.0^{b}	2.11
pH	7.41	6.85	6.73	6.79	6.69	6.50	6.66	6.86	0.06
DM loss (%) of the test substrate	-	75.0	65.8	73.5	61.0	90.2	54.7	55.8	2.38
μmol/ml									
Ammonium	10.5^{a}	22.6^{b}	24.2^{b}	28.6^{b}	23.5^{b}	23.5^{b}	24.8^{b}	24.6^{b}	1.03
L-lactate	0.03^{b}	0.09^{b}	0.17^{b}	0.12^{b}	0.23^{b}	2.59^{a}	0.19^{b}	0.09^{b}	0.13
D-lactate	0.03	0.32	0.34	0.38	0.32	0.88	0.42	0.24	0.05
Acetate	1.32^{a}	9.69^{ab}	9.04^{ab}	12.4^{b}	12.3^{b}	10.4^{b}	10.9^{ab}	8.84^{ab}	0.70
Propionate	0.16^{a}	0.80^{ab}	0.96^{b}	1.31^{ab}	1.95^{b}	1.78^{ab}	1.18^{b}	0.84^{ab}	0.11
i-butyrate	0.05	0.22	0.24	0.24	0.15	0.18	0.21	0.14	0.04
n-butyrate	0.12^{a}	2.23^{ab}	2.63^{b}	2.61^{b}	2.63^{ab}	4.01^{b}	2.13^{ab}	2.06^{ab}	0.21
i-valerate	0.02	0.10	0.05	0.07	0.05	0.08	0.08	0.07	0.01
n-valerate	0.00	0.01	0.02	0.01	0.01	0.05	0.01	0.01	0.00
Total SCFA	1.67^{a}	13.0^{ab}	12.9^{ab}	16.6^{b}	17.1^{b}	16.5^{b}	14.5^{ab}	12.0^{ab}	0.96
Mol %									
Acetate	74.5	74.5	68.8	74.6	72.4	63.0	74.6	72.8	1.12
Propionate	10.9	6.08	7.66	7.67	11.7	10.4	8.37	7.32	0.45
i-butyrate	4.41	2.06	2.46	1.50	0.97	1.21	1.87	1.91	0.44
n-butyrate	8.66^{a}	16.4^{ab}	20.5^{bc}	15.7^{ab}	14.6^{ac}	24.5^{b}	14.4^{ac}	17.2^{ab}	0.88
i-valerate	1.18	0.84	0.40	0.45	0.32	0.53	0.69	0.73	0.10
n-valerate	0.32	0.07	0.14	0.08	0.10	0.33	0.11	0.05	0.04

[1]Incubation without test substrate; same blank control as for the raw test substrates (Table 4).

DFR_m: non-sterile dried food residues with meat; DFR_{ms}: sterile dried food residues with meat; DFR_{wms}: sterile dried food residues without meat; SCFA: short-chain fatty acids; Different letters in the same row indicate significant group differences ($P < 0.05$).

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The acetate and total SCFA concentrations in the blank control were lower when compared to the concentrations after the microbial fermentation of the pre-digested wheat bran, beet pulp and DFR_{wms}. The propionate concentrations in the inocula were low in general, but higher after the microbial fermentation of enzymatically pre-digested DFR_{ms}, carrot pomace and beet pulp when compared to the blank control. Lowest concentrations of n-butyrate were measured in the blank control (mean 0.12 μmol/ml), whereas higher amounts were measured, when pre-digested DFR_{ms}, DFR_{wms} and wheat bran were microbially fermented (means 2.61–4.01 μmol/ml). The concentrations of i-butyrate, i-valerate and n-valerate in the inocula did not differ among the groups. When the mol % of the single SCFA was calculated, group differences were only observed for n-butyrate. Highest relative amounts of n-butyrate were measured after the microbial fermentation of enzymatically pre-digested wheat bran (mean 24.5 mol %; group difference compared to the blank control and pre-digested carrot pomace and beet pulp). In addition, the microbial fermentation of enzymatically pre-digested DFR_{ms} also resulted in high relative amounts of n-butyrate (mean 20.5 mol %), which was higher compared to the blank control (mean 8.66 mol %).

Comparison between the microbial fermentation of the raw and enzymatically pre-digested test substrates

When the microbial fermentation of the raw and pre-digested test substrates was compared, variations in the gas production, DM loss and concentrations of microbial metabolites in the inocula could be observed (Table 6).

Table 6. Comparison (*P* values) between the raw and enzymatically pre-digested test substrates (↑ increase or ↓ decrease when compared to the microbial fermentation of the raw test substrate; → no difference between the microbial fermentation of the raw and enzymatically pre-digested test substrate), for means see Tables 4 and 5.

	Raw versus pre-digested test substrate (*P* value)						
	DFR_m	**DFR_{ms}**	**DFR_{wms}**	**Beet pulp**	**Wheat bran**	**Carrot pomace**	**Brewer´s spent grains**
Gas (ml)	↓ (0.803)	↓ (0.230)	↑ (0.133)	↑ (0.137)	↑ (0.113)	↑ **(0.006)**	↑ **(0.002)**
pH	↑ (0.184)	↑ (0.587)	↑ (0.526)	↑ (0.703)	↓ (0.090)	↓ (0.728)	↓ (0.542)
Dry matter loss (%)	↑ **(0.011)**	↑ **(0.003)**	↑ (< **0.001**)	↑ **(0.007)**	↑ (< **0.001**)	↑ **(0.038)**	↑ (< **0.001**)
μmol/ml							
Ammonium	↓ (0.602)	↑ (0.198)	↑ **(0.001)**	↑ **(0.007)**	↑ (0.175)	↑ **(0.009)**	↑ **(0.016)**
L-lactate	↓ (< **0.001**)	↓ (< **0.001**)	↓ (< **0.001**)	↓ **(0.016)**	↑ **(0.009)**	↓ (< **0.001**)	↓ **(0.004)**
D-lactate	↓ (< **0.001**)	↓ **(0.009)**	↓ (< **0.001**)	↓ (0.090)	↑ (0.174)	↓ (0.067)	↓ (0.149)
Acetate	↓ (0.836)	↓ (0.711)	↑ **(0.003)**	↑ (0.086)	↑ (0.110)	↑ (0.051)	↑ (0.064)
Propionate	↓ **(0.009)**	↓ **(0.017)**	↓ (0.478)	↑ (0.936)	↑ (0.347)	↓ (0.218)	↑ (0.065)
i-butyrate	↓ (0.251)	↓ (0.251)	↑ (0.917)	↑ (0.754)	↑ (0.602)	↑ (0.754)	↑ (0.385)
n-butyrate	↓ **(0.009)**	↓ **(0.035)**	↑ (0.520)	↑ (0.251)	↑ (0.004)	↑ (0.068)	↑ **(0.011)**
i-valerate	↑ (0.465)	→ (0.997)	↑ (0.146)	↑ (0.220)	↑ (0.763)	↑ **(0.007)**	↓ (0.502)
n-valerate	↓ **(0.008)**	↓ (0.113)	↓ (0.071)	↓ (0.738)	↓ (0.602)	↓ (0.447)	→ (0.290)
Total SCFA	↓ (0.233)	↓ (0.218)	↑ **(0.012)**	↑ (0.095)	↑ **(0.047)**	↑ (0.056)	↑ **(0.041)**
Mol %							
Acetate	↑ (0.076)	↑ (0.076)	↑ **(0.002)**	→ (0.989)	↓ (0.204)	↑ (0.133)	↓ (0.117)
Propionate	↓ **(0.016)**	↓ **(0.005)**	↓ **(0.002)**	↓ (0.175)	↓ (0.177)	↓ **(0.016)**	↓ **(0.009)**
i-butyrate	↑ (0.917)	↓ (0.347)	↑ (0.602)	↓ (0.602)	↓ (0.917)	↓ (0.754)	↑ (0.347)
n-butyrate	↓ **(0.006)**	↓ (0.124)	↓ (0.094)	↑ (0.238)	↑ **(0.009)**	↑ (0.227)	↑ **(0.009)**
i-valerate	↑ (0.465)	↑ (0.682)	↓ (0.645)	↑ (0.220)	↓ (0.521)	↑ **(0.024)**	↓ (0.117)
n-valerate	↓ **(0.008)**	↓ (0.245)	↓ **(0.023)**	↓ (0.911)	↓ (0.602)	↓ (0.270)	↓ (0.126)

DFR_m: non-sterile dried food residues with meat; DFR_{ms}: sterile dried food residues with meat; DFR_{wms}: sterile dried food residues without meat; SCFA: short-chain fatty acids.

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For all test substrates, the DM loss was higher after the microbial fermentation of the pre-digested substrates than of the raw test substrates.

The pre-digestion of the DFR_m and DFR_{ms} resulted in lower L- and D-lactate, propionate and n-butyrate concentrations as well as in lower relative amounts (mol %) of propionate in the inoculum compared to the microbial fermentation of the raw DFR_m and DFR_{ms}. In addition, lower total amounts (μmol/ml) of n-valerate and lower relative amounts (mol %) of n-butyrate and n-valerate could be measured in the inoculum after the microbial fermentation of the pre-digested DFR_m compared to the microbial fermentation of the raw DFR_m.

When the enzymatically pre-digested DFR_{wms}, beet pulp, carrot pomace and brewer´s spent grains were microbially fermented, higher concentrations of ammonium and lower concentrations of L-lactate were measured than after the microbial fermentation of the raw test substrates. Additionally, the pre-digestion of the DFR_{wms} resulted in lower D-lactate, propionate (mol. %) and n-valerate (mol %) as well as in higher acetate (μmol/ml and mol %) and total SCFA concentrations than after the microbial fermentation of the raw DFR_{wms}.

The enzymatic pre-digestion of wheat bran increased the concentrations of L-lactate, total SCFA and the relative amount of n-butyrate in the inoculum. Similar effects were observed for the microbial fermentation of pre-digested brewer´s spent grains, with additionally higher total amounts (μmol/ml) of n-butyrate and lower relative amounts (mol %) of propionate as well as a higher gas production in the inoculum.

The microbial fermentation of pre-digested carrot pomace also resulted in a higher gas production, but additionally in higher total and relative amounts of i-valerate and lower relative amounts of propionate in the inoculum when compared to the microbial fermentation of raw carrot pomace.

Discussion

Depending on the pattern of bacterial metabolites produced, the microbial fermentation of undigested nutrients can be beneficial, but also detrimental for gut health. While undigested protein entering the large intestine can favor pathogenic bacteria and harmful metabolites of microbial protein fermentation [15], the bacterial fermentation of non-digestible carbohydrates is considered beneficial due to an increased microbial production of SCFA [16] and balancing effects on the intestinal microbiota [17].

In the present study, different non-digestible carbohydrate sources were microbially fermented, using an *in vitro* batch culture system and canine fecal inoculum. On the one hand, the test substrates included dietary ingredients that are highly to moderately fermentable: *inulin*, a prebiotic oligo- or polysaccharide [18, 19], *beet pulp*, containing pectins, cellulose and hemicellulose [20], *carrot pomace* with insoluble and soluble fibers, particularly pectic polysaccharides, hemicellulose and cellulose [21], *wheat bran*, mainly consisting of cell wall polysaccharides like (glucurono)arabino xylans, cellulose and (1→3, 1→4)-beta-glucans, but also of protein and lignin [22], and *brewer´s spent grains*, a by-product of the brewing industry and characterized by high contents of cellulose, non-cellulosic polysaccharides and lignin [23], as well as protein and lipids [24]. On the other hand, substrates that are not or less fermentable were also included: *cellulose*, an insoluble fiber [25], and *lignocellulose*, which mainly comprises cellulose, hemicelluloses and lignin [26]. Different studies have evaluated the microbial fermentation of these test substrates in dogs, both *in vitro* and *in vivo* (e.g. [6, 13, 27–33]). However, the focus of the present study was to evaluate the fermentative capacity of food residues and to compare the effects with the microbial fermentation of the other test substrates. Moreover, as these reference substrates are non-digestible carbohydrate sources, a pre-digestion might not be necessary for their use in an *in vitro* system to simulate the microbial fermentation in the large intestine. In contrast, it was assumed that DFR might not only contain microbially usable substances, but also enzymatically digestible nutrients. Thus, we compared the microbial fermentation of raw and pre-digested substrates in our study to gain more insights into the nutrient profile of DFR as a potential dietary ingredient.

As a main finding of the present study, the raw DFR_m, DFR_{ms} and DFR_{wms} were highly fermentable, as demonstrated by the highest concentrations of ammonium, lactate, acetate, n-butyrate and total SCFA in the inoculum. Group differences were detected compared to the blank control, but also to other test substrates.

The ammonium concentrations in the inoculum were higher after the microbial fermentation of the raw DFR_m compared to the raw inulin, carrot pomace, beet pulp and DFR_{wms}. Ammonia is produced by bacterial protein degradation [34] and has been demonstrated to reveal toxic effects in the organism [35]. In healthy individuals, ammonia is detoxified to urea in the liver and excreted by the kidneys afterwards [36].

The higher concentrations of ammonium after inoculation of the raw DFR_m might likely result from a higher amount of highly fermentable protein in the raw DFR_m compared to the other test substrates. In addition, although the crude protein concentration of the DFR_{wms} was higher than of the DFR_m, the microbial fermentation of the DFR_{wms} was associated with lower ammonium concentrations in the inoculum. It can therefore be assumed that especially meat protein in the raw DFR_m might have contributed to a higher microbial ammonium

production. However, as meat protein is highly digestible [37], an inclusion of DFR_m in a diet for dogs might not necessarily result in an increased concentration of ammonium in their large intestine. Instead, it can be assumed that meat protein from DFR could be enzymatically digested in the canine small intestine. This assumption is supported by the results of the pre-digestion trials, demonstrating a relatively high crude protein digestibility of the DFR_m. In addition, the microbial fermentation of the pre-digested DFR_m revealed a comparable ammonium production as for the other test substrates, stressing the hypothesis that the raw, but not the pre-digested DFR_m contained notable amounts of highly fermentable protein.

The lactate and SCFA concentrations in the inocula were also higher after the fermentation of the raw DFR_m, DFR_{ms} and, although less pronounced, of the raw DFR_{wms} when compared to most other test substrates. These metabolites result from the bacterial fermentation of non-digestible carbohydrates [38], indicating an intensive microbial degradation of these ingredients of the food residues.

When the enzymatically pre-digested test substrates were microbially fermented, group differences were especially observed compared to the blank control, but marginally between the substrates. Most group differences compared to the blank control were detected after the fermentation of wheat bran, followed by the DFR_{ms}, DFR_{wms} and beet pulp, indicating the highest fermentative capacity for these substrates. As the effects of the bacterial fermentation were more pronounced for the raw than for the enzymatically pre-digested food residues, it can be assumed that the raw food residues contained notable amounts of digestible nutrients, which were also microbially fermented when the raw substrates were inoculated, but which were available to a lesser extent in the pre-digested substrates. This might concern protein, as already discussed above, but also digestible carbohydrates, especially starch.

Interestingly, high concentrations of n-butyrate were measured after the inoculation of both raw and enzymatically pre-digested food residues. Butyrate is the major energy source for colonocytes [38] and also associated with beneficial effects on gut and host health [39]. Thus, the observed increase of n-butyrate when the food residues were microbially fermented can be considered as a positive result. When compared with the bacterial fermentation of the other test substrates, only enzymatically pre-digested wheat bran also increased the n-butyrate concentrations in the inoculum compared to the blank control. This observation is in contrast with results from Bosch et al. [6], where the incubation of beet pulp with canine feces for 72 hours resulted in higher butyrate concentrations than the incubation of wheat fiber. However, Tuncil et al. [40] also measured high butyrate concentrations, when wheat bran was incubated with human feces for 24 and 48 hours. In addition, the authors could demonstrate that the particle size of wheat bran affected its fermentative capacity [40]. Thus, the observed differences between the results of the present study and the study of Bosch et al. [6] might be attributed to differences in the study design or the test substrates used.

In the present study, the test substrates were incubated for 24 hours, which is in accordance with the protocol of Vierbaum et al. [13]. However, the incubation time in comparable studies varied from 3–72 hours [6, 27–30, 33], making data comparison difficult. In addition, as beet pulp, carrot pomace and brewer´s spent grains are by-products of the food industry, their composition might differ depending on the production processes. Although Serena and Bach Knudsen [41] could demonstrate that those by-products showed only moderate variations in the nutrient composition, even minor differences might influence the microbial fermentation of the substrates and should be taken into account when comparing different study results. With regard to food residues, it can be assumed that the composition might vary depending on the collection procedure. In the present study, two different batches of hotel catering leftovers were evaluated, which also differed in their heat treatment (sterilized vs. non-sterilized). For the potential future use of food residues for animal nutrition, a heat treatment might be

necessary in order to improve the hygienic quality of the food residues and therefore to prevent health risks for the animals. In the present study, the sterilization process did not affect the fermentation of the raw food residues. In addition, although the composition differed between the two batches, comparable effects for the microbial fermentation of the raw food residues could be detected. For some variables, however, smaller effects were observed for the raw DFR_{wms}. When the enzymatically pre-digested food residues were microbially fermented, the effects were more pronounced for the DFR_{ms} and DFR_{wms} than for the DFR_{m}. It can be speculated that the heat treatment of the food residues might have affected the nutrient availability, but given the small sample size, this hypothesis should be further investigated in future studies. Both regarding the impact on the intestinal microbiota and the calculation of well-defined diets, compositional variability of food residues should be reduced if considered as a potential "new" ingredient for pet food in the future. In particular, collection and heat treatment procedures should be standardized.

For the interpretation of the results, a potential impact of the donor animals should finally be considered. The composition of the intestinal microbiota of dogs is dependent on animal related (breed, age), but also external (housing, diet) factors [42]. In this context, it has been demonstrated that differences in the *in vitro* fermentation of fiber substrates occurred, when the donor animals were either adapted to a diet with fermentable or non-fermentable fiber [43]. In the present study, feces of dogs kept under the same housing and feeding conditions were used for the *in vitro* experiments. The results, however, require a careful interpretation, taking into account that varying factors might affect the fermentative activity of the intestinal microbiota.

Conclusions

Based on the present *in vitro* fermentation of raw and enzymatically pre-digested food residues, it can be assumed that food residues might contain both enzymatically digestible and microbially fermentable nutrients. In comparison with the other test substrates, the microbial fermentation of food residues was comparable or partially more pronounced, but differences between the two batches of food residues were also observed. A standardization of the collection and processing of food residues might be necessary if considered as a potential "new" ingredient for pet food in the future.

Supporting information

S1 Data.
(XLSX)

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