



Luna Science and Nutrition, LLC

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INVESTIGATOR

Ryan M. Yamka, PhD, MS, MBA, FACN, PAS, Dipl. ACAS

CLIENT

Earth Animal Ventures, LLC

EXECUTIVE SUMMARY

Earth Animal Ventures, LLC requested a third-party consultation for their No-Hide™ chews because of recent questions raised about the composition of No-Hides. Allegations were made that the No-Hide™ products are “*mostly made of beef*”. Microscopy (not a validated method) and DNA analysis were used to support the allegations. Earth Animal elected to utilize both science and observations of the manufacturing process to address the questions that have been raised.

The objective of this consultation was to determine how the product is made and to determine if rawhide is utilized in their finished products. This included reviewing of the ingredients, formulations and observing the manufacturing of the No-Hide™ chews. Additionally, finished products of No-Hide™ and store-bought rawhide were sent out for DNA analysis and formaldehyde analysis (formaldehyde is a rawhide contaminant).

DNA analysis detected multiple animal species in the No-Hide™ products tested (not just beef). Given the manufacturing process and usage of common machinery, racks, etc., these results do make logical sense. Additionally, the products were tested for plant material DNA. The only plant material found in the No-Hide™ product was rice. Again, this finding is logical since brown rice is the only grain used in the facility and is included in the formulations. Our findings clearly indicate that analyzing for beef alone is not sufficient in determining if the product is rawhide or not. Given the results of the No-Hide™ products, it is apparent that the final product contains rice and has multiple animal protein DNA likely because of protein migration. For more details on the findings and results please see the DNA ANALYSIS section of this report.

In addition to the DNA analysis, samples submitted by unknown individuals were also sent to The Leather Lab for visual examination to support the allegations that the majority of the No-Hide™ Chicken chew is made from beef rawhide. After utilizing a series of processes that have not been proven to be scientifically valid, the investigator reviewed the material underneath a microscope. From there the investigator came to the conclusion, that the material must be rawhide because it contains indicators of flesh (blood vessels) and collagen. To those skilled in the art of microscopy, a microscopist generally follows up on their findings with

scientific analysis to support their observations since this technique is an “art”. If the investigator would have known that the product contained chicken (flesh) and gelatin (partially hydrolyzed collagen), I am sure he would have questioned his findings. Since this information was not provided to him or secondary scientific analysis was not performed, his findings become fatally flawed. Lastly, his report immediately jumps to and indicates it must be either beef or horse based because of the physical attributes (thickness and length). Again, before arriving at this conclusion the investigator should have sent the samples for DNA or protein analysis to confirm his findings. He gives no indication on how he would determine the species of the products he reviewed without simply guessing.

Formaldehyde analysis of the No-Hide™ chews further confirms the validity of the No-Hide™ labeled ingredients. Formaldehyde was not detected in any of the No-Hide™ products; however, formaldehyde was present in the store-bought rawhide products. Formaldehyde is a contaminant in the rawhide process. Not finding formaldehyde in the No-Hide™ product indicates rawhide is not present in the formulation.

Finally, on August 24, 2017 I visited the Pony Express Facility located in New Holland, PA to observe the production of No-Hide™ chews. A detailed summary of the ingredients and the manufacturing process are listed in sections PROCESS FOR MANUFACTURING THE NO-HIDE™ CHEW and INGREDIENTS UTILIZED IN NO-HIDE™ CHEW* DURING VISIT. Based on my observations, I did not see rawhide being used in the manufacturing of No-Hide™.

In Summary, based on the DNA analysis, formaldehyde analysis and my visit to the manufacturing site (ingredients utilized and manufacturing process) I can verify that no rawhide is used in the No-Hide™ product.



August 29, 2017

Ryan M. Yamka

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Luna Science and Nutrition, LLC

DNA ANALYSIS

On July 27, 2017 an article entitled *“Is No-Hide Dog Treat Actually Hide?”* was posted on-line. In the article, allegations were made that the majority of the No-Hide™ chew is made from rawhide, when it is not. Test samples, submitted by unknown individuals, to two labs showed the presence of beef DNA which was used to support their claim. There was a misinterpretation of that data in the article. Although beef was identified via DNA in both No-Hide™ chews, the DNA data is not quantitative (i.e. it does NOT measure the percentage of composition). The DNA analysis is only qualitative (i.e. is the specific DNA present or not). Additionally, NSF AuthenTechnologies clearly stated on their report that their analysis was not quantitative.

Since all varieties of the No-Hide™ chews (Pork, Salmon, Chicken and Beef) are made on the same equipment, Earth Animal believed the presence of bovine in the chicken product was the result of protein migration (i.e. all proteins coming into contact with the same surfaces).

To test this hypothesis, we sent four samples to NSF AuthenTechnologies for DNA analysis. This is the same lab used for the testing that was reported in the on-line article. However, instead of just testing for bovine DNA, Earth Animal tested for all four protein sources utilized by Earth Animal. In addition, we requested that they perform DNA analysis for plant materials to confirm the presence of rice since it is an ingredient of the No-Hide™ products. The samples were labeled as Earth Animal 1 to 4. The samples included two different samples of rawhide and two different samples of No-Hide™ chews (Chicken and Pork).

The No-Hide™ Chicken chew and Pork chew DNA analysis confirmed the hypothesis of protein migration since multiple species of animals were detected via DNA (samples 2 and 4 below). Additionally, the DNA analysis of the No-Hide™ identified the presence of rice. This was expected since brown rice flour is a component of the formula.

Additionally, the rawhide products also contained other animal species as well as plant DNA sources. This data would suggest that these products are made in facilities that contain other animal sources in addition to plant materials since rawhide is the only ingredient listed on their labels.

In conclusion, if the original testing had included all four proteins and not just bovine, similar results would have been seen. As a reminder, DNA analysis is qualitative analysis not a quantitative analysis as indicated in the website article.

Reports published in the on-line article:

Notice that the units is N/A since this is not a quantitative analysis.



REPORT OF ANALYSIS

Customer: 

Date Received: 06/05/17
Report Date: 06/08/17

Description: Chicken No Hide
Lab Number: CH13729
Commodity: Wrapper

Analysis	Result	Units	Analyzed
Bovine (beef) DNA by PCR	Positive	NA	06/08/17



REPORT OF ANALYSIS

Customer: 

Date Received: 06/05/17
Report Date: 06/08/17

Description: Salmon No Hide
Lab Number: CH13730
Commodity: Wrapper

Analysis	Result	Units	Analyzed
Bovine (beef) DNA by PCR	Positive	NA	06/08/17

Data Accessed August 25, 2017.

<http://truthaboutpetfood.com/wp-content/uploads/2017/07/DNACHickenandSalmon.pdf>

RESULTS FROM SAMPLES SENT TO NSF AuthenTechnologies FOR EARTH ANIMAL VENTURES TESTING

Sample # 1. Rawhide Sample Brand A Report #460

RESULTS

The sample was analyzed using a universal animal DNA test, which detected *Bos taurus*. DNA from other animal species was also detected in relatively low abundance. Therefore, the sample was reanalyzed using specific *Sus*, *Gallus*, and *Oncorhynchus* tests, which did not detect any DNA for these genera. The sample was also analyzed using a CleanScreen DNA test specific for *Triticum* sp. (Wheat), *Glycine max* (Soy), *Pisum sativum* (Pea), *Zea mays* (Corn Starch), *Solanum tuberosum* (Potato - Maltodextrin), *Oryza sativa* (Rice), *Arachis hypogaea* (Peanut), *Prunus dulcis* (Almond), *Juglans* spp. (Walnut), *Carya illinoensis* (Pecan), and *Corylus avellana* (Hazelnut). DNA from *Oryza sativa* and a *Triticum* species was detected in the sample.

Fig 1. Species Identification Test Results. The Y-axis is the total number of sequences identified for each species; *this value is not representative of weight or volume* of the species. "Other" category (if applicable) represents sequences not specifically identified.

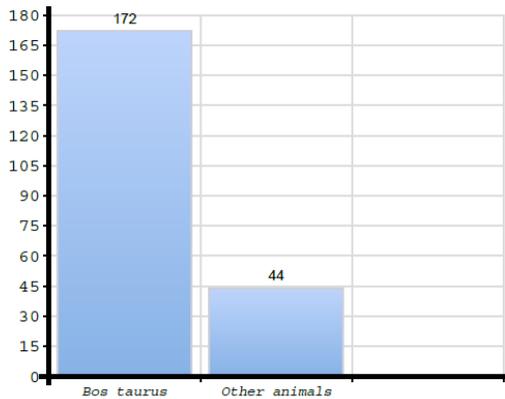
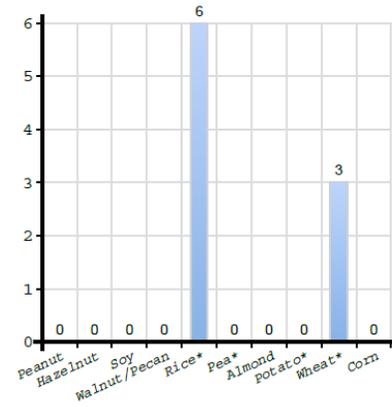


Fig 2. Clean Screen Test Results. The Y-axis is the total number of sequences detected for each species. *LOD = 50 pg DNA.



Sample 2. Chicken No-Hide™ Report #461

RESULTS

The sample was analyzed using a universal animal DNA test, which detected *Bos taurus* and *Gallus gallus*. Therefore, the sample was reanalyzed using specific *Sus*, *Oncorhynchus*, and *Salmo* tests, which only detected *Sus scrofa* but not any *Oncorhynchus* or *Salmo* species. The sample was also analyzed using a CleanScreen DNA test specific for *Triticum* sp. (Wheat), *Glycine max* (Soy), *Pisum sativum* (Pea), *Zea mays* (Corn Starch), *Solanum tuberosum* (Potato - Maltodextrin), *Oryza sativa* (Rice), *Arachis hypogaea* (Peanut), *Prunus dulcis* (Almond), *Juglans* spp. (Walnut), *Carya illinoensis* (Pecan), and *Corylus avellana* (Hazelnut). DNA from *Oryza sativa* was detected in the sample.

Fig 1. Species Identification Test Results. The Y-axis is the total number of sequences identified for each species; *this value is not representative of weight or volume* of the species. "Other" category (if applicable) represents sequences not specifically identified.

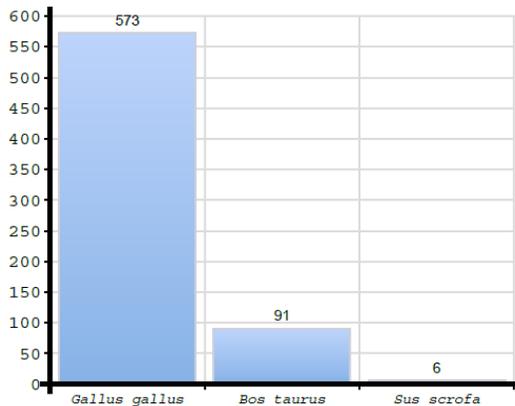
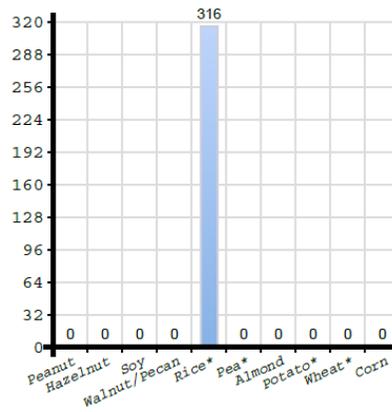


Fig 2. Clean Screen Test Results. The Y-axis is the total number of sequences detected for each species. *LOD = 50 pg DNA.



Sample 3. Rawhide Sample Brand B Report #462

RESULTS

The sample was first analyzed using a universal animal DNA test, which did not detect any DNA. Therefore, the sample was reanalyzed using specific tests for *Bos*, *Gallus*, *Sus*, *Salmo* and *Oncorhynchus*, which confirmed the presence of *Gallus gallus* and *Bos taurus*. No other species within the tested genera were identified in the sample. The sample was also analyzed using a CleanScreen DNA test specific for *Triticum* sp. (Wheat), *Glycine max* (Soy), *Pisum sativum* (Pea), *Zea mays* (Corn Starch), *Solanum tuberosum* (Potato), *Oryza sativa* (Rice), *Arachis hypogaea* (Peanut), *Prunus dulcis* (Almond), *Juglans* spp. (Walnut), *Carya illinoensis* (Pecan), and *Corylus avellana* (Hazelnut). DNA from a *Triticum* species was detected in the sample.

Fig 1. Species Identification Test Results. The Y-axis is the total number of sequences identified for each species; *this value is not representative of weight or volume* of the species. "Other" category (if applicable) represents sequences not specifically identified.

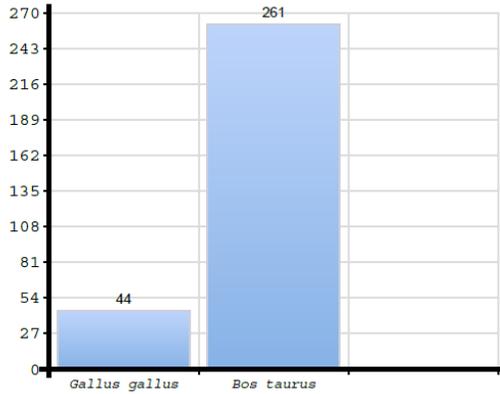
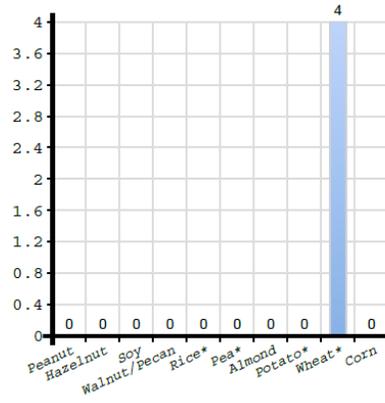


Fig 2. Clean Screen Test Results. The Y-axis is the total number of sequences detected for each species. *LOD = 50 pg DNA.



Sample 4. Pork No-Hide™ Report #463

RESULTS

The sample was first analyzed using a universal animal DNA test, which detected the presence of *Sus scrofa*, *Bos taurus*, and *Gallus gallus*, but not from *Salmo* or *Oncorhynchus* species. DNA from other animals was also detected. The sample was reanalyzed using specific tests for *Salmo* and *Oncorhynchus*, which confirmed the presence of *Salmo salar*. No other species within the tested genera were identified in the sample. The sample was also analyzed using a CleanScreen DNA test specific for *Triticum* sp. (Wheat), *Glycine max* (Soy), *Pisum sativum* (Pea), *Zea mays* (Corn Starch), *Solanum tuberosum* (Potato), *Oryza sativa* (Rice), *Arachis hypogaea* (Peanut), *Prunus dulcis* (Almond), *Juglans* spp. (Walnut), *Carya illinoensis* (Pecan), and *Corylus avellana* (Hazelnut). DNA from *Oryza sativa* was detected in the sample.

Fig 1. Species Identification Test Results. The Y-axis is the total number of sequences identified for each species; *this value is not representative of weight or volume* of the species. "Other" category (if applicable) represents sequences not specifically identified.

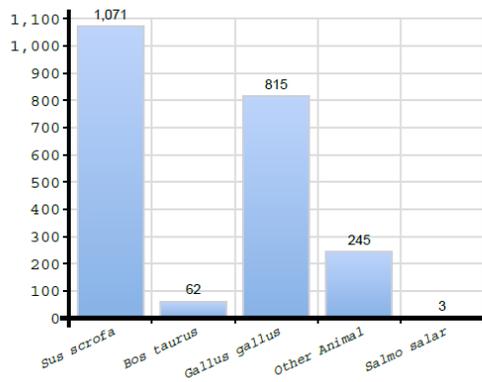
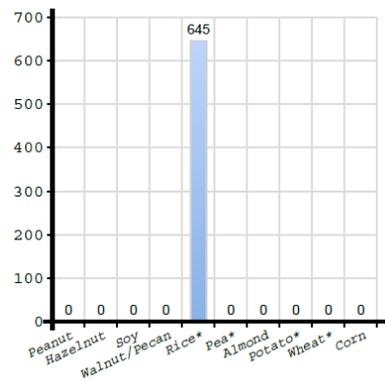


Fig 2. Clean Screen Test Results. The Y-axis is the total number of sequences detected for each species. *LOD = 50 pg DNA.



FORMALDEHYDE ANALYSIS

In addition to DNA analysis, I requested that Earth Animal send in samples to Eurofins for formaldehyde analysis. Formaldehyde is utilized as a cross-linking agent in making collagen fiber reconstituted rawhide and is utilized in the process of making traditional rawhide. Although finished rawhide products go through a washing process, formaldehyde is often present in residual amounts. Samples were sent to Eurofins and labeled as Sample 1 through 5.

The formaldehyde analysis of the No-Hide™ chews further supports that rawhide is not present in their products. As expected, the rawhide samples had detectable levels of formaldehyde.

Sample #	Eurofins Sample Code	Product	Formaldehyde
1	468-2017-0816B152	No-Hide™ Salmon Chew	Not Detected
2	468-2017-0816B153	Rawhide Brand C	6 ppm
3	468-2017-0816B154	No-Hide™ Chicken Chew	Not Detected
4	468-2017-0816B155	Rawhide Brand D	9 ppm
5	468-2017-0816B156	No-Hide™ Beef Chew	Not Detected

MICROSCOPY RESULTS USED IN THE ARTICLE

In addition to the DNA analysis, samples submitted by unknown individuals were also sent to The Leather Lab for visual examination to support the allegations that the majority of the No-Hide™ Chicken chew is made from beef rawhide.

The investigator clearly indicated that it was difficult to determine any differences in structure. As a result, the investigator proceeded to utilize non-validated methods to investigate both the rawhide and No-Hide™ samples. It is unknown why the investigator chose to delime, pickle and tan the samples since these methods are typical for leather (skin) materials not for the materials in question.

Following these methods, the investigator reviewed the material underneath a microscope. From there the investigator came to the conclusion that the material must be rawhide because it contains indicators of flesh (blood vessels) and collagen. To those skilled in the art of microscopy, a microscopist generally follows up on their findings with scientific analysis to validate their observations since this technique is an “art” and not scientifically valid. If the investigator would have known that the product contained chicken (flesh) and gelatin (partially hydrolyzed collagen), I am sure he would have questioned his findings. Since this information was not provided to him or secondary scientific analysis was not performed, his findings become fatally flawed.

Lastly, his report immediately jumps to and indicates it must be either beef or horse based on the physical attributes (thickness and length). Again, before jumping to this conclusion the investigator should have sent the samples for DNA or protein analysis to confirm his findings. He gives no indication on how he would determine the species of the products he reviewed without simply guessing.

Given the flaws in the published report, I am recommending that we find a laboratory with the expertise in this field. Once the data becomes available we can amend this report accordingly with their results.

PROCESS FOR MANUFACTURING THE NO-HIDE™ CHEW

On August 24, 2017 I attended a full production run for the Beef No-Hide™ chew. The purpose of the visit was to understand the processes for manufacturing the finished product and the ingredients used to make No-Hide™ chew and to verify that no rawhide is utilized in the process.

Each step of the process is listed below with pictures.

Step 1: Blended ingredients (proprietary blend, though I inspected each ingredient used and verified that each came from sealed, properly labeled sources) are extruded into flat sheets utilizing a proprietary method (below picture on left). The resulting sheet has many of the visual characteristics of rawhide (below picture on right); however, no rawhide is utilized in the product. This appearance of the No-Hide™ chew below is the result of their proprietary blend of ingredients and manufacturing methods.



Step 2: The flat sheet is cut into smaller sections for the next step of the No-Hide™ chew (pictured below left). Finally, the flat sheet is rolled by hand and placed onto the oven racks (pictured below right).



Step 3: Prior to the products entering the oven, we punched a hole in one of the No-Hides™ chews to ensure we tracked the actual rack that went into oven (pictured below right). Additionally, we put one of the flat sheets (middle picture above) on the rack to see how the product behaves without being rolled. Prior to entering the oven, the product was still very pliable and soft.



Step 4: The No-Hide™ chew was placed in the oven utilizing proprietary conditions (temperature, humidity and duration). Following the cook time, the No-Hide™ chews were removed from the oven. As the product cooled, the product began to set up and became harder and similar in texture to rawhide. This was clearly demonstrated in both the flat sheet and roll (pictured below).



Step 6: Once the rolls were cooled, they were cut into appropriate sizes (pictured below left), hand coated (pictured below middle) and then placed back onto the racks to enter the oven and complete the drying of the finished product (pictured below right).



Finished Product: The resulting finished product has similar characteristics to rawhide (visual, hardness and texture); however, the No-Hide™ chew does not contain rawhide.



INGREDIENTS UTILIZED IN NO-HIDE™ CHEW* DURING VISIT

- Beef
- Brown Rice Flour
- Gelatin
- Olive Oil
- Organic Eggs
- Banana
- Pineapple (Bromelain)

*Actual inclusion rates are proprietary to the client. As a result, inclusion rates are not listed above. The above listed ingredients are the only ingredients that were used to produce the No-Hide™ Chew on the day of production.

INVESTIGATOR BIOGRAPHY

Dr. Ryan Yamka is a native of New Jersey and earned a BS in animal science and a BS in biology from Virginia Polytechnic Institute and State University followed by his MS and PhD at the University of Kentucky. In 2016, Yamka founded Luna Science and Nutrition as an Independent Consultant and Pet Industry Expert with 19+ years of experience in companion animal nutrition research and product development. Yamka's research focused on genomics, weight management, amino acid metabolism, carbohydrate metabolism, fiber utilization, and technologies for alternative testing. He has maintained a critical role in the development and implementation of many new and innovative products for companion animals and was active in the application of new technologies for several health management products. In addition, Yamka also developed a urine pH prediction model for felines that eliminated the need for feeding studies to test urine pH. Yamka also led a highly productive team that successfully developed and launched 250+ new products in the dry, wet, meat roll, treat and litter categories. In 2011, Yamka was the recipient of the Corbin Companion Animal Biology Award. In addition, Yamka is a certified Professional Animal Scientist (PAS), Board Certified with the American College of Animal Sciences in Animal Nutrition (Diplomate ACAS) and Fellow of the American College of Nutrition (FACN). Yamka has more than 70 scientific papers and abstracts in peer-reviewed journals. Additionally, Yamka co-authored 2 book chapters in the 5th edition of Small Animal Clinical Nutrition and has 48 patents granted. Yamka is recognized as an expert in the pet food industry and has presented at Pet Food Forum, American Society for Animal Sciences Annual Meetings, American College of Veterinary Internal Medicine Forum, NLS Food Evolution Summit, Congreso Veterinario de Leon in Mexico and multiple universities.