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Research Article

Comparison of Digestibility, Stool Quality, Preference and Manufacturing Cost of Grain-inclusive and Grain-free Dry Dog Foods

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Abstract: This study aimed to compare nutrient digestibility, preference rate, effects on stool and cost of grain-inclusive and grain-free dry dog foods. Two dry dog foods with and without grain formulated with poultry meal, barley, rice, corn, peas, carrot, potato flour, whey, fat, vitamin and mineral sources were manufactured. Twelve adult Golden Retriever dogs (age 3-4 years, body weight = 22.5±1.7 kg) were divided into two groups for the digestibility trial. A total of 20 Golden Retriever and Kangal (age 4-5 years, body weight = 35.5±1.9 kg) breed dogs were used for a two-pan preference test. Crude fibre digestibility of grain-free food was significantly lower (P<0.05). There was no difference in other nutrient digestibility in foods as determined by total faecal collection method. Presence of grains in food improved the consistency and dry matter of stool(P<0.05). Dogs preferred grain-free food (55.88%) to grain-inclusive food (44.12%) (P<0.05). The manufacturing cost of grain-free food was found to be about three times higher than grain food. The benefits of grain-free dog diets are debated. The effects of grain and grain-free foods on digestibility, stool parameters and canine health should be demonstrated by further studies. Cost must be calculated to produce reliable and suitable quality dog food with different ingredients. Palatability and intake levels, which are among the most important criteria in dog nutrition, should be determined by preference tests.

Keywords: *Digestibility level, Faecal consistency, Grain free dog food, Grain inclusive dog food, Preference test*

Tahıllı ve Tahılsız Kuru Köpek Mamalarının Sindirilebilirlik, Dışkı Kalitesi, Tercih Oranı ve Üretim Maliyetinin Karşılaştırılması

Öz: Bu çalışmanın amacı tahıllı ve tahılsız kuru köpek mamaların besin madde sindirilebilirliği, tercih oranı, dışkıya etkileri ve maliyetini karşılaştırmaktı. Kanatlı unu, arpa, pirinç, mısır, bezelye, havuç, patates unu, peynir altı suyu, yağ, vitamin ve mineral kaynakları ile formüle edilmiş tahıllı ve tahılsız iki kuru köpek maması üretildi. On iki yetişkin Golden retriever köpek (3-4 yaş, canlı ağırlık = 22.5±1.7 kg) sindirilebilirlik denemesi için iki gruba ayrıldı. İki kap tercih testinde toplam 20 adet Golden Retriever ve Kangal ırkı (4-5 yaş, canlı ağırlık = 35.5±1.9 kg) yetişkin köpek kullanıldı. Tahılsız mamanın ham selüloz sindirilebilirliği önemli düzeyde daha düşüktü (P<0.05). Toplam dışkı toplama yöntemi ile belirlenen diğer besin madde sindirilebilirlikleri arasında fark bulunmadı. Mamada tahılların varlığı dışkı kıvamı ve dışkı kuru madde düzeyini iyileştirdi (P<0.05). Köpekler tahılsız mamayı (%55.88) tahıllıya (%44.12) göre daha fazla tercih etti (P<0.05). Tahılsız mamanın maliyeti tahıllıya göre yaklaşık 3 kat daha yüksek bulundu. Tahılsız köpek diyetlerinin faydaları tartışılmaktadır. Tahıllı ve tahılsız mamaların sindirilebilirlik, dışkı parametreleri ve köpek sağlığı üzerindeki etkileri daha fazla çalışmayla ortaya konmalıdır. Farklı ham maddeler kullanılarak güvenilir ve uygun kalitede köpek maması üretimi için maliyet mutlaka hesaplanmalıdır. Köpek beslemede en önemli kriterler arasında olan lezzet ve tüketilebilirlik düzeyi de tercih testleriyle belirlenmelidir.

Anahtar sözcükler: *Dışkı kıvamı, Sindirilebilirlik düzeyi, Tahıllı köpek maması, Tahılsız köpek maması, Tercih testi*

Introduction

Grains such as barley, wheat, corn, rice, sorghum and oats are used as ingredients and economical energy sources in dry dog foods [1]. Most dry foods have carbohydrate levels of 30-60%, which is mainly derived from cereal grains. Although carbohydrates are not one of the essential nutrients for dogs, they are commonly used for economic

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reasons. Starch is an important source of carbohydrates, as it increases the release of glucose after digestion [2]. For these reasons, the nutritional value, palatability and digestibility level of the cereal grains have become an important issue in dog care [3]. Dry dog foods are produced by an extrusion method, which results in the gelatinization of starch present in the ingredients. Gelatinization is described as breakdown of all starch granules by moisture, temperature, pressure and mechanical shear. This gelatinized starch in extruded grains is highly digestible and ranges from 89% to 99% in food products [4]. Due to their important role in the majority of dog diets worldwide, interest in the effects of grains is growing [5].

Dogs willingly eat extruded food, which is rich in cereal grain. In some dog food, rice, barley, oats, wheat or millet are marketed as part of the names of dog food. Of these grains, rice is the most preferred for dog foods due to its high digestibility, low fibre content and hypoallergenic properties. Use of rice does not cause any problems related to digestibility, stool consistency or preference [6]. Whole grains also contribute important nutrients, such as vitamins, minerals and essential fatty acids, in pet foods. Various cereal products can also provide more easily digestible source of protein than some animal proteins sources [1].

Grain-free dog foods are available on the market and have been made more popular by the belief that dogs do not eat cereal grains in nature. Today, commercially available, grain-free pet food options represent more than 40% of available dry dog foods in the United States [7]. Many dog owners believe that grain-free food is best for the health of their animals. However, some owners trying to feed their dogs healthier food are focusing too much on advertisements and labels on commercial foods, such as 'grain-free' and 'gluten-free' [8]. Alternative starch sources, such as potatoes, tapioca and legumes (peas, beans, lentils), are also used in the production of grain-free dry dog foods. Grain-free foods are more expensive than regular grain-inclusive foods in the market because they are considered to be premium dog foods. Despite claims of high quality, only limited studies have reported that grain-free foods are more digestible and palatable for dogs. Chiofalo et al.[9] reported that grain free diets are more suitable for active dogs, but in another study, grain-inclusive foods were shown to be more digestible than grain-free foods in terms of dry and organic matter [3].

Despite the fact that grain-free dog foods are becoming increasingly popular, research on the effects of grain-free dog foods on digestibility, preference, stool consistency and manufacturing costs are limited, and there is not well-established data comparing these parameters with grain-inclusive foods. Therefore, this study aimed to evaluate these parameters in grain-inclusive and grain-free dry dog foods.

Material and Methods

Ethical Statement

The research was carried out with the permission of the Selcuk University, Faculty of Veterinary Medicine, Experimental Animals Production and Research Center ethics committee (Approval no: 2016/74) at the Selçuk University, Faculty of Veterinary Medicine, Application and Research Farm Dog research unit.

Animals

Twelve Golden Retriever dogs (eight females and four males) aged 3-4 years old, with the body weights (BW) of 22.5±1.7 kg and eight healthy adult Kangal breed dogs (four females and four males) aged 4-5 years old (35.5±1.9 kg BW) were used in this study. They were housed in individual concrete kennels with a closed (190 x 190 cm) and open area (510 x 230 cm).

Preparation of Foods

Grain-inclusive and grain-free food formulations were prepared and two experimental diets were created. Nutritionally sufficient foods with balanced energy and protein levels were produced for adult dogs according to FEDIAF guidelines [10]. Poultry meal was used as an animal protein source in the composition of both food types. Cereal grain products such as rice, corn, corn gluten, corn starch and barley were included in the grain-inclusive composition. Potatoes, peas and carrots were included in grain-free formula *(Table 1)*.

Foods were produced at a feed facility operating in Ankara. All of the raw materials in the composition of the foods were supplied by the factory. Raw ingredients were weighed according to the formulations and milled to pass through a 0.4 mm sieve. After homogenization in the mixer, water was added into the conditioner to achieve 20-30% humidity. The mixture was then cooked for 4 min at an increasing temperature between 90-135°C. After four stages in a DG-85 double-screw extruder, wet extrudates were dried in the belt dryer at temperatures of up to 140°C for 30-45 min. Vitamins and minerals were added and oils and fats sprayed into the hot dried foods. After cooling, and packing in airtight bags, food samples were taken from each bag for nutrient analysis and the bags were sealed.

Chemical Analysis

Experimental foods and faeces were analysed for dry matter (DM), ash, crude protein (CP), acid hydrolysed ether extract (EE) and crude fibre (CF); starch analyses were performed using the methods reported in AOAC [11]. Using the results of the analysis, the metabolizable energy of the foods was calculated with the equation below [12]:

Table 1. Ingredient and nutrient composition of dog foods

Food Formulation	Digestibility Trial		Preference Test	
Ingredients	**Grain-inclusive,%**	**Grain-free,%**	**Grain-inclusive,%**	**Grain-free,%**
Poultry meal	18.00	26.00	20.00	26.00
Whey powder	2.00	2.00	2.00	2.00
Corn gluten meal	10.00		12.00	
Barley	10.00		10.52	
Corn	20.00		18.00	
Corn starch	12.00		12.00	
Rice	20.00		18.00	
Pea flour		18.00		30.00
Carrot flour		10.00		5.82
Potato flour		36.30		29.00
Sunflower oil	3.00	3.00	3.00	3.00
Beef tallow	3.53	3.53	3.53	3.53
Aminovit[1]	0.30	0.30		
Zinc proteinate	0.02	0.02		
Calcium iodate	0.0002	0.0002		
Minesol[2]	0.85	0.85		
Vitamin premix[3]			0.30	0.30
Mineral premix[4]			0.30	0.30
Choline chloride			0.05	0.05
Potassium chloride	0.30	0.30	0.30	
Calculated nutrients in 100 g dry matter				
Crude protein,g	23.26	23.14	25.22	25.27
Crude fiber, g	2.27	3.95	2.25	4.04
Ash, g	4.64	6.14	5.16	6.17
Carbohydrate, g	61.15	55.64	58.29	52.05
Calcium, g	0.76	1.11	0.90	1.15
Phosphorus, g	0.67	0.80	0.64	0.76
Ether extract, g	11.44	11.78	11.55	11.75

[1] ***Aminovit (per liter):*** *Vit. A 20.000.000 IU, Vit. D_3 200.000 IU, Vit. E 10.000 mg, Vit. B_1 2.500 mg, Vit. B_2 2.500 mg, Vit. B_6 500 mg, Vit. B_{12} 5 mg, Vit. K_3 500 mg, Vit. H 15 mg, Pantotenic Acid 2.500 mg, Choline Chloride 70.000 mg, L-Arginine 600 mg, L-Cystine 100 mg, L-Leucine 600 mg, L-Valine 600 mg, L-Isoleucine 200 mg, L-Histidine 200 mg, L-Phenylalanine 500 mg, L-Proline 800 mg, L-Serine 100 mg, L-Tyrosine 200 mg, L-Treonine 500 mg, DL-Methionine 500 mg, L-Triptophane 20 mg, L-Lysine 3.000 mg, L-Glutamic Acid 4.000 mg, L-Alanine 1.000 mg)*

[2] ***Minesol (per liter):*** *Phosphorus 75.000 mg, Calcium 20.000 mg, Sodium 1.600 mg, Manganese 600 mg, Potassium 1.050 mg, Ferrous 1.600 mg, Magnesium 3.200 mg, Zinc 650 mg, Copper 250 mg, Cobalt 250 mg, Selenium 10 mg, Methionine 10.000 mg, Lysine 5.000 mg); Potassium chloride; Zinc proteinate; Calcium iodate; Sodium bicarbonate*

[3] ***Vitamin premix (mg/kg):*** *Biotin 250 mg, Folate 2.000 mg, Niacin 50.000 mg, Pantothenic acid 20.000 mg, Riboflavin 8.000 mg, Thiamine 4.000 mg, vit B_6 5.000 mg, Vit. B_{12} 25 mg, Vit. A 3.600 mg, Cholecalciferol 125 mg, Vit. E 50.000 mg*

[4] ***Mineral premix (mg/kg):*** *Mn proteinate 70.000 mg, Zn proteinate 100.000 mg, Fe proteinate 70.000 mg, Cu proteinate 14.000 mg, Iodine 1.000 mg, Co proteinate 350 mg, Se proteinate 140 mg, Mo proteinate 700 mg, Mg oxide 35.000 mg*

ME, kcal/kg = ((5.7 × CP × 10) + (9.4 × EE × 10) + (4.1 × (NFE × 10 + CF × 10))) x (91.2 – (1.43 × CF))/100 – (1.04 × CP × 10)

NFE, % = 100 – (% crude protein + % ether extract + % crude fibre + % moisture + % ash)

ME: Metabolizable energy, CP: Crude protein, EE: Ether extract, NFE: Nitrogen free extract, CF: Crude fibre

Digestibility Trial

The digestibility of the nutrients of two foods was determined using the total collection method [10,11]. Twelve adult Golden Retriever dogs were divided into two groups with equal body weight and gender. Animals were fed daily at 10:00 am for 15 days. Clean drinking water was provided *ad libitum*. Daily metabolizable energy (ME) requirements of laboratory kennel dogs were calculated according to the recommendation of NRC (kcal/day, 132 * $BW^{0.75}$ kg) [12]. After day 10 of the acclimation period, the freshly excreted faeces of all animals were collected daily for 5 days. The faeces were weighed and stored in a deep freezer (-18°C) until analysis. The collected frozen faeces of each animal were thawed at laboratory environment temperature (23-25°C) and mixed homogeneously. Stool samples were dried in an oven set at 55°C for 60 h. Dry faeces were then ground with a laboratory mill to pass

through a 1 mm sieve (Retsch SM100, Germany) and nutrient analyses of stools and foods were performed. Dry matter (DM) and nutrient digestibility were calculated using the following equations [13]:

DM digestibility, % = [(Consumed DM – Faecal DM)/Consumed DM x 100

Nutrient digestibility, % = [(Nutrient intake (g/day) – Nutrient in faeces (g/day))/Nutrient intake (g/day)] x 100

Preference Test

A two-bowl preference test was carried out over 8 days. Two foods (450 g each for Goldens, 750 g each for Kangals) were presented in stainless steel bowls to 20 dogs (12 Golden Retrievers, 8 Kangals) to choose between; total quantity of food consumed from both bowls during 20 min was recorded [14]. Grain-inclusive and grain-free foods were served in the same bowls, while switching the bowls between sides every day to avoid bias. Foods were relocated and the same food was presented to each dog four times on the right and four times on the left side.

Throughout the preference test, the animals were kept in their compartments and were provided with access to fresh water at all times. Food bowls were placed in the same location of kennels. The dogs were kept in the open area of kennels while the food was placed. After feeding, bowls were collected and the remaining amounts were weighed. The right and left side bias of the dogs was also recorded and calculated. The preference rate was calculated according to the following formula [15]:

Preference rate of grain food (PRG), % = Grain food consumption, g /(Grain food consumption, g + Grain-free food consumption, g) **x** 100

Preference rate of grain-free food, % = 100 – PRG

Stool Consistency Scoring

Stool consistency was scored by three researchers just before fresh faeces were collected in the last 4 days of the total faeces collection experiment. A 1–5 scoring system was used as follows: 1 = pasty and shapeless; 2 = soft, lightly shaped; 3 = soft, shaped, moist and leaves a mark on the ground; 4 = well-formed, non-dispersing, no mark on the ground; 5 = well-shaped, solid, dry [16].

Determination of the Production Cost of Foods

Cost of the prepared foods was calculated by taking into consideration the unit prices of the raw materials as well as operating, waste, depreciation, packaging and shipping costs. After calculating the unit costs of food, daily food costs were calculated according to the daily consumption of an adult dog with 20 kg body weight.

Statistical Analysis

Statistical analysis was performed using the statistical package of SPSS version 22.0 (SPSS, Chicago, IL, USA). A Mann-Whitney U test was performed to compare nutrient digestibility results of the two independent groups of the same dog population. The significance of preference rate was determined using the same statistical test on the 20 dogs. An independent sample test was used to compare stool consistency scores.

Results

Nutrient composition and metabolizable energy levels of the foods fed to dogs in the research are given in *Table 2*.

Results of nutrient digestibility, preference rate, faecal dry matter and stool consistency of dogs and cost of foods are shown in *Table 3*. Digestibility coefficient of crude fibre (CF) of grain-inclusive food was statistically higher ($P<0.05$). Dry matter (DM), organic matter (OM), ether extract (EE) and crude protein (CP) digestibility coefficients of grain-inclusive and grain-free foods were not significantly different ($P>0.05$). Bowls were offered in different directions, i.e., left and right sides of kennels, but the dogs did not show right or left direction preferences The grain-inclusive group had higher quantities of faecal dry matter and stool consistency ($P<0.01$). Manufacturing and production costs of grain-free food were 3 times higher than those of grain-inclusive food *(Table 3)*.

Preference rate of each dog was given *(Fig. 1)*. Grain-free food was significantly more preferred ($P<0.05$). Unlike Golden Retrievers, Kangal breed dogs preferred grain-inclusive food *(Fig. 2)*.

Discussion

Due to possible differences between the calculated and determined nutritional values of ingredients in the composition of the foods, the energy level found in the grain-free food was slightly lower, while that of the grain-inclusive food was slightly higher. The energy levels of the food are important, as food intake is essentially controlled by the energy density of dogs' food or diets. Therefore,

Table 2. *Determined Nutrient composition (DM,%) and metabolizable energy levels of foods (kcal, DM)*

Food Type	DM	Ash	EE	CF	CP	ME*	Starch
Grain-inclusive	92.56	5.25	12.65	4.30	24.23	395.2	42.10
Grain-free	91.50	5.48	12.11	6.67	23.45	375.2	41.40

** kcal, calculated with the NRC 2006 equations;* ***DM:*** *Dry matter;* ***EE:*** *Ether extraction;* ***CF:*** *Crude fibre;* ***CP:*** *Crude protein;* ***ME:*** *metabolizable energy*

Table 3. Digestibility coefficients, production costs, preference rates of foods and stool characteristics of dogs

Parameters		Food Type		Significance
		Grain-inclusive	Grain-free	
Digestibility coefficients	DM	80.93	80.65	-
	OM	84.43	80.65	-
	EE	97.35	96.74	-
	CF	61.70	53.23	**
	CP	78.03	78.77	-
Stool characteristics of dogs	Stool consistency	41	36.59	*
	DM of stool	4.56	4.07	**
Production cost of dog foods	Amount of food required,kg/d	0.320	0.320	
	Cost,$/kg	0.82	2.49	
	Cost,$/d	0.26	0.80	
	Ratio	100	308	
Preference rates	Intake,d/day	267	325.85	**
	Preference rate of 20 dogs,%	44.12	55.88	*
	Food preference rate of Golden retriever dogs (n= 12)	38.83	61.16	*
	Food preference rate of Kangal dogs(n=8)	59.99	40.01	*
	Preference of side bias of 20 dogs, %	right side 50.31	left side 49.69	-

*** P<0.05 (Mann-Whitney U test), * P<0.001, -: not significant*

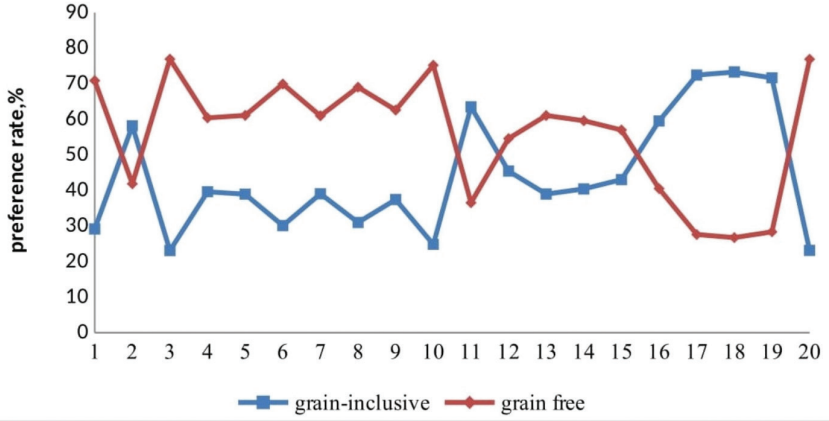
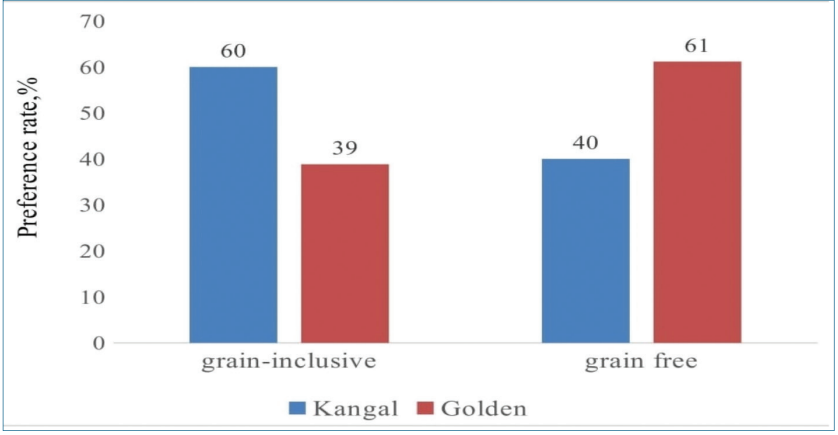


Fig 1. Preference rates of 20 dogs, %

Fig 2. Preference rates of Golden and Kangal breed dogs, %



all nutrients in the food should be relative to the energy content [17]. Modified Atwater factors and NRC equations have moderate accuracy for estimation of the ME for wet pet foods. However, NRC equations are recommended for estimation for dry dog foods [18], and were used to estimate energy level in foods in this study.

Crude fibre (CF) digestion was significantly lower when dogs were fed grain-free food. Contrary to popular belief, grain-free dog foods are generally not low in carbohydrates, with some types even having carbohydrate levels similar to grain-inclusive dog foods [8]. In this study, peas, carrots and potatoes were used as the carbohydrate source in grain-free food, and rice, corn and barley were used in grain-inclusive food. Previously, it has been reported that there is no difference in CF digestibility among dog food based on corn, rice and peas [19]. However, in contrast, one study showed that pea diets had the lowest digestibility level [4]. De-Oliveira et al.[19] reported that CF had the lowest apparent digestibility in dogs because there is a significant correlation between fibre intake and faecal fibre excretion in dogs. Their pea-based diet had a CF level of 8.2%, and this diet had the lowest DM digestibility (76%). In our study, the grain-free diet had 6.67% CF and 80.65% digestibility of DM. The potato flour level was 36% in grain-free food formulation, while the level of carrots was 10%. Although there is no information on the CF digestion of these two vegetables, we believe that the low level of CF digestibility for grain-free foods in this study was caused by the presence of carrots, because carrot peels are generally used as a source of fibre and are composed of more than 75% total dietary fibre level [20]. Kara [21] found that the OM disappearance of carrot-including dog food was 83%, showing one of the lowest coefficients of eight low-priced dog foods he evaluated. Although vegetables are good sources of protein and energy, there is little experimental data regarding their digestibility for domestic dogs [22]. In one study, Kahraman and İnal [3] compared seven grain-free commercial dry foods and 14 grain-inclusive foods, reporting that CF digestion was lower in grain-free foods. Chiofalo et al.[9] found higher protein and fat digestibility, more stable large intestinal fermentation, 13% lower food requirement, reduced stool excretion and higher body condition scores in dogs fed high-protein, low-carb and grain-free food.

Digestibility of DM and OM foods was found to be similar. Due to the formulations of these foods, the nutrient compositions were calculated as similar as possible and DM and OM digestibility results were consistent with those reported by Brambillasca et al.[23]. OM digestibility was close to the average OM digestibility of 38 commercial dry dog foods calculated by Castrillo et al.[24] In this study, OM digestibility found in grain-free food was lower than the figure found for grain-free food by Chiofalo et al.[9]. However, commercial grain-free food used in that study contained 39% CP and 19% EE. Nonetheless, DM digestibility level of their food was quite similar to this study.

In this study, EE digestibility found in both foods was much higher than poultry fat-including foods investigated by Donadelli and Aldrich [25]. This difference could be attributed to the use of sunflower oil and beef tallow in the composition of food in our study. Animal fat and vegetable oils were used in the commercial grain-inclusive and grain-free dry food formulations. Unlike in the current study, Kahraman and İnal [3] reported that fat digestibility was higher for grain-free food than grain-inclusive food. Abd El-Wahab et al.[26] found 87.2-88.0% fat digestibility of 7.7% crude fat-containing food. On the other hand, Kim et al.[27] found 97% fat digestibility in dog food containing 20.5% fat. Here, our foods contained 12% fat and digestibility levels of EE were 96.7-97.3% for grain-free and grain-inclusive foods, respectively. Fat utilization and fat digestibility in dogs is underestimated [28]. Considering that fat level and source could be related to EE digestibility in dogs, this relationship should be investigated in future studies.

Dos Reis et al.[29] found 86% and 87% total tract apparent protein digestibility levels in foods containing 25% and 35% CP, respectively. We report CP digestibility levels of 78.03% for grain-inclusive foods and 78.77% for grain-free foods. The CP digestibility determined here was found to be lower than that found by some previous studies [9,23,24]. This difference could be explained by the fact that CP level can be up to 45% in commercial dry foods. Kahraman and İnal [3] did not find any difference between foods with and without grain in terms of CP digestion. Chiofalo et al.[9] found that CP digestion was significantly higher for grain-free foods compared to grain-inclusive. Because the rate of CP in grain-free food used by Chiofalo et al.[9] was about 60% higher than that in the grain-inclusive dog food of this study. However, high-protein foods do not always have a higher CP digestibility rate. Because high-protein foods can be created with vegetable sources that are rich in protein but low in digestibility.

The consumption rate of a food or diet is the best indicator of overall taste preference [15]. Foods served daily totalled 450 g for Golden Retriever and 900 g for Kangal dogs. On average, 267 g of grain-inclusive food was consumed, while 352.85 g of grain-free food was consumed. In other words, grain-free food was significantly preferred. Dogs had no preference regarding preference for feeding on the right or left side. Various processing techniques and enzyme use affect the preference for canned or cooked meat, soybean or poultry meal in dogs. It is known that previous habits are also important in food preference [27]. However, old habits were not effective here, as the dogs were fed grain-

inclusive food before starting the preference test. Foods were tested for 8 days in this study. In other studies, 4 days of preference testing was reported to be sufficient for dogs to determine palatability [15]. However, increasing the number of days of preference test increases reliability [25]. Callon et al.[22] reported that dogs should be fed a new diet for at least 9 days in order to determine acceptance.

In regard to food preference by dog breed, we saw that 8 Kangal dogs preferred grain-inclusive foods. As Kangal dogs are used in the management of sheep herds in Turkey and traditionally fed with cooked barley or wheat, there may be hereditary reasons that Kangal breed dogs preferred grain foods [30].

Stool consistency scores or faecal DM content affect the stool quality. Although faecal score was considered to be in the optimal range (3-4) for both foods, softer stools were observed in dogs fed grain-free food. This could be explained by the fact that the CF level was higher and CF digestibility was lower for this food type. High fibre levels in the diet limit the interaction between food, enzymes, digestive products and water absorption by increasing transit time through the gastrointestinal tract [23]. Additionally, the stool consistency score (4.07) detected for grain-free food was within the desired intervals for dogs [31]. The result of stool consistency scores of Chiofalo et al.[9] was similar to the current study. Zanatta et al.[32] also reported similar scores and faecal DM in adult dogs fed similar food in terms of nutrient composition. In that study, they fed dogs grain-free and grain-inclusive foods for 84 days. Desired stool consistency scores were observed despite low CF digestibility of grain-free food of this study. This might be the result of insoluble fibre fraction levels of CF in the foods, although this was not determined in this study. Oba et al.[33] found that grain-free dog foods that included potatoes and carrots had higher total insoluble fibre fraction levels, and that these foods had the lowest digestibility. Dhingra et al.[34] also emphasised that most of the total fibre of potatoes and carrots consists of insoluble fibre fraction. The grain-free formula in this study contained potatoes and carrots, and this could be considered to be another cause of the low CF digestibility. Diets prepared in extruded form in this study and by Rashid et al.[35] showed that the insoluble fibre content decreased and the soluble fibre content increased after extrusion. Effects of extrusion on total dietary fibre and insoluble fraction of fibre should be considered in future studies.

In this study, no flavour, digestion, stool consistency enhancers or additives were included in the formulations of the foods. In commercial foods, adsorbent materials can be used to increase faecal consistency or faecal DM. In this study, the faecal DM of dogs was found to be parallel, with faecal scores of 41% (grain-inclusive) and 36.59% (grain-free). Higher stool DM contributed to the stool consistency score.

Commercial grain-free foods are more expensive than grain-inclusive foods on the market. Vegetable flours included in the formula of grain-free food were three times more expensive than grain cereals used in this study. Due to commercial concerns or sales policies, exotic and expensive ingredients are used in the formulations of grain-free foods, and they are generally higher in CP level than regular grain dog foods. Higher dietary protein levels are less sustainable and more expensive. Protein rich diets increase the presence of ammonia by decreasing the number of lactobacilli and enterococci and form carcinogen biogenic amines that decrease digestibility through negative effects on villi height of intestines [36,37].

In conclusion, palatable and nutritionally balanced foods are essential for dog health. There should not be any ingredients or additives that are risky for animals. Regardless of whether a food is grain-inclusive or grain-free, if any nutrient is excessive or deficient, the animal will suffer from it. Grain cereals should not be considered to be unhealthy for dogs. It should always be remembered that dogs can be fed grain cereals, vegetables and animal products. Grains are avoided in foods because owners are trying to protect dogs from allergies or diabetes, but grain-inclusive foods are as digestible as grain-free foods. In grain-free and low-carbohydrate foods, protein and fat levels in particular are considerably higher. Thus, obesity and kidney problems become inevitable. However, grain-free foods can be considered for dogs that are susceptible to grains. Further research on the effects of high-protein and fat-containing grain-free food on obesity, health, digestibility and stool quality parameters should be conducted through long term feeding trials.

Availability of Data and Materials

The authors declare that data supporting the study findings are also available to the corresponding author (O. Kahraman).

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Conflict of Interest

The authors declared that there is no conflict of interest.

Author Contributions

OK: the hypothesis of this study; Fİ: dog food formulating; OK, Fİ, MSA, Şİ, MU and CT: experimental procedure follow-up; MU: dog food formulating; OK and Fİ: literature review, assessment of results, final decision.

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