


CONFIDENTIAL REPORT for Earth Animal.

IN VITRO DOG CHEW DIGESTIBILITY



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Summary

PetSci, LLC, a humane pet product research and testing company located in Stillwater, Oklahoma, tested a unique dog chew called “No-Hide” from Earth Animal (2 flavors, beef and chicken, with two resting times for enzyme activity, 45 and 60 minutes). Samples were designated Beef 45, Beef 60, Chicken 45, and Chicken 60 with the numbers reflecting the minutes of rest time for the enzyme to work during manufacturing. Rawhide squares were also tested for comparison purposes.

The dog 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 for humane reasons, 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 a dog chew in the canine digestive tract to assess product safety. Dog chews that are swallowed whole, or in part, should degrade rapidly in the canine digestive system to prevent potentially dangerous blockage. Procedures developed by Bowser and Abramson (2006) were followed.

Tests were conducted on 10 mm slices of the No-Hide from samples that were provided by the customer and as described in this report. The pieces were cut to simulate chunks that could potentially be swallowed by dogs. Rawhide was cut into 10 mm squares with native thickness. No-Hide chews could not be cut into squares or cubes because of their physical shape and texture.

Results of the tests were plotted individually and brief comments are 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:

Product	Size, shape mm	8-hour Degradation*	
		Gastric	Intestinal
No-Hide beef 45 dog chew	10, slice	68.5 %, very good	76.2%, excellent
No-Hide beef 60 dog chew	10, slice	82.3%, excellent	86.3%, excellent
No-Hide chicken 45 dog chew	10, slice	78.6%, excellent	72.7%, excellent
No-Hide chicken 60 dog chew	10, slice	88.7%, excellent	69.1%, very good
Rawhide	10, square	32%, poor	4%, 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

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Products tested

A description of dog pet chews tested is given in Table 1. Figure 1 shows the No-Hide product samples before they were cut, weighed, and placed in simulated digestive juices. All products were received as factory samples that were packaged in sealed plastic pouches. The rawhide product was tested to provide a performance comparison to the No-Hide dog chews.

Table 1. Products tested.

Product	Source	Lot Number	Shape
No-Hide beef formula dog chew, 45	Earth Animal	NA	10 mm slice
No-Hide beef formula dog chew, 60	Earth Animal	NA	10 mm slice
No-Hide chicken formula dog chew, 45	Earth Animal	NA	10 mm slice
No-Hide chicken formula dog chew, 60	Earth Animal	NA	10 mm slice
Rawhide	Apelsa, Mexico	NA	10 mm square



Figure 1. Product samples of the No-Hide chews, cut and prepared for testing.

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Procedure

Tests were conducted according to the procedure developed by Bowser and Abramson (2006). Briefly, product samples are immersed in containers (one container per sample) filled with either simulated gastric or intestinal juices for a given period of time. In this study, time periods were 1, 2, 4, and 8 hours. The containers were held in a constant temperature oven at 101.5 F, the average canine body temperature. Containers were shaken periodically to simulate movement in the digestive tract. At the end of each time period, the appropriate containers were emptied and the sample recovered. Samples were then dried and weighed. The difference between the dry weights gave the raw data for the calculation of the percent degradation of the pet chew. Percent degradation may include both product dissolution into the digestive fluids and breakdown into fine particulates. Each experiment was repeated in triplicate.

Results and Discussion

All figures with error bars showing product degradation in this section give the maximum and minimum degradation for each time period (top and bottom of bar) and the average product degradation for each time period (shapes connected by lines). Figures 2 and 3 show combined results for gastric and intestinal tests, respectively for all products.

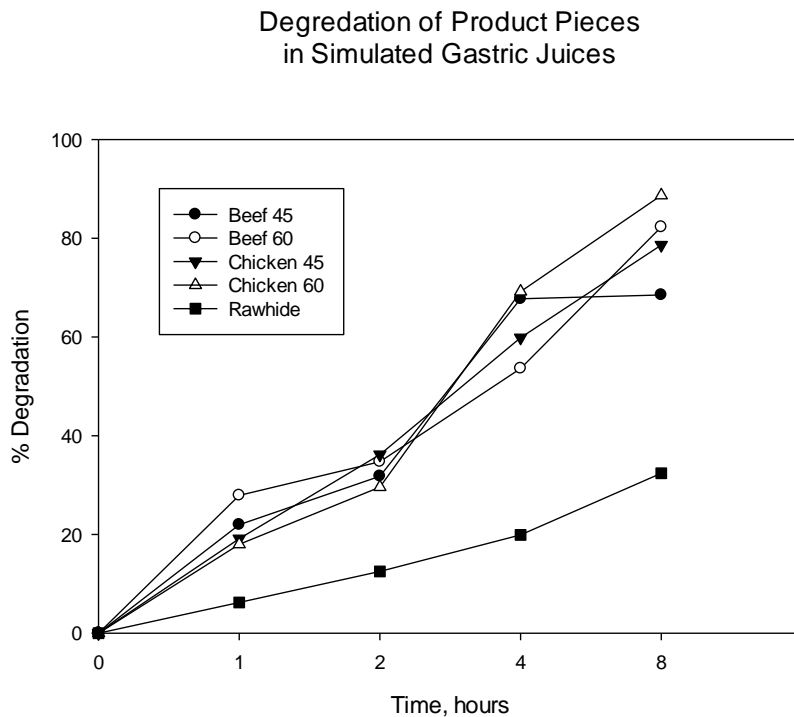


Figure 2. Average degradation of all products in simulated canine gastric juices.

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Degredation of Product Pieces
 in Simulated Intestinal Juices

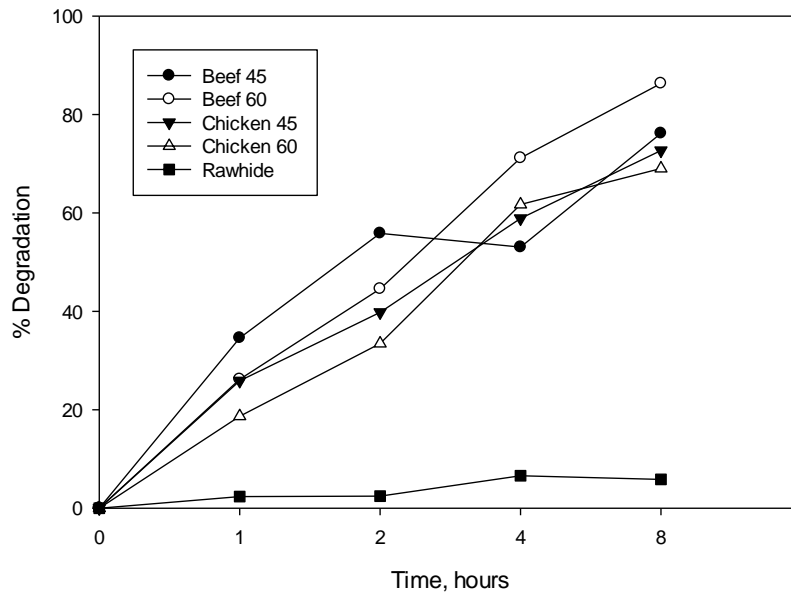


Figure 3. Average degradation of all products in simulated canine intestinal juices.

Figures 4 and 5 show results of degradation tests of No-Hide Beef 45 dog chews in simulated canine gastric juices.

Average Degradation of 10mm Slices of No-Hide Beef 45
 Chews in Simulated Canine Gastric Juice

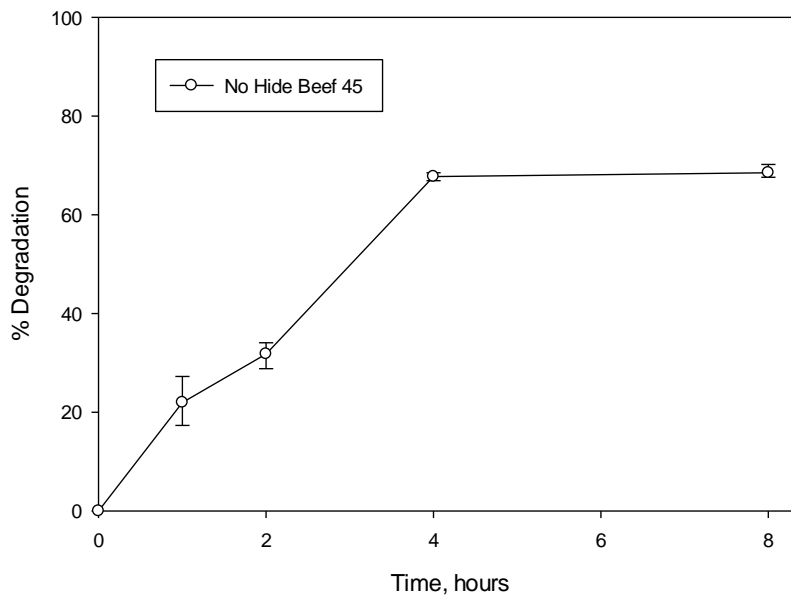


Figure 4. Average degradation of 10 mm slices of No-Hide Beef 45 dog chews in simulated canine gastric juice.

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Figure 5. Image of 10 mm slices of No-Hide Beef 45 dog chews after immersion for 8 hours in simulated canine gastric juice. Samples showed degradation, softening, color loss, particle formation and unravelling.

Figures 6 and 7 show results for degradation tests of No-Hide Beef 45 dog chews in simulated canine intestinal juices.

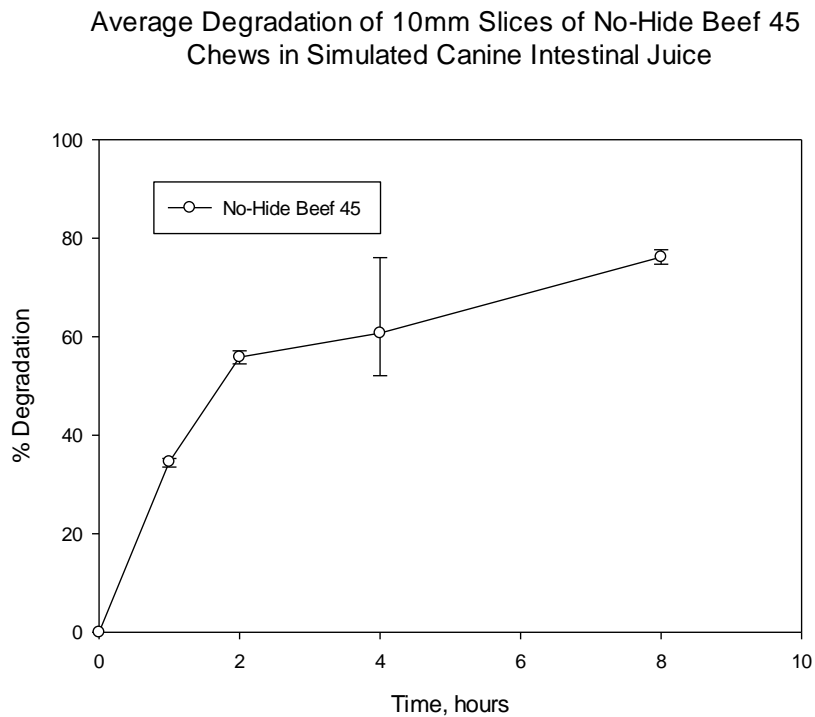


Figure 6. Average degradation of 10 mm slices of No-Hide Beef 45 dog chews in simulated canine intestinal juice.

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Figure 7. Image of 10 mm slices of No-Hide Beef 45 dog chews after immersion for 8 hours in simulated canine intestinal juice. Samples showed good degradation, softening, color loss, particle formation and unraveling.

Figures 8 and 9 show results for degradation tests of No-Hide Beef 60 dog chews in simulated canine gastric juices.

Average Degradation of 10mm Slices of No-Hide Beef 60 Chews in Simulated Canine Gastric Juice

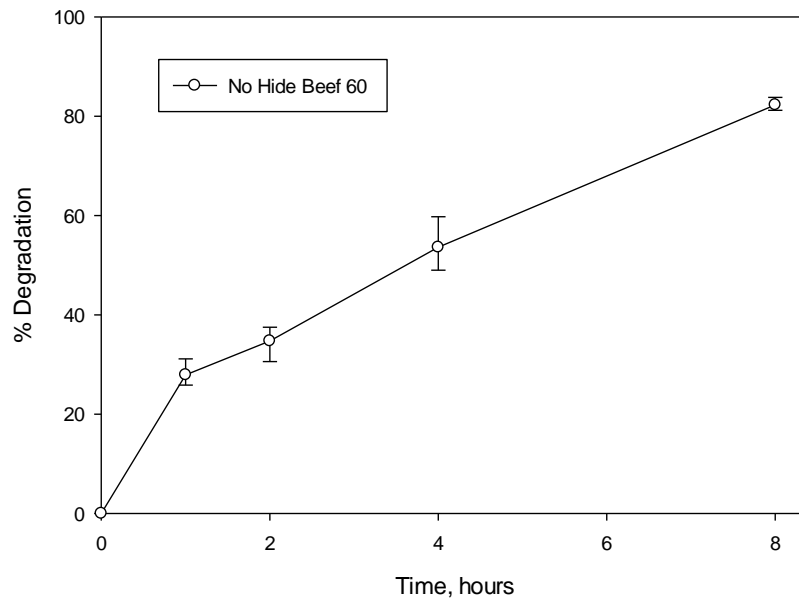


Figure 8. Average degradation of 10 mm slices of No-Hide Beef 60 dog chews in simulated canine gastric juice.

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Figure 9. Image of 10 mm slices of No-Hide Beef 60 dog chews after immersion for 8 hours in simulated canine gastric juice. Samples showed strong degradation, softening, color loss, particle formation and unraveling.

Figures 10 and 11 show results for degradation tests of No-Hide Beef 60 dog chews in simulated canine intestinal juices.

Average Degradation of 10mm Slices of No-Hide Beef 60 Chews in Simulated Canine Intestinal Juice

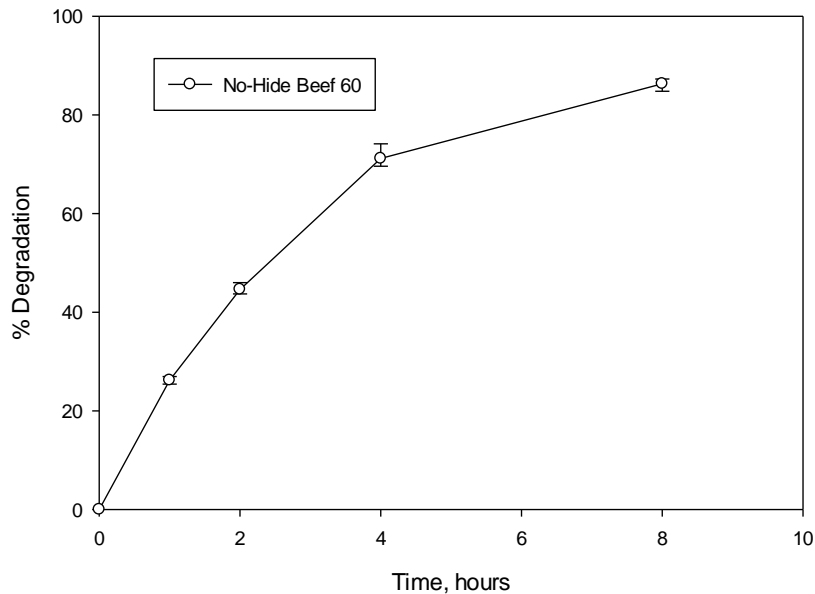


Figure 10. Average degradation of 10 mm slices of No-Hide Beef 60 dog chews in simulated canine intestinal juice.

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Figure 11. Image of 10 mm slices of No-Hide Beef 60 dog chews after immersion for 8 hours in simulated canine intestinal juice. Samples showed very good degradation, softening, color loss, particle formation and unraveling.

Figures 12 and 13 show results for degradation tests of No-Hide Chicken 45 dog chews in simulated canine gastric juices.

Average Degradation of 10mm Slices of No-Hide Chicken 45 Chews in Simulated Canine Gastric Juice

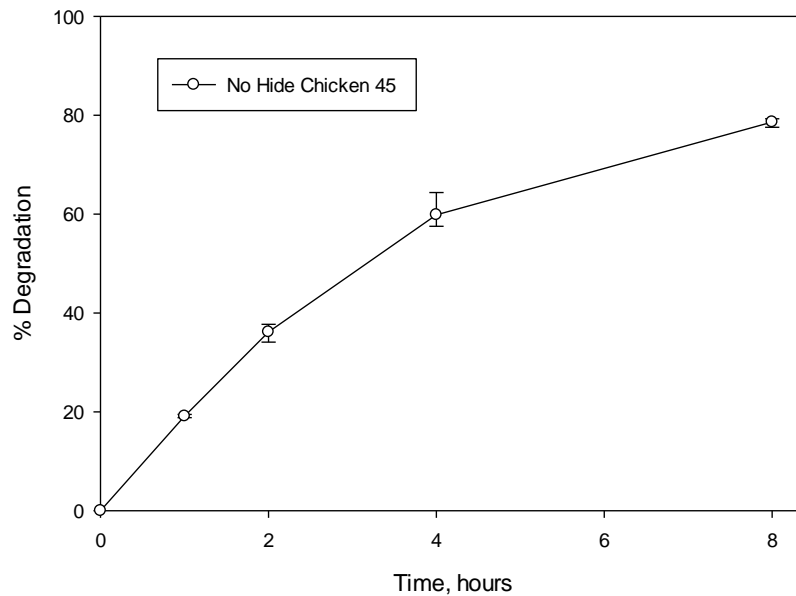


Figure 12. Average degradation of 10 mm slices of No-Hide Chicken 45 dog chews in simulated canine gastric juice.

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Figure 13. Image of 10 mm slices of No-Hide Chicken 45 dog chews after immersion for 8 hours in simulated canine gastric juice. Samples showed degradation, softening, particle formation, unraveling and color loss.

Figures 14 and 15 show results for degradation tests of No-Hide Chicken 45 dog chews in simulated canine intestinal juices.

Average Degradation of 10mm Slices of No-Hide Chicken 45 Chews in Simulated Canine Intestinal Juice

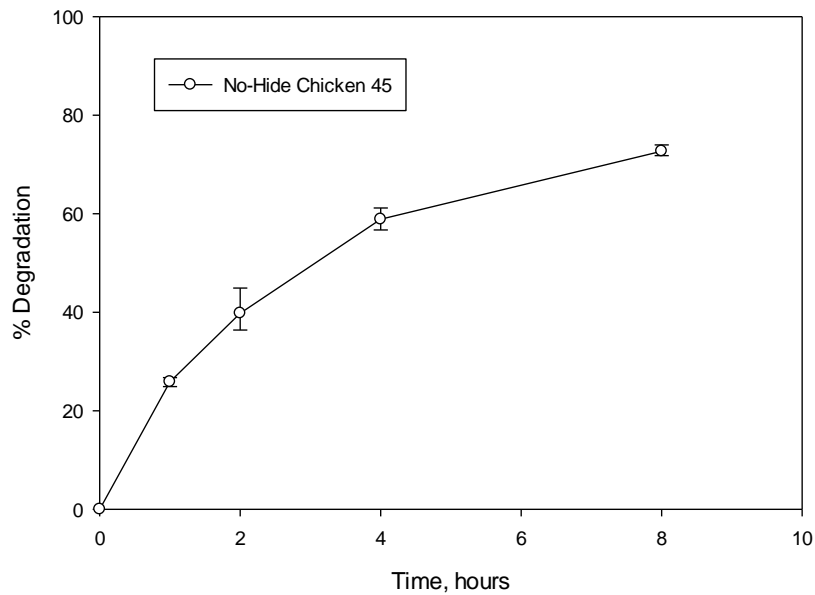


Figure 14. Average degradation of 10 mm slices of No-Hide Chicken 45 dog chews in simulated canine intestinal juice.

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Figure 15. Image of 10 mm slices of No-Hide Chicken 45 dog chews after immersion for 8 hours in simulated canine intestinal juice. Samples showed degradation, softening, particle formation, unraveling and color loss.

Figures 16 and 17 show results for degradation tests of No-Hide Chicken 60 dog chews in simulated canine gastric juices.

Average Degradation of 10mm Slices of No-Hide Chicken 60 Chews in Simulated Canine Gastric Juice

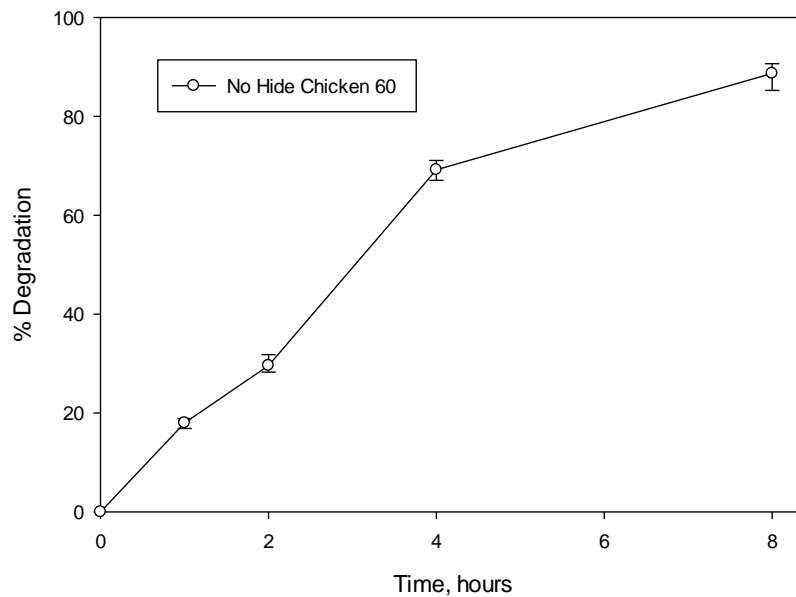


Figure 16 Average degradation of 10 mm slices of No-Hide Chicken 60 dog chews in simulated canine gastric juice.

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Figure 17. Image of 10 mm slices of No-Hide Chicken 60 dog chews after immersion for 8 hours in simulated canine gastric juice. Samples showed strong degradation, softening, particle formation, unraveling and color loss.

Figures 18 and 19 show results for degradation tests of No-Hide Chicken 60 dog chews in simulated canine intestinal juices.

Average Degradation of 10mm Slices of No-Hide Chicken 60 Chews in Simulated Canine Intestinal Juice

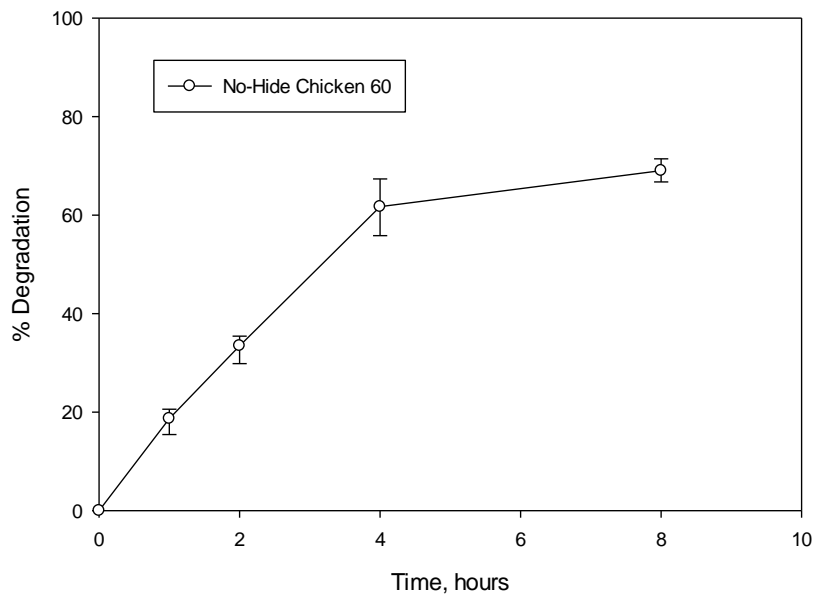


Figure 18 Average degradation of 10 mm slices of No-Hide Chicken 60 dog chews in simulated canine intestinal juice.

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Figure 19. Image of 10 mm slices of No-Hide Chicken 60 dog chews after immersion for 8 hours in simulated intestinal juice. Samples showed strong degradation, softening, particle formation, unraveling and color loss.

Figures 20 and 21 show results for degradation tests of Rawhide dog chews in simulated canine gastric juices.

Average Degradation of 10mm Squares of Regular Rawhide Chews in Simulated Canine Gastric Juice

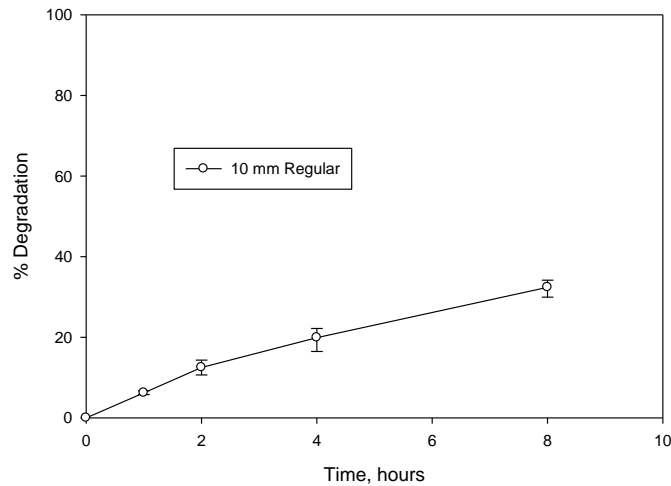


Figure 20 Average degradation of 10 mm slices of Rawhide dog chews in simulated canine gastric juice.

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Figure 21. Image of 10 mm slices of Rawhide dog chews after immersion for 8 hours in simulated canine gastric juice. Samples showed some degradation, some softening and color loss.

Figures 22 and 23 show results for degradation tests of Rawhide dog chews in simulated canine intestinal juices.

Average Degradation of 10mm Squares of Regular Rawhide Chews in Simulated Canine Intestinal Juice

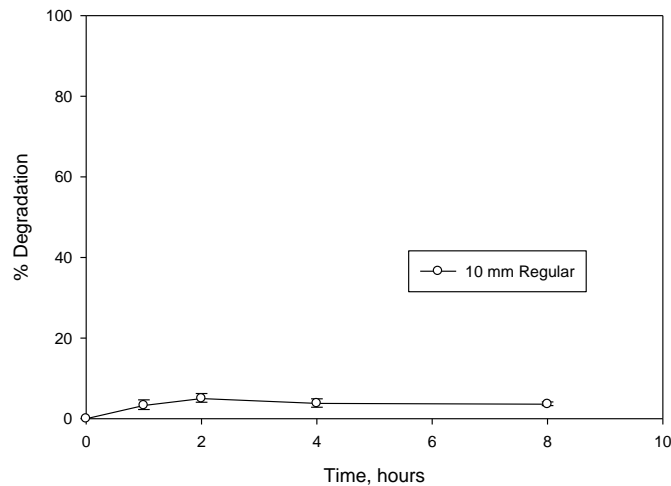


Figure 22 Average degradation of 10 mm slices of Rawhide dog chews in simulated canine intestinal juice.

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Figure 23. Image of 10 mm slices of Rawhide dog chews after immersion for 8 hours in simulated canine intestinal juice. Samples showed little degradation, some softening and color loss.

Conclusions

Digestibility in simulated canine gastric juices

No-Hide Beef 45 dog chew, 10 mm slices –had very good digestibility, reaching an average degradation of 69% within 8 hours.

No-Hide Beef 60 dog chew, 10 mm slices –excellent digestibility, with about 82% degradation within 8 hours.

No-Hide Chicken 45 dog chew, 10 mm slices –had excellent digestibility, reaching an average degradation of 79% in 8 hours.

No-Hide Chicken 60 dog chew, 10 mm slices –had excellent digestibility, with about 89% average degradation within 8 hours.

Rawhide, 10 mm squares –had poor digestibility, reaching an average degradation of 32% in 8 hours.

Digestibility in simulated canine intestinal juices

No-Hide Beef 45 dog chew, 10 mm slices –had excellent digestibility, reaching an average degradation of 76% within 8 hours.

No-Hide Beef 60 dog chew, 10 mm slices –very excellent digestion, with about 86% degradation within 8 hours.

No-Hide Chicken 45 dog chew, 10 mm slices –had excellent digestibility, reaching an average degradation of 73% in 8 hours.

No-Hide Chicken 60 dog chew, 10 mm slices –had very good digestibility, with about 69% degradation within 8 hours.

Rawhide, 10 mm squares –had very poor digestibility, reaching an average degradation of 4% within 8 hours.

Reference

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