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## ROLE OF METHIONINE IN FETAL DEVELOPMENT OF BEEF CATTLE

A Master's Thesis

Presented to

The Graduate College of

Missouri State University

In Partial Fulfillment

Of the Requirements for the Degree

Masters of Science, Agriculture

By

Colin Chalk

May 2019

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## ROLE OF METHIONINE IN FETAL DEVELOPMENT OF BEEF CATTLE

Agriculture

Missouri State University, May 2019

Master of Science

Colin David Chalk

## ABSTRACT

The objective of this study was to evaluate whether total amino acids (AA) or methionine have an effect on fetal programming of calves using 108 Angus Brangus cows. Treatments were 1) Control, limpograss hay with molasses plus urea (16% CP as fed basis) at 2.72 kg./hd/d, 2) Fishmeal, Control plus 0.33 kg./hd/d of fishmeal (methionine 2.85 % of RUP), and 3) Methionine, Control plus 10 g/hd/d of MetaSmart liquid (Addisseo Alpharetta, GA). Fishmeal and Methionine treatments supplied similar amounts of metabolizable methionine. Weight of cows and calves along with body condition score of cows were measured at the start and end of the 120 day supplementation period, and milk yield was measured at 3 time points by weighsuckle-weigh technique. In Year 2, 24 steer calves conceived during the treatment period in Year 1 were fed individually during a metabolism experiment following weaning at approximately 7 months of age. Body weight, feed intake, plasma metabolites, and nutrient digestibility were measured in steers during the metabolism experiment. Body weight and body condition score change of cows were not different among treatments during the treatment period in Year 1. Treatment did not affect calf weight gain even though there was a trend for Methionine dams to have greater energy-corrected milk yield and for Fishmeal and Methionine dams to have greater milk protein content than Control dams. In Year 2, treatment did not affect weaning weight of calves conceived during the treatment period in Year 1. During the post weaning metabolism experiment, Average daily gain, final body weight (FBW), and gain: feed ratio were greater in steers whose dams supplemented with Fishmeal or Methionine during early gestation. Steers born to Control and Methionine dams had greater plasma urea nitrogen concentrations before and after feeding, and tended to have greater change in plasma urea nitrogen concentration than steers born to Fishmeal dams. Steers born to Methionine dams had lower plasma glucose concentration before and after feeding, but greater change in plasma glucose concentration than steers born to Fishmeal dams. There was a trend for treatment to effect Neutral detergent fiber (NDF) and Acid detergent fiber (ADF) digestibility with steers born to Methionine dams having greater digestibility than steers born to Control or Fishmeal dams. In conclusion, methionine is a key nutrient in fetal programming and can be used in conjunction with poor quality forage to improve performance of offspring.

KEYWORDS: beef cattle, methionine, fetal programming, ruminant nutrition, amino acid

## **ROLE OF METHIONINE IN FETAL DEVELOPMENT OF BEEF CATTLE**

By

## Colin David Chalk

A Master's Thesis Submitted to the Graduate College Of Missouri State University In Partial Fulfillment of the Requirements For the Degree of Master of Science, Agriculture

May 2019

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In the interest of academic freedom and the principle of free speech, approval of this thesis indicates the format is acceptable and meets the academic criteria for the discipline as determined by the faculty that constitute the thesis committee. The content and views expressed in this thesis are those of the student-scholar and are not endorsed by Missouri State University, its Graduate College, or its employees.

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#### **INTRODUCTION**

#### **Justification for Study**

Beef cows in the Southeastern United States begin calving in late fall and rebreed during the winter months. During this time, the nutritional value of warm season forages is poor while at the same time nutrient requirements of the cow is greatest. This presents a serious challenge for beef cattle producers, supplementing their cows with an economical and practical feedstuff that also meets the nutritional needs of the lactating beef cows. Nutritional management of pregnant or lactating beef cows impacts not only the cow and her currently nursing calf but also the calf that is developing in utero. Liquid molasses, a by-product of the sugar cane industry, is a high energy feedstuff and an effective feedstuff for beef cattle producers (Pate, 1983; Arthington et al., 2004), although effective it requires supplementationwith a high-protein feedstuff in order to meet metabolizable protein requirements of the cow and potentially fetus in utero.

Essential amino acids are vital to in vivo protein synthesis and diets deficient in them do negatively impact a cow's performance. Methionine is typically first limiting amino acid when cattle rely almost exclusively on microbial crude protein (Richardson and Hatfield, 1978), and is an important methyl donor in the process of DNA methylation that is thought to influence fetal development (Wu et al., 2006). Patton et al. (2010) reported that when supplemental by-pass methionine was added to the diet of dairy cows, milk yield and milk protein content increased in similar to what you might see in lactating beef cows that are fed low quality hay and molasses supplement. Researchers supplemented beef cattle diets with ruminal protected methionine to cows being fed chopped rye hay. The results were cattle fed the ruminal protected protein had increases in total milk yield, protein and fat. This study shows that supplementing with by-pass

methionine can increase milk production (Hess et al., 1998) and improve reproductive performance in beef cows (Clanton and England, 1980). Addition of methionine also has been shown to increase retention of nitrogen in late gestating beef cows, which may suggest that methionine is limiting in their diets (Waterman et al., 2007). Santos et al. (1998) reported that only 17% of dairy cattle saw an increase in milk yield when supplemented with by-pass protein, subsequently the 17% was mostly comprised of fishmeal and treated soybean meal both of which have relatively high levels of by-pass methionine and lysine. Santos research indicates that additional methionine may be required in order to optimize growth in calves.

## **Problem Statement**

Supplemental metabolizable protein appears to be important to maternal nutrition in enhancing fetal development and subsequent offspring performance. However, it is not understood whether total metabolizable protein supply or key individual amino acids are responsible for the improved offspring performance.

## **Objective and Null Hypothesis**

Our null hypothesis is that increasing the methionine supply to lactating cows will not enhance milk production and reproductive performance of cows, as well as fetal development and performance of offspring.

Objective #1: Determine the effect of increased metabolizable protein or metabolizable methionine supply to lactating beef cows consuming low quality hay during early gestation on digestibility, plasma metabolite profiles, and growth performance in subsequent offspring.

Objective #2: Determine the effect of increased metabolizable protein or metabolizable methionine supply to lactating beef cows consuming low quality hay on milk yield and components, pregnancy rates, body weight, body condition score in beef cows, and weight gain in currently nursing calves.

#### LITERATURE REVIEW

#### **Fetal Development in Bovine**

**Early-gestation.** Timeline for early-gestation is from d 0 to d 90 of the fetus life. The process in which sperm penetrates into the ovum sets forth the process known as oocyte activation. The ovum phase is a short process lasting 11 to 14 days and is characterized by rapid cell division (Gerrard and Grant, 2003). The single celled structure develops into many cells uniform in size known as blastomeres. Once completed the structure undergoes compaction with the inner cells become associated. This gives rise to the morula which is a structure of 20 cells and signals the end of the ovum stage of development (Gerrard and Grant, 2003). Morphogenesis is the term that refers the changes in the embryo as directed by structures both internal and external. Two main events occur that progress the embryo to the blastula phase. First, increasing the numbers of blastomeres forms a blastocyst (Gerrard and Grant, 2003). Secondly, the formation of trophoblasts which surrounds the blastocoele (Gerrard and Grant, 2003). This forms the embryonic disc which consists of the epiblast and hypoblast. Gastrulation then occurs, which is a period in which regions begin to exert more control over development rather than the specific cells. Neurulation then occurs with the formation of what will then be the spinal cord. During somitogenesis two ridges develop in the neural tube which are known as paraxial mesoderm and lateral plate mesoderm (Gerrard and Grant, 2003). After embryonic maturing the elongated groups of cells organize into masses known as somites. After the somitocoele formation, two specific populations of somatic cells develop, these two populations are dermomyotome and sclerotome (Gerrard and Grant, 2003). Cells than migrate to the spinal cord and develop the spinal column. Limb formation then begins to occur when cells from somites

move to the closest point of limb formation and migrate to just under the surface ectoderm to form limb buds.

Fetal organogenesis begins simultaneously with the development of the placenta, in beef cattle a heartbeat can be detected in as few as 21 days. At 25 days into pregnancy extremities begin to develop followed by vital organs including the pancreases, liver, adrenals, lungs, thyroid, spleen, brain, thymus and kidney (Hubbert et al., 1972). Although the growth trajectory for each organ is different, nutritional deficiencies have the greatest control on how quickly or slowly organs develop. Other areas where development has documented change is in the reproductive tract of females. By day 60 testicles begin to develop in male calves, and female ovarian development has begun.

**Mid gestation.** Timeline for mid-gestation is from d 91 to d 180 of the fetus life. Mao et al., documented a significant growth of kidneys during mid gestation (2008). Primary myogeneis occurs during d 60 and d 90 of early gestation (Russell and Oteruelo, 1981). Initial myogeneis is minimal skeletal muscle development the majority of skeletal muscle fiber development occurs during mid gestation. Muscle weight increased between 214-fold and 483-fold in biceps femoris muscle from d 90 to d 270 months of age (Mao et al., 2008) Primary muscle fiber development continues until 210 d of gestation in cattle, Mao et al. (2008) concluded that muscle weight to BW ratios increased in mid gestation and continued to grow into late gestation.

Secondary myogeneis myofibers form during this stage in the fetus life. Secondary myogeneis fibers account for most of the skeletal muscle fibers at this point in gestation. Adipogenesis process begins at the tail end of mid gestation.

Late gestation. Timeline for late gestation is from d 181 to parturition. Mao et al., found that during late gestation accelerated growth in both liver and heart occurred in cattle. In cattle, skeletal muscle matures around d 210 of gestation. Adipogenesis and muscle fiber hypertrophy are the main process occurring during late gestation in beef cattle. During late gestation a greater number of mesenchymal cells are directed toward adipogenesis. The amount of fat is determined by the size and number of adipocytes (Du et al., 2010). Both skeletal muscle cells and adipocytes originate from the same group of mesenchymal stem cells. Also during late gestation fibrogenesis occurs where fibroblasts synthesize connective tissue that forms the endomysium, perimysium and epimysium in skeletal muscle (Du et al., 2010). Mao et al. (2008) reported that contrary to muscle weight to BW ratios, fat weight to body ratios significantly increased throughout gestation. This increase indicates that fat accretion in the fetus significantly increases towards the end of gestation. Mao et al. (2008) found that while body weight ratios to organs decreased, body fat increased continuously from 3 to 9 mo of gestation.

## **Fetal Programming**

Epigenetics is the study of heritable but potentially reversible modifications at the molecular level of DNA (Li., 2003). As more research is conducted, it is recognized that epigenetics develops a mechanical link between environment and genetics. Some of the most common forms of epigenetic mechanisms include DNA methylation, histone modifications, nucleosome position along DNA, and the modulation of gene expression by noncoding RNA (Mohr et al. 2011; Niculescu and Lupu 2011; Skinner, 2011).

**Dutch Famine.** During World War II, German troops made their way into the Netherlands setting up a blockade that caused, a vast restriction in the food coming into the

country. In December 1943, the daily ration was 1800 Cal, then it was decreased to 1400 Cal in October 1944, by the height of the famine caloric intake was down to between 400-800 calories per person (Burger, 1948). Those that were most affected by the famine were pregnant women and their subsequent offspring. Children were considered to be prenatally exposed to famine if the mother experienced an average daily ration of under 1000 calories for 13 weeks. Research conducted at the University of Amsterdam in 2012, looked at the 1,423 men and women who were in utero during the Dutch Famine. Researchers looked at long term health issues observed in children who were born during the Dutch Famine (Figure 1.) (Roseboom et al., 2006). In 2013, researchers from the University of Amsterdam conducted an observational study on 150 people who were born during the Dutch Famine. They saw a statistical difference in both grip strength and lower physical performance score (P>0.05) in men, but not women (Bleker et al., 2016).

Exposure to Famine					
In late gestation	In mid gestation	In early gestation			
Glucose Intolerance	Glucose intolerance	Glucose intolerance			
	Microalbuminuria	Atherogenic lipid profile			
	Obstructive airway disease	Altered blood coagulation			
		Obesity (women only)			
		Stress sensitivity			
		Coronary heart disease			
		Breast cancer			

Figure 1. Adapted from Roseboom et al., 2006

### **DNA Methylation**

The development of a fetus from conception to birth is a complex process that involves a collaboration of a multicellular organism to achieve this goal. The primary regulation of genes is predominately encoded in cis regulatory elements and is carried out by transcription factors. Which are stretches of DNA pairs where transcription factors bind and regulate the expression of genes and their rate of transcription. Genes are also regulated by modifications that occur from heritable covalent modifications i.e. DNA methylation. DNA methylation is a process that occurs with the covalent addition of methyl groups to DNA predominately at the 5<sup>th</sup> atom of the cytosine ring which is followed by a guanine nucleotide. Although other nucleotides can also be methylated, methylation of cytosine is one of the most studied and mechanically understood epigenetic modifications.

Folate (Vitamin B9) is the most extensively studied methyl donor in epidemiological DNA methylation research. This not an issue in ruminants, due to the fact that ruminants produce folate to meet their requirements via microbial synthesis. Folate is reduced to dihydrofolate (DHF) and then to tetrahydrofolate (THF) which serves as a donor in single carbon metabolism in the form of 5-methly THF (Anderson et al. 2012). 5-methyl THF functions in one carbon metabolism by donating a methyl group to homocysteine, creating methionine. Other B-vitamins serve as enzymatic cofactors in the process of SAM regeneration: cobalamine, pyrodoxine, and the quasi-vitamin choline (Ba et al., 2011). Reduced folate status decreases global methylation causing permanent phenotypic changes in small intestinal tissue of young women (Shellnutt et al., 2004) and decreased global DNA methylation in liver of offspring (Ly et al., 2011) demonstrating that folate restriction has a key role in global DNA methylation.

The Vitamin B family plays a crucial role in the function of one-carbon transfers. Riboflavin, pyrodoxine and cobalamine play a crucial role in folate and one carbon metabolism. Riboflavin is a precursor for flavin adenine dinucleotides, which is the cofactor for methylenetetrahydrofolate reductase (MTHFR), an enzyme that is responsible for the reduction of 5, 10-methylene THF to 5-methly-THF (Chan et al., 2010). Pyridoxine is a coenzyme for serine hydroxymtheyltransferase which is the enzyme responsible for converting THF to 5, 10methyl-THF in the folate cycle (Perry et al., 2009). Finally, cobalamine is the coenzyme for methionine synthase, which catalyzes the conversion of homocysteine to methionine.

Choline is an indirect donor to one-carbon metabolism (Figure 2). Choline is oxidized to form betaine by the enzyme intermediate betaine aldehyde (Kidd et al., 1997); betaine is the primary methyl group donor converting homocysteine into methionine. Choline deficiency has been linked to changes in neurogenesis along with declines in memory function (Craciunescu et al., 2010; Mehedint et al., 2010). Rats fed choline deficient diets, showed a hypomethylation of promoter genes that are involved in fetal brain development (Niculescu et al., 2006).

#### 5-Methylcytosine



Figure 2. Chemical structure of 5-methylcytosine (Adapted from Bartosik et al., 2016)

#### **Single Carbon Metabolism**

Folate, methionine, and choline are methyl donors responsible for regulation of single carbon metabolism. These are key nutrients in the generation of S-adenosylmethionine (SAM), which is the universal methyl donor responsible for methylation of DNA and histones. When SAM is demethylated it becomes S-adenosylhomocysteine (SAH), if SAH accumulates it can begin to interfere with methylation reactions (figure 3). S-adenosylhomocysteine concentration in the cell is a determining factor of methylation capabilities of certain tissues. Sadenosylhomocysteine is hydrolyzed to homocysteine and adenosine via a reversible reaction that favors SAH synthesis; therefore, removal of SAH is essential for allowing the methionine cycle to function properly (Brosnan, 2006). Methionine is an essential nutrient that has a multitude of functions in the diet of ruminants. Methionine plays a key role in protein synthesis; it also functions as a methyl donor and precursor to a number of antioxidants and other compounds. Research have implicated that close to half of the methionine requirements of rats, cats, dogs, pigs, and chicks can be replaced by cysteine (Chung and Baker, 1992; Baker et al., 1996). While these animals can efficiently convert cysteine to methionine, ruminants specifically cattle, have not observed the same conversion (Campbell et al., 1997) Methionine is often the most limiting amino acid (AA) in beef cattle diet particularly along with lysine and threonine (Richardson and Hatfield, 1978).



Figure 3. Involvement of dietary micronutrients in one-carbon metabolism (1) Vitamin B<sub>6</sub> is a cofactor to serine hydroxymethyltransferase in the conversion of tetrahydrofolate (THF) to 5, 10-methylene THF. (2) Vitamin B<sub>2</sub> is a precursor to FAD, which is a cofactor to methylenetetrahydrofolate reductase (MTHFR) in the conversion of 5, 10-methylene THF to 5-mthyl THF. (3) Vitamin B<sub>12</sub> is a precursor to methionine synthase, involved in the production of methionine from homocysteine and betaine. DHF, Dihydrofolate; FAD, Flavin adenine dinucleotide; DMG, dimethyl glycine; MTHFR, methylenetetrahydrofolate reductase; SAH, S-adenoslyhomocysteine; THF, tetrahydrofolate. Adapted from Anderson et al., 2012

Key vitamins that act as coenzymes in one carbon metabolism are folic acid, riboflavin, pyridoxine and cobalamine. DNA methylation is dependent on methyl donors and cofactors from the diet which are involved in methionine and folate metabolism (Chmurzynska, 2010). Methionine is vital because unlike the vitamins involved in one-carbon metabolism, it cannot be synthesized in the rumen of cattle. In mammal's, protein synthesis and synthesis of Sadenosylmethionine (SAM) compete for the available methionine in the body (Finkelstein 1998). Two process trying to utilize similar nutrients can be detrimental, in situations when methionine is scarce, the animals body will pull methionine from the DNA methylation process.

## Rodents

The liver potentially plays a central role in the fetal programming. Expression of key enzymes in carbohydrate and lipid metabolism such as, phosphoenolpyruvate carboxykinase (PEPCK), acyl-CoA carboxylase-1 (ACC-1) and liver carnitine palmitoyl transferase-1 (L-CPT-1) was decreased in the liver. (Desai et al., 1997; Zhang and Byrne, 2000; Maloney et al., 2003; Rees et al., 2006; Maloney et al., 2007). When rats were fed a low protein diet just before conception researchers observed gender specific effects on hepatic PEPCk and 11B-HSD1 gene expression during fetal development (Kwong et al., 2006).

Bertam et al., (2001) conducted research looking at the effects of protein-restricted (PR) diet to pregnant rats. Rat pups had an increase in glucocorticoid receptor (GR) expression and a reduction in the enzyme that is responsible for the inactivation of corticosteroids,  $11\beta$ -hydroxysteroid dehydrogenase type II, in major organs (i.e., liver, lung, kidney and brain). Research shows that, nutrient restriction while the fetus is in utero in the early stages of pregnancy can have effect on the development of major organs which subsequently can affect the animal's ability to utilize nutrients and function properly.

Previous research has also evaluated the effect diet can have on the development of major organs. Interestingly, nutrient restriction of the dam affected one-carbon metabolism and the concentration of SAM and SAH which led to decreased methylation status of PPARα (Peroxisome proliferator-activated receptor alpha) and GR genes in the offspring's liver (Lillycrop et al., 2005). Even after the offspring were fed a protein sufficient diet, hypo

methylation of GR and PPAR $\alpha$  persisted indicating the potential long-term effects on offspring performance from epigenetic changes in utero

When maternal diets were deficient in folic acid and other methyl donors, researchers saw a reduction in acetyl-CoA carboxylase, the rate limiting enzyme in fatty acid synthesis in rats, (McNeil, 2008). Further research in fetal programming narrowed the scope and specifically looked at how deficiency in methyl donors could affect subsequent offspring. When fed a diet deficient in methyl-donors, studies show that multiple functional groups change in the offspring of dams who received methyl-deficient diets during pre-conception and preimplantation stages (Maloney 2011). At 6 and 12 months of age, key proteins involved in energy metabolism, antioxidant defense, and amino acid and peptide metabolism were expressed different which suggested that maternal diets deficient in methyl donors during the beginning stages of cell division can have lasting effects on metabolic processes (Maloney 2011).

### Sheep

Fetal programming research in sheep has focused on the effects of total nutrient restriction during early- to mid- gestation on fetal development and subsequent performance of offspring. Researchers fed ewes a restricted (50% NRC requirements) or adequate diet (100% NRC requirements); NRC, 2000) from day 28 to 78 of gestation. Once slaughtered (d 78) organ weights of ewes, were recorded and were similar in both control and nutrient restricted ewes (Vonnahme et al., 2003). Plasma glucose concentrations were decreased and plasma T4/T3 ratio increased in nutrient restricted compared to control-fed ewes (Vonnahme et al., 2003).

Ford (2010) conducted similar research with a flock of ewes at the University of Wyoming to evaluate restriction of total nutrients on concentrations of amino acids and

polyamines in maternal and fetal plasma and fetal fluids. Similar to Vonnahme et al., (2003) nutrient restricted ewes were fed 50% of NRC nutrient requirements compared with 100% for the control ewes from day 28 to 135 of gestation. Total amino acid concentrations were substantially reduced, specifically serine, arginine-family amino acids, and branched chain amino acids. Furthermore, polyamine concentrations in maternal and fetal plasma and in fetal allantois and amniotic fluids were decreased during both mid and late gestation (Kwon et al 2004). This research shows that 50% global nutrient restriction decreases total amino acids and polyamines in the ovine fetus could affect key functions in fetal development (Kwon et al., 2004). Results are important to the understanding of the mechanisms responsible for growth retardation in utero and how those retardations can affect adults later in life.

In a second study, Kwon et al., (2004) evaluated the effects of nutrient restriction in a flock of ewes near Baggs, Wyoming that are managed using low input and reared in a low nutrition environment. The objective of the study was to evaluate nutrient restriction in a flock of ewes subjected to low nutritional environment. Interestingly, nutrient restriction of ewes selected under low nutritional environment did not result in decreased fetal amino acid concentrations as was the case with the traditionally managed ewes were records were kept and animals were culled due to poor performance at the University of Wyoming (Kwon et al 2004). Also, contrary to the intensively managed UW ewes in the prior research, fetal growth was not reduced in extensively managed Baggs ewes in response to severe maternal nutrient restriction.

Diet of the damn can affect reproductive tissues as well, ewes that were fed 60% of the NRC recommendations from d 50 to 135 during gestation saw a decrease cellular proliferation rate in primordial follicles compared to the ovaries of those fed 100% of NRC recommendations

(Grazul-Bilska et al., 2009). Researchers speculated that this decrease could affect future fertility.

## Cattle

**Early Development.** Maternal nutritional status is a key factor in programming nutrient partitioning and ultimately the growth, development, and function of the fetal organ systems (Wallace 1948; Wallace et al., 1999; Godfrey and Barker, 2000). Although 75% of the growth in ruminant fetus occur in the final two-month, crucial framework is established in the beginning months (Robinson et al., 1977). The critical events that occur during the early phase of fetal development include: maximal placental growth, differentiation of, vascularization, and fetal organogenesis.

The reproductive tract in cows is a vital part of development of the calf. The placenta is crucial in the development of the ruminant fetus. The placenta attaches itself to the uterine walls using structures called caruncles which are the primary area for exchange of nutrients between mother and fetus. The uteroplacental blood flow is directly related to growth of the fetus in utero by affecting placental nutrient transport and oxygen exchange (Reynolds and Redmer, 1995; 2001). Vonnahme et al. (2007) reported that nutrient restriction from d 30 to d 125 of gestation followed by supplementation from d 125 to 250 affected both placental angiogenesis (i.e. capillary vascularity) and angiogenic factor mRNA in cows. Cows who were nutrient restricted from d 30 to d 125 had decreased vascular permeability and vascular endothelial growth factor (VEGF) mRNA. Restriction was observed in both the caruncular and cotyledonary tissue compared with cows supplemented throughout gestation.

Similar effect was observed by McMullen et al. (2005) when they restricted ewes for 7 days during mid-gestation and measured a decrease in mRNA abundance of the angiogenic factor, VEGF, and placental weights on d 90. However at the day of lambing, placental weight between nutrient-restricted and the control (100% NRC requirements) were not different. In cattle, there was a difference in placental weight on d 125 between nutrient-restricted and control, and continued to be suppressed even after re-alimentation on d 250 (Vonnahme et al., 2007; Zhu et al., 2007). This difference can be correlated to species variation between ovine and bovine. Randuz et al., (2012) saw increases in birth weights and increased carcass weights in calves from cows that were fed hay a low starch diet in late gestation. Reported an increase in marbling score and intramuscular fat content in the carcass of calves whose mothers were fed hay compared to those that were supplemented with corn. Wang et al., (2015) concluded that diet of the dam during late gestation specifically affects postnatal growth and adipose deposition in the cattle.

Additional, research has evaluated maternal nutrition during pregnancy on long-term performance of offspring with effects on progeny growth, glucose tolerance, and carcass composition. Radunz et al., (2012) reported that maternal late gestation energy source (starch versus fiber) altered fetal growth, birth weight, and had long term effects on intramuscular fat deposition of the progeny. Calves born to these cows were lower grading when slaughtered and carcasses analyzed. This research suggests that fetal programming in cattle can affect development of several physiological systems.

Protein restriction of the cows during late gestation decreases fetal muscle fiber number resulting in less muscle cross-sectional area (Larson et al., 2009). Other research demonstrated that calves from nutritionally-restricted cows had reduced body and carcass weight compared to

those that were fed control diets throughout gestation (Greenwood et al., 2004). Larson et al. (2009) reported retail yield of calves from nutrient-restricted cows was greater than calves from nutrient-adequate cows.

Research stated that maternal protein supplementation might affect the integrity of the oocyte or embryo resulting in fewer calves within the first 21 days of calving season (Martin et al., 2007). Martin et al. (2007) also noted that dams that were supplemented during late gestation gave birth to heifers that were more reproductively efficient, with increased pregnancy rates during first pregnancy compared to those whose dams were not supplemented. Supplementing cows not only boosted reproductive performance in subsequent calves, but improvements in calf birth weight, pre-breeding calf weight, and calf 205-d adjusted weaning weight were observed when mothers were supplemented protein while grazing low quality forage prior to calving. Maternal nutritional management also affected feedlot performance post weaning with increased feedlot ADG and increased final weight and HCW, and had a tendency to have a higher marbling: yield grade ratio (Larson et al., 2009).

The majority of research involving cattle and the effects of maternal nutrition was done in the later part of gestation. Because the majority of structural growth of the fetus occurs in the final third of the pregnancy (NRC, 2000), it was hypothesized this is where the greatest differences would be observed. Multiple studies have reported effects on calf birth weight by protein and energy deficiency of the dams (Holland and Odde, 1991). However, nutrient restriction of cows in early gestation could have a pivotal role in the development of vital organs that are responsible for many aspects of future productivity.

Long et al., (2009) tested this hypothesis by evaluating fetal development during nutrient restriction of cows in early gestation. When maternal undernutrition in early-gestation, fetal

intrauterine growth restriction (IUGR) can occur (McMillen et al., 2001; Vonnahme et al., 2003). Long et al., (2009) documented the first decrease in fetal growth although fetal growth restriction only occurred in the NR IUGR group and not the NR non-IUGR group. Necropsy on the calves at d 125 were documented in this study. Fetal weight and empty carcass weight were significantly reduced (p<0.01) in offspring of NR dams compared to offspring of dams that were not restricted, brain and heart weight were increased, left and right atrium thickness also increased and, liver and lungs saw a decrease in weights during development. Although kidney and pancreas weights did not change in nutrient restricted cows, the absolute glomerular number and glomeruli per gram of tissue in kidneys were reduced in nutrient restricted fetuses.

Limesand et al., (2005) saw a decrease in pancreas weight among intrauterine growth restriction (IUGR) fetuses. Reported a reduction of 76% of  $\beta$ -cell mass in fetuses near term which was a result of decreased rates of  $\beta$ -Cell proliferation and neoformation. Limesand et al., (2006) reported decreases in glucose oxidation in IUGR at high concentrations of glucose. Reported impairment of islet oxidative glucose metabolism (Limesand et al., 2006). Thus, even though tissue mass was not impacted, physiological function of the vital organs was changed, which could have long-term consequences on growth of the calf.

Maternal nutrition impacts development of the fetus throughout gestation with the effects somewhat dependent upon the organ systems developing during each stage of gestation. Organ systems can be affected through changes in overall growth and mass, as well as, changes in physiological function. Total nutrient restriction has been well studied in beef cattle, but less research has focused on the role of specific nutrients. Methionine may be one of the specific nutrients that are key in the development of the fetus.

#### **METHODS**

This study was conducted at the University of Florida Range Cattle Research and Education center in Ona, FL. All animal handling techniques were approved prior to data collection from the University of Florida Institutional Animal Care and Use Committee (protocol #201408583).

### Year 1

**Cow Management.** One-hundred and eight Brangus-Angus crossbred lactating beef cows (n=108) were used in this experiment. Cows were stratified by previous calving date and assigned to 1 of 3 treatments (2 herds per treatment; 18 cows per herd) while fed low-quality limpograss (*Hemarthria altissima*) hay and grazing dormant bahaigrass (*Paspalum notatum*) pastures. The treatments consisted of: (1) Control, supplemented with molasses plus urea (16% CP as fed basis) at 2.72 kg/hd/d, (2) Fishmeal, 2.27 kg/hd/d molasses with urea supplement plus 0.33 kg/hd/d of fishmeal (2.85 methionine % RUP; NRC, 2000) to meet metabolizable protein requirement providing an estimated 3.5 g of by-pass methionine, and (3) Methionine, 2.72 kg/hd/d of molasses with urea plus 10 g/hd/d of Metasmart Liquid© (Adisseo, Alpharetta, GA) to provide 3.7 g of by-pass methionine. The base diet (Treatment 1) provided 100% of the metabolizable energy and 87% of the metabolizable protein requirement (NRC, 2000) for lactating beef cows having peak milk yield of 7 kg/d. Treatment 2 was designed to meet or exceed metabolizable protein and methionine requirement as well as other essential amino acid requirements. Treatment 3 was designed to meet or exceed the metabolizable methionine requirements providing similar methionine as Treatment 2. At calving in October and November 2014, birth weight and birth date of calves and body condition score of cows were recorded by two trained personnel. Dietary treatments began on December 8, 2014. On d 35 (early January 2015) and d 120 (end of March 2015) of the treatment period, cows and calves were weighed following overnight withdrawal from feed, and body condition score of cows assessed by 2 trained personnel. On d 120, hay feeding and supplementation ceased, and cows grazed bahaigrass pastures from April to December 2015. Cow herds were rotated among twenty 4-ha pastures such that all herds grazed each pasture during the grazing season. In June 2015, pregnancy status of cows was determined by rectal palpation.

**Milk production.** On d 35 (early January 2015), 70 (mid-February 2015), and 120 (end of March 2015) of the treatment period, milk production was determined on cows using the weigh-suckle-weigh technique (Hess, 1998). Cows and calves were gathered to the working pens in the morning. Calves were separated from dams at 1200h, then allowed to suckle for 30 minutes at 1600h, and again separated from dams overnight. At 0800h, calves were weighed, placed with their dams for 30 minutes to suckle, and weighed again. Each time, calves were weighed using a squeeze chute on load bars with a scale indicator set for 0.454-kg increments (Tru-Test XR3000, Datamars, Mineral Wells, TX). On d 35, 70, and 120 of the treatment period, a sample of milk from the udder of each cow was collected for analysis of milk components.

#### Year 2

**Cow Management**. In December 2015, cows were fed similar to Treatment 1 allowing ad libitum access to limpograss hay while grazing dormant bahaigrass pastures and supplemented with molasses-urea. At the end of March 2016, hay feeding and molasses supplementation ceased and cows grazed bahaigrass pastures.

**Calf Management**. At calving, birth weight and birth date of calves was recorded. At weaning in July 2016, weaning date and body weight of calves was recorded. Weaning weight was adjusted to standard 205 days of age.

#### **Metabolism Trial**

A subset of 24 steer calves (4 per herd; 8 per treatment) were weaned on June 1, 2016 at 7 months of age. After weaning, steers were placed in individual pens and fed a diet of grain and hay (80:20) at 2.2% of BW. Steers were fed concentrate and hay separately with mineral salt added to the concentrate at 0800h each day.

**Body Weight.** On June 17, 2016, steer calves from year 1 cows were weighed and placed in pens. Calves were given a 7-day adaptation period before being put in their individual pens. Calves were limit-fed the growing diet and feed offered was adjusted based on BW. On June 27 (day 0), steers were weighed after overnight withdrawal of feed and water and place in individual pens, feed offered for the next 14 d was adjusted based on BW collected on d 0. Body weight was measured every 14 d to adjust feed offered at 2.2% of BW. Final BW was collected on d 42 after overnight withdrawal of feed and water.

**Nutrient Digestibility.** Determination of apparent total tract digestibility of DM, OM, starch, CP, neutral detergent fiber (NDF) and acid detergent fiber (ADF) was performed using indigestible NDF (iNDF) as an internal marker. Concentrations of iNDF in feed and fecal samples were determined as described by Cole et al. (2011) with modifications proposed by Krizsan and Huhtanen (2013). Diet and rectal fecal samples were collected beginning on d 36 and 37, respectively, for 4 consecutive days. Both feed and fecal samples were collected twice per day, at 0800 and 1700 h. After collection, samples were frozen at -20°C until further

processing and analysis. At the end of the experiment, feed and fecal samples were dried at 55°C for 48 h in a forced-air oven. Then, samples were ground in a Willey mill (Arthur H. Thomas Co., Philadelphia, PA) to pass a 2-mm sieve and pooled within steer, on an equal weight basis per sample, for determination of nutrient and marker concentration.

For determination of feed and fecal sample DM and OM, approximately 0.5 g of sample was weighed in duplicate, dried in a forced-air oven at 100°C for 24 h and ashed at 550°C for 6 h. For determination of the fibrous components, 0.5 g of dry feed and fecal samples were weighed in duplicate into F57 bags (Ankom Technology Corp., Macedon, NY) and analyzed for NDF, using heat-stable α-amylase and sodium sulfite, and subsequently for ADF as described by Van Soest et al. (1991) in an Ankom 200 Fiber Analyzer (Ankom Technology Corp). Concentration of CP in the samples was determined by rapid combustion using a micro elemental N analyzer (Vario Max CN, Elementar Americas Inc., Mt. Laurel, NJ) according to the official method 992.15 (AOAC, 1995). Starch concentration in feed and feces was measured by an enzymatic-colorimetric method as described by Hall (2015).

For the determination of iNDF, 0.5 g of feed and fecal samples were weighed in duplicate into F57 bags (Ankom Technology Corp.), incubated in the rumen of a cannulated steer for 288 h, and the residue analyzed for NDF, as previously described. Apparent total tract digestibility of DM, OM, CP, NDF, and ADF were calculated using the following formula:

$$100 - 100 \times \left[ \left( \frac{marker \ concentration \ in \ feed}{marker \ concentration \ in \ feces} \right) \times \left( \frac{nutrient \ concentration \ in \ feces}{nutrient \ concentration \ in \ feed} \right) \right]$$

**Blood Collection**. On d 28, steers were weighed and blood was collected before feeding. Once the feeding was complete and 4 hrs had passed blood was once again collected. Blood was collected via jugular venipuncture into sodium-heparin (158 USP) containing tubes (Vacutainer, Becton Dickinson, Franklin Lakes, NJ). Blood samples were immediately placed on ice following collection and then centrifuged at 1,200-x g for 25 min at 4°C to separate plasma. Plasma samples were then stored at -20°C until analysis. Commercial quantitative colorimetric kits were used to determine the plasma concentrations of urea nitrogen (B7551; Pointe Scientific Inc., Canton, MI) and glucose (G7521; Pointe Scientific, Inc., Canon, MI). Inter- and intra-assay CV for assays of PUN and glucose were 2.9% and 3.5% and 3.7% and 5.8%, respectively.

### **Statistical Analysis**

All data were analyzed as a completely randomized design. Pasture was used as the experimental unit for cow and calf performance data in both years. Individual animal was considered the experimental unit for data collected during the metabolism trial. All data was analyzed statistically using the PROC MIXED of SAS (version 9.4; SAS Inst. Inc., Cary, NC). For steer performance data during the metabolism trial, initial body weight was evaluated as a covariate. For blood metabolite data during the metabolism trial, pre-feeding plasma glucose or urea nitrogen concentrations were evaluated as covariates for the appropriate parameter. Treatment comparisons were performed using Turkeys-Kramer method of adjusting for multiple pair-wise comparisons. LS means were considered significant at P < 0.05 and tendencies at 0.05 < P < 0.10.

#### RESULTS

**Forage Nutrient Composition.** There was no difference (P > 0.10) between treatments in nutrient composition of dormant bahaigrass pasture or limpograss hay. The mean and standard deviation for each nutrient analyzed in bahiagrass pasture and limpograss hay are presented in Table 1, as well as the nutrient composition of molasses and fishmeal supplements crude protein content of bahiagrass pasture and limpograss hay for Control, Fishmeal, and Methionine were 7.1%, 7.7%, & 7.8% and 8.1%, 8.0% and 8.0% respectively.

**Dam Performance.** Performance data of the dams during the treatment period in early gestation is presented in Table 2. Treatment had no effect on final BW, ADG or final BCS (P > 0.11). There was a trend (P < 0.10) for Methionine dams to have greater ECM and Adj. ECM. Control dams tended (P = 0.08) to have lesser milk protein content than Fishmeal and Methionine dams, but there was no difference in milk fat, urea N, lactose, or somatic cell count. There was no effect of treatment on pregnancy rate (P = 0.45) following the treatment period.

**Pre-Weaning Growth Performance of Calves.** Performance of nursing and fetalprogrammed calves from birth to weaning are presented in Table 3. Calendar day of birth, birth weight, and 205-d adjusted weaning weight did not differ (P > 0.15) among treatments for nursing or fetal-programmed calves.

**Post-Weaning Growth Performance of Fetal-Programmed Steers.** Growth performance of fetal-programmed steers during the post-weaning metabolism trial is presented in Table 4. There was no difference (P = 0.52) in initial BW between treatments, which coincides with the lack of difference in weaning weight of calves. However, ADG and final BW were greater (P < 0.05) in steers whose dams were supplemented with fishmeal or methionine during

early gestation. Dry matter intake and DMI as a percentage of BW did not differ (P = 0.59) among the treatments per experimental design. Feed efficiency was greater (P < 0.05) in steers whose dams were supplemented with Fishmeal or Methionine during early gestation.

	Bahai	Banaigrass		Limpograss		
Nutrient <sup>1</sup>	Mean	SD	Mean	SD	Molasses	Fish meal
DM, %	87.68	0.15	92.18	0.45	77.50	91.90
CP, % DM	7.53	0.52	4.20	0.57	15.10	65.4
NDF, % DM	74.20	1.19	80.28	1.09		
ADF, % DM	46.67	1.00	42.32	1.39		
EE, % DM	1.32	0.12	0.98	0.40	1.60	10.30
TDN, % DM	54.00	1.67	53.50	1.38	58.00	74.00
Lignin, % NDF	5.35	0.80	6.58	0.47		
Ash, % DM	5.61	0.22	2.96	0.75	12.68	19.66

Table 1. Nutrient composition of bahaigrass pasture, limpograss hay, molasses and fishmeal fed to early gestation beef cows during the treatment period in Year 1

<sup>1</sup>DM= dry matter; CP= crude protein; NDF= neutral detergent fiber; ADF= acid detergent

fiber; EE= ether extract; TDN= total digestible nutrients

Item <sup>1</sup>	Control	Fish meal	Methionine	SEM	P-value
IBW, kg	428.6	445.9	448.0	21.4	0.36
FBW, kg	426.2	448.2	441.4	19.5	0.30
ADG ,kgd	-0.05	-0.02	-0.08	0.20	0.72
Initial BCS	4.15	4.55	4.21	0.11	0.15
Final BCS	3.85	4.23	4.01	0.09	0.11
BCS change	-0.29	- 0.30	-0.19	0.07	0.49
MY, kg/d	4.85	4.93	6.50	1.01	0.12
Adj MY, kg/d	5.66	5.76	7.72	1.26	0.12
ECM, kg/d	8.40 <sup>x</sup>	8.96 <sup>xy</sup>	12.32 <sup>y</sup>	0.84	0.07
Adj. ECM, kg/d	9.31 <sup>x</sup>	11.30 <sup>xy</sup>	14.50 <sup>y</sup>	1.10	0.09
Milk Composition					
Fat, %	1.83	2.22	2.30	0.31	0.56
Protein, %	2.84 <sup>x</sup>	3.06 <sup>y</sup>	2.97 <sup>z</sup>	0.05	0.08
Lactose, %	4.98	4.96	4.96	0.04	0.87
MuN, mg/dL	10.82	13.35	11.73	0.92	0.28
SCC, per/mL	69.63	129.73	117.21	31.81	0.43
Pregnancy Rate,					
%	94.0	89.0	82.0	0.7	0.45

Table 2. Performance of dams during the treatment period in early gestation in Year 1

<sup>1</sup>IBW = initial body weight; FBW = final body weight; ADG = average daily gain; BCS = body condition score; MY = milk yield; ECM = energy-corrected milk yield; SCC = somatic cell count

<sup>xyz</sup> LS means within a row with a common superscript differ ( $P \le 0.10$ )

<sup>abc</sup> LS means writhing a row with a common superscript differ ( $P \le 0.05$ )

Item <sup>1</sup>	Control	Fish meal	Methionine	SEM	P-value
Nursing Calf					
DOB	320.40	322.02	323.60	4.44	0.86
BW, kg	34.3	34.1	34.1	2.5	0.99
Adj. WW, kg	204.7	221.3	214.5	10.9	0.18
Programmed Calf					
DOB	323.94	325.21	321.05	3.58	0.70
BW, kg	32.5	32.6	32.1	2.4	0.94
Adj. WW, kg	204.4	211.7	211.5	17.9	0.77

Table 3. Growth performance of nursing calves in Year 1 and fetal-programmed calves in Year 2 from birth to weaning

 $^{1}$ BW = birth weight; WW = weaning weight; DOB = calendar day of birth

**Plasma Metabolites.** Concentrations of plasma urea nitrogen (PUN) and glucose of fetalprogrammed steers before and after feeding during the post-weaning metabolism trial are presented in Table 5. In year 2 saw an effect (P < 0.05) on PUN both before and after feeding, and tended (P = 0.10) to effect the change in PUN from the calves. Steers born to control and methionine dams had greater PUN concentrations, but also greater change in PUN concentration than steers born to fishmeal dams. Treatment also affected (P < 0.05) plasma glucose concentrations both before and after feeding as well as the change in plasma glucose. Prior to feeding, steers born to methionine dams had lesser plasma glucose concentration than steers born to Fishmeal dams with steers born to control dams being intermediate. In contrast, after feeding, steers born to control and fishmeal dams were not different, but steers born to methionine dams had lesser plasma glucose concentrations than the other treatments. Steers born to methionine dams also had greater change in plasma glucose concentrations from before feeding to after feeding than steers born to control or fishmeal dams.

Item <sup>1</sup>	Control	Fish meal	Methionine	SEM	P-value
IBW, kg	222.4	210.7	205.8	24.6	0.52
FBW, kg	247.9 <sup>a</sup>	255.0 <sup>b</sup>	255.4b	5.0	0.04
ADG <sup>2</sup> , kg/d	0.83 <sup>a</sup>	0.99 <sup>b</sup>	1.01 <sup>b</sup>	0.12	0.04
DMI, kg/d	12.05	11.61	11.24	0.60	0.59
DMI, % BW	0.02	0.02	0.02	0.0002	0.60
FE, kg/kg	0.16 <sup>a</sup>	0.19 <sup>b</sup>	0.19 <sup>b</sup>	0.01	0.02

Table 4. Performance of fetal-programmed steers during the post-weaning metabolism trial in Year 2.

<sup>1</sup>IBW = initial body weight; FBW = final body weight; ADG = average daily gain; DMI = dry matter intake; FE= gain: feed

<sup>2</sup>Initial BW was a significant covariate (P < 0.05).

<sup>abc</sup> LS means writhing a row with a common superscript differ ( $P \le 0.05$ )

**Nutrient Digestibility.** Nutrient intake and digestibility for fetal-programmed steers during the post-weaning metabolism trial are presented in Table 6. Treatment did not affect (P > 0.13) intake of feedstuff analyzed, which is expected based on the experimental. Treatment did not have an effect on DM, OM, CP and Starch digestibility (P=0.52). There was a trend (P=0.06) for treatment to effect NDF and ADF digestibility with steers born to methionine dams having greater digestibility than steers born to control or fishmeal dams.

Item <sup>1</sup>	Control	Fish meal	Methionine	SEM	P-value
PUN					
Pre, mg/dL	12.58 <sup>a</sup>	11.20 <sup>b</sup>	12.85 <sup>a</sup>	0.43	0.02
Post, mg/dL	14.60 <sup>a</sup>	12.33 <sup>b</sup>	13.77 <sup>ab</sup>	0.61	0.04
Change <sup>2</sup>	2.33 <sup>x</sup>	0.16 <sup>y</sup>	1.48 <sup>xy</sup>	0.71	0.10
Glucose					
Pre, mg/dL	82.82 <sup>ab</sup>	90.40 <sup>b</sup>	75.25 <sup>a</sup>	4.32	0.05
Post, mg/dL	81.18 <sup>ab</sup>	82.75 <sup>b</sup>	69.61 <sup>a</sup>	3.05	0.01
Change <sup>3</sup>	-1.27 <sup>ab</sup>	1.07 <sup>b</sup>	-13.64 <sup>a</sup>	3.35	0.01

Table 5. Plasma metabolite concentrations of fetal-programmed steers during the postweaning metabolism trial in Year 2

<sup>1</sup>PUN = plasma urea nitrogen; Pre = blood collected prior to the morning feeding; Post = blood collected 4 h following the morning feeding; Change = difference between post-feeding and pre-feeding measurements.

<sup>2</sup>Pre-feeding PUN was a significant covariate (P < 0.05)

<sup>3</sup>Pre-feeding glucose was a significant covariate (P < 0.05)

<sup>abc</sup> LS means writhing a row with a common superscript differ ( $P \le 0.05$ )

<sup>xyz</sup> LS means within a row with a common superscript differ ( $P \le 0.10$ )

Item	Control	Fish meal	Methionine	SEM	P-value
Intake, kg/d					
$DM^1$	5.99	5.27	5.88	0.29	0.17
OM	5.58	4.94	5.47	0.26	0.20
NDF	2.30	2.02	2.34	0.12	0.13
ADF	1.42	1.29	1.43	0.07	0.32
СР	1.10	0.95	1.04	0.07	0.38
Starch	0.50	0.45	0.45	0.05	0.81
Digestibility, %					
DM	76.47	75.99	78.35	1.53	0.52
OM	78.08	77.82	79.94	1.40	0.52
NDF	68.17 <sup>xy</sup>	65.2 <sup>x</sup>	71.84 <sup>y</sup>	1.81	0.06
ADF	69.95 <sup>xy</sup>	67.79 <sup>x</sup>	73.74 <sup>y</sup>	1.83	0.06
СР	79.66	80.15	79.16	1.77	0.92
Starch	92.38	90.71	92.60	2.40	0.52

Table 6. Nutrient intake and apparent total tract digestibility in fetal-programmed steers during the post-weaning metabolism trial in Year 2

<sup>1</sup>DM = dry matter; OM = organic matter; CP = crude protein; NDF = neutral detergent fiber, ADF = acid detergent fiber

<sup>xyz</sup> LS means within a row with a common superscript differ ( $P \le 0.10$ )

## DISCUSSION

Year 1

In the current study, by-pass protein or methionine did not affect body weight or body condition in lactating beef cows. Similar to the current study, previous studies have reported no effect of supplemental rumen undegradable protein on body weight or body condition change in gestating or lactating beef cows (Triplett et al., 1995; Encinias et al., 2005) and lactating dairy cows (Chen et al., 2011). In contrast, protein supplementation to gestating beef cows consuming low to medium-quality forage (6-8% CP) decreased body weight and body condition loss (Stalker et al., 2006, 2007). Waterman et al. (2007) reported that by-pass methionine in addition to urea supplement for cows consuming low-quality forage, tended to increase N retention and N use efficiency. However, the effect of supplemental by-pass methionine was not evident in body weight or condition score change in the current study or Chen et al. (2011).

Energy-corrected milk yield and adjusted ECM tended to be greater for methionine supplemented cows, and milk protein content tended to be greater for cows supplemented with by-pass protein or methionine. Similar results were published by Chen et al. (2011) where dairy cows supplemented with rumen by-pass methionine had greater ECM and protein content of milk. Toledo et al. (2017) also reported greater milk protein content, as well as fat content, in dairy cows supplemented with rumen by-pass methionine, but there was no difference in ECM. In contrast, Triplett et al. (1995) and Encinias et al., (2005) reported no effect of rumen by-pass protein on milk yield or milk components in lactating beef cows. The differences in milk yield could be due to level of production between dairy and beef cows with dairy cows requiring greater amounts of methionine due to increased milk production. Differences could also be due

to supplementation with methionine alone versus total protein. In the current study, cows supplemented with fishmeal had similar milk yield as Control cows which was similar to Triplett et al. (1995) and Encinias et al. (2005), where cows fed methionine had greater milk yield than control cows similar to Chen et al. (2011).

Calf performance during the treatment year was not influenced by dietary treatment even though methionine cows tended to produce more milk. Similar to the current study, Encinias et al. (2005) found that calf growth was not affected by rumen by-pass protein supplementation to cows during early lactation, but milk yield also was not affected in this study. In contrast, Triplett et al. (1995) reported that calves tended to have greater ADG when cows were supplemented with rumen by-pass protein during early lactation, but milk yield was not different. The differences in calf growth among studies is not readily apparent, but could be due to calf access to cow supplement and (or) cow parity. In the current study, calves had access to the supplemental feed offered to cows, whereas in the studies of Triplett et al. (1995) and Encinias et al. (2005) the calves did not have access to cow supplement. Access to the cow supplement could have allowed calves from control and fishmeal cows to consume supplement thus replacing the nutrients not being consumed from greater milk yield as calves from methionine cows. In the study of Triplett et al. (1995) there was a dietary supplement by cow parity interaction where rumen by-pass protein supplementation increased milk yield in primiparous cows, but not multiparous females. Given that primiparous cows constituted 61% of treatment groups (Triplett et al. (1995) may have influenced calf growth for the overall treatment group resulting in an effect on calf growth.

#### Year 2

Fetal-programmed calves had pre-weaning growth performance that was not affected by dietary treatments applied to cows during the periconception period. Similarly, Martin et al., (2007) reported no difference in weaning weight of heifers from cows fed a protein supplement during late gestation. In contrast, several studies (Stalker et al., 2006, 2007; Funston et al., 2008; Larson et al., 2009) reported greater weaning weight for calves from cows that were fed a protein supplement during late gestation. The lack of differences in calf weaning weight in the current study is unclear, but may be related to less replication per treatment. Previous studies report improvements in calf weaning weight of 5 to 9 kg, which is similar to the 7 kg difference in the current study. However, previous studies report SEM of 2 to 5 kg compared with the SEM in the current study of 18 kg.

Post-weaning growth performance of steers was affected by dietary supplement fed to cows during the peri-conception period where calves from cows fed the fishmeal or methionine diet had improved ADG and feed efficiency compared to steers from cows fed the control diet. Stalker et al. (2007) reported a tendency for calves from dams supplemented with protein during late gestation to have greater post-weaning ADG, but no difference in gainfeed ratio. In contrast, several studies (Stalker et al., 2006; Martin et al., 2007; Funston et al., 2010 and Larson et al., 2009) found that maternal nutrition during late gestation had no effect on post-weaning ADG or feed efficiency. This may be related to the stage of gestation in which maternal dietary treatments were applied. Previous work (Mao et al., 2008; Du et al., 2010, 2015) indicates that fetal development follows the pattern of organogenesis during early gestation, myogenesis during mid-gestation, and adipogenesis during late gestation. The current study altered maternal nutrition during the peri-conception period when organogenesis would be expected to occur,

which likely impacted development of important tissues in nutrient metabolism such as liver and gastrointestinal tract. Evidence is shown in the altered glucose and nitrogen metabolism, and nutrient digestibility of steers from cows supplemented with fishmeal or methionine.

Plasma urea nitrogen concentrations were decreased in steers from fishmeal cows, and glucose concentrations were decreased in steers from methionine cows. Additionally, steers from methionine cows tended to have increased apparent total tract digestibility of NDF and ADF. Interestingly, Jacometo et al. (2016) found that calves from dams fed rumen-protected methionine during late gestation had lower blood glucose concentration at birth, and insulin concentrations were greater during the first few weeks of life. In the first few weeks after birth, blood urea concentration was altered by rumen-protected methionine supplementation of the cow, but not consistently across several time points. Interestingly, Jacometo et al. (2016) reported greater mRNA expression of genes involved in gluconeogenesis (phosphoenolpyruvate carboxykinase, fructo-bisphosphatase 1), fatty acid oxidation (carnitine palmitoyl-transferase 1A), and insulin signaling (murine thymoma viral oncogene homolog 2 (AKT2), facilitated glucose transporter 2) in liver. No previous studies have evaluated nutrient digestion in fetalprogrammed calves, but the tendency for improved digestibility could be due to changes in gastrointestinal tract metabolism or microbiome.

#### **CONCLUSION & IMPLICATIONS**

## Conclusion

Supplementation of methionine to lactating beef cows tended to increase energy corrected milk yield through a combination of increased milk yield while maintaining milk fat and protein concentrations. However, cows supplemented with methionine did not lose additional weight or condition score. But, methionine-supplemented cows did not wean heavier calves questioning the added cost of methionine supplementation.

Neither fish meal nor methionine affected pre-weaning growth performance of fetalprogrammed calves. However, steers from cows supplemented with additional by-pass protein or methionine gained more weight and converted feed more efficiently post-weaning. Additionally, steers from methionine supplemented dams tended to have increased fiber digestion and regulate glucose metabolism differently. These results indicate that methionine is the key nutrient affecting fetal development when additional by-pass protein is fed to gestating beef cows.

## Implications

Supplementation of methionine to cows during early gestation would be beneficial when fed poor quality forage that may limit metabolizable methionine supply. More research is required on methionine and fetal programming, specifically furthering our understanding of how one-carbon metabolism functions in ruminants and the supply of key nutrients to the fetus. Additionally, maternal nutrition during the peri-conception period could be used to improve post weaning growth and feed conversion through altered nutrient utilization.

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