EFECT OF ACETATE ADDITION AND HEADSPACE GAS COMPOSITION ON IN VITRO PRODUCTION OF VOLATILE FATTY ACIDS AND GASES

Latisha M. Judd*, Rick A. Kohn
University of Maryland College Park
Department of Animal Science
8127 Regents Drive
College Park, MD 20742

*Email: ljudd@umd.edu

ABSTRACT: In vitro methods have been developed to measure digestibility, but such methods may not accurately estimate volatile fatty acid (VFA) profile. Therefore, new methods are needed to measure the production of VFA in order to better understand the mechanisms of rumen fermentation. The development of in vitro methods would facilitate replication of multiple treatments, and enable isolation of fermentation effects from animal interactions. Gas production and VFA production are stoichiometrically linked, therefore gas pressures could affect VFA profile. The purpose of this experiment is to examine the effects of different ratios of gases (CO₂, CH₄, and H₂) and acetate addition on the VFA profile of an in vitro system. The results should elucidate the importance of headspace gas composition for in vitro techniques. In vitro tubes were subjected to different experimental combinations including 4 different gas profiles, with or without addition of sodium acetate. Experimental design was a 4 × 2 factorial CRD with 4 replicates. In each tube, 0.10 g of Timothy hay was added. Total volume of the in vitro tubes was 20 mL. 100% rumen fluid was used in this experiment to focus on the effect of gas and acetate. Gas mixture combinations were calculated by volume and prepared in 5-L balloons at levels of 100% CO₂, 50/50 CH₄/CO₂, 95/5 CO₂/H₂, and 47.5/47.5/5 CH₄/CO₂/H₂. The volume of each tube was filled with the respective gas mixture and thereafter syringes were attached to the tubes. Tubes were then given 0.5 mL of 1M acetate (NaOAc) or 0.5 mL of buffer solution through a needle into the tubes and 9.5 mL of rumen fluid were added to each tube. The tubes were incubated at 39°C while shaking. Each tube was sampled for liquids and gases at 0, 4, 16, 24, and 48 hours, and VFA and gas volume were determined. Propionate production at 48 hours was highest (P<0.05) for the 47.5/47.5/5 CO₂/CH₄/H₂ mixture (10.60 mmol, SE = ± 0.815 ml). This could be due to the enrichment of hydrogen in the system promoting conversion to propionate. Propionate production for the 50/50 CO₂/CH₄, 100% CO₂, and 95/5 CO₂/H₂ were 8.97, 8.71, and 7.12 mmol (SE±0.789) respectively. Gas mixtures affected (P<0.05) butyrate production at 4 hours. The 50/50 CO₂/CH₄, 100% CO₂, 95/5 CO₂/H₂, and 47.5/47.5/5 CO₂/CH₄/H₂ gas mixtures had butyrate production values of 3.09, 2.96, 2.33, and 1.44 (mmol; SE±0.437) respectively. There was an effect (P<0.01) of the gas mix on gas production at 4 hours. The 50/50 CO₂/CH₄ gas mix produced the most gas (2.70 ml), followed by the 47.5/47.5/5 CO₂/CH₄/H₂, the 100% CO₂, and the 95/5 CO₂/H₂ with values of 1.43, 1.08, and 0.98 (ml) (SE±0.327). Lower starting concentration of CO₂ would have resulted in CO₂ efflux from the buffer. At 24 hours there was a trend (P=0.058) of the gas mix, on the acetate: butyrate (A: B) production ratio. The 100% CO₂, 95/5 CO₂/H₂, and 50/50 CO₂/CH₄ gas mixtures had A: B production ratios of 2.95, 2.87, and 2.77 (mmol) (SE±0.208) respectively, while the 47.5/47.5/5 CO₂-CH₄-H₂ gas mix had the lowest ratio of 2.18 (mmol) (SE±0.208). There was a trend (P = 0.08) for greater acetate production with acetate addition (10.71 mmol) than without (7.11 mmol, SE±1.413). Initial gas composition of in vitro procedures can affect gas production and VFA.
profiles with higher percentage of CH₄ and H₂ in headspace favoring propionate and butyrate over acetate and gas production.
CHARACTERIZATION OF THE PREVALENCE AND MANAGEMENT OF OBESE PONIES AND HORSES IN MARYLAND

Aubrey E. Lowrey* and Amy O. Burk

The University of Maryland
Department of Animal and Avian Sciences
8127 Regents Drive, Bldg. 142
College Park, MD 20742

*Email: alowrey@umd.edu

ABSTRACT: It has been estimated in parts of the U.S. and abroad that 30-40% of the equine population suffers from obesity. Laminitis, a debilitating and potentially fatal hoof condition, is one of the most serious obese-related disorders. Weight management strategies for obese equine include feeding lower quality hay in a dry lot, applying muzzles while grazing, and administering experimental medications. The purpose of this study was to evaluate the prevalence of obesity in pony and horse populations in Maryland, characterize weight management strategies used, and to ascertain whether the strategies impacted the operation. A 25-question internet survey was developed using surveymonkey.com, piloted, and approved by the University’s Internal Review Board (684776-1). Using a multiple mailing strategy, invitations to participate were mailed to 769 licensed horse farm operators in Maryland in the late summer and early fall of 2015. A total of 93 farm operators completed the survey. The average age of participants was 55 with the majority being female (87%) and having more than 21 years horse experience (63%). A total of 238 ponies and 1290 horses were represented with the primary use of farms being boarding followed by lessons. Nearly all operators (96%) indicated they managed at least one obese pony or horse on their farm. Participants indicated that 41% of their ponies (n=97) and 40% of their horses (n=512) were either fat or very fat. Of that population, 24% of ponies suffered from laminitis within the last five years as opposed to 5.3% of horses and 9.3% of ponies suffered from insulin resistance within the last five years compared to 8.6% of horses. Obese horses had a higher prevalence of arthritis compared to obese ponies (12.5% vs. 2.1%, respectively). For obese ponies, 70% were housed in a dry lot and 50.5% wore grazing muzzles while on pasture. Conversely, only 14.1% of obese horses were housed in a dry lot while 16% wore grazing muzzles while on pasture. In both groups, medication was the least used management tool. Of the participants that used dry lots, 75% felt they required more maintenance and 78% felt that using dry lots increased time spent caring for their obese animals. Operators were most satisfied with using exercise for weight management, followed by housing horses in dry lots. They were least satisfied with using grazing muzzles and administering medication. Grain was fed to 27.8% and 11.5% of the obese ponies and horses, respectively, while forage balancer was fed to 12.4% and 4.1%, respectively. Operators reported they spent an average of $434.18 ± $146.30 more each year to manage their obese equines compared to their non-obese equines. In conclusion, a significant portion of Maryland’s horses and ponies are obese with laminitis more prevalent in the pony population. Additionally, an increased effort in farm maintenance, labor, and operational costs were observed when managing obese equines. These findings support the view that prevention of equine obesity remains the best option for promoting optimal health in the horse.
RECOMBINANT BACTERIOPHAGE ENDOLYSIN, PLYC, IS NON-TOXIC AND DOES NOT ALTER BLOOD NEUTROPHIL OXIDATIVE RESPONSE IN LACTATING DAIRY COWS

Cynthia M. Scholte*,1, Daniel C. Nelson2,3, Theodore H. Elsasser4, Stanislaw Kahl4, Erin E. Connor4, Yang Qu1 and Kasey M. Moyes1

1Department of Animal and Avian Sciences, University of Maryland, College Park
2Institute for Bioscience and Biotechnology Research, University of Maryland, Rockville
3Department of Veterinary Medicine, University of Maryland, College Park
4U.S. Department of Agriculture, Agricultural Research Service, Beltsville, MD

University of Maryland-College Park
Department of Animal Science
8127 Regents Dr.
College Park, MD 20742

*Email: cscholte@umd.edu

ABSTRACT: Mastitis is the leading cause of antimicrobial use on dairy farms. The potential for antimicrobial resistance have led to the examination of alternative strategies for controlling mastitis. The streptococcal C1 phage endolysin, PlyC, is the most potent endolysin described to date and causes targeted lysis of the cell wall of Streptococcus uberis. A relatively low concentration (1.0 μg/mL) of recombinant PlyC (rPlyC) can induce lytic activity, suggesting that a low dose may successfully eliminate infection. We evaluated the dose effect of rPlyC on cytotoxicity and oxidative response of bovine blood neutrophils. We hypothesized that rPlyC would be non-toxic and not alter the inflammatory response of neutrophils in vitro. Cells were isolated from plasma obtained from healthy, mid-lactation primiparous dairy cows (n=12) and incubated with various concentrations of rPlyC (0, 1, 10, and 50 µg/mL) for 0.5 and 2 hours. Following incubation, cytotoxicity was measured by non-radioactive, colorimetric assay to quantify lactate dehydrogenase. Oxidative response was measured by chemiluminescence assay of reactive oxygen species (ROS) production in response to 0 and 1.6 μg/mL phorbol 12-myristate-13-acetate (PMA) in addition to rPlyC during incubation. Data were analyzed as ANOVA using mixed model procedures in SAS (version 9.3). As expected, neutrophil cytotoxicity varied across incubation time with greater cell toxicity measured at 2 hours incubation as compared to 0.5 hours (P=0.01; 55 vs. 45±3%) and is primarily attributed to the short half-life of neutrophils. Oxidative response was affected by incubation time (P=0.04) and PMA concentration (P<0.01) with the greatest ROS production at 0.5 hour incubation in the presence of 1.6 μg/mL PMA. Concentration of rPlyC did not affect oxidative response (P=0.73) nor neutrophil cytotoxicity (P=0.41). In summary, varying doses of rPlyC are non-toxic and do not alter ROS production in bovine neutrophils. The use of rPlyC as an alternative therapy for Streptococcus uberis mastitis is promising where rPlyC may not interfere with immune response during mastitis.
EFFECTS OF DIETARY CALCIUM ON ENERGY DIGESTIBILITY AND BIOAVAILABILITY OF CORN AND SOY OIL IN COMMERCIAL BROILER CHICKS

Brittany Singh*, M.E. Persia

Virginia Polytechnic Institute and State University
3060 Litton-Reaves Hall
175 W. Campus Drive
Blacksburg, VA 24061

*Email: bsingh1@vt.edu

ABSTRACT: It has been suggested that dietary Ca may interact with dietary oil causing the formation of lesser digestible soaps; reducing energy digestibility and ultimately performance. The objective of this experiment was to determine the effects of dietary Ca (0.9% or 2.0% Ca) on performance, body composition and energy digestibility when fed to broiler chickens consuming either corn or soybean oil. The four dietary treatments consisted of 0.9 and 2.0% Ca diets fed across diets that contained either corn or soy oil in a 2 x 2 factorial arrangement of treatments. Treatments were applied to 10 replicate cages of five chicks each, resulting in 200 chicks for the 28 day feeding period. Birds were housed in raised wire cages to allow for excreta collection for AMEn determination. Body weight and feed intake were measured over the 28 day period and mortality corrected feed efficiency was calculated. At the end of the 28 d period all remaining birds were euthanized, scalded and plucked to remove feathers before being scanned for lean tissue and fat tissue via Dual-energy X-ray absorptiometry (DXA). There were no main effects or interactions of oil source and dietary Ca on 28 day body weight. Feed efficiency tended to be reduced in the birds fed the 2.0% Ca diets (P≤0.10), but oil source had no effect and there was no interaction. The 2.0% dietary Ca resulted in a reduction in total body fat mass and body fat percentage, as determined by DXA scan, without an oil source main effect or interaction (P≤0.05). No significant effects were seen on total lean mass. The higher Ca concentration did result in lower feed efficiency, total body fat and body fat percentage, although there did not appear to be an interaction between dietary Ca and oil source in these birds as both the soy and corn oils were negatively affected by higher dietary Ca content. It does appear that body composition (total fat mass and body fat percentage) is a more sensitive measurement of bird energy status in comparison to feed efficiency (significance v. trend).
IMPACT OF GRAZING SYSTEM ON FORAGE CARBOHYDRATES AND HORSE METABOLISM

An V. Le1*, Laura B. Kenny1, Amy O. Burk2, Carey A. Williams1

1Rutgers, The State University of NJ
Department of Animal Science
84 Lipman Dr., Bartlett Hall
New Brunswick, NJ 08901

2University of Maryland
Department of Animal and Avian Sciences
1117 Animal Sciences Center
College Park, MD 20742

*Email: anle4@yahoo.com

ABSTRACT: There has been research showing the benefits and drawbacks of rotational (R) vs. continuous (C) grazing in livestock. Due to the horse’s unique grazing behaviors, data derived from studies using other livestock cannot be applied to horses. Equine grazing affects the health of the horses and pasture. There has been a lack of research on the nutritional impact in horses grazing on different pasture systems. The objective of this project was to determine the effects of different grazing systems on forage carbohydrate fractions and on glucose dynamics by evaluating plasma glucose and fecal pH in horses grazing either C or R systems. The hypothesis was that in R systems, the taller grasses would contain higher fiber/lower sugar fractions and therefore the horses grazing R systems would have a lower glucose response than those grazing C systems. This project was conducted at the Rutgers University Ryders Lane Best Management Practice Demonstration Horse Farm using a replicated C and R system, each consisting of 1.6 ha. Horses in the C systems had access to all areas of the pasture, a run-in shed, hay feeders and a water source. Horses in the R systems utilized a central sacrifice area, which contained a run-in shed, hay feeders and a water source and then were allowed access to one of four smaller (0.40 ha) pastures. Only one pasture was accessible at a time, allowing grasses to rest and regrow between grazing bouts. The trial took place in June 2015, on previously established Camas Kentucky bluegrass, Potomac orchardgrass and Jesup MaxQ endophyte-friendly tall fescue pastures. Twelve mature Standardbred mares were split into four groups (n = 3; 0.52 ha per horse) and assigned to either a C or R system. Horses were housed for 12 hrs overnight in stalls prior to sampling and fed a moderate quality grass hay to meet half of their daily requirement. The first sample of blood, forage and feces was taken at 0800. Samples were collected every four hours thereafter. Blood was collected through jugular catheters, aliquoted for plasma and serum and stored at - 80°C until further analysis. Hand-clipped forage samples were placed on dry ice and mailed overnight to Equi-Analytical Laboratories (Ithaca, NY) to be analyzed for acid detergent fiber (ADF), neutral detergent fiber (NDF), ethanol soluble carbohydrates (ESC), water soluble carbohydrates (WSC) and starch. Data was analyzed using a repeated measures ANOVA in SAS with significance set at P < 0.05. There were no significant differences in the ADF, NDF, starch, WSC or ESC between treatments. Overall, plasma glucose was higher in the C horses compared to the R horses (115.6 ± 1.9 mg/dl vs 109.0 ± 1.9 mg/dl, respectively; P < 0.05). The C horses had a corresponding lower fecal pH compared to R horses (6.2 ± 0.1 vs. 6.6 ± 0.1, respectively; P = 0.018). Our hypothesis was supported for glucose response; however, there were no differences in sugar fractions between systems. Further analysis of data (insulin concentration) and more trials are needed to give a more complete picture of the relation between forage carbohydrates and horse metabolism in response to grazing systems.
THE EFFECTS OF FLAME RETARDENT ON HYPOTHALMIC ARCUATE GENE
EXPRESSION AND ENERGY HOMEOSTATIS

Vipa Patel*, Elizabeth A. Krumm, Ali Yasrebi, Troy A. Roepke

Rutgers, The State University of New Jersey
Department of Animal Science
84 Lipman Dr., Bartlett Hall
New Brunswick, NJ 08901

*Email: vjp53@scarletmail.Rutgers.edu

ABSTRACT: Endocrine disrupting compounds (EDCs) are compounds found in the environment that interfere with the normal endocrine system. When EDCs interact with a wide variety of receptors in the hypothalamus, they can cause changes in feeding behavior and energy expenditure. Some EDCs are estrogenic and interact with steroid receptor like the estrogen receptors (ER). ERs are expressed in the hypothalamus to control homeostasis including reproduction and energy expenditure. One group of potential EDCs are flame retardants (FR) because they have been shown to interact with steroid and nuclear receptors in in vitro experiments. FR are persistent in the environment because of their use in plastics, furniture, clothing, toys, and electronics. Because little is known about the effects of FR on mammalian energy homeostasis, we hypothesize the exposure to these compounds would alter energy balance and hypothalamic gene expression. For this project two experiments were conducted - Experiment #1 examined hypothalamic gene expression and Experiment #2 monitored whole animal physiology after 4 weeks of oral exposure to FR. In Experiment #1, four treatments were used; oil: negative control, EE2 (2.5 μg/kg/day): positive estrogenic EDC control, BDE-47 (1 mg/kg/day or 10 mg/kg/day), and a mixture of organophosphate flame retardant (OPFRs, 1 mg/kg/day or 10 mg/kg/day of TPP, TCP, TDCPP). The mice were orally dosed with FR using peanut butter as the vector for 28 days. In Experiment #2, mice were orally dosed using the same method as Experiment #1 but with only the low dose of BDE-47 and OPFR. Body weight and food intake were recorded weekly. At the end, body composition was measured using an MRI and glucose tolerance test and insulin tolerance test were conducted. Terminal plasma was also analyzed for ghrelin, insulin, and leptin. For Experiment #1, Pomc, Insr, Ghsr, and Lepr expression was elevated and Cart, Npy, Agrp, and Esr1 (ERα) expression was suppressed by FR in males. In females, FR increased Insr expression and decreased in Pomc and Cart. For Experiment #2, EE2 and OPFR suppressed body weight in males, while in females, EE2 augmented body weight gain. OPFR suppressed energy intake in males. BDE-47 suppressed lean mass in females. For the GTT, there was no significance in males but in females EE2 and BDE-47 augmented glucose clearance. For ITT, there was no significance in either sex. Fasting glucose levels were elevated in males by FR. FR increased plasma ghrelin but decreased plasma leptin and insulin independent of body weight in males. The results from both experiment show that there are dose-dependent and sex-dependent effects of FR exposure in mice as males were more sensitive to the FR especially in the hypothalamus. The physiological implications were that OPFR reduced male body weight and energy intake and that FR induced hyperglycemia, hypoinsulinemia, hyperleptinemia, and hyperghrelinemia in males, but increased glucose clearance in females. Future studies will examine the role of ERα in mediating these effects using ERα knockout mice.