Protocol for Measuring ABC-4 in Feed and Feed Ingredients

ABC-4 Procedure using a Mettler Toledo SevenCompact pH/Ion S220 meter

Developed by Ethan Stas, Kansas State University,
by modifying procedures used by Lawlor et al., 2015[1]

1. **Sample preparation:** all samples should be ground to at least 400 µm
2. Measure out 0.5 g of ingredient into a weigh boat
3. Suspend sample in 50 mL distilled deionized water in a 100mL beaker. Place beaker with suspended solution onto stir bar platform and stir with magnetic stirrer softly and steadily.
    a. For minerals use 0.5 g sample with 50 mL of distilled deionized water in a 400 mL beaker. This will allow more vigorous stirring to suspend the mineral in solution. It will also prevent overflow of the beaker with the addition of high amounts of acid.
4. **Ensuring pH meter is reading accurately:** remove plastic cover on the pH meter reader, press the "on" button
    a. Ensure the pH meter is on "manual" and not "automatic". The right side of the screen with have a "M" for manual. If the pH meter has an "A", switch it to manual.
5. Prior to sample analysis: ensure pH meter is calibrated
    a. Remove solution cap from pH meter probe
    b. Rinse pH meter probe with distilled deionized water
    c. Dry pH meter probe with Kimtech Kimwipes
    d. Place pH meter probe in yellow buffer solution (pH of 7.00) and hit the green "Read" button.
        i. pH meter should stabilize at 7.00 ± 0.01, otherwise the pH meter must be recalibrated
    e. Remove pH meter probe from yellow buffer solution and rinse with distilled deionized water.
    f. Dry pH meter probe with Kimtech Kimwipes.
    g. Place pH meter probe in pink buffer solution (pH of 4.00).
        i. pH meter should stabilize at 4.00 ± 0.01, otherwise pH meter must be recalibrated.
    h. If pH meter correctly reads each buffer solution, skip ahead the below steps and move to step 6. Otherwise continue below
    i. Recalibration of pH meter: place the pH meter probe in the pink buffer solution (pH of 4.00) and press the purple "Cal" button.
    j. When the stable icon appears over the "M", press the green "Read button.

k. Remove the pH meter probe from the pink buffer solution, rinse with distilled deionized water, and dry with a Kimtech Kimwipe.
l. Place pH meter probe in the yellow buffer solution (pH of 7.00) and press the purple "Cal" button.
m. When the stable icon appears over the "M", press the green "Read" button.
n. Remove the pH meter probe from the yellow buffer solution, rise with distilled deionized water, and dry with a Kimtech Kimwipe.
o. Place pH meter probe in the purple buffer solution (pH of 10.00) and press the purple "Cal" button.
p. When the stable icon appears over the "M", press the green read button.
q. Press "Calculate" on the screen. Scroll down to the bottom of the screen and press "Save" on the screen to accept the calibration procedure.
r. Remove the pH meter probe from the purple buffer solution, rinse with distilled deionized water, and dry with a Kimtech Kimwipe.

6. ABC-4 Titration of sample (all samples should be analyzed in triplicate)

7. Place pH probe into the beaker containing magnetic stir bar and the ingredient sample into solution.
    a. The stir barn should be spinning enough to suspend the sample in solution, but not so aggressively that it creates a vortex. The speed of the magnetic stir bar will depend on the density of the sample.
8. Record the initial pH of the sample once the pH meter is stable
9. Using a micro pipette, add 0.1 N hydrochloric acid in increments of 0.1 to 5.0 mL.
    a. The amount of HCl to add will depend on the ingredient and stage of titrations. Reference previous publications or documents on the expected ABC-4 based on ingredients. Below is a general guide for HCl additions based on ingredient categories
    b. Cereal grains, co products, amino acids, and fiber: only additions of 0.1 mL
    c. Protein sources and milk products: additions of 0.5 to 1.0 mL at the start of the titration. Progressively decreased HCl additions as titration progresses and endpoint becomes closer.
    d. Premixes, carriers, and minerals: begin additions of 5.0 mL. Certain ingredients will take hours, potentially even days (Limestone and ZnO). If titration is not completed, you can find a stopping point, cover the sample with tin foil and continue the titration the following day.
    e. Acidifiers will have a starting pH below 4. Titrations will follow the exact same procedure but will use Sodium Hydroxide instead of HCl to raise the pH. Therefore, the ABC-4 will have a negative value. Acidifier blends with a carrier will require less NaOH than a pure acid.
10. Continue acid additions until a stable pH of 4 is reached. Keep track of mL of acid added to the sample.
    a. For most ingredients, initially the pH of the sample will decrease dramatically with the addition of the acid, however, as the sample begins to come in contact with added acid the pH will rise
    b. The addition of too much acid will result in a lower pH than desired. Pay close attention to how quickly the pH of the sample rises after each acid addition

(the pH will rise slower as you approach the ABC-4 value of the ingredient sample).

11. Once the pH meter becomes stable at a pH of 4 ± 0.04 record the final pH as well as the total amount of acid added to the beaker in mL.
    a. The pH meter must be stable for at least 1 continuous minute to consider the analysis completed. Use a stopwatch to time the sample.
12. Calculate ABC-4 (acid-binding capacity) and BUF-4 (buffering capacity)
    a. ABC-4 = mL of acid added × 0.1N × 2000
    b. BUF-4 = mL of acid added / (initial pH – final pH)
13. Dispose of ingredient solution in the sink designated by the lab technician.
14. Clean pH meter probe with distilled deionized water and a Kimtech Kimwipe.

[1] Lawlor, P. G., P. B. Lynch, P. J. Caffrey, J. J. O'Reilly, and M. K. O'Connell. 2005. Measurements of the acid-binding capacity of ingredients used in pig diet. Ir. Vet. J. 58:447-452. doi:10.1186/2046-0481-58-8-447.