

**Canine Digestibility Assessment of Diet**

**Frozen Chicken Formula**

**Study #PPFDIGC00419**

Submitted to:  
Lindsay Meyers  
Primal Pet Foods  
535 Watt Avenue Suite B  
Fairfield, CA 94534

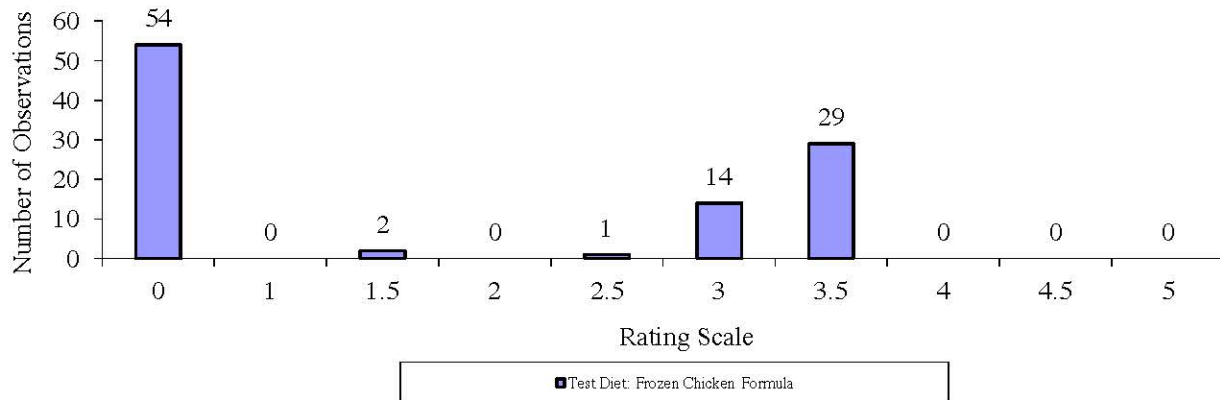
Dates of Performance:  
8/18/2019  
to  
8/27/2019

**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	± 0.68
Protein Digestibility -----	93.5	± 0.69
Fat Digestibility -----	97.1	± 0.24
Caloric Digestibility ----- (Using Atwater calculation)	94.8	± 0.42
Metabolizable Energy (M.E.) kcal/g ----- (Using Atwater calculation)	1.57	± 0.007
Caloric Digestibility ----- (Using Bomb Calorimetry)	93.4	± 0.48
Metabolizable Energy (M.E.) kcal/g ----- (Using Bomb Calorimetry)	1.41	± 0.007

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

### **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 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, 146 patties of test article Frozen 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, four (4) male and two (2) female at least one (1) year of age, were placed on the test diet Frozen 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 Frozen 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 Frozen 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 Frozen Chicken Formula are being held until results are reviewed for submission.

**Study #PPFDIGC00419**  
**Test Diet: Frozen 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
13427	F	11.70	11.61	11.51	11.50	11.52	11.54	11.56	-0.14	-1.20 %
13293	M	9.30	9.27	9.18	9.21	9.22	9.23	9.20	-0.10	-1.08 %
13437	M	11.92	11.85	11.64	11.63	11.57	11.59	11.54	-0.38	-3.19 %
13378	F	10.07	10.01	9.97	9.97	9.94	9.90	9.81	-0.26	-2.58 %
13383	M	10.86	10.75	10.65	10.64	10.58	10.51	10.44	-0.42	-3.87 %
3216005	M	9.96	9.97	9.90	9.88	9.82	9.82	9.66	-0.30	-3.01 %
Mean:									-0.27	-2.49 %
SEM:									0.052	0.460 %

**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
13427	471	457	474	497	525	574	574	574	574	574	2870
13293	400	401	424	424	435	458	459	459	459	459	2294
13437	408	342	482	445	507	554	557	555	555	555	2776
13378	424	423	423	424	530	555	16	556	556	556	2239
13383	449	426	500	525	550	572	534	573	573	573	2825
3216005	420	420	419	420	431	450	450	450	450	450	2250
Mean:											2542
SEM:											126.6

**Table 3: Fecal Weights (g)**

Dog ID	Day 6	Day 7	Day 8	Day 9	Day 10	Total
13427	50	94	40	115	67	366
13293	72	62	36	79	19	268
13437	65	63	57	47	72	304
13378	70	0	44	37	24	175
13383	51	68	46	70	41	276
3216005	55	53	16	46	10	180

**Study #PPFDIGC00419**  
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**Table 4: Diet and Fecal Analysis**

Test Diet	Dog ID						
	13427	13293	13437	13378	13383	3216005	
MOISTURE%	70.80	72.00	68.27	68.07	66.80	70.62	66.03
FAT%	10.42	2.48	3.33	3.19	3.12	2.42	3.55
PROTEIN%	12.38	7.40	9.66	7.63	7.91	6.85	7.59
FIBER%	0.9	2.6	2.6	3.2	3.9	2.9	3.6
ASH%	2.47	10.93	12.09	13.01	12.67	11.81	14.71
PHOSPHORUS %	0.34						
CALCIUM%	0.44						
CAL/PHOS	1.29						
CALORIE (KCAL/G) (from calculation)	1.80	0.84	1.03	0.93	0.97	0.84	0.95
CALORIE (KCAL/G) (bomb calorimetry)	1.66	0.98	1.16	1.10	1.25	0.98	1.08
Carbohydrates (from calculation)	3.93	7.2	6.66	8.1	9.5	8.31	8.13

**Table 5: Calculations**

	Dog ID						Mean	SEM
	13427	13293	13437	13378	13383	3216005		
DRY MATTER (TOTAL) DIGESTIBILITY (%)	87.8	87.3	88.0	91.1	90.2	90.7	89.2	± 0.68
PROTEIN DIGESTIBILITY (%)	92.4	90.9	93.3	95.0	94.6	95.1	93.5	± 0.69
FAT DIGESTIBILITY (%)	97.0	96.3	96.6	97.7	97.7	97.3	97.1	± 0.24
CALORIC aDIGESTIBILITY (%)	94.1	93.4	94.3	95.8	95.5	95.8	94.8	± 0.42
METABOLIZABLE ENERGY a(M.E.) KCAL/G	1.55	1.54	1.56	1.58	1.58	1.58	1.57	± 0.007
CALORIC bDIGESTIBILITY (%)	92.5	91.8	92.7	94.1	94.2	94.8	93.4	± 0.48
METABOLIZABLE ENERGY b(M.E.) KCAL/G	1.39	1.38	1.40	1.42	1.42	1.43	1.41	± 0.007

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

bValues used to calculate these numbers were obtained from Bomb Calorimetry

**Study #PPFDIGC00419**  
**Test Diet: Frozen Chicken 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)
13427	1.66	2870	0.98	366	12.38	7.40	1.25	1393
13293	1.66	2294	1.16	268	12.38	9.66	1.25	1384
13437	1.66	2776	1.10	304	12.38	7.63	1.25	1395
13378	1.66	2239	1.25	175	12.38	7.91	1.25	1415
13383	1.66	2825	0.98	276	12.38	6.85	1.25	1418
3216005	1.66	2250	1.08	180	12.38	7.59	1.25	1426
							MEAN:	1405

**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 Chicken Formula	12.38	10.42	0.9	70.80	2.47	3.03	1.80	1425

**Study #PPFDIGC00419**  
**Test Diet: Frozen Chicken Formula**

**Table 8: Fecal Consistency Observation Ratings**

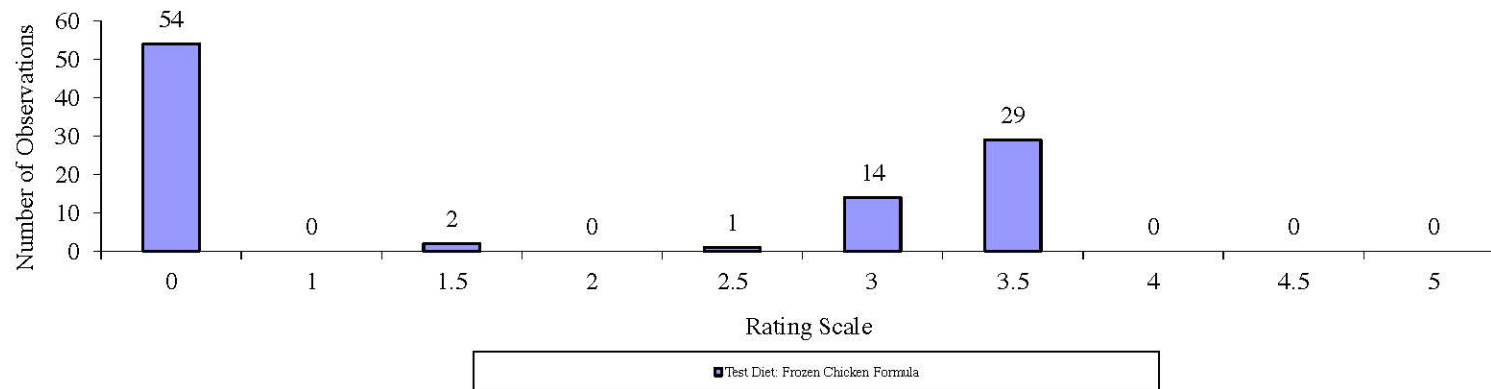
Dog ID	Sex	Day 6		Day 7		Day 8			Day 9			Day 10			Day 11	
		PM	AM	Midday	PM	AM	Midday	PM	AM	Midday	PM	AM	Midday	PM	AM	Midday
13427	F	0	3.5	0	0	3	3	0	3	0	0	3,3	0	0	3.5	0
13293	M	0	2.5	3.5	0	3.5	3.5	0	3.5	0	0	3,3.5	0	0	3.5	0
13437	M	0	3.5	3,3.5	0	3.5	0	0	3.5	0	0	3,3.5	0	0	3.5	0
13378	F	0	3.5	#1.5,3	0	0	0	0	3.5	0	0	3.5,3.5	0	0	3.5	0
13383	M	0	3.5	3.5	0	3,3.5	0	0	3	0	0	3,3	0	0	3.5	0
3216005	M	0	3.5	0	0	1.5,3.5	3.5	0	3.5	0	0	3,3.5	0	0	3.5	0

#Blood in stool

RATING SCALE: Number of observations  
 0 = none 54  
 1 = watery diarrhea 0  
 1.5 = diarrhea 2  
 2 = moist, no form 0  
 2.5 = moist, some form 1

RATING SCALE: Number of observations  
 3 = moist, formed 14  
 3.5 = well formed, sticky 29  
 4 = well formed 0  
 4.5 = hard, dry 0  
 5 = hard, dry, crumbly 0

**Graph 2 -- Total Fecal Consistency Observation Ratings**





Study #PPFDIGC00419

Diet: Frozen Chicken Formula

**Table 9: Calculations**

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

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

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

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

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) =  $\frac{\{(5.65 \times \text{Crude Protein}) + (4.15 \times \text{NFE}) + (9.4 \times \text{Crude Fat})\}}{100}$