

Canine Digestibility Assessment of Diet

Freeze Dried Chicken Formula

Study #PPFDIGC00319

Submitted to:
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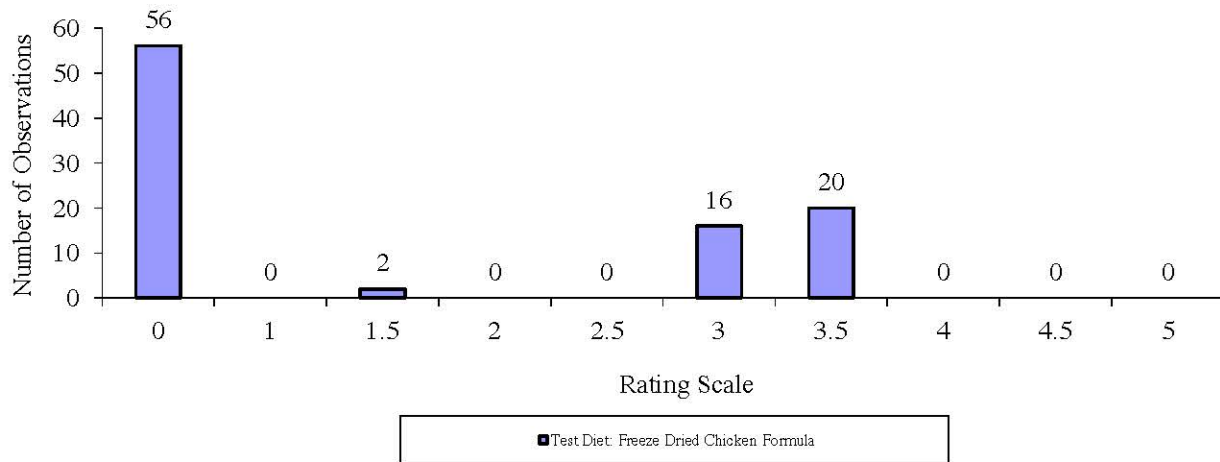
Dates of Performance:
8/18/2019
to
8/27/2019

Summary

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:

	<u>Mean</u>	<u>SEM</u>
Dry Matter (total) Digestibility -----	89.2	± 1.07
Protein Digestibility -----	94.0	± 0.70
Fat Digestibility -----	93.9	± 0.54
Caloric Digestibility ----- (Using Atwater calculation)	93.5	± 0.65
Metabolizable Energy (M.E.) kcal/g ----- (Using Atwater calculation)	1.78	± 0.012
Caloric Digestibility ----- (Using Bomb Calorimetry)	92.3	± 0.78
Metabolizable Energy (M.E.) kcal/g ----- (Using Bomb Calorimetry)	1.58	± 0.014

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



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

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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 Freeze Dried Chicken 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 8/6/2019, 26-14 ounce bags of test article Freeze Dried Chicken 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, five (5) male and one (1) female at least one (1) year of age, were placed on the test diet Freeze Dried Chicken 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 Freeze Dried Chicken 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 Freeze Dried Chicken Formula were used for analytical determination (see Table 4):

- moisture
- protein
- fat
- fiber
- ash
- phosphorus
- calcium
- calories
- carbohydrates

Five hundred grams of test diet Freeze Dried Chicken Formula are being held until results are reviewed for submission.

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Test Diet: Freeze Dried Chicken 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
13069	M	15.10	15.02	14.80	14.73	14.69	14.60	14.51	-0.59	-3.91 %
13328	M	10.98	10.73	10.45	10.38	10.29	10.26	10.24	-0.74	-6.74 %
13139	M	12.50	12.31	12.06	11.98	11.90	11.81	11.83	-0.67	-5.36 %
13266	M	12.09	11.80	11.54	11.48	11.42	11.37	11.31	-0.78	-6.45 %
13177	F	11.33	11.04	10.89	10.77	10.71	10.67	10.75	-0.58	-5.12 %
13017	M	13.70	13.46	13.18	13.06	13.01	12.96	13.05	-0.65	-4.74 %
Mean:									-0.67	-5.39 %
SEM:									0.033	0.433 %

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
13069	167	166	197	222	262	362	362	362	362	362	1810
13328	132	162	212	262	332	432	431	432	432	432	2159
13139	146	174	225	276	336	435	435	434	434	434	2172
13266	142	170	221	272	332	432	430	431	431	431	2155
13177	135	170	201	251	310	410	409	410	410	410	2049
13017	156	193	241	291	361	460	460	460	460	460	2300
Mean:											2108
SEM:											67.8

Table 3: Fecal Weights (g)

Dog ID	Day 6	Day 7	Day 8	Day 9	Day 10	Total
13069	36	81	18	14	46	195
13328	50	50	42	54	70	266
13139	34	87	0	63	58	242
13266	52	73	46	62	63	296
13177	8	32	7	40	17	104
13017	48	67	7	141	37	300

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Table 4: Diet and Fecal Analysis

Test Diet	Dog ID						
	13069	13328	13139	13266	13177	13017	
MOISTURE%	64.70	61.72	67.80	65.66	64.75	59.16	68.69
FAT%	9.92	6.26	4.80	5.33	5.56	7.31	4.75
PROTEIN%	17.19	9.79	8.96	9.32	10.04	9.61	8.51
FIBER%	0.5	2.7	2.5	2.4	2.7	3.2	2.1
ASH%	2.56	14.20	11.72	12.62	12.51	15.39	10.61
PHOSPHORUS %	0.43						
CALCIUM%	0.53						
CAL/PHOS	1.25						
CALORIE (KCAL/G) (from calculation)	2.12	1.36	1.13	1.22	1.27	1.45	1.15
CALORIE (KCAL/G) (bomb calorimetry)	1.93	1.47	1.20	1.31	1.41	1.62	1.25
Carbohydrates (from calculation)	5.63	8.02	6.71	7.08	7.14	8.53	7.45

Table 5: Calculations

	Dog ID						Mean	SEM
	13069	13328	13139	13266	13177	13017		
DRY MATTER (TOTAL) DIGESTIBILITY (%)	88.3	88.8	89.2	86.3	94.1	88.4	89.2	± 1.07
PROTEIN DIGESTIBILITY (%)	93.9	93.6	94.0	92.0	97.2	93.5	94.0	± 0.70
FAT DIGESTIBILITY (%)	93.2	94.0	94.0	92.3	96.3	93.8	93.9	± 0.54
CALORIC aDIGESTIBILITY (%)	93.1	93.4	93.6	91.7	96.5	92.9	93.5	± 0.65
METABOLIZABLE ENERGY a(M.E.) KCAL/G	1.77	1.78	1.78	1.74	1.83	1.77	1.78	± 0.012
CALORIC bDIGESTIBILITY (%)	91.8	92.3	92.4	90.0	95.7	91.6	92.3	± 0.78
METABOLIZABLE ENERGY b(M.E.) KCAL/G	1.57	1.58	1.58	1.54	1.64	1.57	1.58	± 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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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)
13069	1.93	1810	1.47	195	17.19	9.79	1.25	1570
13328	1.93	2159	1.20	266	17.19	8.96	1.25	1581
13139	1.93	2172	1.31	242	17.19	9.32	1.25	1582
13266	1.93	2155	1.41	296	17.19	10.04	1.25	1539
13177	1.93	2049	1.62	104	17.19	9.61	1.25	1639
13017	1.93	2300	1.25	300	17.19	8.51	1.25	1566
MEAN:								1579

Table 7: Proximate Analysis Method

Test Diet: Freeze Dried Chicken Formula	Crude Protein (%) AF	Crude Fat (%) AF	Crude Fiber (%) AF	Moisture (%) AF	Ash (%) AF	NFE (%) AF	Gross Energy (kcal/g)	ME (kcal/kg)
	17.19	9.92	0.5	64.70	2.56	5.13	2.12	1624

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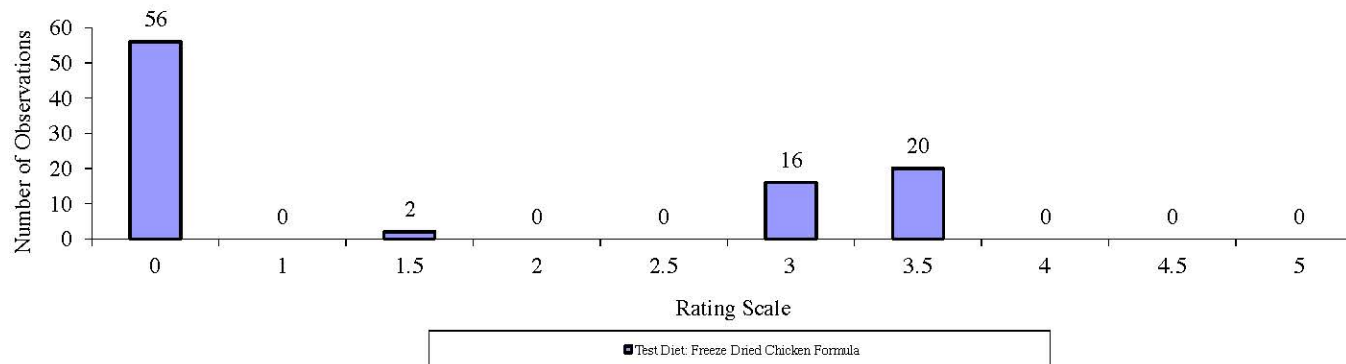
Table 8: Fecal Consistency Observation Ratings

Dog ID	Sex	Day 6			Day 7			Day 8			Day 9			Day 10			Day 11	
		PM	AM	Midday	PM	AM	Midday	PM	AM	Midday	PM	AM	Midday	PM	AM	Midday		
13069	M	0	3.5	0	0	3.5	3	0	0	3	0	3	0	0	3	0		
13328	M	0	1.5,3.5	0	0	3.5	3	0	3.5	0	0	3	0	0	3.5	0		
13139	M	0	3	0	0	0	3.5	0	0	0	0	3	0	0	3.5	0		
13266	M	0	3.5	0	0	3.5	3	0	3	0	0	3,3	0	0	3.5	0		
13177	F	0	1.5	0	0	3.5	3.5	0	3	0	0	3,3.5	0	0	3.5	0		
13017	M	0	3.5	0	0	3.5	3	0	3.5	0	0	3.5,3	0	0	3.5	0		

RATING SCALE: Number of observations
 0 = none 56
 1 = watery diarrhea 0
 1.5 = diarrhea 2
 2 = moist, no form 0
 2.5 = moist, some form 0

RATING SCALE: Number of observations
 3 = moist, formed 16
 3.5 = well formed, sticky 20
 4 = well formed 0
 4.5 = hard, dry 0
 5 = hard, dry, crumbly 0

Graph 2 -- Total Fecal Consistency Observation Ratings



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Diet: Freeze Dried Chicken
Formula

Table 9: Calculations

Dry Matter (Total) Digestibility = $\left[\frac{\{(Total\ Food\ Consumed) \times (\% \text{ Dry Matter of Food})\} - \{(Total\ Weight\ of\ Stool) \times (\% \text{ Dry Matter of Stool})\}}{\{(Total\ Food\ Consumed) \times (\% \text{ Dry Matter of Food})\}} \right]$

Protein Digestibility = $\left[\frac{\{(Total\ Food\ Consumed) \times (\% \text{ Protein of Food})\} - \{(Total\ Weight\ of\ Stool) \times (\% \text{ Protein of Stool})\}}{\{(Total\ Food\ Consumed) \times (\% \text{ Protein of Food})\}} \right]$

Fat Digestibility = $\left[\frac{\{(Total\ Food\ Consumed) \times (\% \text{ Fat of Food})\} - \{(Total\ Weight\ of\ Stool) \times (\% \text{ Fat of Stool})\}}{\{(Total\ Food\ Consumed) \times (\% \text{ Fat of Food})\}} \right]$

Caloric Digestibility = $\left[\frac{\{(Total\ Food\ Consumed) \times (\text{Gross Energy per gram of diet})\} - \{(Total\ Weight\ of\ Stool) \times (\text{Gross Energy per gram of Stool})\}}{\{(Total\ Food\ Consumed) \times (\text{Gross Energy per gram of diet})\}} \right]$

Metabolizable Energy (M.E.) = $\frac{\{Gross\ Energy\ of\ Diet - Gross\ Energy\ of\ Stool - (Grams\ Protein\ Digested \times 1.25\ kcal/g)\}}{Amount\ of\ Food\ Consumed}$

Calculated Gross Energy = $\frac{((9.4 \times \text{Fecal Fat}) + (5.65 \times \text{Fecal Protein}) + (4.15 \times \text{Fecal NFE}))}{10}$

Nitrogen-free Extract (NFE) = $100 - (\% \text{ Crude Protein} + \% \text{ Crude Fat} + \% \text{ Crude Fiber} + \% \text{ Moisture} + \% \text{ Ash})$

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

Gross Energy (kcal/g) = $\frac{\{(5.65 \times \text{Crude Protein}) + (4.15 \times \text{NFE}) + (9.4 \times \text{Crude Fat})\}}{100}$