Is there a nutritional difference when using a different flour base while making dog biscuits?

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#### Abstract

The purpose of this project was to compare the amount of macromolecules that would be absorbed from four different flour based dog biscuits (soy, sorghum, wheat, and brown rice), and determine which base would benefit dogs the most. It was believed that the soy flour based dog biscuits will have a higher digestibility rate and more nutritional absorbency due to the fact of soy flour having the highest amount of protein, as stated by the labels of the flour. The four biscuit types were tested for different macromolecules (starch, complex and simple sugars, protein, and calories) using the lodine, Benedict's, Bradford, and Calorimeter tests after digestion. The lodine test was used to determine the presence of starch; the Benedict's test was used to determine the presence of simple sugars; the Bradford test was used to determine the absorbance of protein, which could then be interpolated on the standard curve graph to determine concentration levels, and the Calorimeter test was used to calculate how many calories are contained in one gram. The data concluded that compared to the other flour bases, the soy flour based biscuits had the best overall nutritional absorbency readings with the highest Benedict's test and the lowest amount of calories calculated by the Calorimeter test. The experiment did support the hypothesis. These results were expected due to the fact of soybeans being a complete protein as well as a main source for other vitamins and minerals, thus transferring into having the most nutritious flour source based off the label. The importance of the project was to learn which flour source would be the most beneficial to dogs as well as being the most cost effective alternative for pet owners.

Keywords: nutrition, soy, dogs, macromolecules

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#### Literature Review

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#### Summary One

In "Glycemic and Insulinemic Responses after Ingestion of Commercial Foods in Healthy Dogs: Influence of Food Composition", a study was conducted to see if there is a correlation between diets causing hyperinsulinemia and obesity. Twelve adult beagles were tested and serum glucose and insulin concentration were calculated after meals. Results concluded that the amount of starch in a diet determines the glucose response, but may vary due to activity levels.

#### **Evaluation One**

This article seems to be credible. It has many websites and a footnote cited, and is published by a nutrition company. This is also a '.org' network, which tends to be more reliable than '.com'. This article seemed in no way opinionated, due to the fact that it presented many facts, and the actual data tables from the study.

#### Summary Two

In "Grain-Free Pet Food Trend a Hoax?", Dr. Donna Solomon, a veterinarian from Chicago, examines the nutritional difference between grain free diets and non grain free diets to determine if there is a benefit. It was mentioned that dogs with many food allergies tend to do better with grains in their diets, especially rice. It was determined that the grain free diets are just a myth.

### **Evaluation Two**

Although the article was written by a professional veterinarian, it was also published on The Huffington Post's website, therefore some bias was detected. However, the information was well presented, and also provides examples of the other side of the argument. In conclusion, some of the information from this source is credible, but some could have been seen as biased.

#### Summary Three

In "Soy Protein Increases Glomerular Filtration Rate in Dogs with Normal or Reduced Renal Function", a study was conducted with dogs that have normal kidney function and dogs that have compromised kidney function to determine if one protein source would help over other sources. The dogs with normal kidney function were given four protein sources, (casein, soy meal, soy flakes, and purified soy protein), while the dogs with reduced kidney function were given three protein sources (casein, purified soy protein, and pork liver). The experiment concluded that soy proteins have the same benefits on kidney function as animal proteins.

### **Evaluation Three**

This article seems to be credible. It is from an '.org' network rather than a '.com' network, which is typically considered to be a more credible source. The article also has citations and footnotes from other published works. This article seems in no way opinionated, due to the fact that it explained every step of the research and experimentation that the authors conducted.

Plan A:

Problem: When looking for a sustainable dog biscuit alternative, is there a nutritional difference between four different flour bases, soy, sorghum, wheat, or brown rice.

Hypothesis: It is believed that the soy flour based dog biscuits will have a higher digestibility rate and more nutritional absorbency due to the fact of soy flour having the highest amount of protein, as stated by the labels of the flour.

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Variables:

Independent: Flour type

Dependent: Amount of macromolecules adsorbed

Controls: Same amount of gastric juice, same cooking time, same baking methods, same testing methods, same size equipment, same amount of ingredients, same digestion time, same cookie cut out, etc.

## Plan B

## Materials:

- Peanut butter
- Bob's Red Mill Soy flour
- Bob's Red Mill Sweet Sorghum flour
- Bob's Red Mill Brown Rice flour
- Enriched white flour
- Quick oats
- Water
- Measuring cups
- Bowls
- Cookie cutter
- Cookie sheet
- Honey
- Eggs
- Baking soda
- Bradford solution
- Benedict's solution
- lodine solution
- Cuvettes
- Pipettes
- Strainer
- Permanent marker
- Test tubes
- Test tube racks
- Filter paper
- Strainer
- Magnetic stirring hot plate
- Magnetic stir bar
- Beakers
- Gastric juice
- Electronic balance
- Mortar and pestle
- Micro well plate
- Spectrophotometer
- Soda can
- Cork
- Bamboo skewer
- Paperclip
- Lighter
- Electronic balance
- Graduated cylinder

- Ring stand with clamp
- Safety goggles
- Thermometer

## Procedure:

Baking the Dog Treat Samples:

- 1. A convection oven was preheated to 176 degrees Celsius (350F).
- 2. In a large mixing bowl, 2 ½ cups of wheat flour was added to 1 tsp of baking soda and 1 egg, and stirred.
- 3. 1 cup of peanut butter was added to the bowl along with 1 cup of water and 2 tablespoons of honey.
- 4. The mixture was stirred and kneaded until a dough like mixture formed.
- 5. 1 cup of oats was added to the dough.
- 6. On a lightly floured surface, a quarter of the dough was rolled out to form  $\frac{1}{2}$  inch treats.
- 7. A floured cookie cutter was then placed into the dough to cut out 10 dog treats.
- 8. The cut out treats were then put on a baking sheet, and put in the oven for 20 minutes.
- 9. The cooked treats were then placed on a sheet of wax paper to cool for testing.
- 10. Steps 1-9 were repeated for each of the following flour sources (soy, sorghum, and brown rice).

Digestion of Dog Treat Samples in Gastric Juice:

- 1. 20g of each dog treat sample was measured out, and added to a beaker with 85mL of gastric juice.
- 2. Samples were placed on a hot plate at 39 degrees Celsius, and a magnetic stir bar was added.
- 3. The stir setting was then set to 300 rpm, and samples were left to sit for 7 hours.
- 4. After 7 hours, samples were then filtered with a fine mesh strainer.
- 5. The filtrate was then used to complete the Carbohydrate and Protein Indicator tests.

Monosaccharide Indicator Standard Test (Glucose):

- 1. In a test tube, 1mL of the wheat dog food treat filtrate solution was added to 1mL of Benedict's solution.
- 2. Samples were then given a quick mix.
- 3. Steps 1-2 were repeated 9 more times with the same filtrate.
- 4. All 10 test tubes were then placed in a boiling water bath for 2 minutes.
- 5. Steps 1-4 were repeated with the other flour bases (soy, sorghum, and brown rice) filtrate solution.
- 6. After 2 minutes, the test tubes were analyzed based on color change (0 = no color change/negative 3 = very strong/ positive), and data was recorded.
- 7. After trials were then compared to known standards.

Complex Carbohydrate Indicator Standard Test (Starch):

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- 1. In a test tube, 1mL of the wheat dog treat filtrate was added to 7 drops of Lugol's lodine Solution.
- 2. Samples were then given a quick mix.
- 3. Steps 1-2 were repeated 9 more times with the same filtrate.
- 4. Trials were then analyzed by color change (0 = no color change/ negative 3 very strong/ positive), and data was recorded.
- 5. Trials were then compared to known standards.
- 6. Steps 1-5 were repeated with the other flour based (soy, sorghum, and brown rice) filtrate solution.

Protein Indicator Standard Test:

- 1. 10 cuvettes were labeled for each filtrate solution.
- 30µL of each filtrate solution was placed in the appropriate cuvettes, and 1mL of Bradford 1X Dye was added.
- 3. Cuvettes were then inverted 3 times.
- 4. Trials then sat for 5 minutes.
- 5. After sitting, the trials were placed one at a time, starting with the known standards, into the Vernier SpectroVis Plus, and absorbance readings were recorded.
- 6. A standard curve graph was created with the given protein concentration versus its absorbance reading.
- 7. For each filtrate sample, the average absorbance reading was then interpolated on the standard curve graph.

Calorimeter Test:

- 1. A paper clip was pushed through the middle of a cork.
- 2. The cork was then placed into the bottom of an empty metal coffee can.
- 3. A small piece of the wheat dog treat sample was weighed using an electronic balance, and mass was recorded.
- 4. 50mL of water was added to a cleaned empty soda can, and the temperature was recorded.
- 5. A bamboo skewer was then used to suspend the soda can on top of the coffee can 2.5 cm above the cork.
- 6. The wheat dog treat sample was then stuck through the paper clip, and a lighter was used to set the sample on fire.
- 7. After the fire went out the remains of the treat sample was then reweighed, and the water in the can was remeasured and recorded.
- 8. Steps 1-6 were repeated for each flour base sample (soy, sorghum, and brown rice).
- 9. Using the equation  $Q = 50 \times \Delta T$  where T equals temperature change (after burning temperature initial temperature), the variable Q was solved for.
- 10. Using the equation Q/1000 = Calories (kilo-calories), the number associated with Q was solved for how many kilo-calories were in the sample.
- 11. The amount of kilo-calories was then multiplied by the  $\Delta$  W where W equals the weight change (initial after burning weight).

- 12. The answer to the final equation is the amount of calories in the dog treat sample.
- 13. Steps 1-11 were repeated for each flour bases (soy, sorghum, brown rice).

Data:

## Bradford's Test (Protein):

Protein Concentration (mg/mL)	Absorbance
2	0.937
1.5	0.798
1	0.579
0.75	0.39
0.5	0.301
0.25	0.105
0.125	0.059

## Standard Curve





Protein Averages (mL/mg)	Absorbance
Wheat	0.602
Brown Rice	0.528
Sorghum	0.409
Soy	0.528

# Benedict's Test (Simple Sugar):

Benedict's Averages	Percentage (%)
Sorghum	3.9
Wheat	3.2
Soy	4.5
Brown Rice	4.2



## Iodine Test (Starch):

Iodine Averages	Percentage (%)
Soy	10
Brown Rice	10
Wheat	10
Sorghum	10



### Calorimeter Test (calories):

Sample Type	Averages (Cal/g)
Brown Rice	0.580429835
Soy	0.048751503
Sorghum	0.055845827
Wheat	0.06465698



### Conclusion:

The data concluded that compared to the other flour bases, the soy flour based dog biscuit had the best overall nutritional absorbency readings with the highest Benedict's test and the lowest amount of calories calculated by the Calorimeter test. The experiment did support the hypothesis. These results were expected due to the fact of soybeans being a complete protein as well as a main source for other vitamins and minerals, thus transferring into having the most nutritious flour source based off the label. One factor that was surprising was that when analyzing the filtration liquid from each sample, the soy based biscuits produced the most amount of filtration, meaning that the dog would absorb the most out of those biscuits compared to the others.

The importance of the project was to learn which flour source would be the most beneficial to dogs, which source would be the most cost effective alternative for pet owners, how to calculate the amount of nutrients absorbed from each serving (1 biscuits) to build a product and small business, and how changing the flour source affects the properties of a dog biscuit.

## MACROMOLECULE COMPARISON OF DOG TREATS

Possible Errors	Improvements
Cuvettes getting smudged, causing inaccurate readings	Make sure to hold cuvettes correctly, and wipe down the sides
Filtrates had to sit overnight, causing them to harden, thus water had to be added to form a liquid in order to perform tests	Try to get all testing done in one day
Heat escaping into the atmosphere during the calorimeter test	Try to develop a secure area for testing
Fire burning out during the calorimeter test, causing a need for relighting, causing possibly inaccurate readings	Try to use a flame hot enough to where relighting is unneeded

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