
457 Ionomics: Mineral nutrition, physiology, and interactions as a biological system. J. Fleet* and D. Salt, Purdue University, West Lafayette, IN.

Inorganic elements (metals, non-metals, transition elements, and electrolytes commonly called “minerals” in nutrition science) are critical to all life processes as enzyme cofactors, stabilizers of proteins, structural components of tissues, second messengers, regulators of acid-base balance, participants in redox reactions, and for the maintenance of cellular electrical potential. For this reason, the required mineral elements are essential for the optimal function of a broad array of physiological systems and dysregulation of their metabolism can influence, or be a marker of, disease processes. While traditional reductionist approaches have revealed many aspects of mineral metabolism and function, significant gaps still exist. For example, we don’t know all of the proteins that mediate mineral metabolism or whose function is altered by changes in mineral status. It is also clear that mineral-mineral interactions exist but we don’t fully understand how they influence the absorption, excretion, storage, and utilization of chemically similar elements. This presentation will explain how understanding the breadth of biological processes influenced by minerals, identifying the genes that control mineral metabolism, and revealing the importance of interactions between inorganic elements can be accomplished by using a new approach that examines the metabolism of minerals in toto, or as an “ionome”.

Key Words: ionome, minerals

458 Trace mineral interactions, known, unknown and not used. G. M. Hill* and J. E. Link, Michigan State University, East Lansing.

Potentially, trace mineral interactions can occur anywhere from the feed to the organ of storage to exogenous secretions etc. If and where they take place or if it makes a difference, also depends on your point of view. Excess dietary trace elements provided by feedstuffs are usually assumed to be non-existent and/or not available for the animal’s use. However, their role in altering the functional outcome for another trace element may be as critical as meeting the animal’s unknown requirement. Using the treatise of Hill and Matrone (1970) we can expect interactions based on the chemical and physical properties of the element. For example in aquatic birds, Miller (1996) found an hepatic Mg-Zn interaction due to the spherically symmetric valence orbitals with similar ion pair formation and both ions involved in catalytic hydrolysis of ATP. Yet, this interaction is seldom studied in livestock. Well known interactions such as Fe and Zn are affected by source, species of the element, dietary concentrations and which element is in excess. However, it is not possible to look at the biochemical markers of these two elements without also considering Cu. In areas where I is deficient, the supplementation of Fe and I is another consideration often forgotten due to the large amounts of Fe found in Ca sources. Since Zn is essential in the conversion of β-carotene to vitamin A, perhaps we need to broaden our interaction model to consider all 5 nutrients. One of the most relevant interactions today due to the varying concentration of S in DDGS is the Cu/Mo/S interaction. While this may be of greater interest in ruminant animals, it should be noted that a Cu/Fe/Mn and S amino acid interaction also occurs in non-ruminants. Few interaction studies have utilized molecular techniques, so the tools of today are needed to understand practical application of interactions. Clearly, the potential for interactions can affect the functional outcome we desire in dietary formulation.

Key Words: trace elements, interactions

459 Macromineral interactions. J. S. Radcliffe*, Purdue University, West Lafayette, IN.

Our understanding of mineral availability, absorption, and utilization is minimal at best. Historically, minerals have been individually studied and requirements determined. However, research has reported numerous interactions between minerals. One of the most noted interactions is between Ca and P in which it has been reported that excess Ca can cause the formation of insoluble Ca-phosphate salts, resulting in decreased P availability. In human medicine this is exploited as a treatment for hyperphosphatemia, where Ca carbonate is used as a phosphate binding agent. However, it is unclear in animal nutrition what should be done with Ca when varying P levels are investigated or when phytase is included in the diet. In reality there are dozens of mineral interactions occurring at any time making it difficult to study any one mineral individually. Another way of stating this is that the concentrations and proportions of every mineral in the diet may impact the results of the mineral being studied. It is impossible to take account for all mineral interactions experimentally, but a better understanding of mineral interactions is needed for proper interpretation of results and requirement estimates.

Key Words: mineral, interactions

Physiology and Endocrinology: Estrous Synchronization of Beef Cattle

460 ASAS Early Career Achievement Award Presentation: Control of the estrous cycle for fixed-time artificial insemination (TAI) in beef cattle. G. C. Lamb*, North Florida Research and Education Center, University of Florida, Marianna.

Early estrus-synchronization protocols focused on regressing the corpus luteum with an injection of prostaglandin F₂α (PG) followed by detection of estrus. Later, estrus-synchronization systems involved the use of exogenous progestins, which (when administered) prevented estrus from occurring. Gonadotropin-releasing hormone was utilized to control follicular waves to synchronize ovulation and luteinization of large dominant follicles. Our research aimed to develop: 1) reliable protocols that relied solely on TAI; 2) protocols that required a maximum of 3 animal handlings; and 3) protocols that are successful in estrous cycling and noncycling females. In cows, insertion of a CIDR during the 7-d interval between the initial GnRH injection and PG enhanced pregnancy rates by 9 to 10%. In a multi-location study, a fixed-timed AI (TAI) protocol yielded similar pregnancy rates as a protocol involving detection of estrus plus a clean-up AI (54 vs. 58%, respectively, for cows and 53 vs. 57%, respectively, for heifers). A meta-analysis of data for a TAI protocol containing a progestin resulted in pregnancy rates of 54.7% and 50.3% for cycling for noncycling cows, respectively. A similar analysis for the same TAI protocol without a progestin resulted in pregnancy rates of 47.1% and 34.0% for cycling and noncycling cows, respectively. Recent work has indicated that human chorionic gonadotropin more effectively caused ovulation of follicles in cycling.
and noncycling females than GnRH, but when administered in a TAI protocol 9 or 10 d before or at the time of TAI fertility was compromised. Development of TAI protocols that reduce the hassle factors associated with ovulation synchronization and AI provide cattle producers efficient and effective tools for improving genetics in their operations. Location variables, however, that may include differences in pasture and diet, breed composition, body condition, postpartum interval, climate, and geographic location, may affect the success of TAI protocols. Acknowledgments to CR Dahlen, JE Larson, G Marquezini, and JS Stevenson.

Key Words: estrus synchronization, artificial insemination, beef cattle

461 Comparison of progestin-based protocols to synchronize estrus in prepubertal and estrous cycling beef heifers. N. R. Leitman, D. C. Busch, D. J. Wilson, D. A. Mallory, M. R. Ellersieck, M. F. Smith, and D. J. Patterson*, University of Missouri, Columbia.

The objective of the experiment was to compare differences between 2 controlled internal drug release (CIDR)-based estrus synchronization protocols on the basis of their ability to facilitate a highly synchronized estrus following treatment in beef heifers. The hypothesis tested was that addition of GnRH in a CIDR-based estrus synchronization protocol is required to facilitate an improvement in synchrony of estrus after PG_{2α} (PG). Beef heifers (n = 285) were assigned to 1 of 2 treatments within reproductive tract scores (2 or 3 = prepubertal; 4 or 5 = estrous-cycling) by age and BW. Heifers assigned to CIDR Select received a CIDR insert (1.38 g progesterone) from d 0 to 14 followed by GnRH (100 μg, i.m.) on d 23 and PG (25 mg i.m.) on d 30. Heifers assigned to Show-Me-Synch received a CIDR insert from d 0 to 14 and PG on d 30. Heifers were fitted with HeatWatch estrus detection system transmitters at PG for continuous estrus detection during the synchronized period, and AI was performed 12 h after the onset of estrus. Estrous response did not differ (P = 0.43) between treatments (94% CIDR Select, 98% Show-Me-Synch). Mean interval to estrus after PG was 7 h shorter (54.4 ± 1.7 h, Show-Me-Synch; 61.5 ± 1.7 h, CIDR Select; P = 0.01) and variance for interval to estrus was reduced (P < 0.01) among Show-Me-Synch compared to CIDR Select treated heifers. Conception rate to AI tended (P = 0.09) to be greater for Show-Me-Synch (67%) compared to CIDR Select treated heifers (58%), and AI pregnancy rate was greater (P = 0.05) for Show-Me-Synch (66%) compared to CIDR Select treated heifers (55%). Final pregnancy rate at the end of the breeding season was similar between treatments (81%, CIDR Select; 81%, Show-Me-Synch; P = 0.94). These results suggest that administration of GnRH 9 d after CIDR removal in the CIDR Select protocol does not facilitate an improvement in synchrony of estrus or pregnancy rate resulting from AI in beef heifers. This project was supported by National Research Initiative Competitive Grant no. 2005-55203-15750 from the USDA Cooperative State Research, Education, and Extension Service.

Key Words: beef heifer, estrus synchronization, progestin


This experiment compared estrous response and synchrony of estrus after administration of two progestin-based protocols to synchronize estrus in beef heifers. Heifers (n = 396) were assigned to 1 of 2 treatments by age, BW and reproductive tract score (RTS). Pretreatment RTS were assigned 14 or 15 d prior to treatment to determine estrous cyclicity status (2 or 3 = prepubertal; 4 or 5 = estrous-cycling). Heifers assigned to the melengestrol acetate (MGA) prostaglandin F_{2α} (PG) protocol (MGA-PG; n = 200) received 0.5 mg/animal•d^{-1} MGA in a grain carrier from d 0 to 13. PG (25 mg, i.m.; Lutalyse) was administered 19 d after the end of MGA feeding (d 32). Heifers assigned to the Show-Me-Synch protocol (Show-Me-Synch; n = 196) were equipped with a CIDR insert (1.38 g progesterone) on d 2; CIDRs were removed on d 16; and PG was administered on d 32. Heifers were fitted with HeatWatch (DDx Inc., Denver, CO) transmitters for continuous estrus detection during the synchronized period (0 to 144 h following PG), and AI was performed 12 h after the onset of estrus. Estrous response after PG was greater (P = 0.04) among Show-Me-Synch (92%) compared to MGA-PG treated heifers (85%). Mean interval from PG to estrus did not differ (P = 0.73) between MGA-PG (57.4 ± 2.5 h) and Show-Me-Synch (56.2 ± 2.5 h) treated heifers. Mean interval from PG to estrus differed (P = 0.04) between estrous-cycling (62.4 ± 2.4 h) and prepubertal heifers (52.4 ± 4.4 h) assigned to the MGA-PG protocol, but was similar (P = 0.75) between estrous-cycling and prepubertal Show-Me-Synch treated heifers (55.4 ± 2.4 h and 57.0 ± 4.4 h, respectively). Variance associated with interval from PG to estrus was reduced (P < 0.01) among Show-Me-Synch compared to MGA-PG treated heifers, and was not influenced by estrous cyclicity status (P > 0.50). In summary, the Show-Me-Synch protocol resulted in a greater (P = 0.04) and more synchronous (P < 0.01) estrus response compared to the MGA-PG protocol.

Estrus synchronization and AI are valuable reproductive technologies for beef producers. Experiment 1 was designed to compare the 7-d and 5-d Select Synch + controlled internal drug release (CIDR) protocols on the basis of timing and synchrony of estrus following treatment. Cows assigned to the 7-d protocol (n = 59) received GnRH (100 μg i.m. Cystorelin) and CIDR inserts (1.38 g PG) on day 0 and prostaglandin F_{2α} (PG; 25 mg i.m. Lutalyse) and CIDR removal on day 7. Cows assigned to the 5-d protocol (n = 58) received GnRH and CIDR inserts on day 0, PG and CIDR removal on day 5, and a second injection of PG 12 h after CIDR removal and the first PG injection. Estrus detection and AI were performed for cows assigned to each protocol during the 144 h synchronized period. There was no difference in estrous response (P = 0.85), interval to estrus (P = 0.09), or variance for interval to estrus (P = 0.75) between treatments, nor were there differences in synchronized conception or pregnancy rates resulting from AI (P = 0.85, P = 0.91). Experiment 2 was designed to compare pregnancy rates resulting from fixed-time AI (FTAI) following administration of the 7-d (n = 209) and 5-d (n = 210) CO-Synch + CIDR protocols. Both treatments were administered the same as in Experiment 1, however cows assigned to the 7-d protocol were inseminated 66 h after PG and CIDR removal and cows assigned to the 5-d protocol were inseminated 72 h after the first PG injection. Cows assigned to both protocols were administered GnRH (100 μg i.m.) at AI. There was no effect of...
treatment (P = 0.85), technician (P = 0.20), or sire (P = 0.25) on pregnancy rates resulting from FTAI. Given these observations, the 5-d protocol provides an effective alternative to the 7-d protocol for use in facilitating FTAI, however beef producers must carefully consider the increased labor and treatment costs associated with the 5 d protocol. This project was supported by National Research Initiative Competitive Grant no. 2005-55203-15750 from the USDA Cooperative State Research, Education, and Extension Service.

Key Words: progestin, postpartum beef cow, estrus synchronization

464 Comparison of follicular dynamics and hormone concentrations between the 7 d and 5 d CO-Synch + CIDR program in two-year old beef cows. G. A. Bridges*,1, M. L. Mussard2, L. A. Helser3, and M. L. Day2,3, 1Purdue University, West Lafayette, IN, 2The Ohio State University, Columbus, 3Select Sires Inc., Plain City, OH.

The objectives of the present study were to compare follicular dynamics, prevulatory estradiol (E2) concentrations, and progesterone (P4) concentrations between the 7 d (7CO, n = 15) and 5 d (5CO, n = 13) CO-Synch + CIDR program in 2-yr old suckled beef cows. On d -7 (7CO) or d -5 (5CO) GnRH (100 μg, OvaCyst®) was administered (GnRH-1) and a CIDR was inserted. On d 0 at h 0 CIDR were removed and cows received PGF2α (25 mg/dose; Lutalyse®) and a CIDR® on d 0. On d 5 (7CO) or d 2 (5CO) GnRH (100 μg) and timed AI either 72 (2XPGF-72) or 84 (1XCLP-84) h after CIDR removal. The interval to timed AI with 1XCLP was postponed 12 h relative to 2XPGF based on previous results. In Expt 3, (n = 48) blood for analysis of progesterone concentrations was taken at h 0, 4, 8, 12, 16, 24, 48, 72 and 96 after the first dose in the 2XPGF or the 1XCLP treatment and estrous detection was performed. In Expt 1, estrus was detected in more (P < 0.05) cows from the 2XPGF (94.0%) than the 1XCLP (77.9%) treatment but the interval to estrus (66.4 ± 1.2 h) did not differ between treatments. In Expt 2, timed AI pregnancy rate tended to be greater in the 2XPGF-72 than the 1XCLP-84 (68.8% vs 57.9%, respectively; P = 0.08). In Expt 3, progesterone concentrations did not differ between treatments from h 0 to 12, but were greater in the 1XCLP than 2XPGF from h 24 to 96 (trt x h, P<0.05). When the 1XCLP treatment was used, fewer cows were detected in estrus with the 5 d Select Synch + CIDR program, timed AI pregnancy rate tended to be lower, even when timed AI was delayed by 12 h in the 5 d CO-Synch + CIDR program, and the incidence of a delay, or failure of luteal regression was increased. In conclusion, two doses of PGF are necessary to maximize timed AI pregnancy rate with the 5 d CO-Synch + CIDR program.

Key Words: cattle, PGF2α, progesterone

466 Efficacy of the 5 day CO-Synch estrous synchronization protocol with or without the inclusion of a CIDR in beef cows. K. C. Culp*,1, R. P. Lemenager1, M.C. Claeyès1, P. J. Gunn1, M. Van Emon1, R. P. Arias1, S. L. Lake2, and G. A. Bridges1, 1Purdue University, West Lafayette, IN, 2University of Wyoming, Laramie.

The objective of this experiment was to compare timed-AI (TAI) pregnancy rates in suckled beef cows synchronized with the 5 d CO-Synch protocol with (5CIDR) or without (5NoCIDR) the inclusion of an EZI-BREED™ CIDR® insert (CIDR). Cows managed at Feldun Purdue Agricultural Center (FPAC, n = 130), Animal Sciences Research and Education Center (ASREC; n = 169) and Voyles Farms (n = 89) were assigned to either the 5CIDR (n = 195) or 5NoCIDR (n = 193) program by breed, age, and calving date. On d 0 all cows received GnRH (100 μg; Cystorelin®) and cows in the 5CIDR treatment received a CIDR. On d 5, all cows were removed (5CIDR) and all cows received PGF2α (25 mg/dose; Lutalyse®) with another dose of PGF2α given approximately 10 h later. Cows were TAI on d 8, 72 h after CIDR removal, concurrent with GnRH (100 μg). At ASREC and FPAC, but not Voyles Farm, blood samples were collected on d -7 and 0 to determine estrous cyclicity (progesterone ≥ 1.0 ng/mL). Timed-AI and breeding season pregnancy rates were determined via ultrasonography approximately 35 d after TAI and 35 d after the end of the breeding season, respectively. There were no significant treatment by location interactions for any of the variables measured; therefore data were pooled across locations. There was a treatment by age classification (2-yr old versus ≥ 3 yr) interaction (P < 0.05) for TAI pregnancy rates. In mature cows (< 3 yr of age), TAI pregnancy rates were similar between the 5CIDR (73.6%, n = 159) and 5NoCIDR (74.5%, n = 157) treatments. In 2-yr old cows (n = 36/treatment), TAI pregnancy rates were greater (P < 0.05) in the 5CIDR (77.8%) than the 5NoCIDR (58.3%) treatment. Estrous cyclicity status at treatment initiation did not influence TAI pregnancy rates. Overall breeding season pregnancy rates were similar between treatments (94.6%). In conclu-
Presynchronization with hCG 7 d prior to estrous synchronization and replacement of GnRH with hCG at fixed-time AI (TAI) in suckled beef cows. G. Marquezini*1, C. R. Dahlen2, S. L. Bird2, B. J. Funnell3, and G. C. Lamb1, 1North Florida Research and Education Center, University of Florida, Marianna, 2Northwest Research and Outreach Center, University of Minnesota, Crookston, 3North Central Research and Outreach Center, University of Minnesota, Grand Rapids.

We evaluated whether hCG administered 7 d prior to initiation of estrous synchronization and replacement of GnRH with hCG at TAI would alter pregnancy rates to TAI, concentrations of progesterone, and follicle diameter. Suckled beef cows were stratified by days postpartum and parity and randomly assigned in a 2 × 2 factorial arrangement of treatments: 1) cows received 100 μg GnRH and a CIDR insert (d -7), followed in 7 d by 25 mg PGF2α and CIDR removal (d 0), followed in 67 h by GnRH and TAI (d 3; CG; n = 29); 2) CG but the second injection of GnRH was replaced by 1,000 IU of hCG (CH; n = 28); 3) CG, plus cows received 1,000 IU of hCG administered on d -14 (HG; n = 29); and, 4) HG but the second injection of GnRH was replaced by 1,000 IU of hCG (HH; n = 29). Use of transrectal ultrasonography determined pregnancy status on d 30 and to evaluate ovaries of cows on d 14, -7, 0, and 3. Blood samples were collected on d -24, -14, -7, 0, 3, 10, 17, and 30 to determine concentrations of progesterone. Pregnancy rate of cows presynchronized with hCG did not differ from controls; however, treatment of cows with GnRH (54.6%) at TAI tended (P = 0.07) to be greater than those receiving hCG (37.6%). On d -7 and 0, concentrations of progesterone differed (P < 0.05) between cows receiving hCG on d -14 (2.89 ± 0.4 and 3.14 ± 0.3 ng/mL for d -7 and 0, respectively) and controls (1.91 ± 0.4 and 2.51 ± 0.3 ng/mL for d -7 and 0, respectively). Concentrations of progesterone were similar for cows receiving either GnRH or hCG at TAI on d 10, 17, and 30. Diameter of dominant follicles on d 0 and 3 were smaller (P < 0.05) for cows receiving hCG (12.1 ± 0.5 and 13.0 ± 0.7 mm for d 0 and 3, respectively) than controls (14.1 ± 0.5 and 14.5 ± 0.7 mm for d 0 and 3, respectively). We concluded that presynchronization with hCG increased concentrations of progesterone and decreased follicle size prior to TAI, but failed to alter pregnancy rates. Replacement of GnRH with hCG at TAI appeared to reduce pregnancy rates, but failed to increase concentrations of progesterone after TAI.

Key Words: human chorionic gonadotropin, estrous synchronization, beef cows

Effect of used CIDR and FSH on estrus expression and pregnancy rate during low breeding season in Nili-Ravi buffaloes. N. Ahmad*1, Z. Naseer1, E. Ahmad1, M. Mushtaq2, and J. Singh3, 1Department of Theriogenology, University of Veterinary & Animal Sciences, Lahore, Pakistan, 2Buffalo Research Institute, Pattoki, Pakistan, 3Department of Veterinary Biomedical Sciences, WCVM, Saskatoon, Canada.

The objective of the present study was to determine the effect of once used CIDR and FSH on estrus expression and pregnancy rate (PR) during low breeding season (March-August) in Nili-Ravi buffaloes. Two experiments were conducted during June-August, 2008. In experiment 1, buffaloes received either a used CIDR (UCIDR, n=26) or a new CIDR (NCIDR, n=22) for 7 d and PGF2α on d 6. Estrus detection was done twice daily. Buffaloes were inseminated, 12 and 24 h after the onset of estrus. Pregnancy diagnosis was performed 30 d post insemination using ultrasonography. Estrus expression was similar (P>0.05) between UCIDR (84.0%) and NCIDR (95.0%) buffaloes. The mean interval to estrus after removal of CIDRs in UCIDR was 34.5±2.2 h compared to 38±12.2 h in NCIDR (P=0.05). The PR did not differ (P>0.05) due to treatment (9/26 in UCIDR vs 8/22 in NCIDR). In experiment 2, buffaloes at unknown stages of estrous cycle received CIDRs on d 0 and PGF2α on d 6. Animals were either treated with two injections of FSH (5mg i/m at 12 hr interval; n=10) starting at CIDR removal on d 7 or remain untreated (Control, n=9). Estrus detection, insemination and pregnancy diagnosis was similar as in experiment 1. FSH treatment did not affect the proportion of buffaloes expressing estrus, mean interval from CIDR removal to estrus and ovulation, size of ovulatory follicle or PR (P>0.05; overall estrus expression rate.

Performance data from early weaned (approximately 90 d of age) Simmental x Angus cross steers (n=140) were used to evaluate the effects of plant extracts in a distiller’s grains-based feedlot diet with monensin. Steers were early weaned and backgrounded on a high-energy diet until allotment to a finishing trial. The basal diet consisted of 40% modified NDF diets. This analysis showed consistent improvements in growth and efficiency for growing ruminants fed a blend of eugenol, cinnamaldehyde, and capsicum oleoresin. Systematic search identified 13 studies organized in 18 trials (884 growing ruminants) with trials on growing sheep (n = 3) and beef cattle (n = 15). Effects of XT treatment was tested in duplicate and in two periods. Fifty millilitres of a tubes supplied with 0.5 g of DM of a 60:40 forage:concentrate diet and 1:1 ruminal fluid-to-buffer solution were introduced into polypropylene tubes. A plant extract mixture consisting of 3 extracts totalling 250 mg/L and composed of (in mg/L) 1) 125G + 125C + 125E; 2) 0G + 250C + 125E; 3) 250G + 0C + 125E; 4) 41.7G + 41.7C + 166.7E; 5) 41.7G + 166.7C + 41.7E; 6) 0G + 0C + 250E; 7) 0G + 125C + 125E; 8) 125G + 0C + 125E; and 9) 125G + 0C + 125E. Total VFA were unaffected by the 3 extract combinations. The effect of combination of eugenol (E), cinnamaldehyde (C) and a garlic botanical standardized for propyl propyl thiosulfonate (G) on in vitro microbial fermentation was determined using a simplex centroid model. Total VFA were unaffected by the 3 extract combinations. The objective of this study was to use meta analysis to evaluate whether a plant extract mixture consistently affected the productive performance of growing ruminants. Research was conducted by Pancosma and ADM on XT (XT) containing 17% eugenol, 11% cinnamaldehyde, and 7% capsaicin oleoresin. Systematic search identified 13 studies organized in 18 trials (884 growing ruminants) with trials on growing sheep (n = 3) and beef cattle (n = 15). Effects of XT were tested in duplicate and in two periods. Fifty millilitres of a tube supplied with 0.5 g of DM of a 60:40 forage:concentrate diet and 1:1 ruminal fluid-to-buffer solution were introduced into polypropylene tubes. A plant extract mixture consisting of 3 extracts totalling 250 mg/L and composed of (in mg/L) 1) 125G + 125C + 125E; 2) 0G + 250C + 125E; 3) 250G + 0C + 125E; 4) 41.7G + 41.7C + 166.7E; 5) 41.7G + 166.7C + 41.7E; 6) 0G + 0C + 250E; 7) 0G + 125C + 125E; 8) 125G + 0C + 125E; and 9) 125G + 0C + 125E. Total VFA were unaffected by the 3 extract combinations. The molar proportion of acetate was decreased by C×G (P = 0.015) and by C×G×E (P = 0.023) whereas the molar proportion of butyrate was increased by C×G (P = 0.004) and by E×C×G (P = 0.024). The molar proportion of valerate was decreased by E×C (P = 0.042), by E×G (P = 0.116) and increased by C×G (P = 0.081) and E×C×G (P = 0.021). Concentration of CH4 for treatments 10, 5 and 4 were lower than CTR (17.96, 18.46, 18.49 and 22.2, respectively; P < 0.001) and higher than MON (5.81, P < 0.001). Concentration of CH4 was affected by the