

Efficacy of Binders on Per- and Poly-fluoroalkyl Substances (PFAS) and Aflatoxin B₁ (AFB₁) Levels under *In Vitro* Ruminal Conditions

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We evaluated a collection of PFAS binders under simulated ruminal conditions using contaminated grass (n= 4 blocks). The binder treatments included clay binder 1 (CLY1) and 2 (CLY2), polysaccharide binder (PLS), carbonaceous binder (CRB), and anion exchange resin 1 (AER1) and 2 (AER2). The sequestration of AFB₁ under simulated ruminal conditions was used as a positive control of binding. Thus, only CLY1 and CLY-2 were tested using alfalfa (n= 3 blocks) spiked with AFB₁. For PFAS treatments, ground grass (3 g) was mixed with 0.05 g for each binder (except AER1, 0.01 g) before adding rumen media. Bottles were shaken at 60 rpm for 48 h at 39°C. For AFB₁ treatments, ground alfalfa (3 g) was mixed with 0.05 g of each binder before adding sterile rumen media. Bottles were shaken at 60 rpm for 2 h at 39°C. A randomized complete block design was used to analyze the AFB₁ and PFOS (perfluorooctane sulfonic acid) results using SAS v.9.4. Only AER2 proved to bind PFOS under ruminal conditions with a relative binding of $52.5 \pm 8.59\%$, higher than any other binder tested ($P < 0.05$). None of the binders tested affected the rate and maximum ruminal gas production ($P > 0.05$). CLY1 and CLY2 bound 80.7 ± 3.8 and $9.45 \pm 3.8\%$ of AFB₁, respectively, with the former being higher than the latter ($P < 0.05$). AER2 was the only binder that effectively sequestered PFOS under in vitro ruminal conditions, and it did so without negatively affecting ruminal gas production kinetics.

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