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## Effects of ruminally protected methionine and/or phenylalanine on performance of high producing Holstein cows fed rations with very high levels of canola meal

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#### ABSTRACT

Canola meal is the second largest protein feed in the Northern latitudes and inclusion levels in dairy rations are expected to increase due to projected large increases in production of canola seed in Canada. However, a recent study (Swanepoel et al., 2014) showed that even though higher inclusions of canola meal (CM) had a positive effect on production when CM directly substituted for high protein corn based dried distillers grains (DDG), that there was an optimum point at 120-135 g/kg of diet dry matter (DM) after which animal performance seemed to decline. Only the amino acids (AA), methionine (Met), phenylalanine (Phe) and leucine (Leu) could have limited production based upon plasma AA concentrations at the highest CM inclusion level. Our objective was to determine if either Met or Phe, or both, was limiting performance of early lactation dairy cows fed a ration containing 180 g/kg of diet DM as CM, by supplementing a calculated target of 7.5 g of intestinally absorbable Phe/cow/day and/or 8.0 g of intestinally absorbable Met/cow/day in ruminally protected (RP) forms to four pens of  $\sim$ 320 early lactation cows/pen in a 4 × 4 Latin square with 28 days experimental periods. Dry matter intake was not affected (avg.:  $27.6 \pm 0.4$  kg/day) by feeding either of the RP AA, or the combination. Phenylalanine supplementation alone had no effect on milk production or composition, and body condition score (BCS) change compared to Control. Supplemental Met alone modestly increased (P<0.01) milk protein and fat content, while decreasing (P<0.01) milk lactose content and yield, but with no impact on BCS change compared to Control. Combination Met and Phe supplementation decreased milk and lactose yields, as well as lactose content (P<0.01), while increasing milk protein content and the BCS change (P<0.01). Urine volume (avg.:  $16.7 \pm 0.31 \text{ L/day}$ ) and flow of microbial protein (MCP) from the rumen (avg.:  $2092 \pm 52.7$  g CP/day) were not affected by any treatment. Plasma Met levels increased (P<0.01) with both Met treatments and plasma tryptophan (Trp) levels decreased (P<0.01) with both Phe treatments. However, plasma Phe levels did not change with any treatment. Results are interpreted to suggest that delivery of Met with RP Met feeding was higher than animal requirements and caused an oversupply of Met. Addition of Phe to the Met supplementation changed the way energy was utilized by the cows, redirecting energy liberated by Met from milk components toward BCS gain.

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*Abbreviations*: AA, amino acid; ADF, acid detergent fiber; ADICP, AD insoluble CP; ADIN, acid detergent insoluble N; AL, allantoin; aNDF, amylase-treated NDF; aNDFom, aNDF free of residual ash; BCS, body condition score; BCAA, branched-chain AA; BW, body weight; CM, canola meal; CP, crude protein; CR, creatinine; DC305, DairyComp 305 management system; DDG, dried distillers grains; DHIA, Dairy Herd Improvement Association; DIM, days in milk; DM, dry matter; MCP, microbial CP; NDF, neutral detergent fiber; NE<sub>L</sub>, net energy for lactation; OM, organic matter; PD, purine derivatives; RDP, rumen degradable CP; RP, rumen protected; SCC, somatic cell count; SG, specific gravity; TMR, total mixed ration; TP, true protein.

It remains unclear if Phe was limiting in the Control ration or if RP Phe was not fed at high enough levels to have a measurable response on production. However, it is clear that AA limitations, requirements and production responses are governed by much more than plasma AA levels. Results further suggest that AA are bioactive metabolites to the extent that they can change animal performance, even when they are not "limiting" *per se*, and that their supplementation to practical dairy cattle diets should be approached with extreme caution for this reason.

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#### 1. Introduction

Canola meal (CM) is the second largest protein feed in the Northern latitudes and inclusion levels in dairy rations are expected to increase due to projected increased production of canola seed in Canada (Growing Great, 2015). The goal of the Canola Council of Canada with this program was to produce 15 million tons of canola seed annually by 2015 but, with record breaking seed production in 2013; annual production currently stands at 18 million tons. Vegetable oil demand worldwide is expected to rise 60% in the next decade (Canola Council of Canada Annual Report, 2013) and, with the increased crushing capacity in Canada, this will have a cascading effect resulting in increased amounts of CM produced and used in North American dairy rations.

A recent study (Swanepoel et al., 2014) showed that higher inclusions of CM in lactating dairy cow rations had a positive effect on production when CM directly substituted for high protein dried corn distillers grains (DDG), but that there was an optimum point at 120–135 g/kg of diet dry matter (DM) after which animal performance started to decline. This agrees with other studies comparing CM to DDG which reported that higher proportions of CM, included at up to 120 g/kg DM, tended to have higher milk and protein yields (Mulrooney et al., 2009). It was clear, however, in Swanepoel et al. (2014) that the high rumen degradable protein (RDP) content of CM, and resultant high rumen ammonia levels, did not limit microbial protein (MCP) production when CM was included in the diet at 200 g/kg DM, suggesting that it may have been the availability of absorbable amino acids (AA), and/or specific AA(s), that limited productive performance of the cows. In Swanepoel et al. (2014), only methionine (Met), phenylalanine (Phe) and leucine (Leu), could have limited production, based upon their declining plasma AA concentrations as the CM inclusion level in the diet increased.

Our objective was to determine if either Met or Phe, or both, was limiting performance of early lactation dairy cows fed a ration containing CM as the sole supplementary crude protein (CP) source, by supplementing Met and/or Phe in ruminally protected (RP) forms.

#### 2. Materials and methods

The experimental design used four pens of  $\sim$ 320 early lactation cows/pen in a 4 × 4 Latin square with 28 days experimental periods, utilizing the William's experimental design (Williams, 1949) to balance for potential carryover of treatment effects. The study took place during winter from 28 December 2012 to 18 April 2013 with temperature ranging between -3.6 and 28.3 °C and humidity between 22.7 and 100.0%. All cows were cared for relative to applicable laws of the state of California and the USA, and were consistent with requirements for "The care and use of animals for scientific purposes", as per the South African National Standard (SANS 10386-2008).

#### 2.1. Farm and management

The same commercial dairy farm (located in Hanford, CA) used in Swanepoel et al. (2014) was selected for this study. Every week cows were randomly allocated to one of four early lactation pens from a single fresh pen and, once confirmed pregnant, cows were moved from these pens to mid lactation pens. At the start of the 1st period, treatments were randomly allocated to each of the four early lactation pens and rotated after each 28 days experimental period consistent with a William's design.

#### 2.2. Diets

Mixing of the total mixed rations (TMR) and all other farm practices were as outlined in Swanepoel et al. (2014). All four of the pens were fed the same base TMR based on alfalfa hay, whole crop winter wheat and corn silages, and corn grain, with a premix containing dry ingredients (*i.e.*, almond hulls, fuzzy and cracked pima cottonseed, wheat straw, liquid molasses, mineral premix, CM), with CM inclusion in all TMR targeted at 180 g/kg of total ration DM (Table 2). Cows were fed *ad libitum* to achieve  $\sim 3\%$  refusals on an as fed basis, with each pen receiving a total of  $\sim 16,000 \text{ kg}$  of as-mixed TMR/day in two feedings. Cows were fed one full 11,000 kg load of TMR (which contained the RP AA) at the 1st feeding, between 04:30 and 07:30 h, to a clean bunk as bunks were cleared of all residual feed while the cows were at morning milking. A second  $\sim 5000 \text{ kg}$  load of TMR was fed at the 2nd feeding between 11:00 and 12:30 h and weights for each load of TMR fed were

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