KANSAS CORN STEM

Kansas Corn:
DDG Nutrient Testing

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KANSAS CORN COMMISSION

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Kansas Corn: DDG Nutrient Testing
Grade Level: Middle School

Overview
The availability of nutrients is important to maintaining the diets of livestock to keep them healthy. Having the correct ratio of nutrients is important to ensure that feed nutrients are not wasted, not overfed, and that feed efficiency is optimized on the farm. Scientists use specialized testing protocols to determine the major components required for healthy growth and the development of livestock.

Fermentation is the process in which sugars are broken down and fed to yeast to produce ethanol and carbon dioxide. All the remaining nutrients: protein, fat, minerals, and vitamins, are concentrated into distillers grains, which is a valuable feed for livestock.

Our job today is to test where the most nutrient availability is located by comparing corn slurry (before fermentation) with fermented corn mash. In this lab we will be focusing on three of these major nutrients: glucose, starch, and protein, which are vital components for healthy livestock.

Kansas College and Career Ready Standards
Science

- **MS-LS1-7.** Develop a model to describe how food is rearranged through chemical reactions forming new molecules that support growth and/or release energy as this matter moves through an organism.
- **MS-LS2-1.** Analyze and interpret data to provide evidence for the effects of resource availability on organisms and populations of organisms in an ecosystem.
- **MS-ETS1-3.** Analyze data from tests to determine similarities and differences among several design solutions to identify the best characteristics of each that can be combined into a new solution to better meet the criteria for success.

English

- **W.7.1.** Write arguments to support claims with clear reasons and relevant evidence
- **W.7.10.** Demonstrate command of the conventions of standard English grammar and usage when writing.
- **W.8.1.** Write arguments to support claims with clear reasons and relevant evidence.

Learning Objectives
- Students will develop a model to describe how food is rearranged through chemical reactions, forming new molecules that support growth and/or release energy as this matter moves through an organism.
- Students will analyze and interpret data to provide evidence for the effects of resource availability on organisms and populations of organisms in an ecosystem.
- Students will analyze data from tests to determine similarities and differences among several design solutions to identify the best characteristics of each that can be combined into a new solution to better meet the criteria for success.

Kansas Corn: DDG Nutrient Testing
Grade Level: Middle School

Materials

- DDG Nutrient Testing PowerPoint (available online at kansascornstem.com)
- DDG Nutrient Testing Student Packet (pg. S1-4, or available online at kansascornstem.com)

Materials for Preparation of Corn Mash: (Note: For stand-alone lab only)

- Beaker (600 ml)
- Distilled water
- Hot plate
- Amylase solution (Mix 2.0 mL of amylase concentrate with 98 mL of distilled water to produce a 2% solution. Stir thoroughly.)
- Buffer pH5 solution
- Glucoamylase solution (Mix 2.0 mL of glucoamylase concentrate with 98 mL of distilled water to produce a 2% solution. Stir thoroughly.)
- Yeast
- Parafilm
- Graduated cylinders (10 ml)
- Cheesecloth for filtering

Materials for Corn Slurry: (Note: For stand-alone lab only)

- Mortar and pestle
- Corn
- Distilled water
- Filter paper
- Beaker (25-50 ml)

Materials for Workstations: (Note: For sequence and stand-alone lab)

- 5 pipettes per station
- 6 test tubes per station
- Test tube rack
- Test tube holder (for handling after water bath)
- Parafilm (or plastic wrap and a rubber band)
- Benedict's Solution
- Lugol's Solution (Iodine)
- Biuret Reagent (Note: Do not heat.)
- Hot plate or water bath
- Beaker (600 ml) (for water bath if using a hot plate)
- Water (for hot water bath)

- Tape (for labeling test tubes)
- Sharpie (for labeling test tubes)

Safety Considerations

- All glassware has a possibility of breaking. Protective eyewear should be worn at all times.
- The indicators being used can cause skin irritations and have the possibility of staining clothes.
- Biuret Reagent, Lugol's Solution, and Benedict's Solution are all possible skin irritants. They should be handled carefully. If contact with skin occurs, wash the area with soap and water.
- Biuret Reagent is combustible at high temperatures and should not be heated.

Procedures for Instruction

Note: The following procedures are what you will use if you are doing this as a stand-alone lab. You will need to make up the mash and slurry prior to starting the lab. You also have the option to perform this lab as part of the sequence in ethanol distillation. *Kansas Corn: Ethanol – Corn Mash and Distillation* (available online at kansascornstem.com) is the recommended unit to perform first in the sequence. The nutrient test can be done before and after fermentation, as well as after distillation is complete, to compare the levels of nutrients in the different stages of production.

Length of time for preparation: 2 days for preparation (Note: For stand-alone activity only)

- Day 1: Prepare corn mash to be used on lab day
- Day 2: Slurry can be made the day of the lab.

Length of time for classroom teaching: 1-2 days of classroom work

- Day 1: For a stand-alone activity, one class period is recommended to introduce background knowledge. The DDG Nutrient Testing PowerPoint presentation is available online at kansascornstem.com.
- Day 2: Students perform lab through observations. Students will also record data and comparisons.
- Day 3: Lab review and post-lab discussion. Lab report if requested.

Preparation Procedure/Instructions

- Day 1: Ethanol Preparation Instructions (Corn Mash) (Note: For stand-alone lab only)
  - Weigh out 100 g of ground corn and add to a 600 ml beaker.
  - Heat 300 ml distilled water between 80°C to 90°C and add it to the ground corn. Stir the corn mixture. Place the beaker on a hotplate. Gently boil the solution and continuously stir for 15 minutes. Be careful not to let the corn mixture burn.
  - After boiling is completed, remove the beaker from the hotplate and allow it to cool to between 55°C and 37°C. (Note: See Teacher Resources in corn distillation lab.)
  - While the corn mash is cooling, measure 100 ml of distilled water and pour into a 250 ml beaker. Shake the amylase solution, then measure 10 ml of the amylase solution into a small graduated

cylinder and add to the 250 ml beaker of water. Stir the resulting mixture and add it to the cooled corn mash. Stir the mixture occasionally with a stirring rod throughout the next 10 minutes.

- At the end of the 10-minute period, measure 35 ml of the pH 5 buffer. Shake the buffer solution and add it to the corn mash to maintain a slightly acidic pH.
- Shake the glucoamylase solution, then measure 10 ml of glucoamylase solution. Add it to the corn mash.
- Add 5 g of yeast to the corn mash and stir the entire mixture well.
- Place a piece of plastic wrap over the mouth of beaker and secure it with a rubber band (fermentation and gas production will occur so do not secure it too tightly). Place your beaker on the counter and allow it to ferment 1-2 days.

- Day 2: Corn Slurry Preparation (For stand-alone lab only):
  - The corn slurry can be made for each group before the class period. For one group, grind 5 g of corn and mix with 20 ml of distilled water.
  - Filter the slurry into a beaker (you will need 5-10 ml for each lab group), keeping the liquid food sample.
  - Filter the corn mash in a separate beaker (you will need 5-10 ml for each lab group), keeping the liquid food sample.

- To prep for each workstation:
  - Provide each group with a small beaker of 5-10 ml of the corn slurry and a separate beaker with 5-10 ml of the fermented corn mash.
  - Provide each group with 5 separate pipets.
  - The indicators can be set out to be used when needed, or about 3 ml of each can be provided for each group.
  - Each group will need 6 test tubes and a test tube rack.

Background Information

In the United States, commercial production of fuel ethanol involves breaking down the starch present in corn into simple sugars, also called glucose. These sugars are then fed to yeast, which begins the process of fermentation. The main product is used as ethanol – it is primarily found as a fuel additive in the gasoline used in our vehicles. Some of the other products include animal feed, corn oil, and carbon dioxide. The remaining nutrients include proteins, fats, minerals, and vitamins that are essential in use in feed for livestock. Our focus is on the nutrient availability of the products before fermentation occurs and after.

We will be using three indicators to test for nutrient availability – Benedict's Solution, Lugol's Solution, and Biuret Solution. Benedict's Solution will detect the presence of glucose, and it will provide an orange to red precipitate (form a solid) after heating. The Lugol's Solution (Iodine) will turn black when starch is present.

Kansas Corn: DDG Nutrient Testing
Grade Level: Middle School

The Biuret Solution will turn purple when protein is present. This process will allow us to see where the nutrients are available at different times of the production of corn products, like ethanol.

Classroom Discussion

Let students discuss their ideas, and guide the discussion without telling them if they are right or wrong.

- Our job is to try and figure out what product will provide us with the highest nutrient availability. We will be comparing a corn slurry and a corn mash after fermentation. Have students answer the following questions on their DDG Nutrient Testing Student Packet (S1-4 or available online at kansascornstem.com):
  - What is fermentation?
  - What role does the yeast play in the fermentation process?

Investigation Question

Which of these, corn slurry (before fermentation) or corn mash (after fermentation), will provide the highest nutritional value to livestock after fermentation has occurred? Why?

Procedure for Lab

Carbohydrate Indicator Test (Glucose)

1. Label a test tube for corn slurry glucose test, and another test tube for corn mash glucose test.
2. Add 2 ml of corn slurry to the properly labeled test tube, then add 2 ml of Benedict's Solution.
3. Cover with Parafilm and mix.
4. Place test tube in a boiling water bath for 2 minutes. (Caution: Use a test tube holder as the test tube will be hot to touch).
5. Record color change in the table.
6. Repeat steps 2 - 5 in a new test tube with the fermented corn mash.

Complex carbohydrate Indicator Test (Starch)

1. Label a test tube for corn slurry starch test, and another test tube for corn mash starch test.
2. Add 1 ml of corn slurry to the properly labeled test tube and add 1 drop of the Lugol's solution.
3. Cover with Parafilm and mix.
4. Do not heat solution.
5. Record color change in the table.
6. Repeat steps 2 - 5 in a new test tube with the fermented corn mash.

Protein Indicator Test (Do not heat; heating will cause the proteins to breakdown which will give a negative test.)

1. Label a test tube for corn slurry protein test, and another test tube for corn mash protein test.

2. Add 1 ml of corn slurry and add 2 ml of Biuret Reagent to the properly labeled test tube.
3. Cover with Parafilm and gently mix.
4. Wait 30 seconds for a color change.
5. Record color change in the table.
6. Repeat steps 2 - 5 in a new test tube with the fermented corn mash.

Teacher Resources

DDG Nutrient Testing Results:

- Benedict's Solution will detect the presence of glucose, and it will provide an orange to red precipitate (form a solid) after heating. The Lugol's Solution (Iodine) will turn black when starch is present. The Buiret Reagent will turn purple when protein is present.

Visit kansascornstem.com for videos and resources to assist with this lab.

Lab Analysis

Have the students answer the following questions:

1. Were your predictions correct?
   - Students will look back at their pre-lab predictions and use the data collected from the lab to show if they were correct or not.
2. What do the results tell you about what happened during the fermentation process?
   - They should be making comparisons of the results from the different stages of production. If used as a stand-alone lab, they will have two comparisons – before fermentation and after fermentation. However, if used with the ethanol distillation lab, they will have three comparisons – before fermentation, after fermentation, and after distillation.

Assessment

The students will complete the DDG Nutrient Testing Student Packet (pg. S1-4, or available online at kansascornstem.com) as an assessment for the lab. After collecting the data with their group, each student is responsible for completing the Reflection and Conclusion section on their own. The students will then complete the lab report on their own, with the data from their lab packet.

Reflection and Conclusion

1. Our livestock are in need of protein. Based on our observations, which solution would be the best for our livestock? Explain.
   - The students will report which solution would be the best for our livestock based on the evidence they collected.

2. Based on our test results, which types of macromolecules are primarily seen after the fermentation process?
    - The students will report which macromolecules are present upon completing the fermentation process.

Taking it Further (Optional)

Protein is vital for the health and upkeep of livestock. Farmers not only need to make sure their livestock is healthy, they also have to do this as economical as possible. If you had a feedlot with 1,000 head of cattle, which type of feed would you choose for your cattle? Do some research to compare the different benefits between using distillers grains (wet (WDG) or dried (DDG)) to other corn-based feeds in the daily diet of livestock. Look for the topics below during your research. Write a two-paragraph summary of your findings.

- Cost of feed
- Availability of feed
- Nutrient supply

Science and Agriculture Careers

- Agricultural Inspector
- Agricultural Specialist
- Chemist
- Food Specialist
- Soil and Plant Scientist

To learn more about agriculture careers visit agexplorer.com. You can also find career profiles at kansascornstem.com.

Sources

- Ohio Corn and Wheat Curriculum – http://ohiocorneducation.org/
- Nutrient Management on Livestock Farms: Tips for Feeding – https://esc.rutgers.edu/fact_sheet/nutrient-management-on-livestock-farms-tips-for-feeding/
- Material Safety Data Sheet Iodine Solution – http://www.sciencelab.com/msds.php?msdsId=9924378
- Material Safety Data Sheet Benedict's Reagent – http://www.sciencelab.com/msds.php?msdsId=9925648
- Material Safety Data Sheet Biuret - http://www.sciencelab.com/msds.php?msdsId=9927459

Any educator electing to perform demonstrations is expected to follow *NSTA Minimum Safety Practices and Regulations for Demonstrations, Experiments, and Workshops*, which are available at http://static.nsta.org/pdfs/MinimumSafetyPracticesAndRegulations.pdf, as well as all school policies and rules and all state and federal laws, regulations, codes and professional standards. Educators are under a duty of care to make laboratories and demonstrations in and out of the classroom as safe as possible. If in doubt, do not perform the demonstrations.

DDG Nutrient Testing Student Worksheet

Grade Level: Middle School

Background information

In the United States, commercial production of fuel ethanol involves breaking down the starch present in corn into simple sugars, also called "glucose". These sugars are than fed to yeast, which begins the process of fermentation. The main product is used as ethanol – it is primarily found as a fuel additive in the gasoline used in our vehicles. Some of the other products include animal feed, corn oil, and carbon dioxide. The remaining nutrients include proteins, fats, minerals, and vitamins that are essential in use in feed for livestock. Our focus is on the nutrient availability of the products before fermentation occurs and after.

We will be using three indicators to test for nutrient availability – Benedict's Solution, Lugol's Solution, and Biuret Reagent. Benedict's Solution will detect the presence of glucose, and it will provide an orange to red precipitate (form a solid) after heating. The Lugol's Solution (Iodine) will turn black when starch is present. The Buiret Reagent will turn purple when protein is present. This process will allow us to see where the nutrients are available at different times of production of corn products, like ethanol.

Pre-lab Check Questions: Answer the following questions with your partners before beginning the lab.

1. What is fermentation?

2. What role does the yeast play in the fermentation process?

Investigation Question

Which of these, corn slurry (before fermentation) or corn mash (after fermentation), will provide the highest nutritional value to livestock after fermentation has occurred?

Materials

Corn Mash (4 ml per group)
5 pipettes per station
Test tube rack
Parafilm
Lugol's Solution (Iodine)
Hot plate or water bath
Water (for hot water bath)
Sharpie (for labeling test tubes)
Corn Slurry (4 ml per group)
6 test tubes per station
Test tube holder (for use with hot bath)
Benedict's Solution
Biuret Reagent Solution
Beaker (600 ml) (for water bath)
Tape (for labeling test tubes)

Procedure for Lab

Carbohydrate Indicator Test (Glucose)

1. Label a test tube for corn slurry glucose test, and another test tube for corn mash glucose test.
2. Add 2 ml of corn slurry to a test tube, then add 2 ml of Benedict's Solution.
3. Cover with Parafilm and mix.
4. Place test tube in a boiling water bath for 2 minutes. (Caution: Use a test tube holder as the test tube will be hot to touch).
5. Record color change in the table.
6. Repeat steps 2 - 5 in a new test tube with the fermented corn mash.

Complex carbohydrate Indicator Test (Starch)

1. Label a test tube for corn slurry starch test, and another test tube for corn mash starch test.
2. Add 1 ml of corn slurry in a test tube and add 1 drop of the Lugol's solution.
3. Cover with Parafilm and mix.
4. Do not heat solution.
5. Record color change in the table.
6. Repeat steps 2 - 5 in a new test tube with the fermented corn mash.

Protein Indicator Test (Do not heat; heating will cause the proteins to breakdown which will give a negative test.)

1. Label a test tube for corn slurry protein test, and another test tube for corn mash protein test.
2. Add 1 ml of corn slurry and add 2 ml of Biuret Reagent to a test tube.
3. Cover with Parafilm and gently mix.
4. Wait 30 seconds for a color change.
5. Record color change in the table.
6. Repeat steps 2 - 5 in a new test tube with the fermented corn mash.

Sample	Carbohydrate Indicator Test (Glucose)	Complex Carbohydrate Indicator Test (Starch)	Protein Indicator Test
Corn Slurry			
Fermented Corn Mash			

Lab Analysis

1. Were your predictions correct?

2. What do the results tell you about what happened during the fermentation process?

Reflection and Conclusion

1. Our livestock are in need of protein. Based on our observations, which solution would best be used for our livestock?

2. Based on our test results, which types of macromolecules are primarily seen after the fermentation process?

Take it further!

Protein is vital for the health and upkeep of livestock. Do some research to compare the different benefits between using distillers grains (wet (WDG) or dried (DDG)) to other corn based feeds in the daily diet of livestock. Look for the topics below during your research. Write a two-paragraph summary or your findings.

- Cost of feed
- Availability of feed
- Nutrient supply

Science and Agriculture Careers

These are a few of the careers that would apply to this type of laboratory specialty. Do a little research and write a short summary on one that would interest you. You may even find one not on the list.

- Agricultural Inspector
- Agricultural Specialist
- Chemist
- Food Specialist
- Soil and Plant Scientist