2021

Metabolomic Pro! le Associated with Pre- Breeding Puberty Status in Range Beef Heifers

Joslyn K. Beard

Waseem Abbas

J. A. Musgrave

R. Funston

Samodha C. Fernando

See next page for additional authors

Authors

Joslyn K. Beard, Waseem Abbas, J. A. Musgrave, R. Funston, Samodha C. Fernando, and Travis Mulliniks

Metabolomic Profile Associated with Pre-Breeding Puberty Status in Range Beef Heifers

Joslyn K. Beard
Waseem Abbas
Jacki A. Musgrave
Rick N. Funston
Samodha C. Fernando
J. Travis Mulliniks

Summary with Implications

A 4-yr study utilizing heifers from March and May calving herds collected serum samples prior to breeding to determine puberty status. Serum samples were used for Metabolomics analysis to investigate differences related to circulating serum metabolites in pubertal and non-pubertal heifers. Metabolomics, which is a shotgun approach analysis of a large number of small metabolites, is an emerging technology that can provide a more robust analysis of metabolism. No differences were observed in heifer ADG, pregnancy rate, or the percentage that calved within the first 21 d between heifers classified as pubertal and non-pubertal at the start of the breeding season. Using metabolomic analysis, metabolite differences associated with energy metabolism and steroid production between pubertal and non-pubertal groups were identified. Results from this study suggest that there is potential to develop a method that identifies efficient, productive females early in the development period and reduce costs for producers.

Introduction

The early part of the life of a heifer is heavily influenced by their metabolism which experiences drastic shifts throughout this critical growth period to ensure proper growth and reproductive competence in her attainment of puberty prior to breeding. These changes affect protein, carbohydrate, and lipid metabolism through altered nutrient requirements, not only during the heifer development stage but subsequent lifetime productivity as a replacement female in the beef herd. Exponential early growth increases metabolic demand and allows for adaptive changes to occur in those pathways associated with metabolism. Metabolomic analysis provides an overview of those metabolic pathways and associated phenotype. This method allows researchers to look at serum metabolite profiles in a complete systems-wide metabolism and biology approach. . Combining biological mechanism with metabolomics holds the potential to identify efficient, productive females to be used as replacements reducing producer costs. Therefore, the hypothesis of this study that the metabolite profiles of serum collected from heifers prior to their respected breeding season will be different among pubertal and non-pubertal groups.

Table 1. Forage quality prior to the breeding season for March and May calving herds over a four-year period[1]

Item, %DM	2011[1]	2012	2013	2014
March[2]				
CP	14.0	10.1	19.3	14.1
TDN	64.3	61.5	79.7	61.6
May[3]				
CP	11.1	10.6	14.7	10.1
TDN	61.2	59.6	71.0	59.0

[1]Nutritional composition of range was collected from esophageal fistulated cows in each year

[2]March= heifers born from the March-calving herd

[3]May= heifers born from the May-calving herd

Procedure

A 4-yr study conducted at the Gudmundsen Sandhills Laboratory, Whitman, Nebraska, developed replacement heifers from 2 calving seasons. March-born (n = 225) and May-born (n = 258), crossbred (5/8 Red Angus, 3/8 Continental) heifers were maintained with their respective calving herds. Nutrient composition (Ward Labs, Kearney, NE) for the pasture is presented in Table 1, noting the quality of the pasture for the breeding season. Puberty status was determined prior to each breeding season by collecting 2 blood samples via coccygeal venipuncture 10 d apart (May for March-born heifers and early July for May-born heifers). Heifers with serum progesterone concentrations greater than 1 ng/mL at either collection were considered pubertal, anything below 1 ng/mL at either time point was considered non-pubertal. Blood samples were placed on ice following collection and centrifuged at 2,500 × *g* for 20 min at 4°C. Following serum removal, samples were frozen at −20°C pending analysis. At breeding, heifers were synchronized with a single 5 mL i.m. injection of $PGF_{2\alpha}$ (Lutalyse, Zoetis, Parsippany, NJ) 5 d after bull placement (1:20 bull-heifer ratio) and bulls successfully completed a breeding soundness exam before a 45 d breeding season. Heifer pregnancy diagnosis was conducted via transrectal ultrasonography 40 d following bull removal. Metabolite data were normalized by sample volume and then a model was used to identify metabolites related to branched chain-amino acids metabolism, lipid metabolism, carbohydrate metabolism, and steroidogenic biosynthesis to be different in pubertal and non-pubertal heifers. Performance data were analyzed using the PROC MIXED and GLIMMIX procedure of SAS. A mixed model ANOVA accounted for correlations within puberty class and puberty class within each calving season.

Table 2. Growth and reproductive performance between pubertal and non-pubertal heifers

Items	Treatments		SE	*P*-value
	Non-Pubertal	Pubertal		
March[1]				
ADG[2], lbs	2.1	1.8	0.4	0.42
Pregnancy Rate, %	83.5	91.3	5.5	0.15
First 21 d[3], %	72.3	77.7	7.6	0.47
Number of calves[4]	2.22	2.31	0.22	0.68
May[5]				
ADG, lbs	1.0	1.1	0.1	0.10
Pregnancy Rate, %	62.9	72.7	7.1	0.17
First 21 d, %	53.1	52.4	2.0	0.78
Number of calves	1.89	2.34	0.23	0.06

[1]March= heifers born from the March-calving herd

[2]ADG= average daily gain of BW during the breeding season

[3]First 21 d= calving within the first 21 d of the calving season indicative of conceiving within the first 21 d of the breeding season

[4]Number of calf crops for each heifer

[5] May= heifers born from the May-calving herd

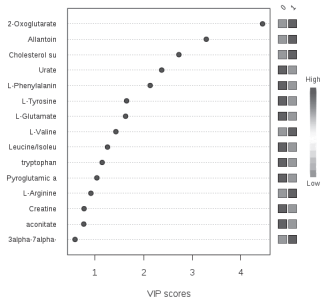

Figure 1. VIP scores of March-born heifers (0 = non-pubertal heifers; 1= pubertal heifers). VIP scores measure the importance of the variable between pre-breeding pubertal status, the greater the VIP number the greater the importance. Color-coded boxes (red = high concentration; green = low concentration) for non-pubertal (0) and pubertal (1) heifers signify the concentration difference of the measure variable.

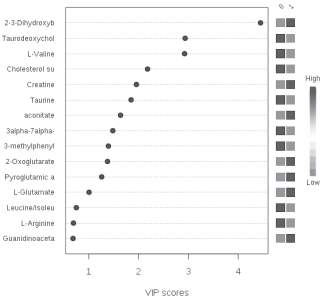

Figure 2. VIP scores of May-born heifers (0 = non-pubertal heifers; 1= pubertal heifers). VIP scores measure the importance of the variable between pre-breeding pubertal status, the greater the VIP number the greater the importance. Color-coded boxes (red = high concentration; green = low concentration) for non-pubertal (0) and pubertal (1) heifers signify the concentration difference of the measure variable.

Models included the effect of treatment, cow age, calving season, and calf sex for all appropriate data. Data are presented as LSMEANS and *P-v*alues ≤ 0.05 were considered significant and tendencies were considered at a $P > 0.05$ and $P \leq 0.10$. Longitudinal data of the serum metabolome were analyzed in MetaboAnalyst 3.0 (Xia and Wishart, 2011). The functions used were principal component analysis (PCA) to depict variation in the data distributed across samples and *t*-test. With distinction of important metabolites classified between the groups using the variable importance projection (VIP) method.

Results

Heifer average daily gain during the breeding season was not different ($P > 0.10$; Table 2) between puberty groups. At the start of breeding, 58% and 66% were classified as pubertal in March- and May-heifers, respectively. However, heifer reproductive performance was not different ($P \geq 0.10$) between puberty classifications prior to the breeding season for final pregnancy rate and the percentage that calved within the first 21 d. These results suggest the later maturing non-pubertal heifers prior to breeding were able to obtain a later puberty with no negative impacts on timing and ability to conceive. Heifer average daily gain was not different between pubertal and non-pubertal groups suggesting that heifers had similar nutrient intake thus body weight did not impact puberty attainment, which challenges current understanding of body weight attainment at time of breeding.

A total of 64 metabolites were identified from pubertal and non-pubertal heifers within each calving season. March-born pubertal heifers had increased ($P \leq 0.01$) concentrations of 2-oxoglutarate compared to non-pubertal heifers (Fig 1). A key molecule in the Krebs cycle (TCA cycle) is 2-oxoglutarate or α-ketoglutarate (AKG). The influence of AKG on the intracellular mechanisms may lead to a greater impact on the neuroendocrine systems, which drives attainment of puberty in heifers. Pubertal heifers with increased blood concentrations of AKG may have increased TCA cycle enzymatic activity, which may increase energy metabolism while stimulating the neuroendocrine activity associated with puberty attainment.

Non-pubertal March heifers had greater ($P < 0.01$) concentrations of creatine and aconitase (Fig 1), which play a role in muscle metabolism, protein breakdown, and catalyzes enzyme reactions for citrate in the TCA cycle. If not used to create energy, creatine is then metabolized to creatinine. The changes of creatine concentration from pre-and post-puberty could be influenced by fluctuation of estrogens during puberty attainment. Aconitate or better known as it's active form aconitase serves as an iron-dependent enzyme catalyst for citrate and iso citrate in the TCA cycle. Therefore, non-pubertal March-heifers with increased aconitase may indicate inefficiencies in the energy metabolism.

May-born pubertal heifers had increased ($P \leq 0.01$) concentrations of 2,3-dihydroxybenzoate (Fig 2) and decreased ($P \leq 0.01$) concentrations of taurodeoxycholate and cholesterol sulfate

(Fig 2) compared to non-pubertal counterparts. This suggests that pubertal heifers with elevated 2,3-dihydroxybenzoate are undergoing bone maturation sooner than the non-pubertal heifers. Taurodeoxycholate acts as a bile salt synthesized in the liver to facilitate excretion, absorption, and transport of fats and sterols in the intestine and liver. Bile salts are key components in regulating enzymes involved in cholesterol homeostasis. This would suggest cholesterol sulfate functions as a regulator of cholesterol side chain cleavage activity and steroid synthesis. Increased cholesterol sulfate concentrations in non-pubertal heifers may suggest decreased steroidogenesis, which may delay the onset of puberty.

Conclusions

In this study, puberty attainment prior to breeding season was characterized by differences in metabolic profiles related to protein, lipid, and carbohydrate metabolism along with steroidogenic biosynthesis. Even though no differences were observed in heifer growth and reproductive performance, this untargeted metabolic analysis identified markers associated with energy efficiency in pubertal and non-pubertal heifers. Overall, this furthers the understanding of the metabolic impact on reproductive efficiency in range beef heifers, which possibly may be utilized as a replacement heifer selection tool for producers.

Joslyn K. Beard, graduate student

Waseem Abbas, graduate student

Jacki A. Musgrave, research technician

Rick N. Funston, professor animal science, West Central Research and Extension Center, North Platte

Samodha C. Fernando, professor animal science, University of Nebraska–Lincoln

J. Travis Mulliniks, assistant professor animal science, West Central Research and Extension Center, North Platte