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Interactions of change in nutrition after AI on plasma metabolites, steroid hormone production, and uterine environment

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Objective

The objective was to evaluate the impact of nutritional changes post artificial insemination (AI) on plasma metabolites, steroid hormones, and uterine environment.

Study Description

Beef heifers (n = 43) were randomly assigned to two dietary treatment groups (High = 161.5% or Low = 77.5% of maintenance energy) for 14 d after AI (post-AI). Post-AI dietary treatments continued until uteri were flushed for embryo recovery (d 14 post-AI). Blood samples were collected on d -3, 0 (day of AI), 3, 6, 9, 12, and 14 for analysis of plasma glucose, proteins, non-esterified fatty acids (NEFAs), and cholesterol using colorimetric assays. Plasma collected on d 0, 3, 6, 9, 12, and 14 was analyzed for progesterone concentrations by radioimmunoassay. Uterine flushes were analyzed for mineral concentrations of Mg, P, S, K, Ca, Cu, Zn, Se, Mn, Co, B, Cr, and Fe by Inductively Coupled Plasma Mass Spectrometry (ICP-MS). Plasma progesterone, NEFAs, protein, glucose and cholesterol (repeated measures) and uterine mineral concentrations were analyzed using the MIXED procedures in SAS. Plasma NEFA concentrations differed between treatments (P = 0.03) with heifers on the low diet treatment having elevated NEFA concentrations. Plasma NEFA concentrations weren't affected by embryo recovery (P > 0.10), treatment by embryo recovery (P > 0.10), and treatment by embryo recovery by day (P > 0.10). Plasma progesterone, glucose, protein, and cholesterol concentrations were not influenced by treatment (P > 0.10), embryo recovery (P > 0.10), treatment by embryo recovery (P > 0.10), and treatment by embryo recovery by day (P > 0.10). Uterine mineral concentrations were affected by embryo presence for Mg (P = 0.02) and S (P = 0.01) a tendency for Ca (P = 0.08) with decreased concentrations in uterine flushes when an embryo was recovered. A tendency for increased concentration of Mn (P = 0.06) was observed in uterine flushes when an embryo was recovered. Additionally, treatment tended to impact Fe concentrations (P = 0.09), with heifers on the restricted diet having reduced uterine Fe concentrations. In conclusion, changing plane of nutrition post-AI had an effect on NEFA plasma concentrations, but no effect on plasma progesterone, protein, glucose, and cholesterol concentrations. The presence of an embryo however affected uterine mineral concentrations.

Take Home Points

Decreasing plane of nutrition post-AI increased NEFA plasma concentrations, but had no effect on plasma progesterone, protein, glucose, and cholesterol concentrations; however, the presence of an embryo did affect uterine mineral concentrations. More work is needed to better understand the relationship between embryo presence and uterine mineral concentrations and the influence of the mineral concentrations on early embryo development.

Introduction

It is widely understood that nutrition has an impact on reproduction in beef cattle. Short and Adams (1988) illustrated whereg reproduction ranks on the nutrient partitioning list; nutrients are used in the following order of importance: basal metabolism, activity, growth, energy reserves, pregnancy, lactation, additional energy reserves, estrous cyclicity, initiation of pregnancy, and excess reserves. Initiation of pregnancy and estrous cycling is toward the end of nutrient allocation; therefore, an adequate plane of nutrition is important to ensure a female's reproductive success.

Management of heifers can have lifetime effects on their reproductive success and efficiency. It is a common management practice to feed replacement heifers a mixture of concentrate and forage diets (greater maintenance energy) prior to artificial insemination (AI) in a confinement system. However, post-AI, heifers are typically placed on pasture and consume solely a forage diet. Perry et al. (2013) reported heifers placed on high forage diets directly after AI experienced a reduced average daily gain, thus indicating a nutrient deficient diet post-AI. Post-AI diet restrictions produced poorer quality embryos, reduced the number of blastomeres in the embryo, and decreased the percentage of live blastomeres (Kruse et al., 2017). Common management practices such as placing heifers on pastures directly after AI can create a nutrient restriction. Post-AI diet restrictions can negatively impact reproductive performance by hindering embryonic development.

Nutrition also can affect reproduction indirectly through blood metabolites. Blood metabolites are indicators of nutrient status and potentially impact reproductive hormones such as FSH, LH, progesterone, and estradiol. It has been shown that restricted planes of nutrition for heifers can decrease certain plasma metabolites such as glucose, insulin, and IGF-1 (Bossis et al., 1999). Cholesterol is a precursor for steroid biosynthesis, which is necessary to produce steroidal reproductive hormones. A decrease in specific steroidal hormones during early gestation could cause early embryonic death. Therefore, diet restriction post-AI that alter blood metabolites could be indirectly affecting reproduction.

Plane of nutrition can also impact a female's uterine environment. An appropriate uterine environment is essential for supporting the conceptus and embryo elongation. Gao et al. (2009) reported that pregnant ewes had increased concentrations of sodium, calcium, and potassium in their uterine lumen flush media compared to cyclic, non-pregnant ewes. Our laboratory found that magnesium, aluminum, potassium, calcium, and sulfur concentrations in the uterine lumen are reduced in pregnant (d 7 embryo) compared to non-pregnant heifers (unpublished, preliminary data), indicating that the embryo may be using the minerals for growth and development.

In conclusion, nutrition and reproduction have an essential relationship. It is understood that the plane of nutrition and metabolic status of a female can impact their reproductive efficiency, however, many of the nutritional mechanisms by which reproduction is mediated are still unknown. The objective of this study was to evaluate the impact of nutritional changes after artificial insemination (AI) on plasma metabolites, steroid hormones, and uterine environment.

Experimental Procedures

Experimental Design

Fifty heifers were provided the same pre-breeding diet to meet crude protein requirements; however, seven heifers were removed from the statistical analysis due to unsuccessful uterine flushes. At time of artificial insemination (AI) half of the heifers were randomly assigned to a restricted energy diet (Low = 77.5% of maintenance energy) post-AI and the other half remained on a high energy diet (High = 161.5% of maintenance energy). Both diets consisted of a total mixed ration of grass hay, straw, and corn silage. Energy content of the diet was changed by intake; heifers on the low treatment were fed 12.1 lb/hd/d and the heifers on the high treatment were fed 25.5 lb/hd/d. Heifers remained on their respective diets for 14 days post-AI.

Feed Analysis

Samples of the dietary treatments were taken twice after assignment post-AI for a total of two samples per treatment. Feed samples were analyzed by wet chemistry at Dairyland Laboratories, Inc. for crude protein, acid detergent insoluble crude protein (ADICP), acid detergent fiber (ADF), neutral ash free detergent fiber (aNDF), ash free aNDF (aNDFom), lignin, fat, ash, calcium, phosphorus, magnesium, potassium, sulfur, sodium, chloride, zinc, iron, manganese, copper, boron, aluminum, total digestible nutrients (TDN), net energy for gain (NEg), and net energy for maintenance (NEm) (Table 1).

Synchronization

Heifer estrous cycles were synchronized across two days using the PG 6-day Controlled Internal Drug Release (CIDR) protocol to facilitate data and tissue collection. The PG 6-day CIDR protocol included administration of $PGF_{2\alpha}$ (Lutalyse, 5 mL i.m.) on day -12, CIDR insertion and GnRH (Factrel, 2 mL i.m.) on day -9, CIDR removal and second dose of $PGF_{2\alpha}$ (Lutalyse, 5 mL) on day -3. Heifers were observed for estrus (day 0) three times daily from day -3 to 0 with the aid of Estrotect patches applied on day -3. Heifers observed in standing estrus (or with ≥ 50% of the Estrotect patch activated) were artificially inseminated approximately 8-12 hours after first observation of estrus activity. Heifers that didn't exhibit estrus by 72 hours post CIDR removal received GnRH (Factrel, 2 mL i.m.) and were AIed at that time. All heifers were bred to the same two sires (evenly distributed among treatments) and by the same technician. Also, at time of CIDR removal, heifers were transrectally ultrasounded to confirm follicular growth and size, and anticipated side of ovulation.

Blood Collection

Blood was collected from all heifers by jugular or tail venipuncture into 10 mL Vacutainer tubes with EDTA. Blood was collected on days -3, 0 (day of AI), 3, 6, 9, 12, and 14. During collections, blood was kept cool until centrifuged. Plasma was harvested following centrifugation at 3,000 x *g* for 30 minutes at 4 ˚C and stored at -20 ˚C until further analyses.

Plasma Analyses

Plasma samples from day -3, 0, 3, 6, 9, 12, and 14 were analyzed for glucose, cholesterol, protein, and non-esterified fatty acids (NEFAs) by colorimetric assays and progesterone concentrations (d 0, 3, 6, 9, 12, and 14) was determined by radioimmunoassay.

Uterine and Embryo Flushing

At the time of uteri flushing, transrectal ultrasound was conducted to confirm ovulation had occurred after AI and identify the side of ovulation (corpus luteum presence). Uteri were flushed for embryo recovery 14 days after AI using a non-surgical technique. Briefly, a flush catheter was placed in the uterine horn ipsilateral to the corpus luteum and 25 mL of flush media was inserted into the uterine horn. Flush media was recovered by gently massaging the uterine horn. Flush media volume was recorded and media was searched for presence of an embryo. If an embryo was not found the procedure was repeated. The initial flush media was used for mineral analysis. Embryos recovered were washed three times in embryo holding media, measured using a ruler, and photographed. Seven heifers were not successfully flushed and were subsequently removed from all analyses.

Statistical Analysis

The effect of treatment on plasma progesterone, NEFAs, protein, glucose and cholesterol were evaluated as repeated measures using the MIXED procedure in SAS. The effect of treatment, presence of an embryo, and their interaction on mineral concentrations were evaluated using the MIXED procedure in SAS. Statistical significance was considered at $P \leq 0.05$ and a tendency when $0.05 < P \leq 0.10$.

Results and Discussion

Plasma Analysis

Plasma NEFA concentrations differed between treatments (Figure 1; $P = 0.03$) with elevated NEFA concentrations among heifers on the low diet treatment. There was no effect of embryo presence ($P > 0.10$), treatment by embryo presence ($P > 0.10$), and treatment by embryo presence by day ($P > 0.10$) on plasma NEFA, progesterone, glucose, protein, and cholesterol concentrations. Furthermore, post-AI diet treatment didn't influence plasma progesterone, glucose, protein, and cholesterol concentrations (P > 10). Also please italicize the P.

Uterine Flushes

Mineral concentrations of uterine flush are reported in Table 2. There was an effect of embryo presence on uterine flush mineral concentrations for Mg ($P = 0.02$) and S ($P = 0.01$) and a tendency for Ca ($P = 0.08$) where decreased concentrations were observed when uterine flushes contained an embryo. There was a tendency for the presence of an embryo to affect uterine mineral concentrations for Mn ($P = 0.06$), with increased concentrations observed when uterine flushes contained an embryo. There was a tendency between treatments to impact uterine flush Fe ($P = 0.09$) concentrations, heifers on the restricted diet had reduced uterine Fe concentrations.

Implications

Post-AI nutrition can impact the metabolic status of bovine females therefore, post-AI nutrition has the ability to affect oocyte and embryonic development. Uterine mineral concentrations fluctuate in the presence of an embryo, potentially indicating that the embryo is utilizing the minerals for development and survival.

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Tables

Table 1. Nutrient analysis (DM basis) of samples collected from the high and low diets post-AI[1].

Item	Low Energy Diet	High Energy Diet
Dry Matter, %	67.59	67.64
Crude Protein, %	9.26	9.23
NEm, Mcal/cwt	54.39	54.21
NEg, Mcal/cwt	28.81	28.64

[1]All values except diet dry matter on a dry matter basis.

Table 2. Effects of an embryo presence on mineral concentrations of uterine fluids.

Mineral	No Embryo	Embryo Present	SEM	P-value
Boron (B)	0.01063	0.00991	0.00115	0.61
Calcium (Ca)	6.209	4.2389	0.89990	0.08
Chromium (Cr)	0.00054	0.00053	0.00052	0.98
Cobalt (Co)	0.00035	0.00023	0.00016	0.55
Copper (Cu)	0.01392	0.01422	0.00119	0.89
Iron (Fe)	0.8119	0.749	0.13680	0.70
Magnesium (Mg)	15.6967	12.5457	0.74760	0.02
Manganese (Mn)	0.00159	0.00291	0.00057	0.06
Phosphorus (P)	10.2541	8.64460	1.01550	0.37
Potassium (K)	112.72	102.46	5.17830	0.11
Selenium (Se)	0.00093	0.00089	0.00028	0.91
Sulfur (S)	110.92	92.15830	6.05310	0.01
Zinc (Zn)	0.07407	0.06577	0.01015	0.51

Figures

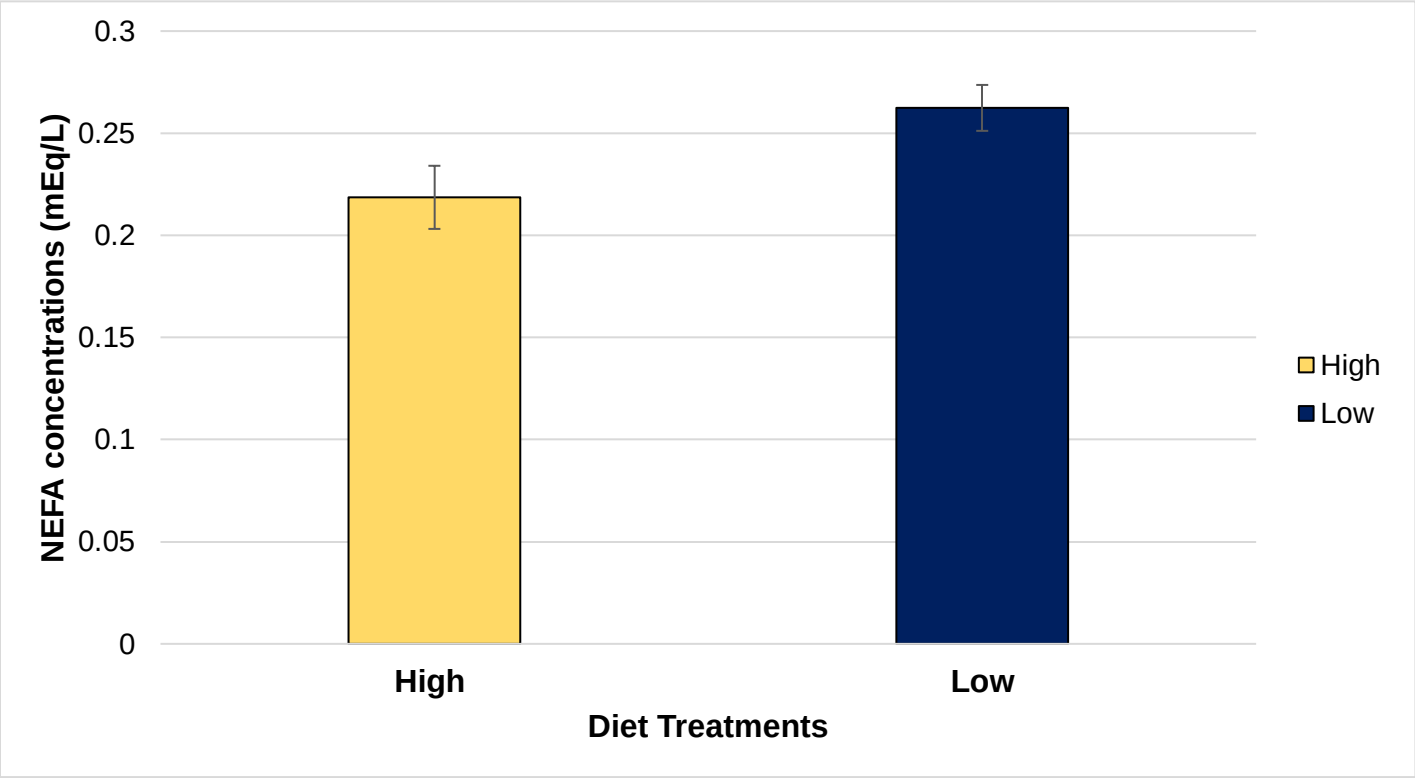

Figure 1. Effects of Post-AI diet treatments on plasma non-esterified fatty acid (NEFA) concentrations in beef heifers.