

CONFIDENTIAL REPORT for Earth Animal.

# *IN VITRO DOG CHEW DIGESTIBILITY*



Head of Food Science/Technical Services  
Southport, CT 06890  
earthanimal.com



Timothy J. Bowser, Ph.D., P.E.  
PetMech, LLC  
Stillwater, Oklahoma

A handwritten signature in black ink that reads "Timothy J. Bowser".

Summary

PetMech, LLC, a humane pet product research and testing company located in Stillwater, Oklahoma, tested unique dog chews for Earth Animal Ventures. The chews were tested for digestibility using in vitro tests in simulated gastric (stomach) and intestinal juices. The term “in vitro” means “outside the body” and “in an artificial environment”. In vitro tests were selected to determine the digestibility of dog chews and were conducted under conditions that were designed to simulate the digestive system of dogs. The purpose of the tests was to estimate the rate of degradation of the products in the canine digestive tract. Procedures designed to evaluate pet chews, developed by Bowser et al. (2006) and de Godoy et al. (2014), were followed.

Tests were conducted on product pieces, cut by PetMech from the chews provided by Earth Animal, as described in this report.

Results of the tests are plotted individually and brief comments made about each test.

Note of caution: The in vitro tests performed were designed to simulate actual digestive conditions in normal, healthy dogs and are based on sound scientific practices and experience. Actual performance of the product will vary in live animals based on individual characteristics and conditions such as age, size, breed, and health.

Summary of Results for products:

Product	6-hour Gastric Digestibility	24-hour Digestibility*
Item A, No-Hide	65%	98%, excellent
Item B, Competitor Gelatin	79%	97%, excellent
Item C, Competitor Collagen	13%	29%, very poor

\*Descriptive scale for evaluating degradation: 0 to 29% => very poor; 30 to 39% => poor; 40 to 49% => acceptable; 50 to 59% => good; 60 to 69% => very good; 70 to 100% => excellent

Products tested

The products tested appeared to be factory samples and were taken from sealed packages provided by the Global Pet Care Group of Spectrum Brands. Product details are listed in Table 1. Figure 1 shows examples of the products, as received for testing. Figure 2 shows some of the cut pieces prepared for testing.

Table 1. Details of the product tested for digestibility.

Product Name	Description	Cut Sample
Item A, No-Hide	Strip of No-Hide material	10-mm square
Item B, Competitor Gelatin	Rod-shaped chew	10-mm cube
Item C, Competitor Collagen	Rolled collagen chew	10-mm slice

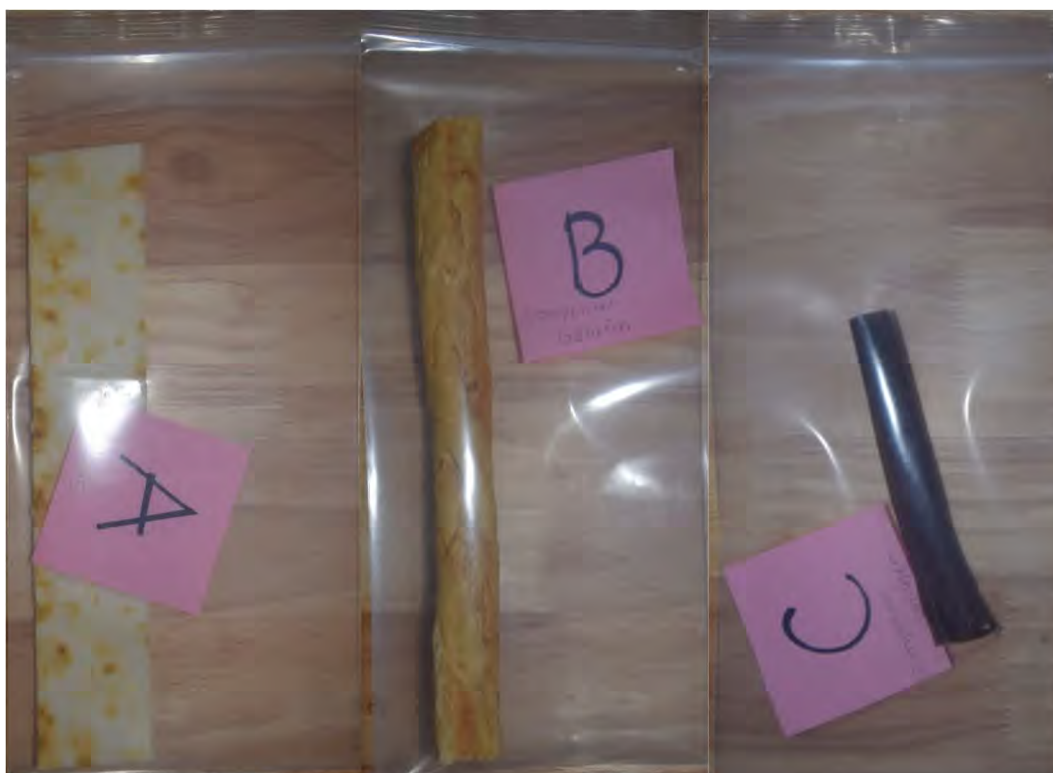


Figure 1. Images of chews, as received.



Figure 2. Examples of chews cut into pieces for testing.

### Procedure

Tests were conducted according to the basic procedures developed by Bowser et al. (2006) and de Godoy et al. (2014), with modifications. Briefly, product samples (shown in figure 2) were immersed in a container (125 ml bottle) filled with either simulated gastric or intestinal juices for three times spanning up to 24 hours. Digestive juices were formulated using Sigma enzymes. In this study, time intervals were 6 hours in gastric fluids followed by immersion in intestinal fluids for an additional 2 and 18 hours. The containers were held in a constant temperature chamber at 101.5 F, the average canine body temperature. Containers were shaken hourly to simulate movement in the digestive tract.

At the end of each time interval (3 total), the appropriate containers were emptied into a #10 sieve, rinsed and the samples recovered and inspected. If any samples remained, they were placed into a dehydration chamber. The difference between the dry weights gives the raw data for the calculation of the percent degradation of the pet treat. Percent degradation may include both product dissolution into the digestive fluids and breakdown into particulates that pass through the collection sieve. Each experiment was repeated in quintuple (except for the Item C, Collagen, which was repeated in quadruple due to the limited amount of material available).

### Results and Discussion

Tables 2, 3 and 4 give the digestibility test results for the chews.

-- CONFIDENTIAL REPORT --  
DOG CHEW DIGESTIBILITY

Table 2. Results of the 24-hour digestibility test for 10-mm squares of Item A, No-Hide.

Hours	Treatment	Avg. degradation, n =3					Overall Avg.
		Rep. 1	Rep. 2	Rep. 3	Rep. 4	Rep. 5	
6	Gastric	70.4	68.3	61.4	62.0	67.3	65.9
8	6 hour gastric + 2 hour intestinal	73.3	82.1	78.3	75.9	84.7	78.9
24	6 hour gastric + 18 hour intestinal	100.0	96.8	96.5	96.7	97.8	97.6

Table 3. Results of the 24-hour digestibility test for 10-mm cubes of Item B, Gelatin.

Hours	Treatment	Avg. degradation, n =3					Overall Avg.
		Rep. 1	Rep. 2	Rep. 3	Rep. 4	Rep. 5	
6	Gastric	82.8	81.4	53.8	93.1	84.1	79.1
8	6 hour gastric + 2 hour intestinal	77.8	89.7	97.4	96.2	97.6	91.8
24	6 hour gastric + 18 hour intestinal	97.2	98.5	93.6	98.8	95.7	96.8

Table 4. Results of the 24-hour digestibility test for 10-mm slices of Item C, Collagen.

Hours	Treatment	Avg. degradation, n =1					Overall Avg.
		Rep. 1	Rep. 2	Rep. 3	Rep. 4	Rep. 5	
6	Gastric	13.7	12.2	13.0	14.1	13.2	13.7
8	6 hour gastric + 2 hour intestinal	17.2	16.5	17.5	18.0	17.3	17.2
24	6 hour gastric + 18 hour intestinal	28.9	28.3	26.1	26.8	27.5	28.9

Figures 3 through 5 are three-point plots (based on average values) of the 24-hour digestibility test data for the three products.

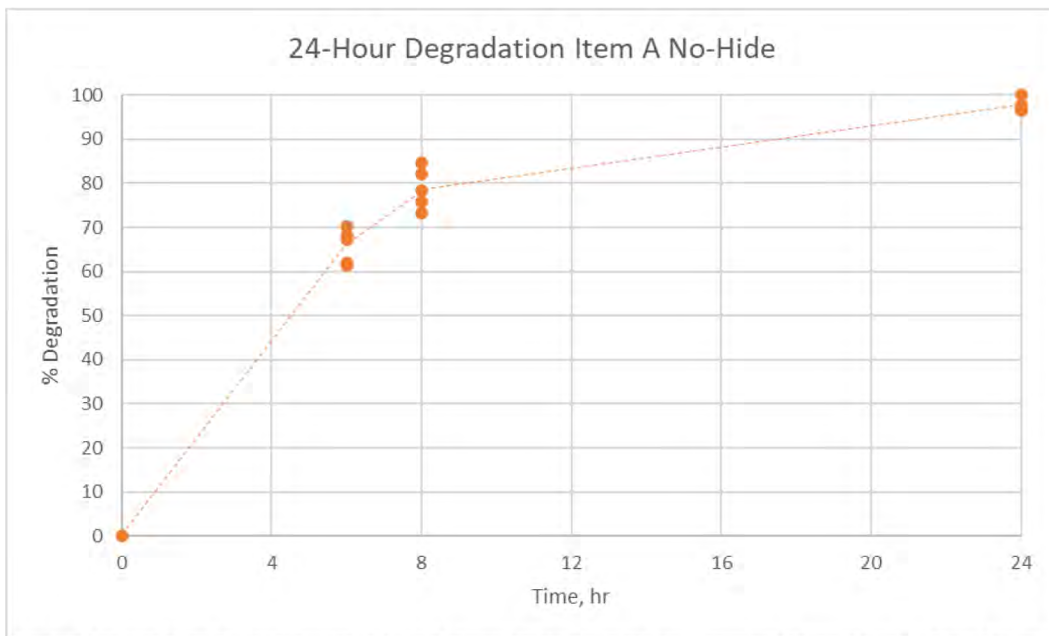


Figure 3. Plot of in vitro digestibility data for 10-mm squares of Sample A, No-Hide.

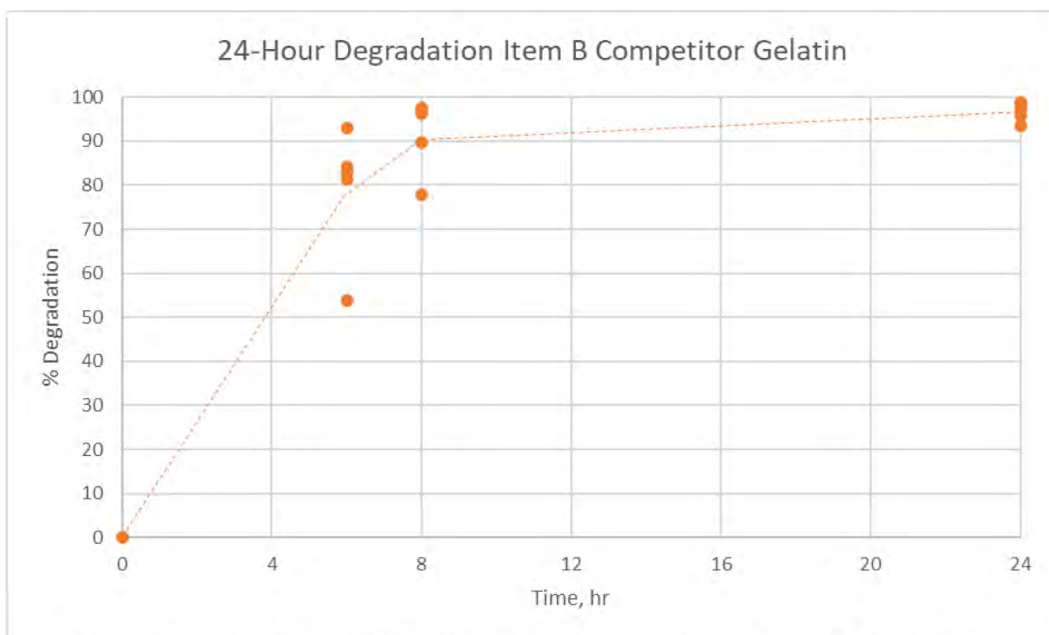


Figure 4. Plot of in vitro digestibility data for 10-mm cubes of Sample B, Gelatin.



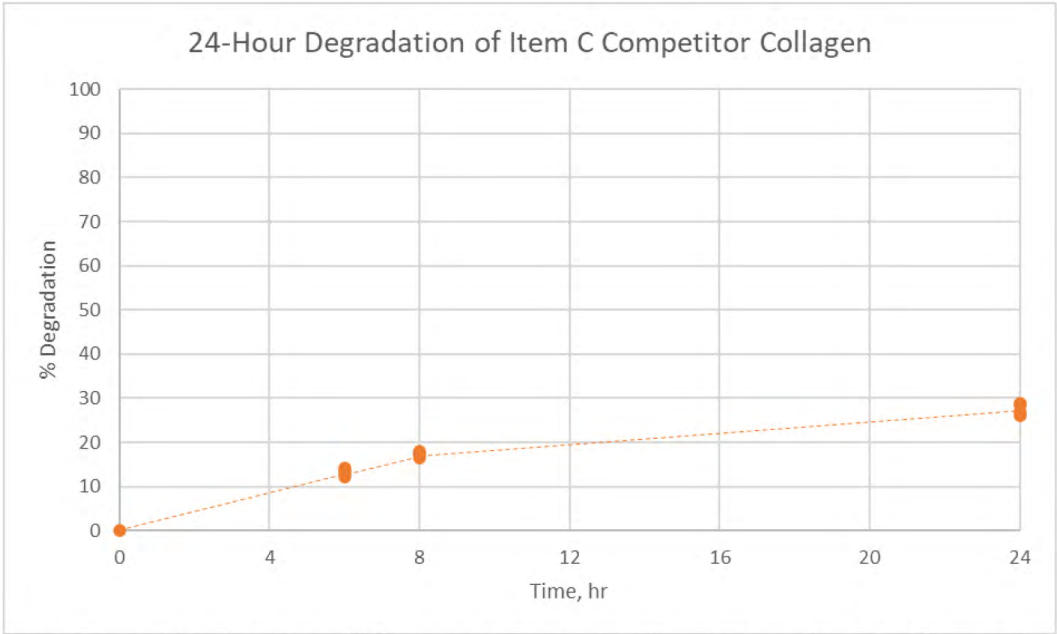


Figure 5. Plot of in vitro digestibility data or 10-mm slices of sample C, Collagen.

Figures 6 through 8 show images of the wet samples (withdrawn from the digestive fluids at the 6<sup>th</sup>, 8<sup>th</sup> and 24<sup>th</sup> hours, from top to bottom, respectively) of the cut pieces of the product samples.



Figure 6. Remains of samples from the 10-mm squares of Item A, No-Hide, after 6, 8 and 24 hours (from top to bottom) in digestive fluids.

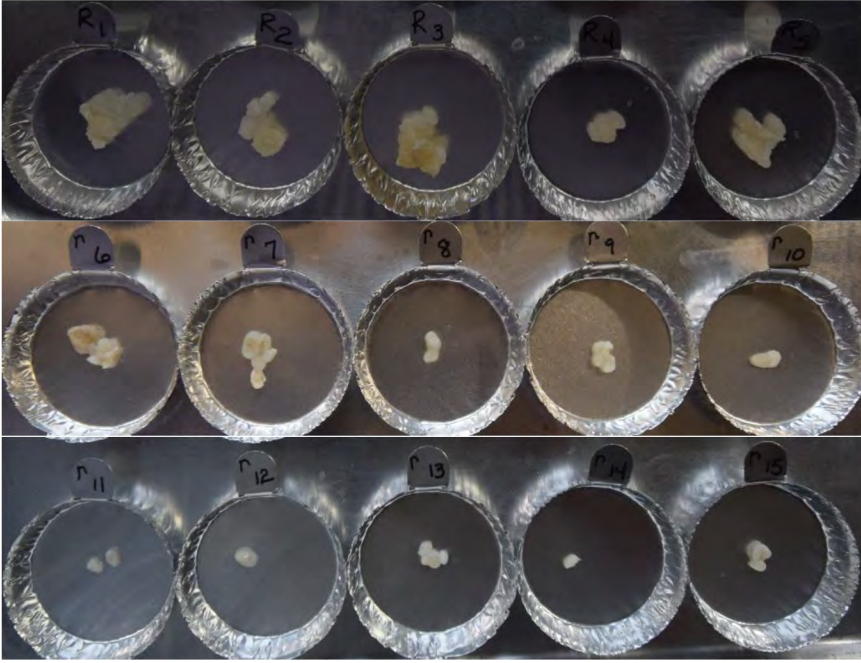


Figure 7. Remains of samples from the 10-mm cubes of Item B, Gelatin, after 6, 8 and 24 hours (from top to bottom) in digestive fluids.



Figure 8. Remains of samples from the 10-mm slices of Item C, Collagen, after 6, 8 and 24 hours (from top to bottom) in digestive fluids.



### Conclusions

*Digestibility in simulated canine gastric and intestinal juices, "3x5-10" protocol:*

Item A, No-Hide chews, were 97.6% degraded by the end of the 24<sup>th</sup> hour (6 hours in gastric juices followed by 18 hours in intestinal juices).

Item B, Competitor Gelatin chews, were 96.8% degraded by the end of the 24<sup>th</sup> hour (6 hours in gastric juices followed by 18 hours in intestinal juices).

Item C, Competitor Collagen chews, were 28.9% degraded by the end of the 24<sup>th</sup> hour (6 hours in gastric juices followed by 18 hours in intestinal juices).

### References

Bowser, T.J., C.I. Abramson and D. Bennett. 2006. Low-cost *in vitro* Screening Method for Digestibility of Pet Chews. American Journal of Animal and Veterinary Sciences 1 (2): 23-26.

de Godoy, M.R.C., R. Vermillion, L. Bauer, R. Yamka, N. Frantz, T. Jia, G. Fahey Jr., K. Swanson. 2014. In vitro disappearance characteristics of selected categories of commercially available dog treats. Journal of Nutritional Science. Vol. 3, e47.