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Dehydrated Natural Feed for Cats: Influence on Apparent Digestibility Coefficient, Feces Scores, and Serum Immune, Antioxidant, and Metabolic Biomarkers

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Abstract

The present study aimed to determine whether natural dehydrated feed (DNF) can replace dry extruded feed while preserving the required daily intake, nutrient digestibility and benefits for immunity and antioxidant response in cats. The animals were divided into two groups of seven animals each. One group received DNF, and the other consumed commercial extruded dry feed (DCF). The experiment lasted 45 days and we measured daily consumption and collected blood and feces samples. The animals in the DNF group showed greater digestibility of fiber and mineral matter than DCF. Cats in the DNF group had a higher volume of feces. DNF cats had lower leukocyte and granulocyte counts and cholesterol levels. Serum enzymatic antioxidant responses and non-enzymatic were higher in DNF cats. Cats in the DNF group showed lower serum acute phase proteins in addition to a tendency toward a higher concentration of transferrin than in the DCF group. Fecal *Escherichia coli* and total coliform counts of cats fed DNF were higher than DCF. We conclude that the daily consumption of cats is adequate. This feed had greater digestibility of fiber and mineral matter, in addition to an improvement in antioxidant and immunological responses.

Keywords

Alternative Feed, Antioxidant, Companion Animals, Health

1. Introduction

Recently, interest in the pet food market has become a research focus. A review of the pet food market revealed that in the last 18 years, there had been an increase of more than 250% in the publication of scientific articles in this area, with 2018 producing the highest number of publications [1]. Nutrition or meeting the requirements of pets is not the only focus of companies; that is, in the last 15 years, intensifying in the last five years, they seek to formulate and produce feed that is aligned with health, longevity, and (mainly) animal welfare [2]. The pet food market has numerous varieties of feed for the consumer; however, the modern owner experiences doubt regarding choice, as there is difficulty in differentiating among available commercial products [3].

The cat, in the context of modern anthropomorphism, is associated with characteristics distinct from its ancestors, becoming sweet and easily adaptable animals kept as family companions, with a disproportionate influence on the well-being of the guardian and the animal [4]. The feed industry has been adapting to the growing demand for production and formulation to meet the physiological and metabolic needs of the species, races, or stages of life; they are based on the available ingredients aiming at a high cost-benefit ratio [5]. However, cats have not changed the typical eating habits and requirements of their ancestors overtime; they are strictly carnivorous, and they need to ingest animal protein and relatively low levels of fat and carbohydrates [6] [7]. Felines can digest carbohydrates, despite the low enzymatic action; however, the type of carbohydrate and the thermal processing can affect digestion [8].

The pet food market is developing practical alternatives to facilitate the use of natural diets and meet the demands of consumers and their pets. The aim is to replace extruded dry feed, the market leader. However, the lack of adequate information to be passed on to the owner causes them to feed their cats potentially harmful diets, including unbalanced feeds in unhealthy proportions [9]. The market, in the quest to facilitate the use of alternative diets, has opted for dehydrated natural feed, and the dehydration process consists of removing moisture from the formulated and produced feed, leaving it with almost zero water activity, intending to maintain the quality, stability, and integrity [10], allowing its use in various situations. Furthermore, its use consists of being more practical and quicker compared to natural frozen diets, facilitating handling by the owner and promoting greater water consumption by the animals, since in most cases, dehydrated feeds are rehydrated (as occurs in the food used in the present study) at the time they are consumed. This process is one of the oldest food conservation methods [11]; however, it remains unexplored in feline diets. Therefore, the present

study aimed to determine whether whole dehydrated feed can replace extruded dry feed, preserve daily intake, nutrient digestibility, and metabolism, and confer benefits in immunity and antioxidant responses in cats.

2. Materials and Methods

2.1. Feed

For the supply of dehydrated natural feed, the following steps were followed: weighing the feed, and then rehydrating it with feed in warm water (72˚C), at a dose of 150 ml of water for each 100 g of feed used, then waiting for 10 minutes and delivered.

2.2. Animals and Experimental Design

The experiment was carried out on the premises of the experimental farm of the University of the State of Santa Catarina, located in the municipality of Guatambu-SC. Fourteen mongrel females were used, centered in an experimental cattery under controlled temperature (22˚C), containing fourteen experimental cages (1 × 1 m) and a communal area with two water sources available *ad libitum*, in addition to four hygienic boxes of sand/sawdust available for the cats, which were cleaned twice a day to avoid an accumulation of excrement. The cats were raised in this facility since they were kittens, keeping their vaccination protocol up to date; all tested negative for FIV and FELV and using a current antiparasitic protocol. The animals had access to an external area during the day, and during the night, they were enclosed in the internal area of the facility.

The animals were divided into two groups for the experimental period, with seven animals in each group; the DNF group: cats that consumed natural dehydrated feed, and the DCF group: cats that consumed commercial extruded dry feed. The cats underwent a five-day adaptation period to the feed until day 0 of the experiment, where the animals were fed twice a day in individual cages with availability for one hour, available, after this time the leftovers were quantified to determine average daily consumption. The amount of feed supplied daily was calculated and established according to the commercial dose indicated on the product label and according to the body weight of each animal, in addition to a comparison being made by calculating the determination of metabolizable energy (ME) requirements with based on the animal's metabolic weight (PCP). The cats had access to water ad libitum.

2.3. Sampling

The weight of the cats was measured, and blood samples were collected on days 1, 20, and 40 of the experiment; in each experimental stage, the cats fasted for 12 h before blood collection. The cats were sedated with a mixture of two commercial sedatives (xylazine at 0.06 to 0.1 mL/kg; and ketamine at 0.1 mL/kg) at the dose indicated by the manufacturer; then, the neck was scraped to facilitate collection. Blood was collected through the jugular vein with a 3-ml syringe fitted

with a 25/7-G needle. The collected blood was placed in Eppendorf tubescontaining 20 µl of anticoagulant solution (EDTA) for hemogram and tubes without anticoagulant for biochemical analysis, antioxidants, and serum proteinogram. Samples were centrifuged (5500 rpm for 10 min) to obtain serum.

2.4. Chemical Composition of the Feed and Feces

Feed samples were collected during the experimental period and stored at −20˚C. A pool was made for composition analysis at the end of four collections at ten-day intervals. The feces were collected between days 41 to 45, total feces collection method was used, and all the excrement that the animal defecated was collected. Feed and feces were thawed, weighed, and placed in an oven to determine moisture (*i.e.* the percentage of dry matter). The analyses of dry matter, mineral matter, ether extract, crude protein, acid detergent fiber, and neutral detergent fiber were carried out following [12].

2.5. Apparent Digestibility Coefficients

Using the chemical composition of feces and feed and the proportion of feed consumed and feces excreted, the apparent digestibility coefficient (ADC) was calculated according to the equation described by [13]: ADC (%) = ((Nutr I (g) – Nutr E (g)) × 100)/Nutr I (g), where Nutr I = ingested nutrient, and Nutr E = nutrient excreted in the feces.

2.6. Hemogram

The number of erythrocytes and leukocytes, hemoglobin concentration, platelets, and hematocrit were obtained using an automatic counter (EquipVET 3000). Leukocyte differential counts were also performed using an automatic counter, as were the mean corpuscular volume, red blood cell count, hemoglobin concentration, mean corpuscular hemoglobin concentration, red blood cell width distribution, and erythrocyte size variability.

2.7. Serum Biochemistry

Total protein, albumin, glucose, cholesterol, triglycerides, and creatinine levels were measured using specific commercial kits (Analisa) and a semi-automatic analyzer (BioPlus 2000). Globin levels were mathematically obtained from the difference between total protein and albumin.

2.8. Oxidant/Antioxidant Status

Serum lipid peroxidation was measured as the amount of thiobarbituric acid reactive substances (TBARS), according to literature [14]. The reaction was read in a spectrophotometer at 535 nm. The result was expressed as nmoles malondialdehyde/ml of serum.

Glutathione S-transferase (GST) activity was measured according to researchers [15]. To measure non-protein thiols (NPSH), the method used to use 5,5-dithiobis-(2-nitrobenzoic) acid (Sigma) was based on literature [16]. The de-

termination of the NPSH content in the samples was measured after deproteinization with trichloroacetic acid (50%). The absorbance readings (405 nm) were performed using a spectrofluorometer (Biotek, Synergy HT).

2.9. Protein Electrophoresis

For the proteinogram, electrophoresis in a polyacrylamide gel containing sodium dodecyl sulfate was performed according to the technique described by researchers [17] using mini-gels (10 × 10 cm). The gels were stained with Coomassie blue and photographed to identify and quantify protein fractions using LabImage 1D software (Loccus Biotechnology). A standard containing fractions with molecular weight between 10 and 250 KD (Kaleidoscope-BIORAD) was used as a reference.

2.10. Fecal Score

The fecal score was determined between days 40 and 45 of the experiment through visual observation of the feces and their characteristics. We used a scale from 1 to 5 proposed by researchers [18]: score 1—very hard and dry feces with small dry pellets; score 2—hard, dry, firm, soft, and well-formed feces; score 3—soft, well-formed, moist feces with preserved shape; score 4—soft feces, with no defined shape; score 5—liquid feces.

2.11. Statistical Analysis

Each animal was considered the experimental unit for all analyses. Data were tested for normality and homogeneity of variance using the Shapiro-Wilk and Levene tests, respectively. All data were analyzed using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC, USA; version 9.4) with the Satterthwaite approximation to determine the denominator degrees of freedom for the test of fixed effects. The results of body weight (BW) changes were tested for fixed effects of treatment and using animal and period as a random variable. All other variables (BW, serum biochemistry, serum antioxidant responses, and hemogram) were analyzed as repeated measures and tested for fixed effects of treatment, treatment × day, and using animal and period as random variables. The results of day 1 were also included as independent covariates in each analysis but were removed from the model when $P > 0.10$. The covariance structures were selected according to the lowest Akaike information criterion. Means were separated using PDIFF, and all results were reported as LSMEANS followed by SEM. The variables related to the animals' behaviors with the firecracker test were analyzed using the Chi-square test. Significance was defined when $P \leq 0.05$, and the tendency to significance when $P > 0.05$ and $P \leq 0.10$.

3. Results

3.1. Chemical Composition of Feed

The chemical composition of the feeds is presented in **Table 1**. The difference

between the feeds tested in terms of chemical composition is small, a numerical increase for the contents of ether extract, crude protein, and acid detergent fiber for the dehydrated natural feed.

Table 1. Chemical composition of dehydrated natural diet and commercial extruded dry feed.

Chemical composition	DNF group	DCF group
Dry matter (g/kg)	928	944
Mineral matter (g/kg of DM)	62.3	69.2
Crude protein (g/kg of DM)	457	344
Ether extract (g/kg of DM)	173.1	128.6
Neutral detergent fiber (g/kg of DM)	161.2	242.5
Acid detergent fiber (g/kg of DM)	69.4	48.5

Note: DNF: Natural dehydrated feed; DCF: Commercial extruded dry feed; **DNF**: One super-premium commercial cat feed was purchased and used as a control. The feed included poultry offal flour, rice grits, wheat gluten, ground whole corn, pea hulls, corn gluten, chicken fat, pork fat, isolated pork protein flour, refined soybean oil, beetroot, dried brewer's yeast, dried egg, zeolite, borage oil, fructo-oligosaccharides, marigold extract, grape polyphenols, green tea 10%, dicalcium phosphate, calcium sulfate, potassium chloride, monosodium phosphate, sodium chloride (common salt), calcium carbonate, monocalcium phosphate, vitamins (A, C, E, D3, B1, B2, B6, B12, PP), pantothenic acid, biotin, folic acid, choline chloride, iron sulfate, sulfate copper, manganese oxide, zinc oxide, calcium iodate, sodium selenite, chicken liver, annatto natural coloring, taurine, L-lysine, DL-methionine, antioxidant (BHA), ground whole corn, and genetically engineered corn gluten modified by *Bacillus thurigiensis* and *Streptomyces viridochromogenes*; Refined soybean oil produced from soybeans genetically modified by *Agrobacterium* sp. **DCF**: We used natural dehydrated feed (SmilePET mix, Tectron). Its composition is based on pork fat, salmomega forte, medium rolled oats, linseed grain (brown), sesame, distilled yeast at alcohol 36.7%, brewer's yeast 40 PB, dehydrated chicken, dehydrated bovine heart, dehydrated chicken liver, bovine lung, dehydrated pork bone trimmings, cassava starch, dehydrated pumpkin in flakes, dehydrated sweet potato in cubes, dehydrated carrot in cubes, dehydrated peas, dehydrated broken peas, whole apple in cubes, meat broth, chicken broth, refined salt with an anti-wetting agent, tricalcium phosphate, guar gum (thickener), xanthan gum, natural antioxidant, sodium hexametaphosphate, L-lysine 80%, DL-methionine 98%, choline chloride 60%, taurine 98%, sodium bisulfate, *Curcuma longa*, liver protein hydrolyzate, probiotics, yucca schidigera extract, vit/min premix, and ACIDMAX.

3.2. Feed Intake and Apparent Digestibility Coefficients

To adapt the cats to the new diet, the DNF was mixed with extruded dry feed in increasing amounts according to the animals' acceptance. From the beginning, the new feed was well accepted by the animals. However, from day 15 of the experiment, one of the cats started to vomit the consumed feed partially. On day 16, she vomitedthe entire consumed diet. We reduced the amount of feed to normalize the situation, and the cat continued to expel what was consumed; as a result, we removed this animal from the treated group of the experiment.

The ADC is presented in Table 2. Lower ADC of the ether extract was found for natural dehydrated feed compared to commercial dry feed ($P < 0.05$). On the other hand, higher ADC for mineral material, fiber neutral detergent (NDF), and fiber acid detergent (ADF) were observed in the DNF group than in the DCF group (control) ($P < 0.05$). There was no significant difference for ADC of dry matter and crude protein ($P = 0.95$; $P = 0.58$, respectively).

Table 2. The apparent digestibility coefficient of feed consumed by cats natural dehydrated feed (DNF) and commercial extruded dry feed (DCF).

Chemical composition	DNF group	DCF group	SEM	P-value
Dry matter (%)	83.4	83.2	0.45	0.95
Mineral matter (%)	67.7[a]	51.5[b]	1.05	**0.01**
Crude protein (%)	82.2	84.6	0.93	0.58
Ether extract (%)	88.1[b]	95.2[a]	0.89	**0.01**
Neutral detergent fiber (%)	86.4[a]	80.2[b]	0.52	**0.01**
Acid detergent fiber (%)	82.1[a]	39.5[b]	1.06	**0.01**

Note: [a,b]Within a treatment, underwriting differs ($P \leq 0.05$) or tends to differ ($P \leq 0.10$).

3.3. Feces

The amount of feces excreted and the fecal score of cats fed with dehydrated natural feed and DCF are presented in Table 3. Animals fed natural chow had more feces excreted (more wet feces than commercial chow) ($P < 0.05$). Stool scores in the DNF group were higher, reaching an average score of 3 in most animals (rare scores of 4), while most in the DCF group had a score of 1 to 2 ($P < 0.05$).

Table 3. Body weight and feces score of cats receiving experimental diets.

Variable	Treatments		SEM	P-value	
	DNF group	DCF group		Treat	Treat × day
***Body weight* (*kg*)**				0.91	0.93
Day 1	2.38	2.29	0.24		
Day 20	2.45	2.44	0.22		
Day 40	2.50	2.47	0.22		
***Weight gain* (*g*)**				0.31	-
Days 1 to 40	120	180	5.96		
Feces score				**0.01**	-
Days 40 to 45	3.13[a]	1.48[b]	0.01		
***Feces MN* (*g*)**				**0.01**	-
Days 40 to 45	212[a]	147[b]	4.12		
***Feces MS* (*g*)**				0.56	-
Days 40 to 45	50.0	47.0	0.85		

Continued

Feed intake (g/ day)				0.98	-
Day 1 to 40	52.5	52.6	1.09		
Feed efficiency				0.25	-
Day 1 to 40	0.057	0.086	0.008		

Note: [a,b]Within a treatment, underwriting differs ($P \leq 0.05$) or tends to differ ($P \leq 0.10$). To analyze the treatment effect, the results of the variables on days 20 and 40, which correspond to the experimental period, were used; the results were presented as an average.

3.4. Body Weight

Results are presented in Table 3. There was no effect of treatment and no interaction between treatment vs. day for BW, feed intake, and feed efficiency ($P > 0.10$).

3.5. Hematologic Analysis

The complete blood count results are shown in Table 4. There was no treatment and treatment vs. day interaction effect for hematocrit, hemoglobin concentration, erythrocyte, monocyte, lymphocyte, and platelet counts ($P > 0.10$). We verified the effects of treatment and treatment vs. day interaction (day 40) for total leukocyte count, with lower values observed in cats in the DNF group compared to the DCF group ($P < 0.05$). Following the same dynamics, the granulocyte count was lower in the cats in the DNF group (day 40) ($P < 0.05$) and had a significant trend between treatments, lower for DNF ($P < 0.10$).

Table 4. Complete blood counts of cats receiving experimental diets.

	Treatments		SEM	P-value	
	DNF group	DCF group		Treat	Treat × day
Hematocrit (%)				0.95	0.93
	33.6	33.8	1.02		
Hemoglobin (g/dL)				0.92	0.88
	9.66	9.73	0.25		
Erythrocytes ($\times 10^6$ µL)				0.81	0.84
	7.18	7.50	0.14		
Leukocytes ($\times 10^3$ µL)				**0.04**	**0.01**
D1	5.69	6.88	0.44		
D20	6.35	7.54	0.39		
D40	5.13[b]	6.82[a]	0.40		
Average	5.74[b]	7.18[a]	0.36		
Granulocytes (/µL)				**0.08**	**0.05**
D1	1.23	1.96	0.52		

Continued

D20	2.07	2.41	0.50		
D40	1.76[b]	2.55[a]	0.32		
Average	1.91[b]	2.48[a]	0.38		
Lymphocytes (/μL)				0.65	0.49
	3.31	3.93	0.41		
Monocytes (/μL)				0.71	0.56
	0.52	0.77	0.35		

Note: [a,b]Within a treatment, underwriting differs (P ≤ 0.05) or tends to differ (P ≤ 0.10). [a,b]In lines without common subscripts, there are differences (P ≤ 0.05) or tendencies to differ (P ≤ 0.10). To analyze the treatment effect, the results of the variables on days 20 and 40, which correspond to the experimental period, were used; the results were presented as an average.

3.6. Serum Biochemical Analysis

The serum biochemistry results are presented in Table 5. There was a treatment effect on serum cholesterol concentrations; animals in the DNF group had lower serum levels of this biomarker compared to the control (DCF group) (P < 0.05). Glucose, triglycerides, creatinine, total protein, albumin, and globulin levels did not differ between groups (P > 0.10).

Table 5. Serum biochemistry and oxidative profile of cats receiving experimental diets.

	Treatments		SEM	P-value	
	DNF group	DCF group		Treat	Treat × day
Glucose (mg/dL)				0.19	0.26
	105.9	91.4	5.14		
Triglycerides (mg/dL)				0.95	0.92
	43.9	44.7	2.41		
Cholesterol (mg/dL)				**0.01**	0.11
	91.2[b]	104.0[a]	2.84		
Total protein (g/dL)				0.89	0.93
	7.01	6.73	0.10		
Albumin (g/dL)				0.96	0.93
	2.22	2.25	0.05		
Globulin (g/dL)				0.83	0.78
	4.78	4.48	0.09		
Creatinine (mg/dL)				0.94	0.95
	1.03	1.10	0.02		
GST (μmol CDNB/min)				**0.01**	**0.01**
D1	585	609	9.36		

Continued

D20	734[a]	672[b]	8.54		
D40	687[a]	591[b]	8.42		
Average	710[a]	632[b]	8.14		
Total tiois (nmol SH/mg)				0.52	**0.05**
D1	103	102	5.36		
D20	88.2	90.2	4.97		
D40	92.1[a]	75.1[b]	4.84		
Average	90.2	82.9	4.68		
TBARS (nmol MDA/mL)				0.97	0.95
	16.4	16.0	1.03		

Note: [a,b]Within a treatment, underwriting differs ($P \leq 0.05$) or tends to differ ($P \leq 0.10$). [a,b]In lines without the common subscript, there are differences ($P \leq 0.05$) or tendencies to differ ($P \leq 0.10$). To analyze the treatment effect, the results of the variables on days 20 and 40, which correspond to the experimental period, were used; the results were presented as an average.

3.7. Oxidant and Antioxidant Status

The results of the oxidant and antioxidant profile are presented in Table 5. We observed treatment effect and treatment vs. day interaction (day 20; 40) for GST levels, with the DNF group being more significant than the DCF group ($P < 0.05$). For total thiols, significance was found in the treatment vs. day interaction (day 40), also more significant for the DNF group ($P < 0.05$). There was no difference in lipid peroxidation (TBARS) in the serum of cats ($P > 0.10$).

3.8. Proteinogram

The results of the serum proteinogram are presented in Table 6. We verified a treatment effect for IgA concentrations, with higher values observed in cats in the DNF group compared to the DCF group ($P < 0.05$). Different from what was found for ceruloplasmin levels, both between treatments and in the interaction between treatment and day (day 40), the DNF group was lower compared to the DCF group ($P < 0.05$). Following the same situation, C-reactive protein levels were lower for the DNF group ($P < 0.05$). Transferrin concentrations were higher in cats in the DNF group (day 40) ($P < 0.05$), and there was still a significant trend between treatments, being higher for DNF ($P < 0.10$). No significant difference was observed for heavy chain IgG concentrations ($P > 0.05$).

3.9. Microbiological Feces

The results of the microbiological feces are presented in Figure 1. We found a higher bacterial count in the feces of cats fed natural dry feed both for *E. coli* ($P < 0.05$) and a trend toward higher total coliform counts when compared to the DCF group ($P < 0.10$).

Table 6. Serum proteinogram of cats receiving experimental diets.

Variable[1]	Treatments		SD	P-value	
	DNF group	DCF group		Treat	Treat × day
IgA (g/dL)				**0.01**	0.12
Average[2]	0.94[a]	0.80[b]	0.02		
Heavy chain IGG (g/dL)				0.14	0.13
Average[2]	1.25	1.08	0.05		
Ceruloplasmin (g/dL)				**0.05**	**0.03**
D1	0.62	0.64	0.07		
D20	0.57	0.65	0.08		
D40	0.48[b]	0.65[a]	0.08		
Mean[2]	0.52[b]	0.65[a]	0.07		
C-reactive protein (g/dL)				**0.05**	0.11
Mean[2]	0.27[b]	0.37[a]	0.04		
Transferrin (g/dL)				**0.10**	**0.05**
D1	0.25	0.28	0.03		
D20	0.29	0.27	0.03		
D40	0.33[a]	0.28[b]	0.03		
Mean[2]	0.31[a]	0.27[b]	0.02		

Note: [1]Immunoglobulin A (IgA), heavy chain immunoglobulin G (IgG). [2]To analyze the treatment effect, the results of the variables on days 14 and 28, which correspond to the experimental period, were used; the results were presented in an average. [a,b]Within a treatment, the subscript differs ($P \leq 0.05$) or tends to differ ($P \leq 0.10$) in the same column. [a,b]In the same line, without the common subscript, there are differences ($P \leq 0.05$) or tendencies to differ ($P \leq 0.10$) between treatments.

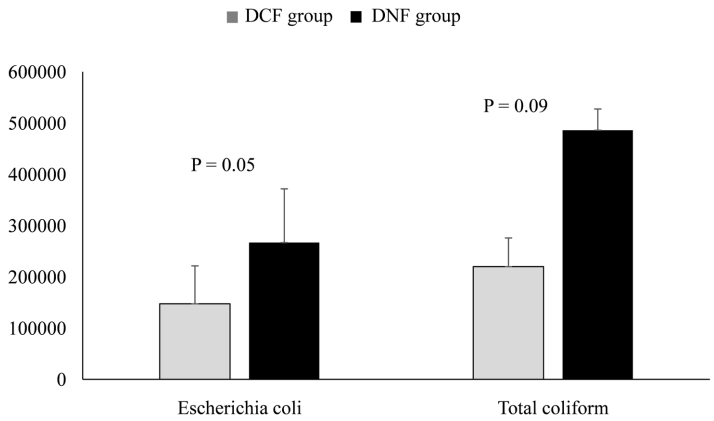

Figure 1. *Escherichia coli* counts and total coliforms in the feces of cats receiving experimental diets. Results presented in colony forming units (CFU).

4. Discussion

The study of pet food comparisons is very controversial due to the discrepancy

of feeds that each product can offer; however, to maintain the integrity of the study, we tried to maintain a similar nutritional base in the feeds tested. The dehydrated natural feed didn't affect the cat feed intake, as they themselves went through an adaptation period so that they could get used to consuming the new diet, highlighting that only an animal had to be removed from the experiment as it didn't accept the new diet. However, numerous factors modify the nutrients in a feed, as we observed that the dehydrated natural feed had higher values of protein and ether extract and lower values of fiber than the extruded feed. This fact is positive because cats are highly demanding animals and can control their intake according to the quality of the feed, consuming feed in several portions during the day [19]. Researchers [20] demonstrated that cats could select and prefer diets with higher values of protein and fat, with protein being the most critical factor in this decision, as it is the nutrient that this species requires nutritionally, in particular, a point that may have been beneficial since the natural feed used had these characteristics.

The digestibility of some nutrients differed between groups of cats, something expected; however, there was an important finding: Even though the feed had a lower value of the mineral matter, the digestibility of this nutrient was more significant for the group that consumed a natural dehydrated diet. However, the diet had higher values of this component for the ether extract, and its digestibility was significantly lower than the extruded dry feed. Regarding digestibility, several factors can interfere because, according to literature [21], the ADC may differ by the composition of ingredients used, the type of processing that the feed may be subjected to, in addition to aspects specific to the animal, such as the stage of life of gastrointestinal flora. However, nutrients have their particularities, including fat, which cats digest well; however, they depend on their saturation load [22], the amount of calcium present in the diet that can cause fat hydrolysis, age of the animal and also losses of endogenous fecal fat [23]. These factors may have beneficially influenced the study, based on the assumption that self-fat levels can harm cats' health in the long term.

Little importance is given to fiber in the diet of cats, but in the present study, the ingredients in the diet directly influenced the existing amount of NDF and ADF in the feed, demonstrating low levels for the natural dehydrated feed, which in turn proved to be highly digestible, as pointed out in the present study. Cats are strictly carnivores by nature; however, a minimal amount of fiber in the diet of these animals can have a positive effect on certain diets that require specific care, such as obesity, and intestinal problems, among other situations that fiber can contribute to this species [24]. This situation is not the case in the present study; however, this finding may influence and stimulate further research to determine the benefits in animals with certain particularities. Protein digestibility was similar for both feeds, remembering that protein levels were higher in the natural dehydrated diet; thus, similar digestibility can be interpreted as beneficial due to the greater availability of amino acids. The lower di-

gestibility of the ether extract may have directly affected blood cholesterol levels, decreasing its concentration in the group that consumed the dehydrated natural feed. Researchers [25] point out that high cholesterol levels can predispose to obesity in dogs and cats, so considering a long-term feeding characteristic, the use of NDF can be metabolically beneficial for the animal predisposed to obesity.

Cats from the DNF group showing wetter feces and consequently in greater quantity, which we believe is related to two factors: a) the dehydrated feed had a smaller amount of fiber, in addition to the type of dietary fiber used in the diet, which aligns with Kerr *et al*. [26], who claim that different types of fiber for felines directly influence the production, consistency, and type of fecal fermentation; another study describes cellulose as the main component of fiber to adjust fecal consistency [24], a component that proved to be highly digestible in the diet in question in the present study; b) another highly relevant factor is that the DNF needs to be hydrated to be supplied; adding water to the diet can directly influence the amount and consistency of the animals' feces.

White blood cell counts differed across diets because feed acts directly in modulating the function of the immune system, in addition to the fact that the components present in the diet can help increase or decrease resistance to possible infections [27]. We highlight the lower presence of white blood cells responsible for our body's defense against infectious agents, being able to associate this result with the serum proteinogram, which showed that the cats that consumed DNF had lower concentrations of inflammatory response biomarkers. We also noticed a higher IgA antibody concentration in the cats, an immunoglobulin that protects the mucosa [28]. It is essential to emphasize that the animals used in the study were healthy. Hence, the differences between diets allow us to believe that the natural diet can improve the immune response without concomitantly generating an inflammatory response.

The diet influenced the antioxidant status, in which the cats in the DNF group had higher levels of enzymatic and non-enzymatic antioxidants, that is, the endogenous activity of GST and those coming from the feed itself (thiols). Antioxidants can delay lipid oxidation and characterize a feed as rancid; however, commercial feeds often use synthetic antioxidants to maintain feed stability and prevent lipid peroxidation [29]; thus, our study demonstrates that dehydrated natural feed, which is rich in natural antioxidants, can beneficially influence protecting the animal organism from possible damage by free radicals and future lipid oxidation of the feed [30]. We hypothesize that it is a cascade effect, where the entire antioxidant system benefits from the positive effects of a natural diet.

We highlighted small changes in acute phase proteins produced by the liver, which are the most critical and sensitive marker of inflammatory response in the body of animals [31]. Lower concentrations of ceruloplasmin were observed for the DNF group, a protein responsible for transporting blood copper in healthy individuals. Ceruloplasmin levels of 0.48 to 0.65 g/dL are considered healthy. This was also seen with C-reactive protein, which was lower in the DNF group;

this protein is typically stimulated by tissue damage linked to inflammatory processes and modulates neutrophil function [32]. Low levels demonstrate that animals are in a normal state, associated with low white blood cell values in the present study. The liver can synthesize the primaryiron-transporting protein, transferrin; in our study, the NDF group had higher levels than DCF. Naturally, blood iron derives almost entirely from erythrocytes that macrophages have phagocytosed; very little blood iron comes from feed [33], and virtually all lost iron is equivalent to how much was absorbed by the diet [34]. Bohn [33] stated that if iron is deficient in the body, transferrin may increase, requiring the iron to be revolved several times by the circulation; however, it is a typical case of a healthy animal unless it is associated with chronic liver disease.

Finally, cats that receiving dehydrated natural feed demonstrated a higher bacterial count measured in *E. coli* and a trend toward the superiority of total coliform counts. One of the factors driving consumers to not adhere to a completely natural diet (which is focused on raw feed) is the fear of bacterial cross-contamination linked to antimicrobial resistance to pathogens [35]. However, a factor to be taken into account is that the natural diet had higher levels of fiber and greater digestibility, and cats will ferment this fiber in the large intestine, thus, it is assumed that there was a higher fermentation rate for digestion and absorption, increasing bacterial levels present in the fecal cake [36]. Even so, when talking about the feed dehydration process, it is assumed that drying the feed, leaving its water activity at minimum levels, can denature the bacterial proteins, alter the bacterial membrane, and cause its extravasation and destruction; *E. coli* is particularly susceptible to this type of damage [10]. Studies focusing on numerous bacterial species, microbiological quality of feed processing and animal excretion can elucidate consumer perspectives regarding dry feed.

During the experiment period, approximately 15 days after using the dehydrated natural feed, one of the animals began to expel all the feed consumed, even though the cat consumed it completely. Vomiting in felines can be a clinical sign indicative of a possible gastrointestinal disease or specific intoxication arising from feed or even an expansion of the stomach caused by feed that leaves it with digestion difficulties [37], the latter cause being more likely to be accepted in the present study, due to the amount of feed increased by the age of the animals. However, the animal was healthy, without characteristic signs of illness or contractions, it only expelled the feed shortly after consuming it. We can also characterize it by a possible regurgitation, which is usually passive expulsion of feed still present in the pharynx or esophagus, which according to literature [38], can happen soon after feed ingestion. We removed this animal from the treatment group and returned to supplying extruded feed and thus the animal's situation immediately normalized.

5. Conclusion

The cats that consumed the dehydrated natural feed had a higher digestibility of

mineral matter, neutral detergent fiber and acid detergent fiber but a lower digestibility coefficient of the ether extract. Cats that consumed natural feed stimulated the antioxidant system, where antioxidants were higher, which may favor the process of controlling oxidative reactions in the body. Lower levels of acute phase proteins, leukocytes, and granulocytes are seen in the blood of cats that have consumed DNF. Therefore, we conclude that both feeds are nutritionally appropriate for growing cats, much like extruded dry feed; moreover, the ingestion of dehydrated natural feed has nutraceutical effects, which are beneficial to animal health.

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Ethics Approval

The ethics committee on the use of animals in research of the Universidade do Estado de Santa Catarina approved all procedures for this project under protocol number 5167240422 and all procedures followed the rules issued by the National Council for Control of Animal Experimentation (CONCEA).

Data Availability Statement

Raw data are held by the authors and may be available upon request.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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