Determination of *in vitro* digestibility and some quality characteristics of fermented sucuk foods produced for dogs

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ABSTRACT

The aim of current study was to determine *in vitro* digestibility, some microbiological properties and shelf life of fermented sucuk foods consisting mixture of animal and vegetable natural foods produced for dogs. Grain-inclusive and grain-free (GF) formulations were prepared. Grain-inclusive group was subgrouped as cooked (CG) and uncooked grain (UCG). *In vitro* digestibility, nutrient composition, pH and thiobarbituric acid reactive substances (TBARS) values of 3 groups of sucuk foods were determined at 0, 1, 3, and 6 months after production. Microbiological characteristics (Aerobic colony number, *E. coli*, coagulase positive Staphylococcus, coliform bacteria, yeast mold, Salmonella spp.) and lactic acid levels of sucuks were determined at the end of 1, 3, and 6-months of storage (+4°C). There were differences in nutrient compositions of groups and storage times within groups ($P<0.05$). All sucuk foods were negative for *E. coli* and Salmonella spp. Count of aerobic colonies were $5.8x10^7$, $3.0x10^7$ and $3.1x10^9$ CFU/g in CG, UCG and GF, respectively. Total yeast-mold counts were between $5.5x10^3$-$9.6x10^4$ CFU/g. The highest pH drop (5.38 to 4.25) and *in vitro* organic matter digestibility (92.02%) were determined in CG sucuk ($P<0.05$). TBARS value of UCG group was the highest at the end of the 6-months storage ($P<0.05$). Lactic acid levels were not different between storage times and groups ($P>0.05$). As a result, healthy and highly digestible sucuk foods were obtained for dogs, which contain sufficient and balanced nutrients and have a long shelf life.

Introduction

Additives and preservatives that claimed to be harmful to health are used in production of commercial dog foods and affect intake, digestibility, appearance, consistency and shelf life (16). The choice of dog food is similar to the choice of food for people's family members (40). Therefore, natural foods have become popular in nutrition of dogs as well as humans (14). Usage of raw meat-containing products in dog nutrition has increased in recent years among pet owners in many countries. A survey conducted in the USA in 2016 revealed that 17% of dog owners feed their dogs with raw or cooked human food (6). However, there is a little information on the evaluation of dog diets prepared as homemade, organic and with products for human consumption.

As a result of chemical preservatives and usage of poor-quality raw materials in commercial dog foods, people want to prepare diets for their dogs on their own. Meat-based natural feeding is preferred for reasons such as using quality natural foods, imitating feeding of dogs in nature, belief of advantages on health, and avoiding the processes applied in commercial food production (23). Such raw diets may be constructed from recipes that do not have nutritional expertise and feeding studies (38). Therefore, homemade diets are susceptible to nutritional imbalances and deficiencies. Inadequate and unbalanced nutrition of dogs is inevitable as a result of the difficulty of preparing a balanced homemade diets (54).

Good quality ingredients can be used in formulation of homemade diets and artificial additives and preservatives could be avoided. This type of diets provide opportunity to preserve natural enzymes and use of herbal sources and whole ingredients which, may provide health benefits that the individual fractionated ingredients or single nutrients cannot provide (14, 35). Properly formulated homemade diets offer pet owners a good

alternative. Ingredients and nutrient composition can be changed according to the physiological state of the animal. They can also be used effectively in dogs showing allergic reactions to commercial foods (46).

Natural raw diets can be beneficial for animals but are risky for both animals and their owners as they can be contaminated with the zoonotic pathogens (Campylobacter, Salmonella and Yersinia). Dogs expel too much bacteria in their feces, live in the same environment with humans, and are likely to be carriers of pathogenic microorganisms. Therefore, even such diets do not cause disease in healthy animals, they have potential to affect human health (37). Limited data known about the prevalence of these pathogens in dog diets (24). Studies on the natural diet of pet animals based on raw meat have generally focused on microorganism contamination (41).

Food fermentation has beneficial effects such as low-cost preservation, improving digestibility, shelf life, nutritional quality, eliminating toxic components, harmful microorganisms, and protecting against infection (52). Allergic reactions caused by excessive use of animal proteins in dog feeding could be resolved by appropriate fermentation. This could be achieved thanks to pre-prandial proteolysis occurring in fermented foods changing the allergen presentation or cleaving the allergenic protein epitopes (18). The appearance, palatability and texture of foods are also improved by fermentation (18). However, it has been determined that fermented foods are less palatable for dogs due to their acidic odor and taste (59). But in a study, fermented chicken meat did not show a negative effect on intake and body weight in dogs (36).

No scientific studies have been conducted on diets in which animal and vegetable products are fermented together for dogs. Therefore, the objectives of the present study were to obtain natural and nutritionally balanced dog food in the form of fermented round sucuk (turkish sausage) with a relatively long shelf life, highly digestible, microbiologically safe and preservative-free.

Materials and Methods

Preparation of sucuk foods: In the study, two different sucuk formulas were prepared as grain-inclusive and grain free. Formulations were prepared in the diet program designed with the Microsoft Office Excel Package Program for dogs. Formulas have been adjusted to meet the nutrient needs of a healthy adult dog according to FEDIAF (21). Rice and barley were ground in mill (Retsch SM100, Germany) using a 0.5 mm diameter sieve and added to sucuk mixture without cooking in one group (uncooked grain-inclusive). Same grains were added to mixture of other group after cooked for 20-30 minutes for gelatinization and dried for at 55°C 48h before grinding (cooked grain-inclusive). Peas, potatoes and carrots were used instead of grains in grain-free sucuk formula (Table 1).

Table 1. Ingredient and chemical compositions of grain-inclusive and grain-free sucuk foods.

Ingredients, %	Cooked grain	Uncoked grain	Grain-free
Beef, 5-10% fat, raw	12	12	12.37
Liver, chicken	7	7	7
Chicken, breast meat, raw	22	22	20
Beef lung, raw	0.05	0.05	1
Beef tripe, raw	11.5	11.5	11
Eggshell	0.4	0.4	0.4
Barley	19.5	19.5	
Peas, green, raw			7
Carrots, raw			10
Potatoes			30
Rice, white, raw	22.88	22.88	
Garlic	0.1	0.1	0.1
Bone meal	0.7	0.7	
Potassium chloride	0.15	0.15	
Iodized salt	0.07	0.07	0.075
Vit-Min. (dog)[a]	0.15	0.15	0.055
Sunflower oil	3.5	3.5	1
Calculated chemical composition			
Crude protein, % DM	25.24	25.24	25.96
Ether extract, % DM	10.94	10.94	13.05
Carbohydrate*	59.84	59.84	29.67
Crude fiber,% DM	4.92	4.92	5.77
ME (kcal/kg DM)**	3912	3912	4045
Calcium, %	0.67	0.67	0.66
Phosphorus, %	0.48	0.48	0.49
Arginine, %	0.9	0.9	0.68
Histidine, %	0.41	0.41	0.31
Isoleucine, %	0.67	0.67	0.52
Methionine, %	0.35	0.35	0.26
Leucine, %	1.08	1.08	0.79
Lysine, %	1.01	1.01	0.85
Phenylalanine, %	0.64	0.64	0.42
Threonine, %	0.52	0.52	0.39
Tryptophan, %	0.17	0.17	0.12
Taurine, %	0.05	0.05	0.06
Linoleic acid, %	3.77	3.77	2.87
Arachidonic acid, mg/kg $BW^{0.75}$	41.69	41.69	94.11
Vitamin A, IU/kg $BW^{0.75}$	1911.32	1911.32	5603.22
Vitamin D, IU/kg $BW^{0.75}$	32.78	32.78	36.79
Vitamin E, IU/kg $BW^{0.75}$	5.68	5.68	4.62

[a] Premix for dogs. Added per kg of food: iron, 120 mg; copper, 15 mg; magnesium, 75 mg; zinc, 150 mg; iodine,2 mg; selenium, 0.3 mg; vitamin A, 18,000 IU; vitamin D3, 1000 IU; vitamin E, 100 IU; vitamin K, 2 mg; biotin, 0.6 mg; thiamine, 20 mg; riboflavin, 10 mg; pantothenic acid, 50 mg; niacin, 75 mg; vitamin B6, 6 mg; folic acid, 4 mg; vitamin B12, 0.1 mg.

*Carbohydrate (NFE), % = 100 - (% crude protein + % ether extract + % crude fibre +% moisture + % ash).

** Metabolizable energy was calculated with NRC (2006) equations.

Chicken breast meat and liver, beef meat, cattle lung and tripe were minced using a grinder (Fakir, minso plus, Türkiye) through a 3 mm plate. Vegetable sources (peas, carrots, potatoes, rice, barley) were dried for 48h at 55°C and ground before mixing and turned into sucuk mixture. All ingredients were mixed homogeneously using a hand type mixer (44). The samples were taken from each mixture and 3000 g mixture was prepared for each three group. Mixtures containing 54-55% dry matter were kept in the refrigerator overnight. High dry matter level was desired. Because sucuk food rounds with high dry matter content were in good shape (hard, not floppy). The mixtures were stuffed into intestine casings by using a hydraulic filling machine (Emir sausage filling machine, Türkiye). Machine was cleaned and sanitized after filling each group. Ten rounds of sucuk food weighing approximately 300 g were prepared from each group. Sucuks were kept for ripening in laboratory environment at 23-25°C and at 75-95% relative humidity (9). They were placed in vacuum bags when the pH was between 4.7-5.4. After this process, all sucuk foods were stored in the refrigerator (+4°C).

***Determination of nutrient composition and pH*:** Samples were taken from all sucuks after 0, 1, 3 and 6-months storage and pH levels were measured (56). Nutrient analyzes were performed on 3 rounds of sucuks (2 parallel each) at the 0, 1st, 3rd and 6th months of storage. After the intestinal casings was stripped, dry matter levels were determined (VWR-Venti-line, USA) at 55°C for 48h. Dried samples were ground with a chopper (Kenwood CH250, England). Dry matter (DM), ash, crude protein (CP), ether extract (EE), crude fiber (CF) and starch analyzes were performed (5). The metabolizable energy (ME) levels of the sucuk foods were calculated using the following 4-step-calculation formula according to NRC (43):

I. Gross energy (GE): GE (kcal) = (5.7 × CP%) + (9.4× EE%) + [4.1× (NFE%+ CF%)]
   Nitrogen-free extract, NFE (%) = DM% – (EE% + CP% + ash% + CF%)
II. Energy digestibility (%) = 91.2 – (1.43 × CF%)
III. Digestible energy: kcal DE = (kcal GE × energy digestibility)/100
IV. Metabolizable energy: ME (kcal) = kcal DE – (1.04 × CP%)

Determination of thiobarbituric acid reactive substances (TBARS) value and lactic acid level: In order to monitor lipid oxidation, TBARS analysis was performed on the sucuks by using the method of Kilic and Richards (32) at the 0, 1, 3 and 6 months after storage. A sample of 20 g was homogenized with 100 ml of 1:1 20% trichloroacetic acid (TCA) (w/v) in 2M phosphoric acid and distilled water. The slurry was then filtered through the Whatman No. 1 filter paper and the volume was completed to 100 ml. After that, 5 ml of the filtrate was mixed with 5 ml of TBA (0.02M) in a test tube. A blind solution was prepared using 1:1 TCA:distilled water. The tubes were incubated at 80°C for 35 min. Finally, the absorbance was measured using a spectrophotometer (Shimadzu, mini-1240, Japan) at 532 nm. The TBARS value was calculated by multiplying the absorbance by 5.2 to express the concentration as mg malonaldehyde/kg samples. Spectrophotometric method was used to determine the lactic acid levels (10).

Microbiological analysis: Total mesophilic aerobic colony, *E. coli* (12), coagulase positive Staphylococcus, coliform bacteria (4, 56) and yeast-mold count (20) on the 1st, 3rd and 6th months of storage in all sucuk food samples was performed. Among the pathogenic bacteria, Salmonella spp. presence was investigated following AOAC (2000) method (4).

Determination of digestibility by in vitro enzymatic method: To determine dry matter (IVDMD) and organic matter digestibility (IVOMD) of sucuk foods in the 0, 1st, 3rd and 6th months of the storage, a 3-phase *in vitro* enzymatic method was followed by using a Daisy^{II} incubator (Ankom Technology Co., Fairport, NY, USA) (Table 2) (39). Six bags were weighed for each sucuk food for each storage time. Each of the digestion jars, which rotates in the Daisy^{II} incubator at constant temperature, were filled with the enzymatic solution. Filter bags (F57, Ankom Technology Corp. Country) used for sample and blank were soaked in pure acetone (99%) to remove substances that could clog pores and inhibit enzyme activity, and then dried prior to use. At the end of the 6h incubation, the bags were removed from the jars and rinsed thoroughly under tap water until clear and dried in oven (VWR VENTI-Line, Germany) at 65°C overnight. After subtracting the blind bag weight change from the sample bag weights, the *in vitro* digestibility of sucuk foods were calculated with the following formula:

In vitro Digestibility (%) = [(initial DM – final DM) / initial DM)] x 100

Table 2. Determination *in vitro* digestibilities of sucuk foods using a Daisy^{II} incubator.

Phases	
	0.5 ± 0.01 g sample was placed in the bags.
1-Gastric digestion	1440 ml pepsin-lipase-HCl solution (HCl 0.075N; pepsin 2g/L; gastric lipase 1g/L) 39˚C, 2 hours
2-Small intestine digestion	1440 ml -pancreatin-bile salt-phosphate buffer solution (10g/L pancreatin 25g/L; bile salt) pH 7.5, 39˚C, 4 hours
3-Collection of undigested sample	F57 bags were washed, dried overnight at 65˚C Ash and dry matter analyzes were performed

Statistical analysis: Statistical analysis was performed using the Statistical Package of SPSS version 22.0 (SPSS, Chicago, IL, USA). The experimental data were subjected to Levene's test to detect the variance homogeneity. The multivariate analyses were implemented for homogeneous variances by General Linear Model procedures to compare the means of nutrient composition, starch, ME, TBA, lactic acid, pH, *in vitro* dry matter digestibility (IVDMD) and *in vitro* organic matter digestibility (IVOMD) values for all groups. Data were analysed using a randomised complete design with sucuk type, storage time and sucuk types × storage time interactions. Storage time differences within each group were also evaluated with same tests. Values were expressed as arithmetic means ± standard deviation. Data were analysed based on the statistical model:

$$Y_{ijk} = \mu + E_i + D_j + ED_{ij} + e_{ijk}.$$

Where, Y_{ijk} = dependent variable; μ = overall mean; E = effect of storage time on the parameters; D = effect of food types on the parameters; ED interaction between the sucuk types and storage time; e*ijk* = the standard error term.

Tukey HSD test was used as a post hoc test for multiple comparison and the level of significance used in all of tests was $P<0.05$.

Results

According to nutrient analysis performed at the 0, 1, 3, and 6 months after storage of sucuks, nutrient levels generally increased in most values in all groups when compared to the day 0. DM, CA and CP levels of GF sucuk group were higher than others. But CG group had the highest level of starch and EE in average evaluation ($P<0.05$). (Table 3).

Table 3. Determined nutrient composition (%DM) and metabolic energy (kcal/kg DM) levels of sucuks at 0, 1, 3 and 6-months of storage (Mean±SD).

Sucuk type	ST	N	DM	Ash	EE	CF	CP	Starch	ME
CG	0	6	54.22±0.05^{d}	2.98±0.07^{b}	13.65±0.13^{b}	7.36±0.16^{a}	24.79±0.42^{b}	38.59±0.44^{c}	3845±20.52^{c}
	1	6	57.12±0.05^{c}	2.72±0.14^{c}	13.83±0.08^{b}	7.04±0.20^{b}	26.69±0.36^{a}	41.53±0.34^{a}	3905±23.29^{b}
	3	6	60.44±0.11^{a}	3.25±0.09^{a}	13.99±0.11^{b}	6.26±0.14^{d}	26.33±0.14^{a}	39.83±0.12^{b}	3969±24.81^{a}
	6	6	57.40±0.14^{b}	2.52±0.09^{d}	16.23±0.45^{a}	6.63±0.19^{c}	26.30±0.18^{a}	41.21±0.73^{a}	4011±52.21^{a}
Tukey HSD									
SEM			0.46	0.06	0.22	0.10	0.17	0.27	18.94
P values			**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
UCG	0	6	55.45±0.17^{b}	3.43±0.16^{a}	13.07±0.06^{b}	6.73±0.15^{a}	25.21±0.15^{d}	35.49±0.37^{c}	3858±26.46
	1	6	64.33±0.28^{a}	3.28±0.13^{b}	13.51±0.16^{a}	6.74±0.14^{a}	26.49±0.16^{c}	37.04±0.17^{b}	3884±24.43
	3	6	54.41±0.30^{c}	3.29±0.08^{b}	13.03±0.15^{b}	6.35±0.17^{b}	28.56±0.11^{b}	39.13±0.21^{a}	3878±21.57
	6	6	56.94±0.10^{b}	2.95±0.11^{c}	13.06±0.19^{b}	6.34±0.16^{b}	28.96±0.12^{a}	37.97±0.20^{ab}	3855±30.68
Tukey HSD									
SEM			0.81	0.04	0.05	0.05	0.31	0.28	5.60
P values			**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	0.163
GF	0	6	55.29±0.32^{c}	5.04±0.09^{b}	12.02±0.16^{a}	10.12±0.19^{a}	30.96±0.08^{d}	26.72±0.36^{a}	3801±28.59^{c}
	1	6	55.62±0.27^{c}	5.23±0.11^{b}	11.77±0.17^{b}	9.48±0.15^{b}	31.02±0.09^{c}	25.27±0.33^{b}	3854±28.60^{b}
	3	6	58.77±0.28^{a}	5.70±0.09^{a}	10.59±0.19^{c}	8.48±0.17^{c}	32.70±0.38^{a}	24.11±0.21^{c}	3843±20.40^{b}
	6	6	56.65±0.42^{b}	5.75±0.10^{a}	12.09±0.27^{a}	7.82±0.15^{d}	31.91±0.25^{b}	25.26±0.21^{b}	3963±20.17^{a}
Tukey HSD									
SEM			0.65	0.101	0.43	0.367	0.41	0.284	20.16
P values			**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**0.002**
CG(average)		24	57.30^{b}	2.87^{c}	14.42^{a}	6.82^{b}	26.04^{c}	40.29^{a}	3933^{a}
UCG(average)		24	57.78^{a}	3.24^{b}	13.17^{b}	6.54^{c}	27.31^{b}	37.40^{b}	3869^{b}
GF(average)		24	56.58^{c}	5.43^{a}	11.62^{c}	8.97^{a}	31.65^{a}	25.34^{c}	3865^{b}
SEM			0.049	0.023	0.042	0.034	0.049	0.071	5.73
ST(average)	0	18	54.98±0.59^{d}	3.82±0.91^{b}	12.92±0.70^{b}	8.07±0.15^{a}	26.99±2.90^{c}	33.60±5.18^{c}	3834±171.93^{c}
	1	18	59.02±3.91^{a}	3.74±1.11^{b}	13.04±0.93^{b}	7.75±0.14^{b}	28.07±2.16^{b}	34.61±7.06^{ab}	3881±177.36^{b}
	3	18	57.87±2.67^{b}	4.08±1.18^{a}	12.54±1.47^{c}	7.03±0.16^{c}	29.20±2.72^{a}	34.36±7.46^{b}	3897±189.86^{b}
	6	18	56.99±0.40^{c}	3.74±1.47^{b}	13.80±1.84^{a}	6.93±0.16^{c}	29.06±2.36^{a}	34.82±7.09^{a}	3943±150.82^{a}
SEM			0.08	0.04	0.06	0.06	0.07	0.10	8.11
P values									
type			**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
ST			**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
type*ST			**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**

DM = Dry matter; EE = Ether extraction; CF = Crude fiber; CP = Crude protein; ME = metabolizable energy as the amount of kcal/kg in DM, ST= storage time, month; CG= cooked grain-inclusive sucuk food; UCG = uncooked grain-inclusive sucuk food; GF = grain-free sucuk dog food, SEM = standard error of the mean.

[a,b,c] Values in the same column that are not sharing a common superscript differ significantly ($P<0.05$).

In vitro dry matter digestibility (IVDMD) and organic matter digestibility (IVOMD) values of sucuk foods were given in Table 4. CG group had the highest coefficients of IVDMD (89.73%) and IVOMD (92.02%), UCG group had the lowest IVDMD and IVOMD ($P<0.05$).

Microbiological characteristics (Aerobic colony number, *E. coli*, coagulase positive Staphylococcus, coliform bacteria, yeast mold, Salmonella spp.) are shown in Table 5.

pH decreases were statistically significant for all sucuk groups determined in 6-months of storage (Figure 1). The highest pH drop was determined in CG group of sucuk foods (5.38 to 4.25) after 6-month storage. It was 5.55 to 5.30 in UCG group and 5.45 to 4.84 in GF group. The highest thiobarbituric acid reactive substances (TBARS) values were determined in UCG sucuk group in the 1, 3 and 6-months of storage ($P<0.05$). TBARS level differences between in-group storage times were insignificant ($P>0.05$) (Figure 2). There was no difference of time and type effects between storage times and main groups in terms of lactic acid ($P>0.05$) (Figure 3).

Table 4. *In vitro* dry matter and organic matter digestibilities of sucuk foods (Mean±SD).

	N	ST	IVDMD	IVOMD
CG	6	0	89.12±0.29^{b}	91.44±0.24^{c}
	6	1	90.32±0.28^{a}	92.91±0.07^{a}
	6	3	90.04±0.13^{a}	92.51±0.22^{b}
	6	6	89.45±0.18^{b}	91.21±0.26^{c}
SEM			0.11	0.15
P			**<0.001**	**<0.001**
UCG	6	0	76.05±0.02^{d}	78.15±0.08^{d}
	6	1	78.16±0.03^{c}	81.03±0.10^{c}
	6	3	81.5±0.07^{a}	84.30±0.11^{a}
	6	6	80.16±0.56^{b}	81.71±0.16^{b}
SEM			0.43	0.45
P			**<0.001**	**<0.001**
GF	6	0	84.96±0.11^{c}	86.08±0.17^{c}
	6	1	87.59±0.12^{b}	89.79±0.30^{b}
	6	3	87.62±0.22^{b}	89.42±0.16^{b}
	6	6	89.48±0.26^{a}	91.26±2.11^{a}
SEM			0.33	0.43
P			**<0.001**	**<0.001**
CG(average)	24		89.73^{a}	92.02^{a}
UCG(average)	24		78.98^{c}	81.29^{c}
GF(average)	24		87.41^{b}	89.26^{b}
SEM			0.053	0.051
ST(average)	18	0	83.37±5.61^{c}	85.22±5.62^{c}
	18	1	85.35±5.36^{b}	87.91±5.18^{b}
	18	3	86.40±3.68^{a}	88.74±3.48^{a}
	18	6	86.37±4.52^{a}	88.23±4.76^{a}
SEM			0.06	0.05
P values				
type			**<0.001**	**<0.001**
ST			**<0.001**	**<0.001**
type*ST			**<0.001**	**<0.001**

^{a,b,c} Values within a row with different superscripts differ significantly at $P<0.05$; IVDMD = *In vitro* dry matter digestibility; IVOMD = *In vitro* organic matter digestibility; SEM = Standard error of the mean.
ST = storage time (month); CG = cooked grain-inclusive sucuk food; UCG = uncooked grain-inclusive sucuk food; GF = grain-free sucuk dog food.

Table 5. Microbiological analysis results of sucuk foods at the end of 1, 3 and 6-months of storage.

Microbiological Analysis	**Group**	**1 month**	**3 month**	**6 month**	
Number of aerobic colonies	Cooked Grain	4.2×10^{8}	7.0×10^{7}	5.8×10^{7}	cfu/g
	Uncooked Grain	1.2×10^{2}	5.0×10^{8}	3.0×10^{7}	
	Grain free	8.0×10^{8}	9.5×10^{8}	3.1×10^{9}	
E. coli	Cooked Grain	<3	<3	<3	EMS/g
	Uncooked Grain	<3	<3	<3	
	Grain free	<3	<3	<3	
Coagulase (+) Staphylococcus	Cooked Grain	<10	<10	<10	cfu/g
	Uncooked Grain	<10	<10	<10	
	Grain free	<10	<10	<10	
Coliform bacteria	Cooked Grain	1.2×10^{2}	<10	<10	cfu/g
	Uncooked Grain	1.5×10^{2}	<10	<10	
	Grain free	7.2×10^{2}	<10	<10	
Number of total yeast and mold	Cooked Grain	2.0×10^{4}	6.0×10^{3}	5.5×10^{3}	cfu/g
	Uncooked Grain	<10	1.1×10^{4}	9.6×10^{4}	
	Grain free	1.7×10^{5}	7.0×10^{4}	6.0×10^{4}	
Salmonella spp.	Cooked Grain	Negative	Negative	Negative	/25 gr
	Uncooked Grain	Negative	Negative	Negative	/25 gr
	Grain free	Negative	Negative	Negative	/25 gr

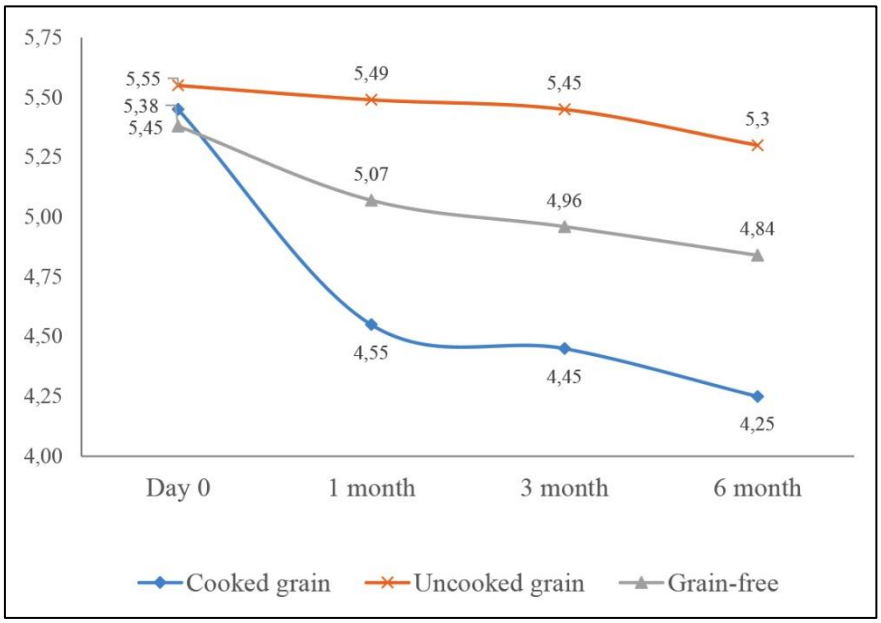

Figure 1. pH changes of sucuk foods in different storage times.

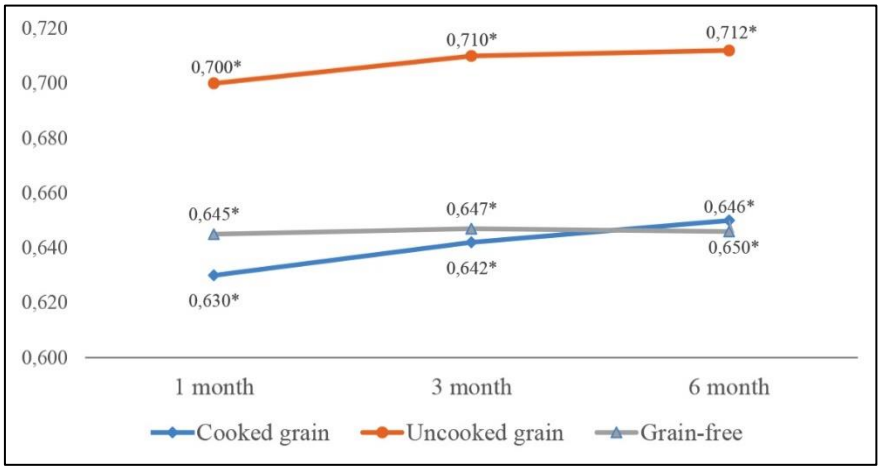

Figure 2. TBARS values of sucuk foods in different storage times (mg malonaldehyde/kg).

*: The values between the groups differ significantly (P<0.05).

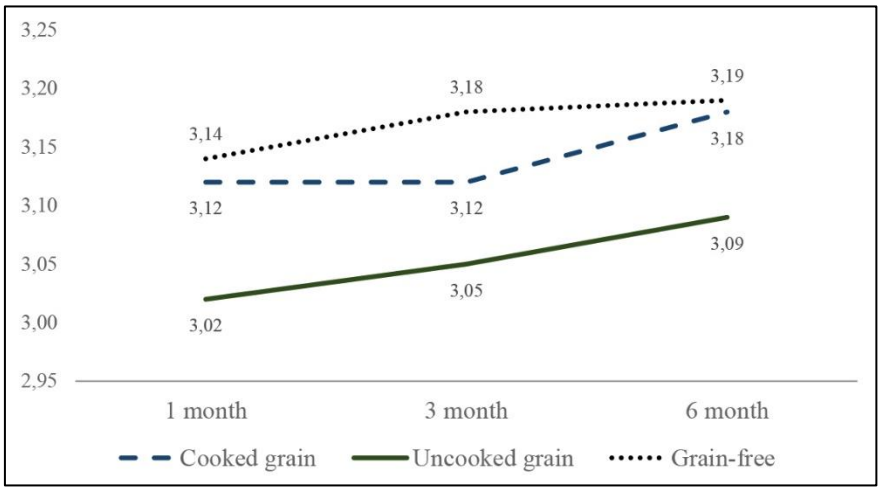

Figure 3. Lactic acid values of sucuk foods in different storage times (% DM).

Discussion and Conclusion

According to nutrient analysis results of the 1, 3, and 6-months after storage of sucuk foods, there were some changes compared with the results of the analysis performed on the first day (day 0). However, despite a storage period of 6 months, all sucuk foods had sufficient nutrients for adult dogs. In a study, there was no nutrient loss or gain as a result of 24-hour fermentation of chicken meats with 9.7% corn starch inoculated with *Pediococcus* spp. as a snack for dogs (36). In this study, nutrient losses were not determined in sucuk foods left to spontaneous fermentation without using starter culture. This is an indication of proper storage conditions without any contamination. The reasons for the increase in nutrients in some sucuk foods in the later stages of storage were that bacteria and yeast breaking down compounds during fermentation and grinding of vegetable ingredients with a diameter of 0.5 mm before mixed into sucuk mixture. By the grinding, the cellulotic structures surrounding the protein and carbohydrate-rich endosperm were physically broken down and nutrients were released (51). In addition, bacteria, yeast and molds also took part in the degradation of the cellulotic structure (26). That was the reason of decreased CF levels at 6th month.

Raw meat diets even have a higher risk of foodborne pathogens are gaining popularity among dog owners. Raw meat has a potential to produce harmful bacteria such as *E. coli*, Salmonella, Neospora, Campylobacter (50). Although healthy adult dogs are sometimes resistant to these pathogens, they can be fatal in puppies with immune system problems (53). Pathogen bacteria like Salmonella and *E. coli* were not detected in sucuk foods analyzed after 1, 3, and 6 months of storage. Sucuks were given nutritional properties and physical, biochemical and microbial changes prevented the growth of various pathogenic microorganisms with fermentation (33). Sucuk foods were also safe for coagulase positive Staphylococcus bacteria. Staphylococcus bacteria are likely to be found in fermented meat products with a pH above 4.2 (58). Since the pH values of the sucuk foods were between 4.2 and 5.5, it is possible that coagulase positive Staphylococcus bacteria were determined at a level of <10 CFU/g in this study. Also, appropriate lactic acid levels help to protect sucuk foods against harmful microorganisms by decreasing pH. The presence of coliform group microorganisms above a certain level is an indication that the sucuks are not fully matured, inadequate hygienic conditions and contamination during production (47). Coliform group bacteria were detected in the sucuk foods prepared without heat treatment in the 1st month after production (1.2-7.2 x 10^2 CFU/g). However, in the next counts (3 and 6-months), this group of bacteria, which is an indicator for sucuk quality, was not detected. Possible reasons for this were decreased water activity during ripening, low pH, competitive flora and bacteriocins (15).

The contribution of yeast to flavor is due to their strong proteolytic activity (48). In fermented sausages, yeasts are preferentially present internally, while molds are present on the surfaces due to the presence of oxygen (49). Different levels of yeast and mold were determined in the sucuk foods in this study. Molds have the ability to produce lipase and protease. It also facilitates dehydration by forming micropores in the intestinal envelope. In the first days of maturation, the number of mold and yeast increases rapidly depending on environmental conditions (11). Yeast and mold growth were detected in UCG sucuk food group. Since no preservatives and additives were used in the study, yeast and mold growth was expected. In some studies yeast and mold detected in fermented sucuks and sausages produced for human consumption (17, 48).

Sausages offered for human consumption consist of almost 100% meat products. Salami-like products for dogs usually contain 80-95% meat and 5-10% rice. In this study, the rate of meat products in sucuk foods prepared for dogs was at the level of 53-56%. The rest consists of vegetable products and vitamin mineral additives. It is thought that the low rate of meat is an important factor in the absence of harmful bacteria. No comparison was made as there were no other studies about a product where meat and vegetable sources were fermented together for dogs. High starch from vegetable products contributed positively to the fermentation of sucuk foods produced without the addition of starter culture (57).

Feeding and digestibility studies of homemade diets in dogs are negligible. In addition, no study was found that reported digestibility of sucuk, sausage and salami type dog foods. A few studies have been conducted about raw meat-based BARF (Biologically Appropriate Raw Diet) diets for dogs. The advantages of BARF diets are based on a few popular publications (13). The sucuk foods produced in this study also consist of high percentage of raw meat, but unlike BARF, sucuks are fermented products. Kara (30) determined the IVOMD values of premium quality lamb meat dry dog foods (n=9) as 76.3-87.9%. IVOMD values of the sucuk foods produced in this study ranged from 78.15% to 92.91%. The DM and OM digestibility rates of the sucuk foods appear to be higher than those of premium quality commercial dry dog foods. However, it should be noted that the methods for determining digestibility and research designs affect the results. However, it has been reported in previous studies that fresh homemade diets are more digestible in dogs (22, 54). Felix et al. (22) determined the *in vivo* DM and OM digestibility values of the homemade diet as 86.8% and 90.1%, respectively. The diet of these researchers consisted of 56.7% puffed rice and 29% fresh meat. In a study, DM digestibility value of wet dog food containing

35.7% CP, 30.3% EE and 19.3% starch was determined as 84.8% with an *in vitro* method similar to this study (8). A positive effect of fermentation on *in vitro* pepsin nitrogen digestibility in dogs has been reported (36). Determination of higher digestibility after 1 month in CG, 3 months in UCG and 6 months in GF groups compared to the day 0 supported this effect. The highest digestibility was determined in CG sucuk foods. Dogs eat and digest diets that high in heat-treated starch (29, 2). It has been determined in previous studies that the OM digestibility rate of cooked starch in dogs is very high (7, 39). The high digestibility of diets with heat-treated starch in dogs has also been confirmed for sucuk foods. Tanprasertsuk et al. (55) found nearly 90% DM digestibility rate of homemade diets containing 30% fresh meat. *In vitro* digestibility coefficients of sucuk foods consisting 53-56% meat was also high in this study.

Lipid peroxidation is considered one of the most important causes of quality deterioration in meat products (44). TBARS changes of the sucuk foods in this study were insignificant at the end of 1, 3 and 6-months of storage and were at the levels of 0.6-0.7 (mg malonaldehyde/kg). Increased TBA values in sucuk foods which produced without nitrate and antioxidant addition were expected to reach the highest levels at the end of 1 month. Because TBARS value increases at a high rate during the fermentation of sucuks due to intense lipid oxidation in ripening period (44). The increase in TBARS value continues at a slower rate during storage with the decomposition of the TBARS into volatile compounds. In a study conducted with pork sausages, a continuous increase in TBARS values was determined in products stored at 4°C for 1 month (60). However, it has been emphasized in a previous study that the TBA increase is lower in sausages that kept in 4°C (42). Karsloğlu et al (31) found that TBARS values between 0.2-0.4 mg malonaldehyde/kg in fermented Turkish sucuks.

The pH decreases of the sucuk foods were significant in all groups during the 6-month storage period. Spontaneous fermentation in meat products is characterized by the presence of lactic acid bacteria. The results of the lactic acid determination analyzes performed on sucuk foods confirmed this. Kurt (34) added carbohydrates at the level of 0.6% to sausages offered for human consumption, and determined that lactic acid levels increased from 4.14% to 14.49% at the end of 9 days. On the other hand, Acton et al. (1) added 1% carbohydrate (dexrose, sucrose) to sausages and determined the lactic acid levels to be similar to the control group. In this study, lactic acid levels were found to be between 3.02-3.19 g/kg. Sucuk foods which have a very high carbohydrate source were prepared without the addition of starter culture had lower lactic acid levels than those offered for human consumption. The lactic acid level of the sucuk foods reached the highest level after 1 month. When starter culture is not used in sausages, the highest lactic acid level is usually reached in 8-10 days (3). The reactions involving carbohydrates (glycolysis), proteins (proteolysis) and lipids (lipolysis) are effective in the formation of taste, aroma, color and texture in fermented sausage products. Gökoğlu et al. (25) determined that the pH value of vacuum-packed beef meat decreased from 5.58 to 4.90. In this study, the pH of CG group of sucuk foods decreased from 5.45 to 4.25, and in the UCG group 5.55 to 5.30. Since the CG group contains the highest percentage of starch, the highest pH decrease was determined in this group. Slow pH decreases in sucuk foods produced without addition of starter culture was also an expected situation (28).

According to the results of this study, it is possible to obtain fermented grain-free and grain-inclusive diets with high digestibility, long shelf life, healthy and consisting of animal and vegetable sources for dogs. Storage of sucuk foods at refrigerator temperature (+4°C) is recommended to avoid bacterial spoilage and reduce lipid peroxidation. Results of this study also indicate that utilization of different source of foods together by fermenting is suitable for dog nutrition. Since homemade diets are difficult to prepare and store, the use of sucuk foods would be practical. The effects of such diets on animal health, stool characteristics and digestibility need to be determined by *in vivo* methods. Such products would be a balanced natural feeding method for dogs when properly prepared and stored under the necessary hygiene conditions. For this reason, it is essential to conduct nutritional trials to determine the intake, palatability, preference and effects on health in future studies.

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Conflict of Interest

The authors declared that there is no conflict of interest. Authors are responsible for all content and writing of this paper.

Author Contributions

OK, Fİ, NG and MSA conceived and presented the idea. Fİ provided to sucuk food's ingredient and chemical formulations. OK and MSA contributed to dog food preparations, laboratory analysis, in vitro digestibility trials and carried out the experiments. Fİ and NG contributed to the interpretation of the results and supervised the work. OK took the lead in writing the manuscript with input from all authors. All authors discussed the results, provided critical feedback and helped shape the research, analysis and manuscript.

Data Availability Statement

The data supporting this study's findings are available from the corresponding author upon reasonable request.

Ethical Statement

This work was approved by the Selçuk University, Faculty of Veterinary Medicine Experimental Animals Production and Research Center Ethics Committee under protocol number 2020/17.

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