UC Davis

UC Davis Previously Published Works

Title

Impacts of substitution of canola meal with soybean meal, with and without ruminally protected methionine, on production, reproduction and health of early lactation multiparous Holstein cows through 160 days in milk

Permalink

<https://escholarship.org/uc/item/5np1m3zv>

Authors

Swanepoel, N
Robinson, PH
Conley, A

Publication Date

2020-06-01

DOI

10.1016/j.anifeedsci.2020.114494

Peer reviewed

Rapid #: -16154042

CROSS REF ID: 13326

LENDER: BOS :: Main Library

BORROWER: CUV :: Carlson Health Sciences Library

TYPE: Article CC:CCG

JOURNAL TITLE: Animal feed science and technology

USER JOURNAL TITLE: Animal feed science and technology

ARTICLE TITLE: Impacts of substitution of canola meal with soybean meal, with and without ruminally protected methionine, on production, reproduction and health of early lactation multiparous Holstein cows through 160 days in milk

ARTICLE AUTHOR: Swanepoel, N

VOLUME: 264

ISSUE:

MONTH:

YEAR: 2020

PAGES: 114494-

ISSN: 0377-8401

OCLC #:

Processed by RapidX: 5/15/2020 8:38:59 AM

This material may be protected by copyright law (Title 17 U.S. Code)

Impacts of substitution of canola meal with soybean meal, with and without ruminally protected methionine, on production, reproduction and health of early lactation multiparous Holstein cows through 160 days in milk

N. Swanepoel^{a,*}, P.H. Robinson^a, A. Conley^b

^a Department of Animal Science, University of California, Davis, CA 95616, USA

^b Department of Population Health and Reproduction, School of Veterinary Medicine, University of California, Davis, CA 95616, USA

ARTICLE INFO

Keywords:

Phyto-estrogens
Isoflavones
Serum estradiol
Progesterone

ABSTRACT

Efficient production by dairy cows depends on optimal protein nutrition. Canola meal (CM) and dried corn distillers' grains (DDGS) have been primary protein meals on Western USA dairies for > 10 years, but a lower relative price of soybean meal (SBM) has created interest in how best to include it in diets containing CM and DDGS. Pens of ~315 multiparous early lactation cows were fed rations with 145 g/kg CM and 0 g/kg SBM or 65 g/kg CM and 65 g/kg SBM of dry matter (DM). A methionine (Met) diet (SBM^{+M}) was created by addition of ruminally protected Met to deliver ~4.6 g of absorbable Met/cow/day. Body condition (BCS) was scored, and milk yields recorded for 5 months after treatment assignment at 13.2 ± 0.11 days in milk (DIM). Blood serum was collected for progesterone (P4), E2 and amino acid (AA) analyses, 24 h prior to 1st and 2nd artificial insemination (AI). The DM intake was unaffected by treatment, but milk yield and components had treatment*DIM interactions which indicated CM cows outperformed SBM cows at earlier DIM. The SBM^{+M} cows produced more milk and milk protein than SBM cows, but at earlier DIM, while SBM^{+M} cows produced more milk fat than SBM cows at later DIM. Rumen microbial protein (MCP) outflow was lower when SBM replaced CM, and MCP was lower in later lactation (i.e., 109 versus 51 DIM). Cows culled for clinical mastitis, lameness and physical injury were not treatment impacted. No aspect of 1st or 2nd AI pregnancy was treatment impacted with an overall 1st AI pregnancy rate of 52/100 cows. The E2 levels in serum collected 24 h prior to 1st AI were unimpacted by treatment or subsequent pregnancy outcome. However, E2 levels were lower in cows open at 32 days post 2nd AI compared to those pregnant. Substituting SBM for CM reduced performance in spite of no apparent Met limitation, with a likelihood that it was metabolizable protein which limited, primarily due to lower MCP outflow. Addition of Met to the SBM diet resulted in improvements, especially to milk protein content and yield, but not to the

Abbreviations: AA, amino acid; ADF, acid detergent fiber; AI, artificial insemination; AL, allantoin; BCS, body condition score; CM, canola meal; CP, crude protein; DDGS, corn distillers dried grains with solubles; DIM, days in milk; DM, dry matter; E2, estradiol; EAA, essential AA; MCP, microbial CP; Met, methionine; MP, metabolizable protein; aNDF, neutral detergent fiber assayed with heat stable α-amylase expressed with ash; aNDFom, aNDF expressed without ash; OM, organic matter; PE, phyto-estrogens; P4, progesterone; RP, ruminally protected; SBM, soybean meal; SCC, somatic cell count; TMR, total mixed ration

* Corresponding author.

E-mail address: nanswanepoel@gmail.com (N. Swanepoel).

<https://doi.org/10.1016/j.anifeedsci.2020.114494>

Received 5 February 2020; Received in revised form 17 March 2020; Accepted 19 March 2020
0377-8401/ © 2020 Elsevier B.V. All rights reserved.

levels of the cows fed the CM ration. If phyto-estrogens in SBM interfered with E2 and P4 metabolism, it did not occur at a level which impacted pregnancy outcomes, suggesting it is of little concern to dairy farmers when fed to early lactation dairy cows, at least at this feeding level.

1. Introduction

The dairy industry in the Western USA has fed canola meal (CM) and corn dried distillers' grains with solubles (DDGS) as main protein meals for ~20 years because alternative protein meals, such as soybean meal (SBM) - used extensively in other areas of the world, has traditionally been more expensive. However, recent and projected changes in protein meal pricing has caused SBM to enter Western US dairy rations.

Swanepoel et al. (2014) showed that the optimal dietary levels of CM and high protein DDGS was ~130 g/kg of dry matter (DM) for CM and ~60 g/kg DM for DDGS for lactating dairy cattle. A large body of research from the Midwestern USA shows a milk production advantage of feeding CM *versus* SBM to lactating dairy cows, but limited data exists on effects of substitution of CM with SBM concomitant with DDGS feeding. However, a recent study (Gauthier et al., 2019) incrementally substituted SBM for CM in lactation diets, all with 75 g/kg DM of DDGS, and showed that SBM inclusion > 35 g/kg of diet DM depressed animal performance. This depression occurred with a substantial reduction in plasma Methionine (Met) concentrations, thereby suggesting that a reason for the lower performance may have been due to a Met deficit.

Although research on feeding CM has repeatedly demonstrated advantages to milk yield and components, responses have generally been measured over short periods of diet exposure (*i.e.*, 3–4 weeks) in cross-over designs. While milk production responses are important, general health, reproductive soundness and fertility are also important since re-breeding success of lactating dairy cows is critical to sustainability of commercial dairy farms. However, there are no data on effects of CM *versus* other protein meals as they relate to reproduction and health. This is unfortunate as it is known that SBM includes secondary compounds which could impact reproduction in dairy cows. Phyto-estrogens (PE), such as isoflavones, in soybean products can exhibit estrogenic activity (Woclawek-Potocka et al., 2013), mimicking effects of estradiol (E2), which could interfere with regulation and physiological control of reproductive processes with a fertility loss (Reinhart et al., 1999; Woclawek-Potocka et al., 2013). Reinhart et al. (1999) summarized how PE could have effects on the reproductive and immune systems, stimulating cytokine release in the oviduct, possibly leading to failed implantation or ectopic pregnancies.

Phyto-estrogens have recently drawn attention due to increased substitution of animal proteins with plant proteins in human diets, as well as SBM being a major protein source in animal diets. This has led to research into possible effects on human and animal health (*e.g.*, potent antioxidants, reduced risk of mammary cancer, prevention of cardiovascular disease, stop progression of atherosclerosis, positive effects in post-menopausal woman), but also adverse effects on reproduction (Rosselli et al., 2000). Indeed, the 1st indications of PE causing reproductive problems was investigated by Bennetts et al. (1946) when sheep grazing subterranean clover exhibited infertility, dystocia and uterine prolapse, which was presumed due to estrogenic substances in the clover. This issue resurfaced when Thain (1966) reported development of cystic ovaries, anoestrus and a high proportion of non-pregnant cows due to feeding subterranean clover, and Kallela et al. (1984) reported fertility disturbances when silage prepared from pure red clover, determined to have a high estrogenic isoflavone content, was fed to a herd of dairy cattle. Indeed, Woclawek-Potocka et al. (2008) showed that prolonged exposure to estrogenic pastures can cause permanent infertility in cattle, just as long term feeding of soy-based diets can have detrimental effects on reproduction. Even though PE effects of clover are well documented, little is known about effects of feeding PE derived from soybeans on dairy cows, and even less research is available on whether feeding SBM to dairy cattle could have similar deleterious effects on reproduction when fed for prolonged periods.

Objectives were to investigate potentially negative effects reported by Gauthier et al. (2019) on productive performance of lactating dairy cows by substituting CM with SBM in their diets, to determine if supplementation of Met to the SBM diets has beneficial effects on production, while assessing impacts on reproductive performance and health, all through ~160 days in milk.

2. Materials and methods

The study was conducted from December 2018 through July 2019. All cows were cared for in compliance with all applicable laws of the state of California and the USA. The study was completed on the dairy farm reported in Swanepoel and Robinson (2019a) using the same experimental design and, for the most part, the same methods thereby resulting in substantial text overlap between the following descriptions and those of Swanepoel and Robinson (2019a). For urine and fecal samples, methods are fully described in Swanepoel and Robinson (2019b).

2.1. Cows and housing

The commercial dairy farm used is located near Hanford (CA, USA) and milks a total of ~5000 Holstein cows three times a day. This farm has 4 pens of ~315 multiparity early lactation cows housed in one fully roofed free-stall barn, with each pen structurally very similar. However, for the purpose of this study, only 3 of these pens were used.

2.2. Treatments, feeds and feeding

The two base study rations were formulated to be iso-nutritious for crude protein (CP), neutral detergent fiber (aNDF), starch and fat in order to allow comparison of substitution of CM with SBM without confounding treatment effects with other diet proximate nutrient changes. To accomplish this, treatment diets were created by varying the dietary inclusion levels of CM with a combination of SBM, wheat silage and almond hulls. Other ingredients were the same among treatments. In order to emulate results obtained by Gauthier et al. (2019), treatment diets were designed to be: (CM) 145 g/kg DM of CM and 0 g/kg of SBM, (SBM) 65 g/kg of CM and 65 g/kg of SBM on a DM basis. The Met supplemented treatment (SBM^{+M}) was created by addition of 7.9 g/cow/day of a ruminally protected (RP) Met product (Smartamine M; Adisseo USA Inc. Alpharetta, GA, USA) estimated to deliver ~4.6 g intestinally absorbable Met/cow/day with the SBM diet to ~315 multiparity early lactation cows.

Cows were fed total mixed rations (TMR) prepared immediately before feeding as described in Swanepoel and Robinson (2019a). There were two premixes, one with and the other without SBM. The TMR loads for the CM fed pens were prepared from the CM premix and the TMR loads for the SBM and SBM^{+M} pens were prepared from the SBM premix, with the RP Met added to the SBM^{+M} loads from pre-weighed bags. To confirm ration feeding accuracy, a microgrits tracer (1.0–1.4 mm corn cob grits, coloured with food dyes; Micro-Tracers Inc., San Francisco, CA, USA) was added to every premix load containing CM (red dye) or SBM (orange dye) as described by Swanepoel and Robinson (2019a). Each pen was fed ~16,000 kg of TMR/day, in two loads (*i.e.*, 1st feeding of 11,000 kg between 6:30 and 8:30 am while cows were at milking with a 2nd feeding of ~5000 kg between 11:00 am and noon). Weights of each ingredient in each load of premix and TMR, and the amount of TMR fed to each pen in each feeding were recorded with daily refusal weights by pen to “TMR Tracker” (Digi-Star LLC, Fort Atkinson, WI, USA).

Individual feed and fresh pen TMR samples were collected during 3 discrete time blocks of 21 days when the fresh pen contained cows that would later be assigned to study pens. Similarly, individual feed and study pen TMR samples were collected during 3 discrete time blocks of 8 days when the 3 study pens contained all study cows. Feed and TMR samples were frozen at -20 °C for chemical analyses. Daily DM intakes/cow by pen were calculated from the amount of TMR delivered to the pens for each period, minus total refusals, multiplied by the DM of the TMR samples collected, and divided by the average number of cows in the pen during that period.

2.3. Assignment of cows to treatments and study design

A total of 566 cows which moved from the common fresh cow pen to one of the three study pens in 9 weekly assignments from December 4 (2018) to February 19 (2019) were eligible for study inclusion. Criteria for excluding cows from assignment were those that moved from the fresh pen > 22 days in milk (DIM; 78 cows excluded) or if they failed to remain in their assigned pen for at least 20 days after assignment (19 cows excluded), thereby leaving 469 assigned cows. To avoid confounding treatment with pen in the statistical analysis, cows and diets were rotated simultaneously among treatment pens every 4 or 5 weeks (*i.e.*, immediately after milk

Fig. 1. Graphic depiction of 9 cow assignments over 11 weeks to each treatment pen in order to create 3 allocations for statistical purposes.

tests).

Cows were assigned weekly to study pens in assignments of ~ 60 from a common fresh pen at 13.2 ± 0.11 DIM. Thus, over 11 weeks based upon a predetermined sequence (*i.e.*, CM, SBM, $SBM^{+M} - SBM$, SBM^{+M} , CM – SBM^{+M} , CM, SBM), each pen received one assignment of cows in each of weeks 1–3, 5–7 and 9–11, with no assignments during weeks 4 and 8. These groups were considered as 3 allocation groups for statistical analysis (Section 2.8 and Fig. 1). The overall schematic of the study overlaid on temperature and humidity measures is in Fig. 2.

The study was completed using the same 3 physical pens and the same experimental design as Swanepoel and Robinson (2019a), including the simultaneous rotation of cows and diets among the 3 pens as described in Swanepoel and Robinson (2019a).

2.4. Animal parameters

2.4.1. Lactation performance and milk composition

Cows were milked thrice daily, and monthly milk yields recorded (Tru-Test WB110 meters, Tru-Test Ltd., Auckland, New Zealand) by California DHIA personnel (Hanford, CA, USA).

2.4.2. Body condition score

Body condition (BCS) was scored on a sub-group of ~ 40 cows in each of the 9 assignments of cows immediately after each milk test by one trained evaluator on a 1–5 scale with increments of 0.25 units, but using intermediate scores when a cow's BCS could not be clearly allocated.

2.4.3. Blood samples

Blood samples analyzed for physiological amino acids (AA), estradiol (E2) and progesterone (P4) were collected 24 h prior to breeding from all cows with DIM of 68–74 DIM at 1st service and 103–109 DIM at 2nd service. Samples were collected from the tail (*i.e.*, coccygeal) vein into 10 ml red-top (no anti-coagulant) evacuated glass test tubes (BD Vacutainer, BD Diagnostics, Franklin Lakes, NJ, USA) during normal cow lockup after morning milking. Samples coagulated for 30–60 min at $\sim 15^\circ\text{C}$ prior to centrifugation at $1750 \times g$ (Sorvall ST 16, Thermo Fisher Scientific, Waltham, MA, USA) for 10 min at 18°C to separate serum, which was stored at -20°C until assay.

2.4.4. Urine and fecal samples

Urine samples were collected by manual stimulation for each assignment at an average of 51 ± 3 and 109 ± 3 DIM and placed on ice. From all collected samples, 12 were selected from each assignment, based on visual assessment to eliminate samples with fecal or other contamination, for sub-sampling. Specific gravity (SG) was measured within 2 h of sampling using a digital refractometer (Atago USA Inc., Bellevue, WA, USA) after which 10 ml of each of the 12 samples was combined and mixed to create 1 composite (12 cows/composite) urine samples per assignment. Aliquots of 7 ml were transferred to 3 replicate tubes containing 1.5 ml of 100 ml/L

Fig. 2. Temperature and humidity patterns during the study relative to periods of cow assignment, breeding and milk sampling.

sulphuric acid, thereby reducing the final pH < 2 to prevent bacterial destruction of allantoin (AL), and diluted with deionized water to a final volume of 35 ml and frozen at -20°C .

Fecal samples were collected rectally from a sub-group of the same 12 cows per assignment at an average of 82 ± 3 and 109 ± 3 DIM. Samples were frozen at -20°C , and later thawed and composited in a procedure which resulted in 2 composite (6 cows/composite) fecal samples per assignment.

The purpose of compositing urine and fecal samples into these cow composite groups was to support the assumption that the chemical composition of the TMR consumed by the composite groups was represented by the TMR sampled for chemical analysis (Swanepoel et al., 2018).

2.5. Reproductive performance, culling and survivorship

Cows were bred using a Double-OvSynch program (Swanepoel and Robinson, 2019a), and entered the program (weekly) if they were > 40 DIM on that day (i.e., cows bred for the 1st time were 68–74 DIM). Pregnancy diagnosis used a portable ultrasound unit in cows 32 days post-artificial insemination (AI), with diagnoses entered to Dairy Comp 305 software (DC305). Cows determined pregnant at this time were classified as '1st service conceivers' and tracked electronically through ~160 DIM (i.e., through their 5th monthly milk test) for pregnancy loss (i.e., abortion) as determined by two subsequent confirmatory ultrasound examinations. Cows which showed a heat prior to the 32 day pregnancy check were bred on that heat, but cows classified as not pregnant to 1st service (which had already re-entered the OvSynch program) were re-bred 3 days later (bred for the 2nd service at 103 and 109 DIM) and then pregnancy checked 32 days later. Again, cows which showed a heat prior to 2nd pregnancy check were bred on that heat. Cows determined pregnant to 2nd service were classified as '2nd service conceivers' whereas those found not pregnant were classified as 'non-pregnant' for study purposes. Due to the short time between the 32 day pregnancy check to 2nd service and 160 DIM (i.e., the approximate time that cows completed the study), no data on abortion to 2nd service were collected.

To be classified as a 'survivor', cows needed to 'survive' (i.e., complete the study) by meeting all the criteria described in Swanepoel and Robinson (2019a). Main reasons to be classed as a 'non-survivor' (i.e., not completing the study) were mastitis, lameness and physical injury, with miscellaneous reasons being pneumonia, stomach pain and lactation failure. These reasons for non-survivorship were treated as experimental outcomes.

2.6. Weather records

Two portable weather stations (Onset, Bourne, MA, USA) were placed in 2 of the pens on a pole in the center of each pen for hourly measurements of temperature and humidity. A rain gauge (12.7 cm capacity glass tube, Cedmon) was mounted 1.22 m high in an open area (at least 15 m from any buildings or structures) in order to record the amount of precipitation.

2.7. Analytical methods

2.7.1. Feed and TMR assays

Feeds and TMR were dried at 55°C for 48 h, air equilibrated for 24 h, and ground on a model 4 Wiley Mill to pass a 1 mm screen. Moisture content was determined by gravimetric loss of free water by heating a sub-sample in a forced air oven for 2 h at 105°C . Residue after heating samples at 550°C for 8 h was considered ash. Fat assay was according to Association of Official Analytical Chemists (AOAC, 2006). The N fractions in feeds and TMR, as well as in the acid detergent fiber (ADF) residue, were determined in a N gas analyzer (Leco FP-528, St. Joseph, MI, USA) according to AOAC (2006). Starch was determined as total glucose minus free glucose multiplied by a factor of 0.9 as described by Smith (1969). The aNDF assay included addition of a heat stable amylase to remove starch (Van Soest et al., 1991) and to inactivate potential contaminating enzymes while sodium sulfite was used to aid in the removal of some nitrogenous matter (AOAC, 2006; #2002-04), whereas ADF was analyzed according to AOAC (2006). Fiber levels (i.e., aNDF and ADF, were expressed including residual ash with aNDFom expressed exclusive of residual ash. Lignin(sa) was quantified utilizing the sulfuric acid procedure (AOAC, 2006).

The TMR and feed samples were digested in a solution of nitric acid and hydrogen peroxide utilizing a microwave digestion system, and concentrations of Ca, P, K, S, Mg, were quantified with atomic absorption spectrometry or inductively coupled plasma atomic emission spectrometry (Meyer and Keliher, 1992; Sah and Miller, 1992).

Isoflavone profile analysis used fresh (not dried) TMR samples (N.P analytical laboratories, St. Louis, MO, USA). Samples were extracted using a methanol:water solution followed by filtration, dilution and analysis by HPLC using a C18 reverse phase column and UV detection at 260 nm (Seo and Morr, 1984). Twelve isoflavones as glycosides, glycoside esters and aglycones were separated and quantified based on standards injected to the HPLC.

2.7.2. Milk assays

Milk samples were analyzed for somatic cell counts (SCC; AOAC, 2006; #975.16), fat, and true protein using mid-infrared spectroscopy (Foss 303 Milk-O-Scan®; Foss Foods, Inc., Eden Prairie, MN, USA) according to AOAC (2006) at the DHIA laboratory in Hanford (CA, USA).

Milk energy (MJ/kg) was calculated according to Tyrrell and Reid (1965) as:

$$\left(\left(\left(41.63 * \frac{\text{g}}{\text{kg}} \text{fat} \right) + \left(24.13 * \frac{\frac{\text{g}}{\text{kg}} \text{TP}}{0.934} \right) + \left(21.6 * \frac{\text{g}}{\text{kg}} \text{lactose} \right) - 11.72 \right) * 4.185 \right) * 2.204$$

where 0.934 converts true protein (TP) to crude protein (CP), 4.185 converts Mcal to MJ and 2.204 converts Mcal/lb to Mcal/kg. Milk energy output (MJ/d) was calculated: Milk energy (MJ/kg) $\times$ milk yield (kg/d).

2.7.3. Blood assays

The concentration of E2 was analyzed by extracting 0.8 mL of serum with 4 mL of methyl tert-butyl ether into 16 $\times$ 125 mm glass tubes followed by vortexing for 3 min. Serum was frozen in a methanol dry-ice bath and ether supernatant decanted into 12 $\times$ 75 mm glass tubes, which were stored in the dark overnight. A 9 point standard curve was created (0.625–100 pg/tube) using estradiol-17 β (#E1132, Sigma Chemical Co., St. Louis, MO, USA) in ethanol which was added to tubes and dried, then re-constituted in 200 μ l of buffer (0.01 M phosphate, 0.9 % NaCl, 0.5 % bovine serum albumen, 0.01 M EDTA, pH 7.5). A commercial assay kit (Beckman Coulter Ultra Sensitive RIA DSL-4800) was used with some modifications to the manufacturer's protocol. Briefly, all incubations were conducted at 20 °C after diluting the antibody solution as supplied 1–2 parts with buffer. The diluted antibody was added (100 μ l) to each tube, vortexed for 5 s and incubated for 1 h. Undiluted tracer was added (100 μ l) to each tube, vortexed for 5 s and incubated for 2 h. Kit precipitating agent (1 ml) was added to each tube, vortexed for 5 s and incubated for 20 min. Tubes were centrifuged (1750 $\times$ g) for 20 min at 4 °C, placed in a gripper rack and decanted by inversion. Finally, tubes were transferred to gamma counting racks on a Packard Cobra Quantum instrument and counted for 1 min. Sensitivity (defined as 95 % binding) was < 0.625 pg/mL. Recovery of spiked serum averaged 87 %. According to the manufacturer, relative cross-reactivity of primary antiserum with estrone and estriol is 2.4 and 0.6 %, respectively, < 0.5 % with multiple other estrogens and not detectable with testosterone or dehydroepiandrosterone. All samples were analyzed in the same run.

The P4 was analyzed using the competitive enzyme immunoassay assay of Munro and Stabenfeldt (1984) as described in Gomez et al. (2018). Levels of AA in blood serum were assayed at the Molecular Structure Facility (University of California, Davis, CA, USA) as described in Swanepoel and Robinson (2019a).

2.7.4. Urine and fecal assays

Urine samples were chemically analyzed for AL according to Chen and Gomes (1995), based on the method of Young and Conway (1942), and is fully described in Swanepoel and Robinson (2019b). Standards were prepared to create working concentrations of 20, 40, 60 and 80 mg/L AL. Urine samples were thawed and centrifuged at 1200 $\times$ g for 15 min at 20–22 °C to remove precipitate which could influence colorimetric reading. Samples were diluted 60 times to fit the standard curve. Duplicate standard curves were at the start and end of each run, and 2 inter-run standards were in each run to assess variation among runs but, as all inter-run standards were within 0.05 of the average over all runs, all run values were accepted without inter-run correction. Each sample was analyzed in duplicate with the average being the final concentration.

Urine volume (L/day) was calculated using an equation derived from data of Burgos et al. (2005) as:

$$332.66 * (((SG - 1) * 1000))^{-0.884}$$

while microbial CP (MCP) production (g/day) was estimated as described in Swanepoel and Robinson (2019b).

Fecal samples were frozen at –20 °C and subsequently analyzed for DM, ash, aNDF, ADF, lignin(sa) and N after drying and grinding as described earlier for feed and TMR samples.

Whole tract apparent digestibility (g/kg) was calculated as:

$$1 - \left(\frac{\text{TMR} \frac{\text{Lignin (sa)} * 0.95}{\text{Nutrient}}}{\text{Feces} \frac{\text{Lignin (sa)}}{\text{Nutrient}}} \right)$$

assuming that lignin(sa) in the TMR was 950 g/kg indigestible (Stensig and Robinson, 1997).

2.8. Statistical analysis

Treatment differences were accepted if $P \leq 0.05$, and tendencies accepted if $0.05 < P \leq 0.10$.

2.8.1. Lactation performance and body condition score

Animal based response parameters including milk production and composition, BCS and blood parameters were analyzed using the MIXED option of SAS (2016). The statistical model included effects of allocation (see Section 2.3 and Fig. 1) and treatment (i.e., CM, SBM, SBM^{+M}) as fixed effects, with time (i.e., milk test/DIM) as a repeated effect, as well as the time*treatment interaction. Individual cows within allocation were a random effect since individual cows were accepted as being metabolically independent and so meet the criteria of experimental replicates. For milk production and composition, the statistical analysis was completed on all survivors (see Section 2.5), which totaled 403 of the 469 cows which were assigned to the study. For BCS, only survivors which had been scored on their 1st and 5th scoring date, and had only missed 1 intermediate score, were included – which totaled 342 cows.

Contrasts were used to identify significant differences of the CM versus SBM diets and SBM versus SBM^{+M} diets, as planned *a priori*. In cases of statistically significant treatment*time interactions, differences of treatments within times were identified with the PDIF

option of SAS (2016). Main effects are in all tables but, where an interaction was significant, figures are used to illustrate the nature of the interaction.

2.8.2. Blood parameters

Serum P4 and E2 were analyzed for 1st and 2nd services with the MIXED option of SAS (2016) using a statistical model which included treatment (*i.e.*, CM, SBM, SBM^{+M}) and subsequent pregnancy status (*i.e.*, pregnant or open) as fixed effects, as well as the interaction. For this analysis, 2 pregnant and 2 non-pregnant cows were randomly selected from each assignment for each of the 2 services, resulting in 72 cows. Contrasts were used to identify significant differences of CM *versus* SBM diets, as well as SBM *versus* SBM^{+M} diets, as planned *a priori*.

Blood serum AA were analyzed using the MIXED option of SAS (2016). The statistical model included treatment (*i.e.*, CM, SBM, SBM^{+M}) and breeding (*i.e.*, 1st or 2nd service) as fixed effects, as well as the interaction. Three cows were randomly selected from each assignment, within each service, resulting in 54 cows. Contrasts were used to identify significant differences of CM *versus* SBM diets, as well as SBM *versus* SBM^{+M} diets, as planned *a priori*. In cases of statistically significant interactions, differences of treatments were identified with the PDIF option of SAS.

2.8.3. Dry matter intake and TMR composition

Dry matter intake and composition of the treatment TMR collected were analyzed using the GLM option of SAS (2016) with sampling period and treatment as fixed effects.

2.8.4. Apparent whole tract digestibility

Apparent whole tract digestibility was analyzed using the GLM option of SAS (2016) with treatment and sampling period as fixed effects. The experimental unit was the 6 cow composite fecal samples. Contrasts were used to identify differences of CM *versus* SBM diets, as well as SBM *versus* SBM^{+M} diets, as planned *a priori*.

2.8.5. Urine parameters and rumen microbial outflow

All urine parameters were analyzed using the MIXED option of SAS (2016). The statistical model included treatment (*i.e.*, CM, SBM, SBM^{+M}) and DIM (*i.e.*, 51 and 109 DIM) as fixed effects, as well as the interaction. For this analysis, the experimental unit was the 12 cow group collected from each assignment at each DIM. Contrasts were used to identify differences of CM *versus* SBM diets, as well as SBM *versus* SBM^{+M} diets, as planned *a priori*.

2.8.6. Culling and conception

Conception (calculated as the proportion of cows within each of the 9 assignment groups that displayed the characteristic) and reasons for culling were analyzed using the GLM option of SAS (2016) with allocation group (see Section 2.3 and Fig. 1) and treatment as fixed effects.

3. Results

3.1. Weather patterns

Temperatures and humidities (Fig. 2) were relatively constant through the period of cow assignment to the study. Temperatures

Table 1

Chemical composition (g/kg dry matter) of the silages^a included in the total mixed rations (TMR) fed during the study, and to all cows while in the common fresh pen.

	Study TMR Silages				Fresh Cow TMR Silages	
	Corn	Wheat	Sorghum	Alfalfa	Corn	Wheat
Dry matter (DM)	351	362	264	436	341	356
Crude protein (CP)	83	122	103	229	75	103
ADICP ^b (g/kg CP)	68	72	110	50	69	76
aNDF ^c	410	507	502	306	406	480
aNDFom ^d	398	458	470	276	391	442
ADF ^e	244	357	342	262	243	337
Lignin(sa) ^f	24	36	29	46	22	35
Ash	72	155	136	138	71	133

^a Based on analysis of 2 pooled (by sampling period) samples collected on 2 occasions in each sampling period.

^b Acid detergent insoluble CP (g/kg of CP).

^c Neutral detergent fiber assayed with heat stable amylase, expressed inclusive of residual ash.

^d Neutral detergent fiber assayed with heat stable amylase, expressed exclusive of residual ash.

^e Acid detergent fiber, expressed inclusive of residual ash.

^f Lignin assayed with the sulphuric acid residual organic matter procedure.

did not vary substantively during 1st service, or while DM intake was measured, ensuring that all cows experienced similar conditions during breeding, and feed intake measurement. There were only a few small rain events during the study.

3.2. Feedstuffs and total mixed ration composition

Composition of silages used (Table 1) is consistent with NRC (2001) values. The ingredient composition of the fresh cow TMR (Table 2) is consistent with contemporary rations in the study area and, in the case of nutrient composition (Table 3), consistent with NRC (2001) guidelines.

There were very few differences in the ingredient profiles of the TMR among treatments (Table 2), with the exception of the planned increases ($P < 0.01$) in levels of SBM and both grades of almond hulls, with a corresponding decline ($P < 0.01$) in the CM level in order to maintain equality of nutrient levels between the CM and SBM based TMR. The amount of wheat silage included increased in the SBM based TMR, but only numerically ($P = 0.19$) due to higher among load variation. Other changes, such as the slight decrease in corn grain level between CM and SBM based TMR, were not judged large enough to be biologically important. The SMB⁺ TMR had similar levels of ingredients to the SBM based TMR, with the only difference being addition of RP Met to the SBM TMR. Overall, dietary levels of SBM were slightly lower than the targeted level (i.e., 63.45 versus 65.00 g/kg DM).

All TMR nutrient levels (Table 3) were within NRC (2001) recommended ranges. Nutrient profiles of TMR among treatments differed minimally, with the exception of CP, which was slightly lower for the CM versus SBM based TMR (170 versus 175 g/kg DM; $P < 0.01$). Small differences in the K, Mg and S levels between the CM and SBM based TMR diets were not judged large enough to be biologically important. There was an approximate 11 fold increase in the isoflavone level of the SBM versus CM based diet reflecting an approximate 27 fold increase in the isoflavone level of SBM (4270 ppm) versus CM (156 ppm).

3.3. Productive performance

Milk fat yield, lactose content and milk energy (MJ/d) was lower ($P < 0.01$) for the SBM treatment compared to CM. Milk and

Table 2

Ingredient profiles^a (g/kg dry matter) of total mixed rations (TMR) fed to cows during the study, as well as the fresh cow TMR fed to all cows while in the common fresh pen prior to treatment assignment.

	Study TMR				P		Fresh cow TMR
	CM	SBM	SBM ⁺	SEM	CM vs SBM	SBM vs SBM ⁺	
Alfalfa silage, whole crop	2.6	7.1	7.5	2.36	0.31	0.93	–
Corn silage, whole crop	126.9	121.0	121.0	3.03	0.30	0.99	178.0
Alfalfa hay, medium ^b quality	–	–	–	–	–	–	93.4
Alfalfa hay, high ^b quality	81.0	80.4	79.4	2.34	0.90	0.80	95.8
Wheat silage, whole crop	77.2	103.0	101.6	10.09	0.19	0.94	58.9
Sorghum silage, whole crop	49.4	40.5	40.2	6.92	0.48	0.97	–
Methionine, rumen protected ^c	0.0	0.0	0.4	0.01	1.00	< 0.01	–
Premix							
Cottonseed, whole upland	64.5	44.8	44.9	0.53	< 0.01	0.95	44.4
Almond hulls, prime grade ^d	47.6	51.5	51.6	0.12	< 0.01	0.75	33.2
Almond hulls, pollinator grade ^d	48.3	52.3	52.3	0.12	< 0.01	0.75	33.6
Cottonseed, cracked Pima	9.4	24.5	24.5	0.46	< 0.01	0.97	–
Mineral, lactation premix	21.5	21.2	21.2	0.11	0.21	0.89	26.7
Canola, meal pellets, solvent	143.2	66.3	66.3	0.55	< 0.01	0.93	150.0
Soybean meal, solvent	0.0	63.4	63.5	0.11	< 0.01	0.70	–
Rumen inert fat, Nurisol ^e	13.5	13.6	13.6	0.03	0.07	0.74	6.4
Wheat, straw	16.0	15.9	15.9	0.31	0.82	0.98	18.8
Corn distillers dried grains	67.8	67.7	67.8	0.13	0.69	0.73	67.4
R1200 ^f	–	–	–	–	–	–	10.9
Molasses, liquid	9.9	9.8	9.8	0.03	0.55	0.84	5.2
Corn grain, flaked	201.5	197.1	199.3	0.77	0.03	0.15	177.2
Citrus, wet pulp mixed fruit	19.7	19.9	19.1	0.50	0.83	0.37	–
Water ^g	–	–	–	–	–	–	0.1

^a Based on average ingredient composition during each of the 3 sampling periods as recorded by the “EZ Feed” feed management system. Feeds are listed in the sequence they were mixed in the treatment and fresh cow TMR and in the Premix which went into the TMR.

^b As assessed by the dairy farm manager.

^c Ruminally protected Met (Smartamine M, Adisseo USA Inc., Alpharetta, GA, USA). Fed at 7.9 g RP Met/cow/day to deliver ~4.6 g intestinally absorbable Met.

^d ‘Prime’ is the highest grade of almond hulls (i.e., lowest stick and shell contamination) whereas ‘pollinator’ is late harvest with higher stick and shell contamination.

^e Global Agri-Trade Organization, Rancho Dominguez, CA, USA, contains about 50 g/kg 16:0, 35 g/kg C 18:1 n9 and 8 g/kg 18:2 n6.

^f Dry cow mineral, MidWestern BioAg, Madison, WI, USA.

^g Water was assessed a DM content of 1 g/kg for calculation purposes.

Table 3

Nutrient profiles^a (g/kg dry matter) of total mixed rations (TMR) fed to cows fed during the study, as well as the fresh cow TMR fed to all cows while in the common fresh pen prior to treatment assignment.

	Study TMR			SEM	P		Fresh cow TMR
	CM	SBM	SBM ⁺ M		CM vs SBM	SBM vs SBM ⁺ M	
Dry matter (DM)	568	559	562	13.0	0.71	0.90	582
Organic matter (OM)	911	912	912	1.6	0.10	0.52	899
Crude protein (CP)	170	175	174	1.6	< 0.01	0.03	174
ADICP ^b (g/kg CP)	69	59	63	0.8	0.14	0.53	64
aNDF ^c	306	298	295	2.6	0.15	0.38	309
aNDFom ^d	290	284	281	2.1	0.23	0.39	293
ADF ^e	210	204	200	2.6	0.08	0.72	219
Lignin(sa) ^f	45	40	39	1.3	0.70	0.73	46
Starch	191	185	179	9.5	0.47	0.98	184
Fat	50	48	48	0.9	0.91	0.96	37
Calcium	8.3	8.0	7.6	0.26	0.52	0.41	12.2
Phosphorus	4.3	4.0	3.9	0.08	0.08	0.63	4.2
Potassium	15.8	17.0	17.4	0.24	0.04	0.36	15.6
Magnesium	3.3	3.0	3.1	0.05	0.04	0.48	3.9
Sulfur	3.2	2.9	2.9	0.06	0.05	0.75	3.3
Zinc (ppm DM)	81.5	77.5	76.0	1.44	0.17	0.54	953
Manganese (ppm DM)	49.3	46.1	45.2	1.14	0.16	0.64	550
Iron (ppm DM)	396.3	366.0	388.0	59.97	0.77	0.83	4017
Copper (ppm DM)	9.9	10.8	10.8	0.44	0.31	0.93	12
Total isoflavones ^g (ppm)	17	184	–	–	–	–	–

^a Based on analysis of 3 pooled (by sampling period) samples collected on 2 occasions in each of the 3 sampling periods.

^b Acid detergent insoluble CP (g/kg of CP).

^c Neutral detergent fiber assayed with heat stable amylase, expressed inclusive of residual ash.

^d Neutral detergent fiber assayed with heat stable amylase, expressed exclusive of residual ash.

^e Acid detergent fiber, expressed inclusive of residual ash.

^f Lignin assayed with the sulphuric acid residual organic matter procedure.

^g Assay of one sample of the CM and SBM based TMR. Total isoflavones of one pool sample of canola meal and one pool sample of soybean meal were 156 and 4270 ppm respectively.

component yields (kg/day), except fat and energy (MJ/d), had treatment*DIM ($P < 0.01$) interactions (Table 4 and Fig. 3a). In general, CM fed cows outperformed SBM fed cows at 22 and 53 DIM ($P < 0.05$), with no differences in milk production at later DIM. The SBM⁺M fed cows produced more ($P < 0.05$) milk than the SBM fed cows, but only at 22 DIM. This differed from milk fat content (Fig. 4a) where SBM⁺M fed cows produced more ($P < 0.05$) fat than SBM fed cows at 83 and 111 DIM, but not at earlier DIM with no difference at any DIM for CM versus SBM. However, for milk protein content (Fig. 4b), SBM⁺M fed cows outperformed ($P < 0.05$) SBM fed cows at all DIM with no difference between CM and SBM treatments, except at 22 DIM. This, and the difference in milk yield, led to a large difference in milk protein yield (Fig. 5a) between SBM and the other treatments at 22 and 53 DIM, but not at later DIM. There was no treatment effect on milk SCC or BCS (Table 4), and no interaction of treatment*DIM with similar BCS values for all treatments through the study.

3.4. Dry matter intake, digestibility and ruminal microbial flow

The DM intake (Table 5) averaged 28.3 kg/cow/day and was not treatment impacted. Whole tract apparent digestibility of aNDF and aNDFom were also not treatment impacted and there were no treatment*DIM interactions. Yet apparent digestibility of organic matter (OM) tended to be lower ($P=0.06$), while CP digestibility was lower ($P=0.02$), for CM versus SBM fed cows.

Urine AL concentrations ($P < 0.01$), and calculated rumen MCP outflow ($P=0.02$), were both lower when SBM replaced CM in the ration. Lower urine SG ($P < 0.01$) resulted in a higher ($P < 0.01$) calculated urine volume for the SBM treatment. Addition of Met to the SBM treatment resulted in increasing urine AL ($P = 0.02$) and urine SG ($P < 0.01$) with a resultant decrease in calculated urine volume ($P < 0.01$). However, MCP was not impacted by addition of Met to the SBM treatment. In addition, all urine parameters were impacted by DIM, with urine SG ($P < 0.01$), AL concentration ($P=0.02$) and MCP flow ($P < 0.01$) being lower, and urine volume higher ($P < 0.01$), in later lactation (109 versus 51 DIM).

3.5. Serum AA concentrations

Levels of serum AA were within normal ranges for high producing lactating dairy cows (Swanepoel et al., 2016) and there were no differences (Table 6) between cows fed the CM versus SBM diets, except for tendencies for arginine serine to be lower in CM versus SBM. Cows fed the SBM⁺M diet had higher ($P = 0.04$) serum Met levels than SBM, while those of arginine and serine were lower ($P < 0.01$ and $P=0.04$ respectively). Serum AA levels were generally more impacted by service number (samples were collected

Table 4

Milk production (kg/day) and composition (g/100 g), as well as body condition scores (BCS) and BCS change as influenced by feeding canola meal (CM) or soybean meal (SBM) without or with added ruminally protected methionine, as well as days in milk (DIM).

	Treatments (Trt)				Days in milk (DIM)					SEM	P				
											Trt		DIM		
	CM	SBM	SBM ⁺ M	SEM	22	53	83	111	141	SEM	CM vs SBM	SBM vs SBM ⁺ M	Lin	Quad	Trt*DIM
<i>n</i> = 403 cows															
Yield (kg/day)															
Milk	51.31	49.55	49.93	0.474	50.30	52.68	50.26	49.63	48.44	0.408	< 0.01	0.57	< 0.01	< 0.01	< 0.01 ^d
Fat	1.78	1.71	1.75	0.019	1.97	1.78	1.64	1.69	1.66	0.019	< 0.01	0.16	< 0.01	< 0.01	0.51
True protein	1.45	1.38	1.44	0.012	1.47	1.45	1.41	1.40	1.39	0.011	< 0.01	< 0.01	< 0.01	0.37	< 0.01 ^d
Lactose	2.50	2.39	2.42	0.022	2.44	2.57	2.44	2.41	2.34	0.020	< 0.01	0.43	< 0.01	< 0.01	< 0.01 ^d
Energy (MJ/d)	147.5	141.0	144.3	1.26	153.8	144.1	139.9	141.0	138.3	1.22	< 0.01	0.06	< 0.01	< 0.01	0.07
Content (g/100 g)															
Fat	3.48	3.46	3.51	0.033	3.93	3.37	3.27	3.41	3.44	0.028	0.58	0.22	< 0.01	< 0.01	0.02 ^d
True protein	2.84	2.80	2.90	0.016	2.94	2.76	2.83	2.84	2.87	0.011	0.09	< 0.01	0.05	< 0.01	< 0.01 ^d
Lactose	4.88	4.83	4.85	0.009	4.85	4.88	4.86	4.85	4.83	0.006	< 0.01	0.25	< 0.01	< 0.01	0.39
Energy (MJ/kg)	2.88	2.85	2.90	0.015	3.07	2.82	2.79	2.85	2.86	0.012	0.21	0.03	< 0.01	< 0.01	0.02 ^d
SCC ^a (1,000)	101	121	124	12.9	70	109	133	139	125	13.1	0.29	0.85	< 0.01	< 0.01	0.65
<i>n</i> = 342 cows															
BCS ^b	2.98	2.98	2.97	0.042	3.14	2.84	2.90	2.98	3.02	0.028	0.98	0.79	0.05	< 0.01	0.86
BCS ^b change ^c	-0.026	-0.038	-0.032	0.018	-	-0.310	0.052	0.088	0.042	0.020	0.90	0.96	< 0.01	< 0.01	0.53

^a Somatic cell counts.

^b Body condition score.

^c Change in BCS adjusted to 30 days (units/30 days). Values listed are the change during the period ending in the column DIM.

^d Interactions depicted as figures: Milk yield – Fig. 3a, Protein yield – Fig. 5a, Lactose yield – Fig. 5b, Fat content – Fig. 4a, Protein content – Fig. 4b, Energy density – Fig. 3b.

24 h prior to 1st and 2nd AI) than treatment, with Isoleucine, leucine and 3-methylhistidine all tending ($P < 0.10$) to be higher, while phenylalanine and aspartic acid levels were higher ($P \leq 0.05$), in cows at 2nd service. Only aspartic acid showed a treatment*service interaction due to the higher ($P = 0.03$) value for 1st versus 2nd service serum concentrations within the SBM⁺M treatment.

3.6. Culling and reproductive performance

Cows culled for clinical mastitis, lameness, physical injury and overall were not impacted by treatment (Table 7), except for the tendency of lameness to be higher for SBM⁺M versus SBM ($P = 0.08$). No measured aspect of 1st or 2nd service pregnancy were treatment impacted with an average 1st service pregnancy rate of 52 cows/100 cows and combined 1st and 2nd service pregnancy rate of 72 cows/100 cows. The E2 levels (Table 8) in serum collected 24 h prior to 1st service for OvSync bred cows were not impacted by treatment or subsequent pregnancy status. However, E2 levels were lower ($P < 0.01$) in cows found open at 32 days post 2nd service compared those found pregnant ($P < 0.01$). The P4 levels were lower ($P < 0.01$) for CM versus SBM fed cows, as well as in cows open at 32 days post 1st service compared those found pregnant ($P < 0.01$), but were not impacted by pregnancy outcome or diet.

4. Discussion

4.1. Productive performance

4.1.1. Dietary substitution of CM with SBM

Numerous previous studies (Broderick et al., 2015; Paula et al., 2017; Gauthier et al., 2019), and a meta-analysis by Martineau et al. (2013), have demonstrated increased milk production and milk protein content and yields with CM as the major protein source in diets of dairy cows, mainly compared to SBM. Although the reason for this is unclear, it has generally been attributed to a 'better' AA profile in CM or 'improved' post-ruminal effects and DM intake (Paula et al., 2017) compared to SBM. The advantage of CM over SBM was again demonstrated in our study where SBM substituted for CM at a level of 63.5 g/kg DM resulted in a 1.8 kg/day reduction in milk yield, similar to the 2.2 kg/day decline in Gauthier et al. (2019) using similar dietary levels of CM and SBM. The concomitant

a)

b)

Fig. 3. Statistical interactions for milk yield (a; kg/day) and milk energy density (b; MJ/kg) as influenced by feeding canola meal (CM) or soybean meal (SBM), without or with added ruminally protected methionine, as well as days in milk (values within days in milk with different superscripts differ; $P < 0.05$).

reduction in milk fat, protein and lactose yields resulted in a considerable decline in milk energy output (141.0 versus 147.5 MJ/day). However, it is clear that most of the milk response occurred prior to ~70 DIM, with CM outperforming SBM at 22 and 53 DIM (Fig. 3a), similar to Gauthier et al. (2019) who used early lactation (< 100 DIM) cows.

Reduced milk and component yields with the SBM diet occurred in spite of an unplanned increase in dietary CP (g/kg DM), and the higher ($P=0.02$) whole tract digestibility of CP may be inconsistent with reports that CM is more ruminally degradable than SBM with a lower post-ruminal digestibility of rumen escaping CP (NRC, 2001). This also agrees with higher CP (Maxin et al., 2013b) digestibility of SBM versus CM diets based on an *in situ* bag procedure and 48 h gas production, respectively.

Blood AA concentrations were similar to Gauthier et al. (2019) and mostly within 'normal' ranges for high producing Holstein dairy cattle (Swanepoel et al., 2018). Only glutamine was lower than in Swanepoel et al. (2016), but comparable to values later reported by our group (Swanepoel and Robinson, 2019a), while arginine appeared almost 2x higher than levels reported previously. Reasons for higher concentrations of these AA in rations seemingly so similar in nutrient and ingredient composition is unclear. Indeed, at 27.2 µg/mL (15.6 µmol/dL), our serum arginine concentrations were higher than those reported in most studies using early lactation cows (Christen et al., 2010; Appuhamy et al., 2011; Haque et al., 2012), all of which were similar to Swanepoel et al. (2016) survey values, but similar to a study that fed CM and SBM diets (Piepenbrink et al., 1998). Serum arginine concentrations are influenced by factors other than requirements for protein synthesis and accretion, including *de novo* synthesis and oxidation rates, functions in the urea cycle and uses for synthesis of other metabolites (Ball et al., 2007).

It has been suggested that CM has a favorable AA profile for milk production (Mulrooney et al., 2009; Huhtanen et al., 2011;

a)

b)

Fig. 4. Statistical interactions for milk fat (a) and protein (b) content (g/100 g) as influenced by feeding canola meal (CM) or soybean meal (SBM), without or with added ruminally protected methionine, as well as days in milk (values within days in milk with different superscripts differ; $P < 0.05$).

Martineau et al., 2013) with many essential AA (EAA) being lower in blood of cows consuming SBM (Piepenbrink et al., 1998). However, there were no differences in any serum AA concentration between our CM and SBM diets, but many tendencies for AA concentrations to be higher at 2nd service. This could be due to cows being later in lactation (avg. 106 versus 71 DIM) at 2nd service with lower AA needs and higher accumulation in blood.

Contrary to expectations based upon Gauthier et al. (2019), there were no reductions in serum Met concentrations, or in concentration of Met as a proportion of total EAA, with SBM feeding. It seems unlikely that this was due to the slightly lower feeding level of SBM (63.5 versus 70 g/kg), but agrees with Maxin et al. (2013a) who found that SBM resulted in the lowest Met concentrations amongst all protein sources examined. It has been suggested that accumulation of unutilized AA in blood could be indicative of an AA limitation resulting in the reduction in milk yield (Swanepoel et al., 2014). However, since there was no such pattern of general AA increase in serum of SBM fed cows, the reduction in milk yield cannot be attributed to a Met limitation. Indeed, the reduction in milk yield in the absence of a Met decrease, suggests that it may have been a metabolizable protein (MP) deficit that limited milk production, possibly due to lower availability of microbial CP, which is discussed in Section 4.1.3.

4.1.2. Supplementing the SBM diet with RP methionine

The increase in milk protein yield due to addition of Met to the SBM diet is consistent with expectations (Robinson, 2010), as is the trend ($P = 0.06$) to higher milk energy output. However, most of the milk yield response to Met occurred prior to ~70 DIM, with

a)

b)

Fig. 5. Statistical interactions for milk protein (a) and lactose (b) yield (kg/day) as influenced by feeding canola meal (CM) or soybean meal (SBM), without or with added ruminally protected methionine, as well as days in milk (values within days in milk with different superscripts differ; $P < 0.05$).

the impact tending to become negative after 90 DIM. There is a similar improvement in milk protein yield (Fig. 5a) for the SBM^{+M} versus SBM treatment, also in earlier lactation (i.e., 22 and 53 DIM), due to a higher milk protein content (Fig. 4b). However, this pattern is reversed with milk fat content (Fig. 4a), where the difference occurs after ~70 DIM, where SBM^{+M} outperforms SBM, possibly due to lower milk yield and an unnecessarily high Met supply allowing an energetic shift to milk fat production. This is consistent with the discussion of Swanepoel et al. (2015), where Met supplementation seems to consistently enhance milk component yields, especially milk protein, regardless of its apparent need as a limiting AA. However, as noted earlier, serum Met was not lower with the SBM versus CM diets and so cannot be considered to be limiting. Thus Met supplementation was likely not required, which is reflected in the higher serum Met concentration (3.62 versus 3.25 $\mu\text{g/mL}$; $P = 0.04$) for the SBM^{+M} versus SBM treatment, presumably due to accumulation of unutilized Met in blood. Indeed, this could be supported by the lower blood arginine concentration with the SBM^{+M} treatment since arginine is known to be removed by the mammary gland in amounts greater than required for milk protein synthesis, and mainly hydrolyzed to urea. Thus, the lower concentration of arginine could indicate its utilization in the urea cycle to remove excess Met, while the higher concentration of arginine with the SBM diet could reflect a possible MP limitation where less arginine is required for utilization in the urea cycle (Ball et al., 2007).

A reduction in serum serine ($P = 0.04$), one of the most gluconeogenic AA (with glycine and alanine) due to Met supplementation supports a potential role of Met in metabolic regulation of hepatic gluconeogenesis, and resultant blood glucose levels (Tavares et al., 2016). As serine is involved in decreasing synthesis of choline, which stimulates milk fat synthesis, this could explain higher milk fat

Table 5

Dry matter intake, apparent whole tract digestibility and rumen microbial protein (MCP) outflow as influenced by feeding canola meal (CM) or soybean meal (SBM) without or with added ruminally protected methionine, as well as days in milk (DIM).

	Treatment (Trt)				Days in milk (DIM)			P			
	CM	SBM	SBM ^{+M}	SEM	82/51 ^a	109	SEM ^a	CM vs SBM	SBM vs SBM ^{+M}	DIM	Trt ^a DIM
<i>n</i> = 3 pens											
Dry matter intake (kg/d)	28.49	28.22	28.19	–	–	–	0.332	0.64	0.96	–	–
<i>n</i> = 18 composite (6 cow) fecal samples (see Section 2.4.4)											
Apparent digestion (g/100 g)											
Organic matter	65.7	67.3	67.6	0.59	67.4	66.3	0.46	0.06	0.74	0.11	0.89
Crude protein	62.0	65.4	63.6	0.90	64.7	62.6	0.71	0.02	0.22	0.05	0.86
aNDFom ^b	46.0	45.8	47.5	0.84	46.8	46.1	0.63	0.84	0.22	0.52	0.98
aNDF ^c	40.0	40.7	42.1	1.18	40.8	41.0	0.85	0.71	0.24	0.89	0.98
<i>n</i> = 18 composite (12 cow) urine samples (see Section 2.4.4)											
Urine specific gravity	1.0265	1.0254	1.0270	–	1.0272	1.0254	0.00042	< 0.01	< 0.01	< 0.01	0.12
Urine volume (L/day)	18.4	19.1	18.1	–	18.0	19.1	0.25	< 0.01	< 0.01	< 0.01	0.19
Urine allantoin (mg/mL)	2.90	2.59	2.85	–	3.05	2.51	0.122	< 0.01	0.02	0.02	0.79
Rumen MCP outflow (g/d)	1802	1659	1737	–	1871	1595	66.0	0.02	0.19	< 0.01	0.97

^a 82 days for apparent digestion but 51 days for urine measurements. Overall for dry matter intake and urine measures but by factor for fecal measures.

^b Neutral detergent fiber assayed with heat stable amylase, expressed exclusive of residual ash.

^c Neutral detergent fiber assayed with heat stable amylase, expressed inclusive of residual ash.

Table 6

Free amino acid (AA) concentrations (µg/mL) in serum of cows as influenced by feeding canola meal (CM) or soybean meal (SBM), without or with added ruminally protected methionine, sampled 24 h prior to 1st and 2nd service.

	Treatment (Trt)				Service (S) ^a			P			
	CM	SBM	SBM ^{+M}	SEM	1st	2nd	SEM	CM vs SBM	SBM vs SBM ^{+M}	S	Trt ^a S
<i>n</i> = 54 cows											
Essential AA											
Threonine	10.7	12.2	12.5	0.74	11.6	12.0	0.60	0.20	0.76	0.61	0.54
Valine	34.1	32.1	34.9	1.53	32.6	34.8	1.22	0.41	0.15	0.20	0.85
Methionine	3.22	3.25	3.62	0.154	3.24	3.49	0.13	0.90	0.04	0.11	0.61
Isoleucine	15.6	15.4	16.3	0.79	15.1	16.4	0.66	0.91	0.35	0.09	0.33
Leucine	24.8	23.5	25.3	1.07	23.4	25.6	0.85	0.44	0.17	0.07	0.72
Phenylalanine	8.14	8.37	8.10	0.261	7.88	8.53	0.209	0.58	0.43	0.03	0.77
Lysine	12.0	12.4	11.9	0.68	11.7	12.5	0.57	0.67	0.46	0.20	0.42
Histidine	7.57	7.97	8.42	0.342	7.80	8.17	0.274	0.45	0.30	0.34	0.68
Arginine	26.8	29.6	25.2	1.03	26.4	28.0	0.82	0.09	< 0.01	0.17	0.70
Non-essential AA											
Aspartic acid	1.67	1.69	1.66	0.090	1.65	1.71	0.072	0.85	0.77	0.05	0.03 ^c
Tyrosine	9.19	9.36	9.92	0.623	9.18	9.81	0.532	0.84	0.41	0.28	0.72
Serine	8.52	9.50	8.52	0.421	8.89	8.81	0.356	0.09	0.04	0.85	0.23
Glutamic acid	7.02	7.40	7.06	0.263	7.15	7.17	0.210	0.36	0.31	0.94	0.25
Glutamine	39.9	37.0	37.9	1.85	37.7	38.7	1.48	0.32	0.69	0.62	0.87
Glycine	29.2	29.0	27.9	1.57	28.9	28.5	1.38	0.90	0.48	0.77	0.28
Alanine	24.5	24.5	24.3	1.20	24.2	24.6	0.98	0.99	0.92	0.75	0.95
Asparagine	6.37	7.06	7.08	0.302	6.82	6.85	0.241	0.14	0.96	0.94	0.99
Proline	13.0	12.8	13.2	0.55	13.0	12.9	0.44	0.81	0.61	0.99	0.79
3-MH ^b	0.62	0.54	0.60	0.033	0.55	0.62	0.026	0.16	0.17	0.07	0.67

^a Cows sampled 24 h prior to breeding, with days in milk (DIM) range 68–74 DIM at 1st service and 103–109 DIM at 2nd service.

^b 3-Methylhistidine.

^c Interaction due to the cows sampled immediately prior to 1st service having a higher value (1.81) versus cows sampled immediately prior to 2nd service (1.51) within the SBM^{+M} group ($P < 0.05$).

synthesis during later lactation when serine decreases with Met supplementation.

In addition, supplementation of Met did not allow production levels to rise to the levels obtained with the CM treatment. This supports the discussion of Swanepoel et al. (2015) suggesting that the level of Met supplemented relative to its requirement is more important than the overall Lys:Met ratio of the diet fed. Indeed, correcting the ratio from 3.82 in the SBM treatment to 3.29 with SBM^{+M} did not result in it reaching CM treatment production levels with its Lys:Met ratio of 3.73.

4.1.3. Rumen MCP outflow as influenced by SBM and RP met

The reduction in rumen MCP outflow for the SBM versus CM treatment is consistent with Gauthier et al. (2019), and possibly

Table 7

Culling and pregnancy rates as influenced by feeding canola meal (CM) or soybean meal (SBM), without or with added ruminally protected methionine.

	Treatments			SEM	P	
	CM	SBM	SBM ^{+M}		CM vs SBM	SBM vs SBM ^{+M}
<i>n</i> = 403 cows						
Culling (cows/100 cows)						
Mastitis	5.9	9.9	5.5	1.27	0.12	0.10
Lameness	2.6	1.7	3.8	0.53	0.38	0.08
Physical injury	2.5	2.3	2.5	1.19	0.90	0.91
Miscellaneous	2.1	1.9	1.3	0.42	0.32	0.43
Total	13.1	15.8	13.1	1.37	0.29	0.29
Pregnancy						
1st Service						
Pregnant (cows/100 cows bred)	52.7	49.9	52.9	4.80	0.74	0.72
Abortion ^a (cows/100 cows pregnant)	6.7	10.9	8.2	2.99	0.43	0.60
Adjusted 1st Service ^b (cows/100 cows bred)	48.9	44.7	48.5	4.34	0.58	0.62
2nd Service						
OvSynch bred (cows/100 cows bred)	55.9	65.2	54.4	8.33	0.53	0.47
OvSynch bred pregnant (cows/100 cows bred OvSynch)	41.0	40.0	42.5	8.59	0.94	0.87
Heat bred (cows/100 cows bred)	44.6	34.8	45.6	8.33	0.53	0.47
Heat bred pregnant (cows/100 cows bred heat)	50.7	36.1	32.5	7.05	0.27	0.77
Total pregnant (cows/100 cows bred)	42.1	38.8	40.1	5.47	0.73	0.89
1st and 2nd Service						
Combined Pregnant (cows/100 cows bred)	72.6	69.4	71.8	4.21	0.66	0.74
Combined adjusted ^{b,c} (cows/100 cows bred)	68.9	64.2	67.4	3.29	0.43	0.58
Pregnant non-aborting survivors (cows/100 cows bred) ^d	59.9	54.0	58.6	3.42	0.35	0.46

^a Cows per 100 cows conceived to 1st service but not pregnant at next pregnancy check.

^b Adjusted for 1st service abortions.

^c Abortions were not recorded to 2nd service.

^d Cows successfully completing the study at ~160 DIM and pregnant at that time.

Table 8

Estradiol (E2) and progesterone (P4) levels in serum 24 h prior to breeding cows to an OvSynch protocol (1st and 2nd services) as influenced by feeding canola meal (CM) or soybean meal (SBM), without or with added ruminally protected methionine.

	Treatment (Trt)			Preg Outcome (PO) ^a			P			
	CM	SBM	SBM ^{+M}	Preg	Open	SEM	CM vs SBM	SBM vs SBM ^{+M}	PO	Trt*PO
<i>n</i> = 36 cows/service										
<i>1st Service^b</i>										
Estradiol, pg/mL	6.41	4.72	3.71	5.16	4.72	1.441	0.21	0.45	0.69	0.51
Progesterone, ng/mL	0.19	0.38	0.47	0.42	0.27	0.072	< 0.01	0.14	< 0.01	0.12
<i>2nd Service^c</i>										
Estradiol, pg/mL	7.64	7.26	8.96	9.70	6.20	1.606	0.79	0.26	< 0.01	0.17
Progesterone, ng/mL	0.25	0.23	0.20	0.24	0.21	0.040	0.80	0.50	0.45	0.86

^a Where 'PO' is subsequent pregnancy outcome (*i.e.*, pregnant (preg) or not pregnant (open)) to that service.

^b Sampled between 68–74 DIM.

^c Sampled between 103–109 DIM.

causative to the lower milk production with SBM feeding. To our knowledge there are no studies documenting changes in MCP outflow with SBM *versus* CM diets other than Gauthier et al. (2019). The first review examining impacts of SBM on rumen MCP outflow (Polan, 1988) stated that protein sources with more rumen undegradable protein would reduce MCP, which corresponds to a 12-year review published by Santos et al. (1998). Polan (1988) also noted that SBM tended to reduce MCP production when compared to other protein sources, probably due to a negative effect of additional soy protein. Indeed, a recent meta-analysis attributed positive responses with CM feeding to increased efficiency in N metabolism with improved MCP production and thus a higher supply of MP from rumen undegradable protein (Maxin et al., 2013a). This is entirely consistent with our results, and suggests that milk production of cows fed the SBM diet was limited by a MP, rather than an AA, deficit due to reduced MCP outflow when SBM substituted for CM in the diet. However, this contradicts the conclusion of Maxin et al. (2013a) that SBM provides an inferior quality MP compared to CM due solely to its limitation in Met, while supporting discussion that most feed tables erroneously report a higher MP value for SBM, compared to CM, such that feed evaluation systems consistently underestimate MP supply with CM diets (Martineau et al., 2013; Maxin et al., 2013b).

The numerical increase in MCP with the SBM^{+M} *versus* SBM diet suggests a possible rumen response. Smartamine M is designed to solubilize at low pH, usually after passing from the rumen (pH ~6.2) to the abomasum (pH ~2.5). However, ruminal stability is

affected by TMR mixing (which may weaken the coating and reduce stability) and rumen retention time and associated ruminative chewing. Thus it seems inevitable that some ruminal Met release occurred in the rumen. Indeed, if rumen degradation of Smartamine M, reported at 220–370 g/kg (Swanepoel, 2009), occurred in our study then feeding Met at 7.9 g/day would result in 1.3–2.2 g/day of ruminal Met release. While it seems unlikely that such a low level of Met could be sufficient to impact MCP synthesis, a previous study reported improved production and efficiency of MCP synthesis when 216–432 g/kg of RP Met was ruminally degraded (Vázquez-Añón et al., 2001).

4.2. Reproductive performance and health as influenced by SBM feeding

An assay of composited samples suggested average levels of 4270 ppm total isoflavones in SBM, compared to 156 ppm in CM. The SBM value is much higher than reported by Třinácý et al. (2009) in extruded full-fat soybeans for daidzein and genistein (1640 *versus* 151 and 2400 *versus* 223 respectively), resulting in a mean intake of isoflavones of 7686 mg/day in our study, which is 50 % higher than that in Třinácý et al. (2009). However, it is comparable to the range of 1200–4200 mg/kg DM reported by Dusza et al. (2006) for unprocessed soybeans.

Since dairy cow diets are formulated to contain ingredient combinations which allow utilization of complementary nutrients, it was unclear whether inclusion of SBM in a diet at a 'normal' level would have the detrimental effects on fertility reported in studies which fed higher levels. At our SBM feeding level (~1.8 kg SBM/cow/day on DM basis), the total isoflavone concentration (mainly genistein and daidzein) in the TMR was 184 ppm, considerably lower than the 1900 ppm in Woclawek-Potocka et al. (2005) that reduced pregnancy rates in cows fed 2.5 kg of 'bruised soy'. Indeed, it is actually lower than the 300 ppm reported by the same group for their standard (no soy) diet, from which no phytoestrogens were detected in blood or urine. This suggests that the level of phytoestrogens in our SBM and SBM⁺ diets was not enough to impact reproductive performance. In this context, it is not surprising that there were no impacts of CM replacement by SBM on reproductive performance to 1st or 2nd service (Table 7). And, contrary to the suggestion of Reinhart et al. (1999) that PE are immunotoxic and may therefore disrupt the immune system, there was no impact on the overall health of the cows as assessed by culling.

4.3. Serum estradiol (E2) and progesterone (P4) levels as influenced by SBM feeding

It has been suggested that PE has the ability to bind to estrogen receptors, modulate E2 metabolism, act as a partial agonist thereby inhibiting E2 responses, and reduce concentrations of circulating active E2 (Reinhart et al., 1999; Woclawek-Potocka et al., 2005), which leads to disturbance in follicle development or lack of estrus. Even though not statistically significant, there was a lower level of E2 in the serum for the SBM diets compared to CM (4.2 *versus* 6.4 pg/mL). This may support Reinhart et al. (1999) in their view that PE can reduce circulating active E2, but only at 1st service – as the difference in E2 levels among treatments did not repeat for 2nd service cows. However, there was a higher concentration of E2 in serum of later pregnant cows *versus* later open cows for 2nd (9.7 *versus* 6.2 pg/mL) and 1st service (5.2 *versus* 4.7 pg/mL), which corresponds with studies reporting that fertility may be limited by circulating E2 levels in blood (Souza et al., 2007).

Concentrations of E2 in our study compared well with values reported previously for lactating Holstein cows producing ~46 kg milk/day (Sartori et al., 2004), where serum E2 concentrations peaked at 7.9 pg/mL. The reason for the higher levels of E2 in our cows at 2nd *versus* 1st service is not clear, but could be due to the clearance rate of steroids from blood, as well as level of intake and milk production. As cows at 2nd service were later in lactation (106 *versus* 71 DIM), and therefore past peak-production, this could have resulted in a lower metabolic rate and slower clearance rate of steroid hormones (Sartori et al., 2004; Martins et al., 2018). However, the opposite effect on P4, which would be subject to similar effects of changes in clearance, argues that clearance was not likely to be a major determinant of differences in steroid concentrations.

It has been suggested that PE can cause abortion (*i.e.*, early embryonic mortality) by decreasing P4 production during pregnancy through inhibition of luteinizing hormone secretion which disrupts corpus luteum function to maintain pregnancy (Woclawek-Potocka et al., 2013; Martins et al., 2018). While serum samples were not collected during pregnancy, the proportion of 1st service abortions for SBM fed cows was numerically higher than for CM fed cows (9.6 *versus* 6.7; Table 7), which may support this theory. Indeed, if the PE in SBM tended to negatively impact E2 levels, thereby limiting fertility, the higher P4 concentration for the SBM fed cows (0.43 *versus* 0.19 ng/mL) would support this possibility since the presence of P4 at insemination could be related to reduced pregnancy rates as an indication of the cows' readiness to be bred. Since overall P4 levels were all below 1 ng/mL, which is generally accepted as the value below which cows are considered to have no luteal tissue left and can therefore be successfully bred (Smith et al., 1975; Woclawek-Potocka et al., 2005), the lower P4 concentration for the CM treatment could indicate that the timing of AI was more favorable to the likelihood of fertilization.

5. Conclusions

Substituting CM for SBM at 63.5 g/kg DM reduced dairy cow performance, especially relative to milk yield and its components. This occurred in spite of no apparent Met limitation, with the resultant suggestion that it was an overall lack of MP which limited production, primarily due to lower rumen MCP outflow with SBM feeding.

In addition, the SBM phyto-estrogens did not impact pregnancy outcomes, suggesting that feeding this level of SBM is of little concern to dairy farmers, at least relative to the reproductive performance of their cows. Indeed, the negative impact of SBM on milk production is of more concern and, even though supplementation of Met to the SBM diet resulted in some improvement of

performance, especially of milk protein yield, it did not rise to levels achieved by cows fed the CM diet.

Overall, results suggest that it was not the AA profiles of the protein sources, but rather their ruminal impact on rumen MCP yield that determined their impacts on milk production, limiting the use of SBM, even with additional Met which would add additional diet costs, over CM in early lactation rations.

CRediT authorship contribution statement

N. Swanepoel: Conceptualization, Methodology, Data curation, Writing - original draft, Writing - review & editing. **P.H. Robinson:** Conceptualization, Methodology, Data curation, Writing - original draft, Writing - review & editing. **A. Conley:** .

Declaration of Competing Interest

None.

Acknowledgements

The authors thank Rebecca Cotterman for providing time and expertise in establishing and validating the E2 assay, as well as conducting final analysis, required for this study. Many thanks to Mr. William Van Die and his crew (Junior, José, Edgar, Pedro, Alejandro, Norberto) for care and assistance, as well as Mr. Ed Vink, Ms. Kari Furtado and the DHIA Hanford crew for careful sampling and assay of milk samples. We also thank Mr. Aaron Highstreet for ration formulation.

References

- AOAC, 2006. Official Methods of Analysis of AOAC International (2006), 20th ed. AOAC International, Arlington, VA, USA.
- Appuhamy, J.A.D.R.N., Knapp, J.R., Becvar, O., Escobar, J., Hanigan, M.D., 2011. Effects of jugular-infused lysine, methionine, and branched-chain amino acids on milk protein synthesis in high-producing dairy cows. *J. Dairy Sci.* 94, 1952–1960.
- Ball, R.O., Urschel, K.L., Pencharz, P.B., 2007. Nutritional consequences of interspecies differences in arginine and lysine metabolism. *J. Nutr.* 137, 1626S–1641S.
- Bennetts, H.W., Underwood, E.J., Shier, F.L., 1946. A specific breeding problem of sheep on subterranean clover pastures in Western Australia. *Aust. Vet. J.* 22, 2–12.
- Broderick, G.A., Faciola, A.P., Armentano, L.E., 2015. Replacing dietary soybean meal with canola meal improves production and efficiency of lactating dairy cows. *J. Dairy Sci.* 98, 5672–5687.
- Chen, X.B., Gomes, M.J., 1995. Estimation of Microbial Protein Supply to Sheep and Cattle Based on Urinary Excretion of Purine Derivatives – an Overview of the Technical Details. Rowett Res. Institute, Bucksburn, Aberdeen AB2 9SB, UK, pp. 1–21.
- Christen, K.A., Schingoethe, D.J., Kalscheur, K.F., Hippen, A.R., Karges, K.K., Gibson, M.L., 2010. Response of lactating dairy cows to high protein distillers grains or 3 other protein supplements. *J. Dairy Sci.* 93, 2095–2104.
- Dusza, L., Ciereszko, R., Skarżyński, D.J., Nogowski, L., Opalka, M., Kamińska, B., Nynca, A., Kraszewski, O., Słomczyńska, M., Wocławek-Potocka, I., Korzekwa, A., Pruszyńska-Oszmałek, E., Szkudelska, K., 2006. Mechanism of phytoestrogens action in reproductive processes of mammals and birds. *Reprod. Biol.* 6 (Suppl. 1), 151–174.
- Gauthier, H., Swanepoel, N., Robinson, P.H., 2019. Impacts of incremental substitution of soybean meal for canola meal in lactating dairy cow diets containing a constant base level of corn derived dried distillers' grains with solubles. *Anim. Feed Sci. Technol.* 252, 51–63.
- Gomez, N.A., Conley, A.J., Robinson, P.H., 2018. Effects of long-term, near-term, and real-time energy balance, and blood progesterone concentrations, on the pregnancy rate of contemporary dairy cows. *Anim. Reprod. Sci.* 189, 136–145.
- Haque, M.N., Rulquin, H., Andrade, A., Faverdin, P., Peyraud, J.L., Lemosquet, S., 2012. Milk protein synthesis in response to the provision of an "ideal" amino acid profile at 2 levels of metabolizable protein supply in dairy cows. *J. Dairy Sci.* 95, 5876–5887.
- Huhtanen, P., Hetta, M., Swenson, C., 2011. Evaluation of canola meal as a protein supplement for dairy cows: a review and a meta-analysis. *Can. J. Anim. Sci.* 91, 529–543.
- Kallela, K., Heinonen, K., Saloniemi, H., 1984. Plant oestrogens; the cause of decreased fertility in cows: a case report. *Nord. Vet. Med.* 36, 124–129.
- Martineau, R., Ouellet, D.R., Lapierre, H., 2013. Feeding canola meal to dairy cows: a meta-analysis on lactational responses. *J. Dairy Sci.* 96, 1701–1714.
- Martins, J.P.N., Wang, D., Mu, N., Rossi, G.F., Martini, A.P., Martins, V.R., Pursley, J.R., 2018. Level of circulating concentrations of progesterone during ovulatory follicle development affects timing of pregnancy loss in lactating dairy cows. *J. Dairy Sci.* 101, 10505–10525.
- Maxin, G., Ouellet, D.R., Lapierre, H., 2013a. Effect of substitution of soybean meal by canola meal or distillers grains in dairy rations on amino acid and glucose availability. *J. Dairy Sci.* 96, 7806–7817.
- Maxin, G., Ouellet, D.R., Lapierre, H., 2013b. Ruminant degradability of dry matter, crude protein, and amino acids in soybean meal, canola meal, corn, and wheat dried distillers grains. *J. Dairy Sci.* 96, 5151–5160.
- Meyer, G.A., Keliher, P.N., 1992. An overview of analysis by inductively coupled plasma-atomic emission spectrometry. In: Montaser, A., Golightly, D.W. (Eds.), *Inductively Coupled Plasmas in Analytical Atomic Spectrometry*. VCH Publishers, New York, NY, USA, pp. 473–516.
- Mulrooney, C.N., Schingoethe, D.J., Kalscheur, K.F., Hippen, R., 2009. Canola meal replacing distillers grains with solubles for lactating dairy cows. *J. Dairy Sci.* 92, 5669–5676.
- Munro, C., Stabenfeldt, G., 1984. Development of a microtitre plate enzyme immunoassay for the determination of progesterone. *J. Endocrinol.* 101, 41–49.
- National Research Council, 2001. Nutrient Requirements of Dairy Cattle, 7th edition. National Academy of Science, Washington, DC, USA.
- Paula, E.M., Monteiro, H.F., Silva, L.G., Benedetti, P.D.B., Daniel, J.L.P., Shenkoru, T., Broderick, G.A., Faciola, A.P., 2017. Effects of replacing soybean meal with canola meal differing in rumen-undegradable protein content on ruminal fermentation and gas production kinetics using 2 in vitro systems. *J. Dairy Sci.* 100, 5281–5292.
- Piepenbrink, M.S., Schingoethe, D.J., Brouk, M.J., Stegeman, G.A., 1998. Systems to evaluate the protein quality of diets fed to lactating cows. *J. Dairy Sci.* 81, 1046–1061.
- Polan, C.E., 1988. Update: dietary protein and microbial protein contribution. *J. Nutr.* 118, 242–248.
- Reinhart, K.C., Dubey, R.K., Keller, P.J., Lauper, U., Rosselli, M., 1999. Xeno-oestrogens and phyto-oestrogens induce the synthesis of leukaemia inhibitory factor by human and bovine oviduct cells. *Mol. Hum. Reprod.* 5, 899–907.
- Robinson, P.H., 2010. Impacts of manipulating ration metabolizable lysine and methionine levels on the performance of lactating dairy cows: a systematic review of the literature. *Livest. Sci.* 127, 115–126.
- Rosselli, M., Reinhart, K., Imthurn, B., Keller, P.J., Dubey, R.K., 2000. Cellular and biochemical mechanisms by which environmental oestrogens influence reproductive function. *Hum. Reprod. Update* 6, 332–350.
- Sah, R.N., Miller, R.O., 1992. Spontaneous reaction for acid dissolution of biological tissues in closed vessels. *Anal. Chem.* 64, 230–233.

- Sartori, R., Haughian, J.M., Shaver, R.D., Rosa, G.J.M., Wiltbank, M.C., 2004. Comparison of ovarian function and circulating steroids in estrous cycles of Holstein heifers and lactating cows. *J. Dairy Sci.* 87, 905–920.
- SAS, 2016. SAS User's Guide: Statistics, Version 9.4 Edition. SAS Inst., Inc., Cary, NC, USA.
- Seo, A., Morr, C.V., 1984. Improved high-performance liquid chromatographic analysis of phenolic acids and isoflavonoids from soybean protein products. *J. Agric. Food Chem.* 32, 530–533.
- Smith, D., 1969. Removing and analyzing total nonstructural carbohydrates from plant tissue. Wisconsin Agric. Exp. Sta. Res. Report 41.
- Smith, J.F., Fairclough, R.J., Payne, E., Peterson, J., 1975. Plasma hormone levels in the cow: I. Changes in progesterone and oestrogen during the normal oestrous cycle. *New Zeal. J. Agric. Res.* 18, 123–129.
- Souza, A.H., Gümen, A., Silva, E.P.B., Cunha, A.P., Guenther, J.N., Peto, C.M., Caraviello, D.Z., Wiltbank, M.C., 2007. Supplementation with estradiol-17 β before the last gonadotropin-releasing hormone injection of the ovsynch protocol in lactating dairy cows. *J. Dairy Sci.* 90, 4623–4634.
- Stensig, T., Robinson, P.H., 1997. Digestion and passage kinetics of forage fiber in dairy cows as affected by fiber-free concentrate in the diet. *J. Dairy Sci.* 80, 1339–1352.
- Swanepoel, N., 2009. Effects of Rumen Protected Amino Acid Supplementation on Performance of Holstein Cows Fed Rations Containing High Levels of Canola Meal. MSc. Thesis. University of Pretoria, Pretoria, South Africa. <https://pdfs.semanticscholar.org/5075/84197bedbf7e6a672fdb052b0ca8d3ef0284.pdf>.
- Swanepoel, N., Robinson, P.H., 2019a. Impacts of feeding a flax-seed based feed supplement on productive and reproductive performance of early lactation multiparous Holstein cows. *Anim. Feed Sci. Technol.* 251, 134–152.
- Swanepoel, N., Robinson, P.H., 2019b. Impacts of feeding a flax-seed based feed supplement on production and health of mid through late lactation multiparous Holstein cows on a commercial dairy farm. *Anim. Feed Sci. Technol.* 258, 114318.
- Swanepoel, N., Robinson, P.H., Erasmus, L.J., 2014. Determining the optimal ratio of canola meal and high protein dried distillers grain protein in diets of high producing Holstein dairy cows. *Anim. Feed Sci. Technol.* 189, 41–53.
- Swanepoel, N., Robinson, P.H., Erasmus, L.J., 2015. Effects of ruminally protected methionine and/or phenylalanine on performance of high producing Holstein cows fed rations with very high levels of canola meal. *Anim. Feed Sci. Technol.* 205, 10–22.
- Swanepoel, N., Robinson, P.H., Erasmus, L.J., 2016. Rumen microbial protein flow and plasma amino acid concentrations in early lactation multiparity Holstein cows fed commercial rations, and some relationships with dietary nutrients. *Livest. Sci.* 190, 58–69.
- Swanepoel, N., Robinson, P.H., Erasmus, L.J., 2018. Production responses of high producing Holstein cows to ruminally protected phenylalanine and tyrosine supplemented to diets containing high levels of canola meal. *Anim. Feed Sci. Technol.* 243, 90–101.
- Tavares, C.D.J., Sharabi, K., Dominy, J.E., Lee, Y., Isasa, M., Orozco, J.M., Jedrychowski, M.P., Kamenecka, T.M., Griffin, P.R., Gygi, S.P., Puigserver, P., 2016. The methionine transamination pathway controls hepatic glucose metabolism through regulation of the GCN5 acetyltransferase and the PGC-1 α transcriptional coactivator. *J. Biol. Chem.* 291, 10635–10645.
- Thain, R.I., 1966. Bovine infertility possibly caused by subterranean clover: further report and derd distories. *Aust. Vet. J.* 42, 199–203.
- Trínáctý, J., Křížová, L., Schulzová, V., Hajšlová, J., Hanuš, O., 2009. The effect of feeding soybean-derived phytoestrogens on their concentration in plasma and milk of lactating dairy cows. *Arch. Anim. Nutr.* 63, 219–229.
- Van Soest, P.J., Robertson, J.B., Lewis, B.A., 1991. Methods for dietary fiber, neutral detergent fiber and non-starch polysaccharides in relation to animal nutrition. *J. Dairy Sci.* 74, 3583–3597.
- Vázquez-Añón, M., Cassidy, T., McCullough, P., Varga, G.A., 2001. Effects of Alimet on nutrient digestibility, bacterial protein synthesis, and ruminal disappearance during continuous culture. *J. Dairy Sci.* 84, 159–166.
- Woclawek-Potocka, I., Bah, M.M., Korzekwa, A., Piskula, M.K., Wiczowski, W., Depta, A., Skarzynski, D.J., 2005. Soybean-derived phytoestrogens regulate prostaglandin secretion in endometrium during cattle estrous cycle and early pregnancy. *Exp. Biol. Med.* 230, 189–199.
- Woclawek-Potocka, I., Piskula, M.K., Bah, M., Siemieniuch, M.J., Korzekwa, A., Brzezicka, E., Skarzynski, D.J., 2008. Concentrations of isoflavones and their metabolites in the blood of pregnant and non-pregnant heifers fed soybean. *J. Reprod. Dev.* 54, 358–363.
- Woclawek-Potocka, I., Mannelli, C., Boruszewska, D., Kowalczyk-zieba, I., Wasniewski, T., Skarzynski, D.J., 2013. Diverse effects of phytoestrogens on the reproductive performance: cow as a model. *Int. J. Endocrinol.* 2013, 1–15.
- Young, E.G., Conway, C.F., 1942. On the estimation of allantoin by the Rirmmi-Schryver reaction. *J. Biol. Chem.* 142, 839–853.