Canine Digestibility Assessment of Diet

Frozen Lamb Formula

Study #PPFDIGC00119

Submitted to:
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Dates of Performance:
1/22/2019
to
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<u>Summary</u>

Digestibility analysis was performed according to the recommended protocol for use in the determination of metabolizable energy of dog food as defined by Method 1 of the Association of American Feed Control Officials (AAFCO). The analysis results were as follows:

	Mean	**SEM**
Dry Matter (total) Digestibility	89.6	± 0.71
Protein Digestibility	94.3	± 0.55
Fat Digestibility	92.7	± 0.54
Caloric Digestibility (Using Atwater calculation)	93.3	± 0.52
Metabolizable Energy (M.E.) kcal/g (Using Atwater calculation)	1.71	± 0.009
Caloric Digestibility (Using Bomb Calorimetry)	93.4	± 0.63
Metabolizable Energy (M.E.) kcal/g (Using Bomb Calorimetry)	2.02	± 0.014

The following is a graph of the total fecal consistency observations:

Test Diet: Frozen Lamb Formula
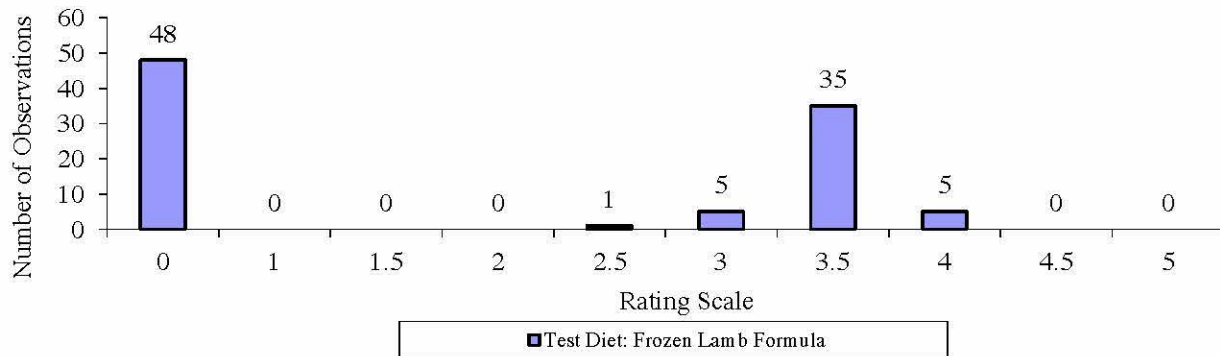
0=none, 1=watery diarrhea; 1.5=diarrhea; 2=moist, no form; 2.5=moist, some form; 3=moist, formed; 3.5=well formed, sticky; 4=well formed; 4.5=hard, dry; 5=hard, dry, crumbly

Methods and Procedures

The kennel facility is registered with the USDA   under the Animal Welfare Act. The kennel had a 12-hour-light/12-hour-dark cycle. Every attempt was made to keep temperature ranges within targeted conditions (from 50° to 85°F) in accordance with the Animal Welfare Act.

The purpose of the study was to assess the digestibility of test diet Frozen Lamb Formula. The Sponsor owns the study including raw data, results and final reports.
agrees to keep all aspects of this study and report confidential. All data created for this study will be stored in   archives for a five (5) year period.

On 1/15/2019, 13 bags with 12 patties of test article Frozen Lamb Formula were received.

Six Beagles identified by ear tattoo and cage numbers were placed on the study. The dogs were housed individually and presented with the test diet on an individual basis. Cages and bowls were cleaned daily and sanitized in accordance with the Animal Welfare Act.

Six dogs, one (1) male and five (5) female at least one (1) year of age, were placed on the test diet Frozen Lamb Formula for 10 days. The test diet was the sole source of food for the length of the test. The dogs were fed once daily at the same time each day. Body weights were recorded on Days 1 through 6, and on Day 10. The first five (5) days of the test were considered an acclimation period. Food consumption was recorded daily. Days 6 through 11 were fecal collection days. Stool quality observations were measured and recorded as according to a photo grading sheet a minimum of three (3) times daily during the collection period. A summary of body weights, food consumption, and grams of fecal output can be found in Tables 1 through 3 respectively. Fecal consistency observation ratings can be found in Table 8 with the frequency distribution in Graph 2. After the final fecal collection, each of six (6) individual fecal samples was sent to Eurofins US, Des Moines, Iowa, for analytical determination as appears in Table 4. A sample of test diet Frozen Lamb Formula was also analyzed and the results of the analyses can be found in Table 4.

The results of the analyses on the feces and the test diet were used to calculate dry matter, protein, caloric digestibility and metabolizable energy as presented in Table 5. Actual equations for the calculations are presented in Table 9. Comparison of metabolizable energy between proximate analysis method and digestibility trial method can be found in Tables 6 and 7.

Parameters to be Measured

Body weights (see Table 1):
- Daily during the acclimation period
- Day 1 of the collection period
- Final

Daily food consumption (see Table 2):

Fecal consistency observation ratings (see Table 8 and Graph 2):
- a minimum of three (3) times daily during the collection period for each dog

Fecal material excreted (see Table 3):
- Collected a minimum of three (3) times daily or as often as needed during the collection period to ensure a clean sample for each individual dog
- Weighed daily during the collection period for each individual dog

Disposition of fecal sample, sent to Eurofins US for laboratory analysis (see Table 4):
- moisture
- protein
- fat
- fiber
- ash
- calories
- carbohydrates

Food analyses, 500 grams of test diet Frozen Lamb Formula were used for analytical determination (see Table 4):
- moisture
- protein
- fat
- fiber
- ash
- phosphorus
- calcium
- calories
- carbohydrates

Five hundred grams of test diet Frozen Lamb Formula are being held until results are reviewed for submission.

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Test Diet: Frozen Lamb Formula

Table 1: Body Weights (kg)

Dog ID	Sex	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 10		Change	% Change
2818290	F	10.34	10.18	10.14	10.08	10.09	10.09	10.09		-0.25	-2.42 %
13195	F	10.15	10.08	10.09	10.10	10.12	10.10	10.14		-0.01	-0.10 %
13536	F	10.89	10.77	10.82	10.81	10.83	10.83	10.83		-0.06	-0.55 %
12794	M	10.56	10.45	10.49	10.39	10.48	10.54	10.55		-0.01	-0.09 %
1483904	F	8.71	8.50	8.43	8.38	8.46	8.42	8.36		-0.35	-4.02 %
13172	F	13.20	12.80	12.88	12.84	12.87	12.93	12.90		-0.30	-2.27 %
									Mean:	-0.16	-1.58 %
									SEM:	0.063	0.648 %

Table 2: Daily Food Consumption (g)

Dog ID	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 6 - 10 Total
2818290	408	429	448	473	498	523	522	523	522	523	2613
13195	402	422	432	458	458	483	484	483	482	482	2414
13536	424	445	454	480	479	504	504	504	504	504	2520
12794	414	434	444	468	494	493	494	493	494	494	2468
1483904	358	379	399	429	453	478	478	478	478	478	2390
13172	490	510	531	560	585	611	610	610	610	610	3051
										Mean:	2576
										SEM:	100.4

Table 3: Fecal Weights (g)

Dog ID	Day 6	Day 7	Day 8	Day 9	Day 10	Total
2818290	55	56	50	61	40	262
13195	67	50	66	33	85	301
13536	53	79	49	50	58	289
12794	42	20	51	31	34	178
1483904	57	30	42	40	34	203
13172	79	60	68	51	96	354

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Table 4: Diet and Fecal Analysis

	Test Diet	Dog ID					
		2818290	13195	13536	12794	1483904	13172
MOISTURE%	67.30	67.85	67.37	65.93	64.93	64.11	69.45
FAT%	10.72	6.50	7.77	6.99	8.16	9.07	7.69
PROTEIN%	14.38	6.78	8.05	9.42	8.35	8.17	7.24
FIBER%	0.8	3.6	3.8	2.7	3.5	3.3	2.9
ASH%	2.08	11.63	10.28	10.64	11.91	11.77	9.04
PHOSPHORUS %	0.38						
CALCIUM%	0.44						
CAL/PHOS	1.15						
CALORIE (KCAL/G) (from calculation)	2.02	1.15	1.30	1.37	1.37	1.46	1.28
CALORIE (KCAL/G) (bomb calorimetry)	2.35	1.38	1.59	1.71	1.51	1.55	1.41
Carbohydrates (from calculation)	5.52	7.23	6.53	7.01	6.64	6.88	6.58

Table 5: Calculations

	Dog ID						Mean	SEM
	2818290	13195	13536	12794	1483904	13172		
DRY MATTER (TOTAL) DIGESTIBILITY (%)	90.1	87.6	88.1	92.3	90.7	89.2	89.6	± 0.71
PROTEIN DIGESTIBILITY (%)	95.3	93.0	92.5	95.8	95.2	94.2	94.3	± 0.55
FAT DIGESTIBILITY (%)	93.9	91.0	92.5	94.5	92.8	91.7	92.7	± 0.54
CALORIC aDIGESTIBILITY (%)	94.3	92.0	92.2	95.1	93.8	92.6	93.3	± 0.52
METABOLIZABLE ENERGY a(M.E.) KCAL/G	1.73	1.69	1.69	1.75	1.72	1.70	1.71	± 0.009
CALORIC bDIGESTIBILITY (%)	94.1	91.6	91.7	95.4	94.4	93.0	93.4	± 0.63
METABOLIZABLE ENERGY b(M.E.) KCAL/G	2.04	1.98	1.99	2.07	2.05	2.02	2.02	± 0.014

aValues used to calculate these numbers were obtained using calculated gross energy

bValues used to calculate these numbers were obtained from Bomb Calorimetry

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Test Diet: Frozen Lamb Formula

Table 6: Digestibility Trial Method

Dog ID	Gross Energy Food AF (kcal/g)	Food Intake AF (g)	Gross Energy Feces (kcal/g)	Fecal Output (g)	Protein Food AF (%)	Protein Feces (%)	Correction Factor Dog	ME (kcal/kg)
2818290	2.35	2613	1.38	262	14.38	6.78	1.25	2040
13195	2.35	2414	1.59	301	14.38	8.05	1.25	1985
13536	2.35	2520	1.71	289	14.38	9.42	1.25	1988
12794	2.35	2468	1.51	178	14.38	8.35	1.25	2069
1483904	2.35	2390	1.55	203	14.38	8.17	1.25	2047
13172	2.35	3051	1.41	354	14.38	7.24	1.25	2017

MEAN: 2024

Table 7: Proximate Analysis Method

	Crude Protein (%) AF	Crude Fat (%) AF	Crude Fiber (%) AF	Moisture (%) AF	Ash (%) AF	NFE (%) AF	Gross Energy (kcal/g)	ME (kcal/kg)
Test Diet: Frozen Lamb Formula	14.38	10.72	0.8	67.30	2.08	4.72	2.02	1580

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Table 8: Fecal Consistency Observation Ratings

Dog ID	Sex	Day 6 PM	Day 7 AM	Day 7 Midday	Day 7 PM	Day 8 AM	Day 8 Midday	Day 8 PM	Day 9 AM	Day 9 Midday	Day 9 PM	Day 10 AM	Day 10 Midday	Day 10 PM	Day 11 AM	Day 11 Midday
2818290	F	0	3.5	3.5	0	3.5	3.5	0	3.5	3.5	0	3.5,3.5	0	0	3.5	0
13195	F	0	3.5	3.5	0	3.5	3.5	0	3.5	3.5	0	3.5	0	0	2.5,3.5	0
13536	F	0	3.5	3.5	0	0	3.5	0	3.5	3.5	0	4,3.5	0	0	4	3.5
12794	M	0	3	*3.5	0	3.5	0	0	3	3	0	3.5	0	0	3	0
1483904	F	0	3.5	0	0	3.5	0	0	3.5	0	0	4	0	0	4	0
13172	F	0	3,3.5	0	0	3.5	0	0	3.5	0	0	3.5	0	0	4	3.5

*Food vomit

RATING SCALE:	Number of observations		RATING SCALE:	Number of observations
0 = none	48		3 = moist, formed	5
1 = watery diarrhea	0		3.5 = well formed, sticky	35
1.5 = diarrhea	0		4 = well formed	5
2 = moist, no form	0		4.5 = hard, dry	0
2.5 = moist, some form	1		5 = hard, dry, crumbly	0

Graph 2 -- Total Fecal Consistency Observation Ratings

Test Diet: Frozen Lamb Formula

Table 9: Calculations

Dry Matter (Total) Digestibility = [{(Total Food Consumed) x (% Dry Matter of Food)} — {(Total Weight of Stool) x (% Dry Matter of Stool)}] ÷ {(Total Food Consumed) x (% Dry Matter of Food)}

Protein Digestibility = [{(Total Food Consumed) x (% Protein of Food)} — {(Total Weight of Stool) x (% Protein of Stool)}] ÷ {(Total Food Consumed) x (% Protein of Food)}

Fat Digestibility = [{(Total Food Consumed) x (% Fat of Food)} — {(Total Weight of Stool) x (% Fat of Stool)}] ÷ {(Total Food Consumed) x (% Fat of Food)}

Caloric Digestibility = [{(Total Food Consumed) x (Gross Energy per gram of diet)} — {(Total Weight of Stool) x (Gross Energy per gram of Stool)}] ÷ {(Total Food Consumed) x (Gross Energy per gram of diet)}

Metabolizable Energy (M.E.) = {Gross Energy of Diet — Gross Energy of Stool — (Grams Protein Digested x 1.25 kcal/g)} ÷ Amount of Food Consumed

Calculated Gross Energy = ((9.4 x Fecal Fat) + (5.65 x Fecal Protein) + (4.15 x Fecal NFE))/10

Nitrogen-free Extract (NFE) = 100 — (% Crude Protein + % Crude Fat + % Crude Fiber + % Moisture + % Ash)

Modified Atwater M.E. (kcal/kg) = 10 x {(3.5 x Crude Protein) + (8.5 x Crude Fat) + (3.5 x NFE)}

Gross Energy (kcal/g) = {(5.65 x Crude Protein) + (4.15 x NFE) + (9.4 x Crude Fat)} ÷ 100