administration, and withdrawal time (WDT) for each drug is obtained from the FARAD website; www.farad.org and EWDT are determined for higher doses following single and multiple doses in target species. To determine how an increased single dose from the label dose affects plasma concentrations, the number of dose doublings are calculated using the following equation:

Number of dose doublings = log2 (dose2/dose1) Equation 1

Where dose 2 = extra-label dose; dose 1 = label dose.

Following multiple administrations of the drug, dose doublings are calculated using the equation 2.

Number of dose doublings = log2((dose2×accumulation factor of 2)/(dose1×accumulation factor of 1))

Equation 2

EWDT = label withdrawal time (WDT) + (# of doublings × tissue half-life) ---Equation 3

Results

Drug plasma concentrations in the animal's body depend upon the amount of drug administered and the rate at which it is cleared from the body. An increased dose from the FDA approved label dose increases concentrations of the drug in the plasma and tissues of the treated animal, and has the potential to cause drug residues in their food products, therefore, need arises to determine EWDT. Examples of scenarios commonly encountered by food animal practitioners in field and calculated EWDT are given below.

1. Drug A label: approved for cattle, beef, non-lactating dairy, 3 to 5 mg/lb (6.6 to 11 mg/kg)/day, IM/IV, do not exceed volume 10 mL per injection site and FDA approved WDT is 22 days.

Extra-label use of Drug A: beef cattle, 9.1 mg/lb (20 mg/kg)/day, IM, 7 doses, volume 15 mL per injection site, EWDT = 42 days.

2. Drug B label: approved for cattle, 1.14 mg/lb (2.5 mg/kg), SC, single injection, don't exceed volume10 mL per injection site and WDT is 18 days.

Extra-label use of Drug B: cattle, 2.27 mg/lb (5.0 mg/kg)/day, SC, 2 doses, q 24h, EWDT = 30 days.

3. Extra-label use of Drug B: goat, 2.27 mg/lb (5.0 mg/kg)/day, SC, 2 doses, q 24h, EWDT = 65 days.

EWDT for Drug B is longer for goat (65 days) than cattle (30 days) when used at the same extra-label dosage schedule because there is no MRL/ tolerance for this drug in goats.

Significance

In compliance with the stipulations set forth by AMDUCA, extended withdrawal intervals are required to ensure that food originated from food animals treated with extra label use of antimicrobials is free from harmful residues. Scientific approaches can be used to establish extended withdrawal times following extra label drug use and provide safe food to consumers.

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Effects of Tri-Lution® on serum volatile fatty acids, electrolytes, and trace mineral status in commercial veal calves

L.A. Krueger, MS; **G.A. Ayangbile**, PhD
Agri-King, Inc, Fulton, IL 61252

Introduction

Commercial veal calves in the United States may be deficient in iron as an outcome of consuming primarily milk or milk replacer with limited grain intake. Iron deficiency promotes the desired coloration of veal, but may also predispose calves to metabolic stress. Tri-Lution® contains proprietary, probiotic strains of *Saccharomyces cerevisiae* and *Enterococcus faecium*, which is a lactic acid producing bacterium. Together with prebiotic nutrients, these probiotic microbes are hypothesized to sequester coliform bacteria and promote reductive fermentation in the intestine. In January 2017, a commercial veal calf grower in the midwestern United States reported to us that calves fed Tri-Lution® showed improved

dietary intake, manure consistency, and thrift compared with control calves during an internal performance trial. The calves in that trial became the subjects of the present study, of which the objective was to quantify metabolic differences between control and Tri-Lution®-fed calves.

Materials and Methods

Calves were subject to either 1 of 2 dietary treatments, which were the control diet (CTRL) or the control supplemented with Tri-Lution® (TL). The basal diet was whole milk obtained from a milk processing facility as well as a complete grain mix (approximately 12.1% CP, 59.0% starch DM) fed at approximately 2.2 lb (1.0 kg/d). Supplemented calves received Tri-Lution® DF at a rate of 2.0 g per hd/d with approximately 7.0 x 10^9 cfu of combined lactic acid bacteria and yeast per g. Calves were housed indoors in compliance with the American Veal Association resolution for group housing with 8 calves per pen. When calves were approximately 17 weeks old, 1 calf from each of 15 CTRL pens and 20 TL pens was sampled for serum and plasma. Glucose, non-esterified fatty acids, and betahydroxybutyrate were measured in plasma by colorimetric assay, whereas volatile fatty acids were measured in serum by gas chromatography, and minerals in plasma were measured by inductively coupled plasma (ICP) spectroscopy. Upon slaughter at approximately 20 weeks of age, livers were obtained from 15 randomly selected calves from each group. Minerals were quantified in liver tissue dry matter by ICP. Data were summarized (Statistix10) as mean ± standard error and differences between means were tested for significance ($P < 0.05$) or a statistical trend ($P < 0.1$) with a 2-sample t-test.

Results

Concentration of total volatile fatty acids in serum tended to be greater in TL (994 ± 45 μM) than CTRL (862 ± 46 μM) calves ($P < 0.1$), whereas acetic and isobutyric acids were significantly greater ($P < 0.05$) in TL (632 ± 34 μM acetate and 17.3 ± 1.9 μM isobutyrate) than CTRL (536 ± 26 μM acetate and 10.7 ± 2.4 μM isobutyrate). No differences in glucose, NEFA, or BHB were detected between groups. Differences between CTRL and TL, respectively ($P < 0.05$) were detected for total Na (2901 ± 11.3 ppm and 2999 ± 33.7 ppm), K (810 ± 8.3 and 881 ± 12.9 ppm), Ca (104 ± 0.7 and 108 ± 1.1 ppm), P (140 ± 3.6 and 151 ± 1.5ppm), and S (889 ± 11.9 and 948 ± 15.0 ppm), but no difference was detected for Cl. Of note, EDTA used to prepare plasma is known to chelate calcium, but only the measure of ionized Ca is affected; our measurement of total Ca in plasma is valid. Upon slaughter at approximately 20 weeks of age, significant differences ($P < 0.05$) for minerals in liver tissue were noted for Fe (64.7 ± 3.2 and 90.4 ± 7.0 ppm) and for Mn (4.56 ± 0.14 and 5.21 ± 0.18 ppm).

Significance

The increased concentrations of volatile fatty acids in serum indicates increased production of organic acids (namely acetate and isobutyrate) during digestion. Concomitant with increased acidity was markedly increased deposition of Fe and Mn in hepatic tissue, as well as increased concentration of electrolytes in plasma at the time of sampling. The results lead to a single hypothesis that Tri-Lution® promoted the fermentation of feeds for improved acidity, which favored the dissociation and greater bioavailability of mineral salts.