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Effects of rumen-protected methionine and choline supplementation on vaginal discharge and uterine cytology of Holstein cows

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ABSTRACT

Methionine is one of the most limiting amino acids in dairy diets and low feed intake around the time of calving could lead to decreased synthesis of phosphatidylcholine. An alternative pathway for phosphatidylcholine is to have choline as a precursor. The objective of this study was to determine the effects of feeding rumen-protected methionine and choline pre – and postpartum on reproduction of Holstein cows. Seventy-two Holstein cows were randomly assigned to four treatments from 21 days before calving to 30 days in milk (DIM): supplementation with rumen-protected methionine (MET; n = 20, received 0.08% of the dry matter (DM) of the diet/d as methionine, Smartamine M® to a Lys:Met = 2.9:1), rumen-protected choline (CHO; n = 17, received 60 g/d choline, Reassure), both rumen protected methionine and choline (MIX; n = 19, received 0.08% of the DM of the diet/d as methionine to a Lys:Met = 2.9:1 and 60 g/d choline), or no supplementation to serve as control (CON; n = 16, fed total mixed ration with a Lys:Met = 3.5:1). Cows were evaluated at 4, 7, 10, 13, 15, 17, and 30 d after calving for the presence of secretion using the Metricheck® device. On 15, 30, and 72 d after calving, the uterine endometrium of all cows was sampled using a cytological brush and streaked onto slides for analysis of the presence of polymorphonuclear neutrophils (PMN). We hypothesized that cows supplemented with methionine would have lower metricheck smell scores and lower rates of PMN than non-supplemented cows. On d 30, a treatment difference was detected using the metricheck score and smell ($P < 0.04$), with treatment MIX (score = 0.38) having a lower score than CHO (score = 2.11). Supplementing cows with rumen-protected methionine may have a beneficial effect on cows' uterine health.

1. Introduction

Cows usually experience negative energy balance after calving, which leads to fatty liver and ketosis [1,2]. Cows that develop ketosis are at risk for other metabolic disorders, impaired reproduction, and endometritis [3,4]. During this transition period, amino acids (AA) are needed to export fat away from the liver in the form of very low density lipoproteins, formed by phosphatidylcholine, which is made from methionine [5]. Supplementing rumen-protected methionine has been shown to improve milk production and composition, increase dry matter intake (DMI), reduce lipid accumulation postpartum, and promote liver function [6–8]. A study by Osorio et al. [7] also reported a faster recovery rate from negative energy balance and a tendency for a lower incidence of ketosis when supplementing methionine [7]. However, methionine is one of the most limiting AA in dairy diets [9] and low feed intake around the time of calving could lead to decreased synthesis of phosphatidylcholine. An alternative pathway for phosphatidylcholine is to have choline as a precursor. Choline supplementation before and after calving has been shown to reduce fatty liver and incidence of ketosis and mastitis [10].

Reducing the risk of metabolic disorders by improving liver function and increasing immune function in the transition period

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is a key to better reproductive health. Retained placenta (RP), metritis, and endometritis are diseases from impaired immune function and can have lasting negative effects on uterine health [11]. In the absence of clinical illness, metritis is defined as purulent uterine discharge within 21 d postpartum and endometritis is defined as either clinical: purulent discharge 21 d or more postpartum and mucopurulent discharge more than 26 d postpartum, or subclinical: endometrial inflammation determined by cytology in the absence of clinical endometritis [12].

The majority of cows develops metritis within the first 14 d after calving with a peak around 5 to 7 DIM, so targeting specific days within this time frame in combination with a physical examination is efficacious in diagnosing cows with metritis [13]. Diagnosing subclinical endometritis has been effectively done by using a cytology brush in both cows and mares and can be superior to other techniques [14–16]. The objective of this study was to determine the effects of feeding rumen-protected methionine and choline pre – and postpartum on reproduction of Holstein cows through the assessment of vaginal discharge and uterine cytology. We hypothesized that cows supplemented with methionine would have lower metricheck smell scores and lower rates of PMN than cows that were not supplemented.

2. Materials and methods

2.1. Experimental design and dietary treatments

The University of Illinois Institutional Animal Care and Use Committee (IACUC) approved all following experimental procedures. Seventy-two (n = 72) pregnant Holstein cows entering their 2nd or greater lactation (parity 3.1 ± 1.2 and body weight (BW) 773 ± 16 kg) were enrolled in a randomized complete block design. Cows were blocked with regard to lactation number and previous lactation 305-days milk yield to ensure minimal influence of these variables on the experimental outcome. Cows were housed in tie stalls bedded with sand at the University of Illinois Dairy Cattle Research Unit (Urbana, Illinois). All cows were fed the same fresh cow diet during the transition period and a high cow diet from 31 to 72 DIM to meet but not exceed 100% of the energy requirements as outlined by NRC 2001. Cows were milked three times daily. During the experimental period, cows were fed for *ad libitum* intake.

At −21 ± 2 d relative to calving, cows were randomly assigned to one of four treatments, given to each cow individually as a top-dress on a total mixed ration (TMR): supplementation with rumen-protected methionine (MET; n = 20, received 0.08% of the DM of the diet/d as methionine, Smartamine M®, Adisseo, Alpharetta, GA, USA, to a Lys:Met = 2.9:1), rumen-protected choline (CHO; n = 17, received 60 g/d choline, Reassure, Balchem Corporation, New Hampton, NY), both rumen protected methionine and choline (MIX; n = 19, received 0.08% of the DM of the diet/d as methionine to a Lys:Met = 2.9:1 and 60 g/d choline), or no supplementation to serve as control (CON; n = 16, fed TMR with a Lys:Met = 3.5:1).

After calving from 30 ± 1 DIM to 72 ± 1 DIM, cows were randomly re-assigned to two new treatments: control (CON; n = 36, fed basal diet with a Lys:Met = 3.4:1) and methionine (MET; n = 36, fed basal diet plus methionine to a Lys:Met = 2.9:1). Therefore, after 30 DIM there were 8 treatments: CON-CON (n = 6), CON-MET (n = 10), MET-CON (n = 10), MET-MET (n = 10), CHO-CON (n = 11), CHO-MET (n = 6), MIX-CON (n = 9), and MIX-MET (n = 10). A schematic of the treatment designs after parturition is shown in Fig. 1.

Dry matter intake was determined daily throughout the dry period and first 72 d post-calving. Refused feed from the previous

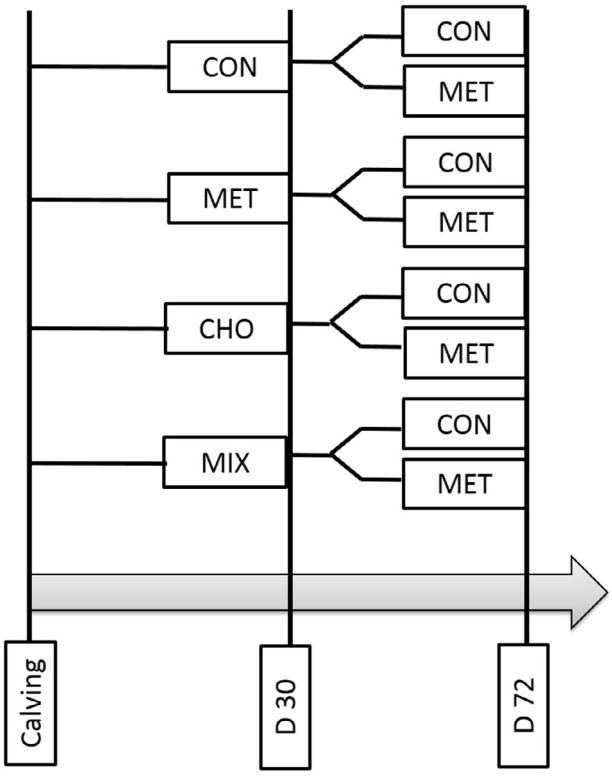

Fig. 1. Schematic of treatment design in chronologic order through the experiment.

day was weighed for each cow daily before feeding. Body weight and body condition scores (BCS; scale of 1 = emaciated to 5 = obese [17]) were obtained weekly throughout the study. Body condition score was assigned in quarter-unit increments by two individuals each time and the average of the score was used for that week. Health disorders were recorded for RP, displaced abomasum (DA), clinical ketosis, mastitis, hypocalcemia, hoof problems, and fever. Retained placenta was defined as a placenta that failed to deliver completely longer than 12 h after calving; Displaced abomasum was diagnosed by a veterinarian; ketosis was diagnosed by farm staff or a veterinarian by urinalysis strip (Ketostix, Bayer Corp. Diagnostics Division, IN); mastitis was diagnosed by altered milk composition and confirmed by positive microbiological culture; hypocalcemia was diagnosed by trained farm staff and veterinarians; hoof problems were defined as cows with abnormal hoof disorders that required extra hoof care such as warts, ulcers, punctures or other injury, abscesses, etc.; fever was defined as cows having a temperature of greater than 39.5 °C on d 4, 7, 10, 13, 15, 17, or 30 relative to calving.

2.2. Metricheck exploration of vaginal content

Cows were evaluated at 4, 7, 10, 13, 15, 17, and 30 d after calving for the presence of vaginal secretions by inserting a device into the vagina of the cow (Metricheck®, Simcro, New Zealand). The Metricheck® (MC) device consists of a 50 cm long stainless steel rod with a 4 cm rubber hemisphere tip that is used to collect vaginal contents. The MC was disinfected before each use with chlorhexidine diacetate disinfectant (Nolvasan Solution, Zoetis Animal Health, Florham Park, NJ). To minimize contamination, the tails of cows were held aside and the vulva was cleaned with Nolvasan solution and dried with paper towels. Sterile lubricant (Therio-gel, Agtech, Inc., Manhattan, KS) was applied to the convex part of the rubber tip before insertion. The MC was inserted

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