Final Report

USE OF CANOLA MEAL AS A PROTEIN SOURCE IN PELLETED STARTER MIXTURES FOR DAIRY CALVES

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ABSTRACT

Canola meal use in starter mixtures for calves is limited. The objective of this study was to evaluate the use of canola meal as a protein source in starter mixtures for Holstein calves around weaning and to evaluate strategies that may enhance canola meal use and improve gastrointestinal development. Four studies were conducted to address the previously mentioned objectives. The first study evaluated the impact of heating canola meal on in situ digestibility and estimated intestinal digestibility. We found that heating canola meal to 110°C increased the rumen undegradable fraction without negatively affecting the intestinal digestibility. In the second study, canola meal was either not heated or heated as described for Study 1. The starter mixtures incorporated canola meal (not heated vs. heated) with or without glycerol. A total of 28 Holstein bull calves were sourced for this study and randomly assigned to 1 of the 4 starter mixture treatments in a 2 x 2 factorial treatment arrangement. We observed that heat-treating canola meal tended to decrease average daily gain and broadly reduced GIT tissue mass. Interestingly, glycerol inclusion, in general, had a positive effect on growth, ruminal fermentation, insulin concentration, and may alter GIT development. Study 3 contained metabolic and growth performance components. Treatments included canola meal or soybean meal as the protein source with or without microencapsulated sodium butyrate. Our findings suggest that, relative to soybean meal, canola meal may negatively affect starter mixture intake and body weight gain. However, we also found that microencapsulated sodium butyrate stimulated starter mixture intake and intestinal development. Studies 1 to 3 suggested that replacing soybean meal with canola meal may decrease starter mixture intake and that heat-treating canola meal exacerbated the effect. However, both glycerol and microencapsulated sodium butyrate had positive effects. The final study was conducted to evaluate how canola meal inclusion affects production responses. In this study (Study 4) we substituted 0, 50, or 100% of the soybean meal with canola meal. We found that full replacement of soybean meal with canola meal reduced starter intake but, replacement of 50% of the soybean meal was acceptable for calves. Altogether, the results of this study suggest that high-quality canola meal can be used in starter mixtures for dairy calves and that the inclusion of glycerol and microencapsulated sodium butyrate stimulate starter intake, growth performance, and aspects of gastrointestinal development.

LIST OF ABBREVIATIONS

ADF	Acid detergent fibre
ADG	Average daily gain
AQP3	Aquaporin 3
ATB0	Neutral and basic amino acid transporter
BHBA	β-hydroxybutyric acid
BW	Body weight
CM	Canola meal without MSB
CM-MSB	Canola meal with MSB
CP	Crude protein
CRA	Cranial sac of rumen
D	Potentially degradable fraction
DM	Dry matter
EAAC1	Glutamate transporter
ED	Effective degradability
ESC	Ethanol soluble carbohydrates
GIT	Gastrointestinal tract
GPR41	G protein coupled receptor 41
GPR43	G protein coupled receptor 43
HCl	Hyhdrochloric acid
IgG	Immunoglobulin G
ILE	Ileum; [2]
Kd	Rate of degradation
KP	Rate of passage
MCT1	Monocarboxylate transporter 1
MCT4	Monocarboxylate transporter4
MR	Milk replacer
MSB	Microencapsulated sodium butyrate
NDF	Neutral detergent fibre
NEFA	Non-esterified fatty acids
OM	Organic matter
PBS	Phosphate buffered saline
PEPT1	Peptide transporter 1
PEPT2	Peptide transporter 2
PROX	Proximal jejunum
PS	Protein source
PS×MSB	Interaction between main effects
RUP	Rumen undegradable protein
SCFA	Short-chain fatty acids
SM	Soybean meal without MSB;
SM-MSB	Soybean meal with MSB
U	Undegradable fraction
UT-B	Urea transporter B
wk	Week

INTRODUCTION

Weaning is an important period in the life of every mammal. It is the time of transition from dependency on milk for delivering all the necessary nutrients to a solid feed source independent of the dam. It is a crucial change especially for livestock animals as it is necessary to balance the welfare of the animal and potential cost. Time of weaning is different for every species and depends on the ability of gastrointestinal tract (**GIT**) to digest the solid feed provided to the animal. Weaning calves is complicated compared with monogastric animals, due to additional changes that need to occur to adapt GIT. Naturally, this process in beef cattle takes place between 7 to 14 mo of age (Enríquez et al., 2010). However, on dairy farms, calves are separated from their dam at birth and fed milk or milk replacer. Given the labour demands and high cost of feeding, strategies have been investigated in order to reduce these costs. Often the dietary change from a milk-fed calf to one that relies on solid feed consumption is too rapid as evidenced by a reduction in growth of the calves (Sweeney et al., 2010). Conventional methods of weaning cease providing milk to calves at certain age, ranging from 12 weeks to as young as 3 or 4 weeks. However at such an age consumption of solid feed might not be adequate, therefore resulting in reduced weight gain after weaning, as well as greater risk of respiratory disease or diarrhea (Roth et al., 2009). It has also been shown that improved preweaning growth performance is associated with greater milk yield during first lactation of the heifer (Soberon at al., 2009). Another approach is weaning based on adequate starter intake, which takes into account individual consumption of starter mixture of the calf. Daily intake of starter at the level of 0.68 kg for 3 consecutive days has been established to be an indicator for adequate concentrate consumption for weaning calves that are approximately 4 weeks of age (NRC, 2001). The use of concentrate intake as a criteria for weaning rather than using calf age mitigates some of the negative effects imposed by weaning at a young age (Kertz et al., 1979; Roth et al., 2009).

The ability of calves to utilize solid feed depends on morphological and functional adaptation of the gastrointestinal tract. For example, milk consumed via suckling generally by-passes the rumen via the reticular groove, thereby entering the omasum and being digested in the abomasum and small intestine. There, due to activities of brush border enzymes, specifically lactase, maltase and sucrase, milk is digested and can be absorbed in the form of glucose, galactose or fructose. The protein from solid feed can first be digested in the rumen by local microflora, unless they by-pass the rumen, entering abomasum where, similarly to protein from milk, they would be broken down to shorter peptide chains and then to the small intestine where further degradation occurs, as well as absorption from through the intestinal epithelium. Weaning causes changes in the activity profiles of brush border enzymes, especially for the disaccharidases (decrease of lactase, increase of maltase and sucrase), affecting only slightly peptidases (Zabielski et al., 2002). As solid feed intake increases, the rumen becomes the site of digestion.

In order to provide a smooth transition for calves from at weaning, it is necessary to provide them with good quality starter, that will promote the rumen development (Žitňan et al. 2005). There are various components influencing the GIT development. Short chain fatty acids (**SCFA**) stimulate the development of the reticulo-rumen by promoting papillae growth thereby increasing the absorptive surface area, increasing the absorptive function of the epithelium (Žitňan et al., 1999), and by stimulating key metabolic processes such as ketogenesis (Baldwin et al., 2004). It has been proven that provision of

low doses (0.6% as fed in starter feed, 0.3% as fed in milk replacer) of sodium butyrate have a positive effect on these processes (Górka et al., 2011b). However, the rumen is not the only region of the gastrointestinal tract that may benefit from sodium butyrate supplementation as the use of microencapsulated sodium butyrate (**MSB**) has also been reported to enhance intestinal development when supplemented in milk replacer (Górka et al., 2014). In fact, development of the small intestine preceded ruminal development. In the small intestine, sodium butyrate is considered to have various effects on enterocytes. It can stimulate the proliferation, differentiation and maturation of enterocytes, decrease apoptosis of normally functioning cells, while possibly increasing apoptosis of cancerous cells (Guilloteau et al., 2010a). Butyrate also increase daily pancreatic secretion and as a consequence, improved protein digestion (Guilloteau et al., 2010b). It also has positive effect on structural development of small intestine, including tissue weight, villus size and crypt depth (Górka et al., 2014). Sodium butyrate also has overall beneficial effects on performance and health (Guilloteau et al., 2010a).

Considering potential of small intestinal development aiding in the rumen development, other strategies incorporating the former should be considered. Two potential stimulants for intestinal development may be glutamine and glutamate. These two amino acids are considered to play an important role in small intestinal development as indicated by an increase in villi and microvilli size (Potsic et al., 2002) , as well as providing an energy substrate for enterocytes (Windmueller and Spaeth, 1974). Supplementation of glutamine has been proven to avert the jejunal atrophy in weaned piglets (Wu et al., 1996), although its effect in ruminants at the timing of weaning is not known. Glutamate is a substrate used for production of other amino acids such as proline and arginine, as well as the antioxidant glutathione (Reeds et al., 2000). Through all these actions, glutamine and glutamate contribute to the development of the small intestine. It has been also shown that it is not only systemic glutamate that is responsible for those processes with dietary glutamate playing an equally important role (Reeds et al., 2000). Interestingly, high concentrations of glutamine and glutamate can be found in canola meal (Newkirk et al., 2003; Borucki Castro et al., 2007).

Canola is one of the main oilseed plants in the world, second only to soybean (USDA, 2001). It is produced in East Asia (China and India), Europe, and Canada. Different species of rapeseed are used in production, though the most abundant ones are *Brassica napus* L. and *Brassica rapa* L. (Newkirk, 2009; Raymer, 2002). Its seeds contain at least 40% of oil and the meal, left after the oil extraction, contains 35 to 40% of protein (Raymer, 2002). Term “canola” was registered in the 1970s and can be used for the cultivars of rapeseed that produce oil containing less than 2% of erucic acid and a meal with less than 30 μmol of glucosinolates/g (Newkirk, 2009; Raymer, 2002). Both erucic acid and glucosinolates are antinutritional factors. Despite efforts to improve the quality of canola meal and its documented benefits in terms for mature cattle (Spörndly and Åsberg, 2006), palatability and digestibility are low for calves around weaning (Fiems et al., 1985; Khorasani et al., 1990). Therefore, the use of canola meal as a feed source for calves is limited. However, there may be an opportunity for the use of canola meal in diets for calves.

Canola meal contains a high proportion of rumen degradable protein (Bell, 1993). As such, the rumen microbial community modifies and utilizes much of the amino acids available, thereby potentially decreasing the beneficial impacts arising from the high

glutamine and glutamate concentrations reaching the small intestine. However, applying heat to canola meal can increase the amount of by-pass protein, which can shift the digestion to the small intestine (McKinnon et al., 1991; Wright et al., 2005). Providing that heat treatment does not affect the relative concentrations of glutamine and glutamate, there could be potential benefits of feeding canola meal to calves. Moreover, heating also inactivates myrosinase, an enzyme that can convert glucosinolates into toxic metabolites in mammals, improving the quality of the meal (Bell, 1993).

The low palatability of canola meal could be masked by including highly palatable feeds within the starter. For example, glycerol, a by-product from bio-diesel production, has been shown to highly palatable and helps to improve pellet (Schröder & Südekum 1999). Moreover, inclusion of glycerol is rapidly fermented in the rumen (Garton et al., 1961; Rémond et al., 1993)and therefore may have a promoting effect on rumen development. As such, strategies that are designed to optimize the supply of key nutrients to the small intestine (i.e. glutamine, glutamate, butyric acid) and readily fermentable substrate to the rumen (i.e. glycerol) may improve the gastrointestinal development in calves at weaning.

The objectives of this program were:

1) To evaluate the effectiveness of canola meal as a protein source (and source of glutamine and glutamate) in pelleted starter mixtures for newborn calves (Study 1, 2, and 3).

2) To evaluate suitable methods of increasing canola meal digestibility and palatability (use of glycerol and/or sodium butyrate) when fed to newborn calves (Study 2 and 3).

3) To compare canola meal and soybean meal in terms of their effectiveness to stimulate gastrointestinal development in calves at weaning (Study 3 and 4)

Although only 2 studies, were planned, we conducted a total of 3 full studies and 1 pilot study in this project as we were able to obtain additional funding in Poland.

EXPERIMENTAL APPROACH AND RESULTS

Note: All tables and figures are shown in the Appendix.

Study 1. Evaluating the effect of heating temperature on ruminal protein digestion kinetics and estimated intestinal digestibility of canola meal.

Introduction

Development of GIT in calves plays an important role especially during weaning period when the transition from liquid to solid feeds occurs. It has been established that development of small intestine is as important as rumen development (Górka et al., 2011b). Canola meal is not commonly used in feeding of calves due to its low palatability and digestibility. However, heat treatment has been proven to decrease protein degradation in the rumen (McKinnon et al., 1991), therefore theoretically allowing it to be digested and absorbed in the small intestine. It is necessary to evaluate the ruminal and estimated intestinal digestibility of canola meal that has been subjected to heat treatment, which will be the objective of this experiment. We hypothesize that heating will cause lower rumen digestibility without compromising intestinal digestibility of the canola meal.

Materials and Methods

Four sub-samples of canola meal (100 kg each) were used during the experiment. A 25-kg portion of each sub-sample was subjected to 1 of 4 treatments: remain untreated (CON) or heat treated to 100°C (100), 110°C (110) or 120°C (120). Heat treatment was done in tumble dryer (POS, Saskatoon). Temperature was steadily increased from room temperature until a desired one was reached and then the meal was held in that temperature for set amount of time (10 minutes). Afterwards the meal was cooled down to 50°C and packaged. More information about the heat treatments is provided in Table 1.

Rumen degradability

Polyester bags (40 to 60 µm pores) were dried to 55°C for 1 h, cooled down in desiccators for 15 min and their weight was recorded. Seven grams of dry feed were weighed into each bag and bags were sealed using double zip-ties. An additional 4-bags/replication were incubated for 12 h in rumen followed by a 3-step in vitro procedure to measure estimated intestinal digestibility. All bags for 0 h of incubation, were soaked in warm (37 to 39°C), distilled water for 30 min. The sequential in all-out procedure, as recommended by NRC (2001), was utilized. Upon insertion into the rumen, bags were placed within a laundry bag fitted with 1 kg of weight.

Upon removing the bags from the rumen, bags were immediately placed in cold water (4°C) and washed 5 times. For each wash, 60 bags were placed in 15 L of cold water. After washing, bags were placed on a flat pan and frozen at -20°C for 24 h. Following this step, bags were removed from the freezer, placed in cold water, and rinsed 1 additional time to reduce microbial contamination. The bags were then dried at 55°C until achieving a constant weight (approximately 72 h). Afterwards, bags were removed from the oven, placed in desiccators for 15 minutes, and the weight of the bag was

recorded. The residual feed at each time point from each replicate was pooled for chemical analysis. Dry matter (**DM**) content was analyzed through drying of the samples in the oven at 135° for 2 hrs. Crude protein (**CP**) content was measure using the Kjeldahl method, which consisted of first digesting samples with concentrated sulfuric acid in the catalyst presence (potassium sulfate) at 420° for 1 hr. Afterwards the nitrogen concentration was determined by titrating ammonium borate against hydrochloric acid in the presence of bromocreson green and methyl red. AAFCO samples were analyzed as well as an internal standard.

Intestinal digestibility

Three step estimated intestinal digestibility was conducted according to procedure detailed by (Calsamiglia and Stern, 1995). The pooled residuals from the 12 h rumen incubation were subjected to the pepsin/HCl digestion for 1 hr at 38°C in shaker water bath, followed by neutralization by sodium hydroxide. Afterwards they were subjected to pancreatin digestion for 24 hrs (with vortexing every 8 hours in the same temperature and conditions as mentioned before. At the end of experiment, all enzymatic activity within samples was stopped by addition of tetra-chloric acid. Samples were then stored in walk-in fridge for a couple of days, until CP in supernatant was analyzed afterwards, by Kjeldahl procedure, as detailed above.

Calculations and Statistical Analysis

In situ residual data for both DM and CP were analyzed as completely randomized design with single 25 kg sample of canola meal as an experimental unit. Degradation rates were analyzed using PROC NLIN (SAS 9.4) following Orscov's model (Ørskov and McDonald, 1979) with the equation:

$$R(t) = U + D \times e^{-K_d \times (t - t_0)}$$

Where R(t) is residue at given incubation time-point (t) (%), U is undegradable fraction (%), D is potentially degradable fraction (%) and K_d is degradation rate of D (%/h).

Effective degradability (ED) was calculated following the equation:

$$ED = S + \frac{(D \times K_d)}{K_p + K_d}$$

Where S is soluble fraction, which was washed out from 0 h incubation bags (T_0 samples); K_p is the rate of passage (assumed K_p = 5%/h).

The degradation rate and fractions, effective degradability for DM and CP, as well as estimated intestinal digestibility were analyzed using PROC MIXED (SAS 9.4) with polynomial contrasts which were used for determination of linear or quadratic relationships between provision of additional heat and rumen degradability and intestinal digestibility. Significance level was declared at $P < 0.05$.

Results and Discussion

The results are summarized in Table 2. The rate of DM disappearance in the rumen has decreased with increasing temperature of heat treatment (quadratic, $P = 0.02$). The rate of CP disappearance was not affected by heat treatment ($P \geq 0.14$). Degradable fractions for both DM and CP decreased with increase of temperature (quadratic, P <

0.01), with values for DM: 60.0 and 34.1 and CP: 79.0 and 22.3 for CON and 120 treatments respectively. Assuming a constant passage rate of 5%/h, the effectively degradable DM decreased with heat treatment (quadratic, $P < 0.01$) from 54.2 for CON to 31.7 % for 120 and the effectively degradable CP decreased as well (quadratic, $P < 0.01$) from 55.5 to 25.4 %. The estimated intestinal digestibility had a quadratic response ($P = 0.03$) with digestibility increasing from CON (45.9%) to 110 (51.0%) and decreasing for 120 (37.2%).

The results presented above suggest that a temperature of 110°C was most suitable for heat treating canola meal. That temperature allowed to significantly decrease the degradable fraction for both DM and CP, as compared with control, while the estimated intestinal digestibility has also shown the highest value from all used treatments.

Study 2. Evaluating whether heat-treated canola meal and glycerol inclusion affect gastrointestinal development in Holstein calves at weaning

Introduction

Canola meal, though not commonly used as an ingredient in calf starters, has high concentration of glutamine and glutamate. Through the means of heat treatment, the proteins containing those amino acids can by-pass the rumen and be digested and absorbed in the small intestine while positively influencing the development of the tissues there. Feeding glycerol, which is easily digestible in rumen, could help increase the intake of starter through better palatability and digestibility, as well as stimulate growth of the rumen.

We hypothesized that heat treated canola meal, with increased RUP fraction, will promote the development of the small intestine by enhancing the supply of glutamine and glutamate, and it will indirectly influence rumen development. Moreover, the beneficial effect of heat-treated canola meal can be enhanced by feeding glycerol to promote rumen development and overall feed digestibility and palatability. The objective of this part of the project is to evaluate the effect that heat treating canola meal will have on small intestine and rumen development. Also, assessment will be made whether glycerol will influence the palatability and digestibility of the starter.

Experimental approach

Animals, housing and feeding regiment

Twenty-eight newborn bull calves, sourced from a single commercial herd (Plum Blossom Farm, Osler, SK) were used in this study. All calves were separated from their dam and provided a commercial milk replacer at birth to supply 180 g of IgG (Headstart Bovine Dried Colostrum, The Saskatoon Colostrum Co. LTD.). Prior to arrival at the U of S, milk replacer was fed according to the commercial protocol from Plum Blossom Farm (4 L of milk replacer/d in 2 equal feedings). Within one week, calves were transported to Livestock Research Building at the U of S. Upon arrival they were weighed and placed in individual pens (1.5 × 3 m) with wood shavings as bedding. Fresh bedding will be added daily.

After arrival at the U of S and at 8 d of age, all calves were provided a common milk replacer feeding protocol (Figure 1). The amount of milk replacer provided was

adjusted to actual body weight (**BW**) through the trial and as follow: 4 L/d until d 7 of age, 10% BW on d 8 and 9, 11.5% BW on d 10 and 11, 13% BW on d 12 and 13, 15% BW for d 14 until the start of wk 5 at which time the amount of milk provided was reduced to 10% of BW for wk 5, 5% BW for wk 6, and 2.5% BW for 1 wk (wk 7). In all cases, 150 g (DM basis) of milk replacer powder was dissolved into 1 L of water. The step-down weaning procedure was used to encourage solid feed intake. For wk 1 to 4, calves were fed in 3 equal feedings at 0800, 1200 and 1600 h, while for wk 5 and 6 calves were fed in 2 equal feedings at 0800 and 1600 h, and were only provided 1 feeding at 0800 h during wk 7. The milk provision for calves ceased on d 50 of age. The health status of calves was monitored daily (see health score rubric – health scoring template file) and body weight was recorded once a week at 0700 h.

Calves were blocked by birth date and within block, randomly assigned to 1 of 4 treatments, using BW at 8-d of age as a secondary blocking factor. Calves were offered 1 of 4 different starter pellets arranged in a 2 × 2 factorial arrangement. Factors included the use of canola meal that was heated or not heated and the inclusion of glycerol. The canola meal was heat treated in a tumble dryer (POS, Saskatoon) at 110°C for 10 min, prior to pelleting with other ingredients of the starter. Calves were fed common milk replacer with their respective starter mixture offered ad libitum. Starter was offered starting from d 8 at a rate of 400 g/d and the amount was adjusted daily to ensure *ad libitum* intake. Refusals of the starter mixture were removed and recorded daily before morning feeding with fresh starter being offered after morning feeding. Refusal samples were pooled weekly and analysed for DM by drying at 55°C until achieving a constant weight (minimum of 48 h). Starter mixtures were designed to be isonitrogenous and isoenergetic and provided fresh daily. The starter mixture compositions are presented in Table 3. No other feeds will be included in this study in order to eliminate additional influence on GI tract development. The consumption of milk replacer and starter feed was measured daily. Samples of milk replacer were collected from each new bag: 200 g for chemical analysis and 200 g for DM analysis (135°C for 2 h). Samples for chemical analysis were composited monthly (with 5 samples in December, 24 samples in January, 21 samples in February and 3 samples in March) and 200 g of composite was allocated for chemical analysis. Samples of the starter (200 g) were collected weekly. Those samples were analyzed for DM content (55° for 48 h) and composited monthly (4 weekly samples per each month starting from 12 December) ground on 1 mm sieve and, along with MR samples, sent to Cumberland Valley Analytical Services for chemical composition analysis including: OM, DM, CP, ether extract, ADF, NDF, starch, ethanol soluble carbohydrates (**ESC**), and mineral analysis.

We acknowledge that calves may have consumed a portion of the wood-shavings used for bedding. That said, providing wood shavings is common in commercial production and in a recent study at the U of S, the use of wood shavings helped to reduce the incidence and severity of scours.

Pre-slaughter data and sample collection

Blood samples (two samples of 10-mL each) were collected on arrival and on d 22 (15% BW milk provision), d 43 (after 5% BW of milk provision) and d 51 (after weaning) of age from the jugular vein at 1000 h (2 h post-feeding). Upon arrival, plasma was collected for measurement of total protein concentration in order to access the

passive transfer of IgG from colostrum. The mean total protein concentration in plasma was 6.0 g/dL (±0.50), with maximum value 7.0 and minimum 5.0. Two calves that had total protein content of 5.0 g/dL, have been prophylactically treated with antybiotics. For remaining time points, samples for plasma (Na-heparin) and serum (no anticoagulant) were collected. Plasma samples were placed on ice and centrifuged for 15 min at 2600 × g at 4°C. Serum samples were allowed to clot for 1 h prior to centrifugation at the same conditions as plasma samples. Supernatant from both samples were then transferred into vials and frozen at -20°C until being analyzed for glucose, β-hydroxybutric acid (**BHBA**), urea and insulin.

Post-slaughter data and sample collection

After weaning, all calves were killed via captive bolt stunning, followed by exsanguination (d 51 at 1400 h or at 1330 h and 1430 h if two calves were killed during one day). The whole gastrointestinal tract was dissected for morphometric measurements. The weight, both with and without digesta, was measured for following sections of GIT: reticulo-rumen, omasum, abomasum, duodenum, jejunum, ileum, cecum and colon. Weight of liver and spleen was also recorded. For the intestine tissues, length measurements were conducted as well for each region. For the reticulo-rumen, digesta was placed in a container, mixed and pH was measured (in duplicate). A representative sample of digesta was strained through 2 layers of cheesecloth and the supernatant was collected (10 mL) and mixed with 2 mL of meta-phosphoric acid (25% wt/v). The sample was stored at -20°C until being analyzed for SCFA concentration using gas-chromatography. A second sample of rumen fluid (10 mL) was collected and mixed with 2 mL of sulfuric acid and frozen at -20°C until being analyzed for ammonia concentration.

Identification of regions of the GIT for sample collection. The rumen was cut open through the dorsal sac and digesta was deposited in separate container. Tissue samples were collected from the caudal ventral blind sac, central ventral sac, and cranial ventral sac. Abomasum tissue samples were collected from the main body of the abomasum, in body of the fundus. Tissue samples from the small intestine included: duodenum (with the end determined by the ligament of Treitz), 3 regions of jejunum (proximal [at 25% of total length], middle [at 50% of total length] and distal [at 75% of total length, with the end based on the ileocecal fold; which also marks the beginning of the ileum], and ileum [with end at the ileocecal junction]. All tissue samples were gently washed in ice-cold sterile PBS in order to remove digesta, before any further processing.

Tissue collection for gross morphological and histological assessment.

Reticulo-rumen. Samples of whole-tissue (~1 cm^2) were collected from each of the 3 sites (caudal, central, and cranial ventral sacs) and placed in 50 mL of formalin solution, for later morphometric and histometric measurements, which included: villus/papillae length and density, crypt depth, tunica mucosa and tunica muscularis thickness. Around 5×5 cm tissue fragment from the ventral sac will be placed in plastic bag and frozen at -20° for DM analysis.

Abomasum. Sample of whole thickness (~5×5 cm) tissue were placed in plastic bag and frozen at -20° for DM analysis.

Duodenum. Whole thickness tissue sample (~1 cm long) were collected from undamaged part of the gut, rinsed with PBS and transferred into the 30 mL of

formalin solution. Another whole thickness tissue sample (~5 cm long) was placed in plastic bag and frozen for DM analysis.

Jejunum. Samples from three separate sites of jejunum were collected as described above. From each region, a 1 cm long sample of whole tissue was collected, rinsed and preserved in formalin. Another sample, approximately 5 cm long was collected from the middle part of the jejunum and at -20° for further DM analysis.

Ileum. A whole thickness tissue sample (~1 cm long) was collected from undamaged part of the ileum as described previously, rinsed with PBS and transferred into the 30 mL of formalin solution. Another whole thickness tissue sample (~5 cm long) was placed in plastic bag and frozen for DM analysis.

Although tissues were collected, the tissues were too fragile for preservation and histological evaluation. Thus, there is no data regarding tissue morphology and histology.

Tissue collection for brush-border enzyme assays. The epithelium of each of the described above regions of the small intestine were scraped using sterile glass slides on a clean surface on top of ice layer. Tissue was put into empty 2-mL tubes, snap frozen in liquid nitrogen and then transferred to -80°C storage until analysis of brush border enzymes activities: lactase, maltase, dipeptidase IV, aminopeptidase A and N.

Tissue collection for gene expression. Representative tissues from the rumen, abomasum and five regions of small intestine were analyzed for gene expression of important nutrient transporters, including short chain fatty acids (MCT1, MCT4); in rumen urea transporters (UTB and AQP3); in the small intestine peptides (PEPT1 and PEPT2) and amino acids transporters (EAAC1 and ATB0). Small pieces of mucosal tissue (2 per region) were taken using sterile equipment, rinsed in sterile, ice-cold PBS and transferred to 2-mL test tubes with 1.8 mL of RNA-later solution (Applied Biosystems), stored for 24 h at 4°C and then frozen at -20°C for further reverse transcription analysis. Samples from cranial ventral sac of the rumen, as well as proximal jejenum and ileum were analyzed for gene expression.

Statistical analysis

Data were analyzed using the mixed model of SAS (SAS Institute, Cary, NC) as a 2 x 2 factorial design. The model included the fixed effects of canola meal heat treatment, glycerol inclusion, and the interaction. For variables collected over time (day or week), time was included as a repeated measure and the covariance error structure that yielded the lowest Akaike's and Bayesian Information Criterion was used. Least square means are presented with the standard error of the mean for the main effects. When interactions are presented, the interaction standard error of the mean is shown. For the interaction, means were separated using the Bonferroni method. Differences were declared when $P < 0.05$ and tendencies are discussed when $0.10 > P < 0.05$.

Results

There were no effects of heat treatment of canola meal or glycerol inclusion on starter intake, milk replacer intake, or calf BW (Table 5). However, ADG tended ($P = 0.07$) to be reduced for calves fed heat-treated canola meal (0.57 vs. 0.47 kg/d) and tended ($P = 0.09$) to be increased with glycerol inclusion (0.48 vs. 0.56 kg/d). This

suggests that over-heating canola meal decreases ADG and that inclusion of glycerol may improve ADG.

Ruminal pH was not affected by heat treating canola meal; however, glycerol inclusion decreased (P = 0.04) ruminal pH by 0.26 units (Table 6). Likewise, the total concentration of ruminal SCFA was not affected by heat treatment, but was increased with glycerol inclusion. The molar proportion of the main SCFA (acetate, propionate, and butyrate) were not affected by heat treatment or glycerol inclusion. The reduced ruminal pH and increased SCFA concentration are not likely to be problematic given the low ruminal pH commonly observed for calves around weaning.

Heat treatment reduced ruminal tissue and ruminal digesta mass (Table 7) while glycerol had no effect. Omasum, abomasum, and duodenum tissue and digesta mass were not affected by heat treatment of canola meal. However, jejunum tissue mass was reduced when canola meal was heat treated and jejunum length tended to be reduced for calves fed heat treated canola meal. Cecal digesta mass was reduced and colon length was reduced when fed heat treated canola meal. Moreover, liver weight was reduced and spleen weight tended to be reduced for calves fed heat-treated canola meal. Glycerol increased digesta mass in the duodenum, jejunum, and cecum and increased liver weight.

The concentration of NEFA (Table 8) were not different at 22 d of age (a time when milk was offered), but tended to be reduced for calves fed heated canola meal and also for those fed glycerol at d 43 (during the weaning process). While NEFA was reduced, the concentrations of NEFA are low and are not indicative of a negative energy balance. No differences were detected for NEFA following weaning (d 51). Plasma urea nitrogen and glucose did not differ among treatments, regardless of the day of age. However, glycerol inclusion decreased insulin concentration at d 22 and d 43 of age. Plasma BHBA (an indicator of ruminal ketogenesis) tended to be reduced and was less for calves fed heat treated canola meal at 22 and 43 d of age, respectively. Calves fed glycerol tended to have lower BHBA concentrations at d 51 of age.

Activity of aminopeptidase A, and N did not differ among treatments (Table 9). Dipeptidase tended to be greater in the jejunum for calves fed glycerol but, did not differ in the ileum. Lactase and maltase activity were not affected by treatment.

Expression of monocarboxylate transporter 1 (MCT1) was affected and MCT4 tended to be affected (P = 0.07) by the interaction between heating canola meal and glycerol inclusion (Table 10; Figure 2). The interactions were a result of glycerol reducing expression when canola meal was heated relative to when glycerol was not included with heated canola meal. Urea transporter B was not affected in the rumen, but Aquaporin3 (an aquaglyceroporin capable of transporting both water and urea) was reduced when glycerol was included with heat treatment relative to when glycerol was not included (Figure 3). In the proximal jejunum, glycerol inclusion increased expression of MCT1 and MCT4, but did not affect peptide transporters. No treatment effects on gene expression were detected in the ileum except an interaction for EAAC1 (glutamate transporter) where inclusion of glycerol in the heat-treated canola diet increased expression relative to the heat treated canola diet that did not contain glycerol.

Conclusion

Heat treated canola meal negatively affected ADG and GIT tissue mass and while contrary to our hypothesis, points out that heat damaged canola meal may have negative

effects when used in starter mixtures for Holstein calves at weaning. Glycerol inclusion, in general, had a positive effect on growth, ruminal fermentation, insulin concentration, and may alter GIT development. Thus, starter mixtures for calves can include canola meal providing it is not over-heated. Glycerol inclusion appears to have beneficial characteristics as an ingredient in starter mixtures for dairy calves.

Study 3. Effect of including canola meal or soybean meal in a pelleted starter mixture for calves when combined with microencapsulated sodium butyrate and their effect to stimulate gastrointestinal tract development in calves at weaning and following weaning.

The objective of this study was to determine the possibility to further enhance the canola meal use in a pelleted starter mixture for newborn calves through supplementation of sodium butyrate. Specifically, it was hypothesized that microencapsulated sodium butyrate will promote rumen and small intestine development, increase digestibility and solid feed intake, which will result in better performance of calves around and after weaning. Furthermore, in this study canola meal use was compared with soybean meal. Based on the results of previous studies, non-heated canola meal was used in this study and glycerol was included in the starter composition.

Materials and Methods

The study was conducted at the University of Agriculture in Kraków, Poland under the direct supervision of Dr. Górka and was divided into to Part A (metabolism) and Part B (performance).

PART A. Metabolism study

Animals, housing and feeding regiment

Twenty-eight newborn bull calves (8.7 ± 0.8 day of age and 43 ± 4.4 kg; mean ± SD) were allocated to the metabolism study. Calves were assigned to 1 of 4 experimental treatments. The treatments included starter mixtures containing canola meal or soybean meal as the main protein sources, both non-heated with the inclusion of glycerol. The second treatment factor was the inclusion or lack of microencapsulated sodium butyrate in the starter mixture. Thus, the experiment was conducted as a 2 × 2 factorial treatment arrangement with 7 calves/treatment. Starters were formulated to be isonitrogenous. Additionally, based on amino acid composition of the components and basal mix of the starter feed, the amount of lysine and methionine was equalized among treatments by methionine supplementation in starter mixture containing soybean meal. This approach allowed for the assessment of whether additional glutamine and glutamate from canola meal will have a positive effect compared to soybean meal, while eliminating the confounding effect of lysine and methionine intake.

Calves were sourced from two local commercial dairy farms belonging to Top Farms Głubczyce (Głubczyce, Opolskie, Poland), both located in close proximity to the calf barn. Calves were separated from the dam immediately after birth, moved to individual hutches, and provided with 4 L of colostrum within 2 h of life and 2 L of colostrum for second feeding within 12 h of life. On day 2 and 3 of life, calves received

transition milk in the amount of 2 L in 2 feedings a day. Starting with day 4 of life, calves were fed 2 L per feeding of whole milk. During that period of life calves had no access to starter feed. Calves were transported from the farm of birth to the calf barn twice-a week (Mondays and Thursdays) at 8 to 10 day of age around 1000-1100 h. After transport, they were housed in a calf barn belonging to Top Farms Głubczyce Sp. z o.o. Calves were randomly assigned to 1 of the 4 treatments based on body weight, farm of origin, and transport age (blocks were either assigned within one transport or two consecutive transports within the week). Only calves weighing between 35 and 50 kg on the transport day were accepted for the study. Calves were housed in individual pens (1.5 × 1.2 m) with wood shavings as bedding material. We acknowledged that calves may consume part of wood shavings; however, it is a common practice within the industry. Upon arrival, calves were prophylactically treated with wide-spectrum injectable antibiotic (Zactran, MERIAL, Lyon, France) to prevent pulmonary diseases, which was a common practice on the farm. Shortly after arrival calves received 2 L of electrolytes (Rehyvet, Univit, Olomouc, Czech Republic; Rehydrat, Biowet, Puławy, Poland). For further routine treatments, calves were given a coccidiostatic (Baycox 5%, Bayer, Leverkusen, Germany) on d 15 of the experiment as coccidiosis has been an issue in the past in the calf barn and was also a routine treatment on the farm.

Calves were fed commercial milk replacer (Polmass Milk Red Full Instant, Polmass S.A., Bydgoszcz, Poland) twice daily (3 L/feeding) at 0730 h and 1700 h. For the study purposes, the original formula of the milk replacer was modified by the manufacturer to remove butyrate sources. The milk replacer was mixed at a rate of 150 g of milk replacer powder as is per 1 L milk. Refusals were measured and recorded after each feeding. To ease the transition from the whole milk to milk replacer, for the 1700 h feeding (first feeding) on the day of the transport (1 experimental day), calves received 2 L/feeding of milk replacer. On the 2nd experimental day, calves received 2.5 L/feeding, which was increased to 3 L/feeding on the 3rd day. This feeding protocol (3 L/feeding) continued until one week before weaning. At the 36th experimental day, calves were limited to one feeding per day at 0730 h. Weaning took place on the 43rd experimental day, which was the first day with no milk provision (51.7 ± 0.8 day of age).

Ingredient composition of starters is presented in Table 11. The microencapsulated sodium butyrate (BIOLEK Sp. z o.o., Macierzysz, Ożarów Mazowiecki, Polska) contained 30% of sodium butyrate and 70% of triglyceride matrix. Furthermore, chromium oxide was included in the starter that was used as a digesta marker.

Starter was offered ad libitum. Daily, after morning milk replacer feeding, refusals were recorded and fresh starter was presented to the calves. The initial amount fed was 500 g/day and it was adjusted daily with 500 g increase whenever half of the previously eaten starter was consumed for the first increase and when less than 500 g was left for the following ones. Starter feed samples were collected twice a week (200 g/sample), while milk replacer samples were collected upon opening of a new bag (200 g/sample). All samples were composited monthly, dried, ground, and analyzed for chemical components.

All health issues and medical treatments were recorded. The fecal scores of the calves were recorded daily using 4-point scale (1 = normal, 4 = diarrhea).

Pre-slaughter data and sample collection

Blood samples were collected 4 times during the study: 1, 21, 42, 63 experimental days at 1000 h, with the exception of the first one which was taken shortly after being transported into the calf barn. Both serum and plasma samples were collected from jugular vein. Plasma samples was placed on ice and centrifuged for 15 min at 1000 × g. Serum samples were allowed to clot for 1 h prior to centrifugation at the same conditions as plasma samples. Supernatant from both samples was then transferred into 2-mL vials and frozen at -20°C until being analyzed for plasma glucose, β-hydroxybutric acid, urea and amino acids concentration and serum insulin and IGF-1 concentrations.

Around 9 wk of age, digestibility was measured using chromium oxide as a digesta marker. This part of the trial was conducted in weekly periods, always beginning on the same day of the week. Between 47 and 51 day of the study calves were assigned to the digestibility trial. Fecal sampling was done over the period of three days (Friday, Saturday and Sunday) each week, resulting in actual age of calves 57.2 ± 2.0 days. Samples were collected every 9 hours, with time-points being at 0700 and 1600 h on day 1, 0100, 1000, and 1900 h on day 2, and 0400, 1300, and 2200 h on day 3. At each sampling time point, 100 g of sample (fresh weight) was collected through manual stimulation of calf's rectum and then frozen at -20°C until the end of sampling period. Then, the samples were thawed and dried at 55°C until achieving constant weight (72 h) and composited per animal on DM basis. Further the composited samples were ground and analyzed for chromium concentration, DM, CP, NDF, ADF, and ether extract. Samples of starters (100 g) were taken during each sampling day. Refusal samples (100 g) were collected daily during three sampling days, composited per animal and analyzed for dry matter (55°C for 48h). All composited feed samples were ground and analyzed for chemical components.

Post-slaughter data and sample collection

Three weeks after weaning, calves at 71 to 73 days of age (72.1 ± 0.9 days of age) were transported to a nearby slaughterhouse at 0830 h, where they were killed via captive bolt stunning, followed by exsanguination, starting with first calf at 0900 h and with next one following as soon as the previous one is complete. Order of killing of calves from different treatments was randomized. The whole gastrointestinal tract was dissected for measurement of development. The weights, both with and without digesta, were measured for following sections of the gastrointestinal tract: reticulo-rumen, omasum, abomasum, duodenum, jejunum and ileum. For the intestine tissues, length measurements were conducted for each region. For the reticulo-rumen, digesta was placed in a container, mixed and pH was measured (in duplicate). A representative sample of digesta was strained through 2 layers of cheesecloth and the supernatant was collected (10 mL) and mixed with 2 mL of meta-phosphoric acid (25% wt/v). The sample was stored at -20°C until being analyzed for short-chain fatty acids concentration using gas-chromatography. A second sample of rumen fluid (4 mL) was collected and mixed with 0.2 mL of saturated $HgCl_2$ and frozen at -20°C until being analyzed for ammonia concentration. The digesta from omasum, abomasum and intestine was discarded.

Rumen tissue samples were collected from the caudal ventral blind sac and cranial ventral sac. Omasum tissue samples were collected from first order (large) laminae. Abomasum tissue samples were collected from the pyloric region of the abomasum (10

cm from the pylorus). Tissue samples from the small intestine included: duodenum (with the end determined by the ligament of Treitz), 3 regions of jejunum (proximal [at 25% of total length], middle [at 50% of total length] and distal [at 75% of total length, with the end based on the ileocecal fold; which also marks the beginning of the ileum]), and ileum (with end at the ileocecal junction). All tissue samples were gently washed in ice-cold sterile saline in order to remove digesta, before any further processing.

Tissue collecting for histometric measurements

Rumen: Whole-tissue (~ 4 cm^2) was collected from each of the 2 sites (caudal blind and cranial ventral sacs), rinsed with sterile saline and placed in 50 mL of formalin solution. *Omasum:* Five tissue samples from central portion of large omasal laminas (from the tip to the bottom, 2 cm thick) were taken, rinsed with sterile saline and placed in 50 mL of formalin solution. *Abomasum:* Whole-tissue (~ 4 cm^2) was collected 7 cm from the pylorus, rinsed with sterile saline and placed in 50 mL of formalin solution. *Small intestine:* Whole thickness tissue samples (~ 4 cm long) were collected from mentioned above and undamaged part of the intestine, rinsed with sterile saline solution and transferred into the 50 mL of formalin solution.

All tissue samples were stored in formalin solution for 24 h and then were transferred to 70% ethanol.

Tissue collection for gene expression

Representative tissues samples from the rumen (cranial sac), proximal jejunum, and illeum were collected. At the time of collection, the ruminal epithelium was manually separated from the muscle layer. Whole-tissue collected from the small intestinal epithelium was scraped using sterile glass slide. All tissues were rinsed in sterile, ice-cold saline and transferred to 2 mL test tubes with 1.8 mL of RNAlater solution (Applied Biosystems). Tissues were then chilled for 24 h at 4°C and then stored frozen at -20°C. Prior to RNA extraction, samples were pulverized using a mortar and pestle under liquid nitrogen. Concentration and integrity of RNA was estimated spectrophotometrically using NanoDrop (Thermo Fisher Scientific, Waltham, MA, USA) and verified electrophoretically. Samples of total RNA of good quality (OD_{260nm}/OD_{280nm} between 1.8 and 2.2) and without signs of degradation were immediately subjected to the reverse transcription reaction. Reverse transcription reaction was conducted using 2 µg of total RNA, High Capacity cDNA Reverse Transcription Kit with RNase Inhibitor (Thermo Fisher Scientific, Waltham, MA, USA) and Thermocycler Eppendorf AG (Eppendorf, Hamburg, Germany). Obtained cDNA was stored at –20°C until further use for gene expression analyses (GPR41, GPR43, MCT1, MCT4, PEPT1, PEPT2, AQP3, UT-B, ATB0, EQQC1 and 3 reference genes). Target and reference gene mRNA expression was analyzed at least in duplicate using StepOnePlus™ Real-Time PCR System (Life Technologies Corporation, Carlsbad, California, USA). PowerUp™ SYBR® Green Master Mix (Thermo Fisher Scientific, Waltham, MA, USA), 900 nM of each primer (forward or reverse), 1 µl of cDNA sample and filled up to 10 µl with Nuclease-free Water (Thermo Fisher Scientific, Waltham, MA, USA). To ensure correct product of analysis, melting curve analysis was performed for each sample and each time. Additionally, product of reaction for each primers was sequenced and run in the agarose gel. Relative expression was calculated based on the $2^{-\Delta\Delta C_T}$ approach (Livak and Schmittgen, 2001).

Tissue collection for brush-border enzyme assays

The epithelium of each of the described above regions of the small intestine was scraped using sterile glass slides and transferred into empty 2 mL tubes and put in liquid nitrogen, until transported to -80°C freezer where the samples were stored until analysis of brush border enzymes activities: lactase, maltase, dipeptidase IV, aminopeptidase A and N. Scraping procedure were conducted on clean surface on top of ice layer.
After tissue samples collection, ruminal, omasal and abomasal tissue was repeatable rinsed with tap water until clear, then water was pressed by hand and shaken down 2 times, and tissue was weighed.

Sample analysis and calculations

Nutrient composition of feeds and feces was preformed as previously described by Hadam et al. (2016) whereas Cr concentration was analyzed using analyzed using flame atomic absorption spectrophotometry (ISO, 2000). Amino acid composition was determined using AA analyzer AAA-400 (INGOS, Czech Republic), following with hydrolysis of the protein in 6N HCL (110^{o}C, 24 h). Sulfur-containing AA were determined after performing acid oxidation. Short-chain fatty acids in the reticulo-ruminal digesta were determined by gas chromatography (3400 CX, Varian Star, Palo Alto, CA, USA) equipped with a flame ionization detector (FID) as described previously by Górka et al. (2017) using DB-FFAP column (30 m × 0.5 mm, J&W Scientific, Folsom, CA, USA) and argon as a carrier. Ammonia concentration the reticulo-ruminal digesta was analyzed as describe by Conway (1962).

Plasma glucose and BHBA were determined by a commercial laboratory (WDL, Gietrzwałd, Poland) on an automatic chemical analyzer (Hitachi 902, Hitachi, Japan) as previously described by Górka et al. (2017). For glucose, BioSystems (Barcelona, Spain) kit was used, whereas for BHBA Diagnostic System Laboratories Inc. (Singheim, Germany) kit was used. Serum insulin and IGF-1 concentration was analysed using radioimmunoassay and commercial set of reagents (INS-IRMA and IGF-1-RIA-CT for insulin and IGF-1 determination, respectively; DIAsource, Louvain-La-Neuve, Belgium). Plasma amino acids concentration was analyzes using Pico-Tag amino acid analysis system (Waters, Milford Massachusetts, USA).

Nutrient digestibility was calculated using following formula: nutrient digestibility % = 100 – 100 × (marker content in feed/marker content in feces × nutrient content in feces/nutrient content in feed).

One square centimeter of rumen tissue that was preserved in formalin was used to determine rumen papillae length, width and density and rumen muscle thickness. All papillae were cut off at the base using forceps and scissors under SteREO Discovery.V12 ZEISS microscope with PlanApo S 0.63x FWD 81mm ZEISS lens and subsequently length and width (middle point) of each papilla was measured using AxioVision 40 V 4.8.2.0 (ZEISS) software. The mucosa surface (mm^2/cm^2) was determined as the length × width × density × 2 (Malhi et al., 2013). The remaining portion of the tissue (~ 2 cm^2) was cut into four 0.5 to 1 cm thick pieces, positioned on one edge, and muscle layer thickness was also measured in four locations on each pieces of the tissue, resulting in 20 measurements for each sample. Abomasum tissues samples were divided into four 0.5 to 1 cm thick pieces. Using the same microscope that was used for rumen papillae and rumen muscle measurements, 4 measurements for epithelium thickness and muscle layer

thickness in abomasum were done on each piece of tissue, resulting in 20 measurements for each parameter and sample. Small intestine tissue samples preserved in formalin solution and stored in ethanol were dehydrated in a graded ethanol series, cleared in xylene and embedded in paraffin and villus length, crypt depth, tunica mucosa and tunica muscularis thickness were measured as previously described by Górka et al. (2011). Brush border enzymes activity was analyzed as previously described by Górka et al. (2011), with minor modifications.

Total RNA from ruminal (cranial ventral sac), abomasal, proximal small intestine and ileal tissues stored in RNAlater was isolated using method of Chomczyński and Sacchi (1987). Concentration and integrity of RNA was estimated spectrophotometrically using NanoDrop (Thermo Fisher Scientific, Waltham, MA, USA) and verified electrophoretically. Samples of total RNA of good quality (OD_{260nm}/OD_{280nm} between 1.8 and 2.2) and without signs of degradation were immediately subjected to the reverse transcription reaction. Reverse transcription reaction was conducted using 2 µg of total RNA, High Capacity cDNA Reverse Transcription Kit with RNase Inhibitor (Thermo Fisher Scientific, Waltham, MA, USA) and Thermocycler Eppendorf AG (Eppendorf, Hamburg, Germany). Obtained cDNA was stored at –20°C until further use for gene expression analyses (GPR41, GPR43, MCT1, MCT4, PEPT1, PEPT2, AQP3, UT-B, ATB0, EQQC1 and 3 reference genes). Target and reference gene mRNA expression was analyzed at least in duplicate using StepOnePlus™ Real-Time PCR System (Life Technologies Corporation, Carlsbad, California, USA). PowerUp™ SYBR® Green Master Mix (Thermo Fisher Scientific, Waltham, MA, USA), 900 nM of each primer (forward or reverse), 1 µl of cDNA sample and filled up to 10 µl with Nuclease-free Water (Thermo Fisher Scientific, Waltham, MA, USA). To ensure correct product of analysis, melting curve analysis was performed for each sample and each time. Additionally, product of reaction for each primers was sequenced and run in the agarose gel. Relative expression was calculated based on the $2^{-\Delta\Delta C}_{T}$ approach (Livak and Schmittgen, 2001).

PART B. Performance study

Animals, housing and feeding regiment

Sixty newborn Holstein heifer calves (9.1 ± 0.8 days of age and 43.2 ± 4.2 kg; mean ± SD) were allocated to the performance study. Experimental designee, ingredient and chemical composition of starters resembled those used for the metabolism study (PART A). Briefly, calves were randomly assigned to 1 of 4 experimental groups and fed a starter mixture containing canola meal or soybean meal as a main source of protein, both non-heated with the inclusion of glycerol. The second treatment factor was inclusion or lack of microencapsulated sodium butyrate. Thus, the experiment was conducted as a 2 × 2 factorial treatment arrangement with 15 calves/treatment.

For this study, calves were also sourced from two local commercial dairy farms (Top Farms Głubczyce) located in close proximity to the calf barn. Calves were separated from the dam immediately after birth and provided with 4 L of colostrum within 2 h of life and 2 L of colostrum for second feeding within 12 h of life. For 2 and 3 day of life calves received transition milk in the amount of 2 L in 3 feedings a day. Starting with day 4 of life calves were fed 2 L per feeding of milk replacer that was offered 3 times a day. During that period of life calves had no access to starter feed. After transport, calves were

housed in a calf barn belonging to a commercial dairy farm (Top Farms Głubczyce Sp. z o.o.). Calves were transported from the farm of birth to the calf barn twice a week (Mondays and Thursdays) at 8 to 10 day of age around 1000-1100 h. Calves were block by week of transport to the calf barn. Blocking also took into account farm of origin that was balanced between treatments. Only calves weighting between 35-50 kg on the transport day were accepted in the study. Within block calves were randomly assigned to 1 of the 4 treatments based on the body weight. Calves were housed in individual pens (1.5 × 1.2 m) with straw as bedding. Upon arrival, calves were prophylactically treated with wide-spectrum injectable antibiotic (Zactran, MERIAL, Lyon, France), as is common practice on the farm. Shortly after arrival calves received 2 L of electrolytes (Rehyvet, Univit, Olomouc, Czech Republic; Rehydrat, Biowet, Puławy, Poland). For further routine treatments, calves were given a coccidiostatic (Baycox 5%, Bayer, Leverkusen, Germany) as coccidiosis has been an issue in the past in the calf barn and was also a part of prophylactic progrogram at the farm. All health issues and treatments were recorded. The fecal scores of the calves were recorded daily as describe for PART A of the study.

Once allocated to the study and kept in calf barn, calves were fed milk replacer twice daily (3 L/feeding) at 0730 h and 1700 h. The milk replacer was mixed at a rate of 150 g of MR powder as is per 1 L milk. The milk replacer used was made on order by Trouw Nutrition Poland (Grodzisk Mazowiecki, Poland) and did not contain any sources of butyrate. Refusals were measured and recorded after each feeding. On the day of the transport (1 experimental day) to ease the transition from the whole milk, for the 1700 h feeding, calves received 2 L/feeding of milk replacer. On the 2nd experimental day, they received 2.5 L/feeding, and the amount increased to 3 L/feeding on the third day. This feeding protocol (3 L/feeding) continued from until two weeks before weaning. At the 36th experimental day, calves were limited to one feeding per day at 0730 h. Weaning took place on the 50th experimental day, which was the first day with no milk provision (59.1 ± 0.8 d of age).

Starter pellet was offered ad libitum. The composition of starters is presented in Table 12, and it differed from the one used in PART A in that it did not contain chromium oxide, which was replaced by barely. For PART B, the same microencapsulated sodium butyrate was used as for PART A. Daily, after morning milk replacer feeding, refusals were recorded and fresh pellet was presented to the calves. The initial amount fed was 500 g per day and it was adjusted daily with 500 g increase whenever half of the previously eaten starter was consumed for the first increase and when less than 500 g was left for the following ones.

Starter feed samples and milk replacer powder samples were collected weekly (200 g/sample). All samples were composited by month, dried, ground, and analyzed for chemical components.

Sample analysis and calculations

Nutrient composition of feeds was performed as described for PART A.

Statistical analysis

For both, PART A and PART B, data were analyzed as a 2 × 2 factorial design using PROC MIXED of SAS (version 9.4). Block of calves was considered in the

statistical model as a random term and, when appropriate, initial body weight of calves was included in the model as a covariate. Repeated measures on one calf were analyzed as repeated measurements by including REPEATED statement in the model.

Results and discussion

PART A. Metabolism study

Nutrient composition of feeds is presented in Table 13. Although starters were formulated to isonitrogenous, crude protein content was slightly greater in starters containing soybean meal than canola meal. Lysine and methionine contents were also slightly greater then formulated in starters containing soybean meal compared to starters containing canola meal. As opposed to what was expected, glutamate and glutamine content in starters containing canola meal was lower than glutamate and glutamine content in starters containing soybean meal (33.90 vs. 39.06 g/kg of dry matter). This was due to lower inclusion of grains (e.g. barley, corn) in starters containing canola meal as compared to starter containing soybean meal: abundant sources of this amino acid.
One calf from CM treatment died before weaning. As a result, data presented correspond to 6 calves from this treatment. Furthermore, two other calves, one from SM treatment and the second from SM-MSB treatment suffered from bloat shortly before the conclusion of the experiment and their data have been eliminated from the post-weaning analysis.

Soybean meal use in the starter mixture resulted in greater ($P = 0.01$) starter mixture dry matter intake pre-weaning whereas microencapsulated sodium butyrate inclusion tended ($P = 0.06$) to increase starter mixture dry matter intake pre-weaning; however, average daily gain of calves was not affected by protein source or microencapsulated sodium butyrate inclusion, with exception of a tendency ($P = 0.10$) for greater average daily gain at weaning for calves fed starter with soybean meal (Table 14). At weaning, and in the whole study period, fecal score was lower for calves fed starters containing canola meal ($P \leq 0.03$). The same was true for the post-weaning period with exception that microencapsulated sodium butyrate inclusion in starter containing canola meal decreased fecal score (protein source × microencapsulated butyrate inclusion interaction, $P = 0.04$). The number of days with diarrhea (fecal score ≤ 3) was not different between treatments.

Fiber (NDF and ADF) digestibility for many calves was negative, indicating that bedding consumption (wood shavings) was substantial. Although it was expected that calves could consume some bedding, we decided to use it in this study. Our experience from previous studies showed that it allows better managing health problems of calves, particularly diarrhea, which could have greater impact on the accuracy of the results of the study than bedding intake itself. Furthermore, current study was conducted during winter season. As a result, bedding was justified from animal welfare point of view. Nevertheless, after removing two calves with extremely low dry matter digestibility coefficients, dry matter digestibility tended ($P = 0.08$; Table 14) to be lower for calves fed starter mixture with canola meal compared to calves fed starter mixture with soybean meal, suggesting that canola meal use in pelleted starter mixture may limit nutrient digestibility, apparently due to greater fiber content in the starters containing canola meal. On the other hand, microencapsulated butyrate use in starter mixture did not affect

nutrient digestibility, which was against our hypothesis. However, digestibility results should be interpreted with caution due to the confounding effect of bedding consumption.

Plasma urea tended ($P = 0.08$, day 21 of study) to be greater for calves fed starter with canola meal pre-weaning but lower ($P = 0.06$) post-weaning (day 63 of study; Table 15). On the other hand, plasma urea was lower at weaning (day 42 of study; $P = 0.02$) and tended ($P = 0.06$) to be lower after weaning for calves fed starters with microencapsulated sodium butyrate. Serum insulin post-weaning tended ($P = 0.06$) to be greater for calves fed starter mixture with soybean meal, most likely due to greater starch intake.

Many differences in plasma amino acids concentrations were found between treatments (Table 16). Of the most important, plasma glutamine concentration was greater for calves fed starters containing soybean meal ($P < 0.01$) but glutamate concentration tended ($P = 0.07$) to be greater for calves fed starters containing canola meal. Furthermore, plasma methionine concentration tended ($P = 0.07$) to be greater for calves fed starter mixture containing canola meal.

Lower plasma urea for calves fed starter mixture with canola meal post weaning corresponded with lower ($P = 0.08$) rumen ammonia but rumen ammonia was not affected by microencapsulated butyrate inclusion in starter mixture (Table 17). Ruminal pH and SCFA concentration in the rumen did not differ between treatments, with exception to greater valerate concentration ($P = 0.02$) in calves fed starter mixture with soybean meal compared to starter mixture with canola meal.

Neither protein source nor microencapsulated butyrate inclusion in the starter mixture affected reticulo-rumen tissue and digesta mass (Table 18). On the other hand, abomasal tissue mass tended ($P = 0.06$) to, jejunal tissue mass was ($P = 0.05$), and jejunal length tended ($P = 0.07$) to be greater for calves fed starter mixture with canola meal compared to calves fed starter mixture with soybean meal. Microencapsulated sodium butyrate did not affect gastrointestinal tract measurements, with exception to lower omasal digesta mass when this feed additive was included in the starter mixture ($P < 0.01$).

Ruminal papillae length in the caudal blind sac of the rumen varied greatly between calves. This made it impossible to measure papillae length, width, and also surface area in this location of the rumen using one technique. On the other hand, as opposed to what was expected, microencapsulated butyrate inclusion in the starter mixture resulted in lower mucosal surface area in the ventral sac of the rumen, particularly when combined with canola meal (protein source × microencapsulated butyrate inclusion interaction, $P = 0.05$; Table 19). Intestinal villi were found to be damage for many of collected samples and thus this analysis was omitted. Due to shown impact of protein source on abomasal tissue mass and importance of this region of the gastrointestinal tract for protein digestion in calves, more focus was placed on the development of abomasum in this study, both at histological and molecular level. Abomasal epithelium thickness was greater for SM-MSB and CM compared to SM and CM-MSB (interaction between main effects, $P = 0.04$).

Brush border enzyme activity was not affected by protein source in starter mixture but aminopeptidase A tended ($P = 0.06$) to be increased in duodenum and was increased ($P = 0.01$) in ileum and aminopeptidase N tended ($P = 0.07$) to be increased in ileum by microencapsulated sodium butyrate use in starter mixture (Table 20). In the proximal

jejunum, lactase activity was increased by microencapsulated sodium butyrate use in starter when combined with soybean meal but not canola meal (protein source × microencapsulated butyrate inclusion interaction, $P = 0.01$).

Gene expression data is presented in Table 21. In cranial sac of the rumen, MCT1 mRNA expression was greater for MSB treatments ($P < 0.01$) and UT-B greater for CM treatments ($P = 0.04$). In the proximal jejunum, MCT4 expression was greater for treatments without MSB supplementation ($P = 0.05$) and PEPT2 expression greater for SM treatments ($P = 0.03$). In ileum, SM treatments resulted in higher expression of ATB0 ($P = 0.04$) and tendency to greater expression of GPR41 ($P = 0.10$). There were no differences observed for the reminder of the analysed genes.

Altogether, results of this study suggest that canola meal use in calf starter mixture may decrease solid feed intake pre-weaning and compromise body weight gain at weaning. Inclusion of microencapsulate butyrate use in starter mixture may promote starter intake pre-weaning. Furthermore, lower mucosa surface area in the rumen, greater digesta mass in the omasum, lower plasma urea, and greater brush border peptidases activities for calves fed starters with microencapsulated sodium butyrate, without compromising average daily gain and feed intake, suggest that microencapsulated butyrate may stimulate post-ruminal nutrient digestion.

PART B. Performance study

Nutrient composition for starters is presented in Table 22. Neither starter intake, nor average daily gain was affected by protein source and microencapsulated butyrate and also feed efficiency was not different between treatments post-weaning (Table 23). There was a tendency ($P = 0.06$) for greater fecal score for calves fed soybean meal in starter mixture (that was opposite to what observed in PART A) and number of days with diarrhea was the lowest for CM-MSB treatment (protein source × microencapsulated butyrate inclusion interaction, $P = 0.05$).

Results of this study suggest that canola meal can be acceptable source of protein in pelleted starter mixtures for calves and microencapsulated sodium butyrate inclusion in the starter mixture may decrease number of days with diarrhea during rearing period.

Study 4. Effect of canola meal use as a protein source in a starter mixture on feeding behavior and performance of calves during the weaning transition

Introduction

Canola meal (CM) is commonly discouraged as a protein source in starter mixtures (SM) for calves. This is mainly a result of concerns with low palatability and digestibility of CM because of presence of unpalatable compounds (tannins, phenolic acids), antinutritional factors (erucic acid, glucosinolate, trypsin inhibitor, phytates), and relatively high fiber content in CM (Fiems et al., 1985; Khorasani et al., 1990). Even though these concerns were not confirmed in all studies (Fisher, 1980; Claypool et al., 1985), some showed lower feed intake and nutrient digestibility when CM was used as a protein source in calf starters (Fiems et al., 1985; Khorasani et al., 1990), at least when compared with soybean meal (SBM). However, the negative effect of CM on feed efficiency in calves may not only be a result of its chemical constituents, but also its

effect on feeding behavior of calves. It has been shown, for example, that corn grain endosperm type and some feed additives used in diets for cattle affect feeding time, frequency, and rate (Lunn et al., 2005; Taylor and Allen, 2005; DeVries and Chevaux, 2014), which itself may affect the rumen environment and in consequence, feed efficiency (Allen, 1997). In reference to calf nutrition, the use of dried distillers grains with solubles in SM increased SM intake rate in the first weeks of life, which was associated with lower rumen pH (Laarman et al., 2012). The effect of SM composition on feeding behavior of calves, and in consequence efficiency of feed digestion and utilization, may be especially apparent during weaning transition, a period when SM intake increases rapidly (Quigley et al., 2006; Laarman et al., 2012). We hypothesized that CM use in SM would affect the feeding behavior of calves during the weaning transition and in consequence, reduce their performance.

The aim of this study was to determine the effect of CM and its inclusion rate in SM on frequency (no./d), time (min/d), and rate (g/min) of eating SM as well as frequency and time of drinking water during the weaning transition period.

Materials and methods

Experimental procedures were reviewed and approved by the Local Ethical Committee (Krakow, Poland) before onset of the study. A total of 36 Holstein female calves with a mean age of 14.9 ± 1.6 d and BW of 40.1 ± 4.2 kg (mean ± SD) were used for the study. Calves were collected from 3 dairy barns belonging to one operator Top Farms Głubczyce Sp. z o.o., Głubczyce, Poland), once per week (Monday), and transported 3 to 15 km to a naturally ventilated calf barn.

Before initiating the study, calves followed the routine procedure for newborn calves adopted at each farm. This included immediate separation from the dam and feeding 3 L of colostrum within the first 3 h of life. Colostrum feeding was continued for the first 2 d of life, and thereafter transition milk was offered, followed by milk replacer (MR; Polmass Milk, Polmass S.A., Bydgoszcz, Poland) feeding beginning on d 5 of life. Liquid feeds were offered in an amount equal to 5 L/d. During that period of life calves were kept in individual hutches bedded with straw and no hay or SM was offered.

The required number of calves for the study was collected over a period of 4 wk, resulting in 4 blocks of 9, 9, 15, and 3 calves. Upon arrival to the calf barn, calves were weighed, treated with a broad-spectrum antibiotic (Zactran, Merial, Lyon, France), placed in individual pens, and allocated to 1 of 3 experimental treatments (12 calves per treatment) differing in the main source of protein comprising the pelleted SM. Allocation to treatments accounted for place of birth (original farm) and initial BW.

The treatments were (1) SBM as the main source of protein in the SM (TSBM); (2) SBM and CM as main sources of protein in the SM (TSBM/ TCM); and (3) CM as the main source of protein in the SM (TCM). Starter mixtures were formulated to be similar for CP content. For the TSBM/TCM, the same amount of CP was provided with SBM and CM. Because of lower content of CP in CM, as compared with SBM, CM was included into SM in expense of barley grain. Detailed ingredient and chemical composition of SM is presented in Table 24.

Calves were fed 450 g of MR (as fed) twice a day (0700 and 1400 h) from d 1 to 35 of the study and once a day (0700 h) from d 36 to 42 of the study, and were completely weaned on d 43 of the study (57.9 ± 1.6 d of age; mean ± SD). The MR

(Polmass Milk, Polmass S.A.) was reconstituted at the rate of 150 g of MR powder in 1 L of water and offered from buckets in amount equal to 3 L per feeding. The SM was fed once a day (0900 h). Initially calves were offered 500 g of SM, and this amount was increased by an additional 500 g each time when less than 200 g of refusals remained. Milk replacer and SM intake was monitored daily. Calves were weighted weekly after the morning feeding (1100 h). Fecal fluidity (4-point scale: 1 = normal; 4 = diarrhea) was determined daily according to Larson et al. (1977), and every abnormal health condition and veterinary treatment was documented. Day with diarrhea was defined when fecal fluidity was ≤ 3.

During the whole study, calves were kept in 1.5 × 1.2 m individual pens bedded with straw. Each calf was in the study for 56 d. Calves from the first and second block (6 calves per treatment) were continuously recorded during weaning transition on d 34 to 35 (before MR step-down), 41 to 42 (at MR step-down), and 48 to 49 (after weaning) of the study using a digital video recorder (model BCS-0404LE-AN, Dahua Technology Co., Hangzhou, China) equipped with 4 high-resolution color day/night video cameras (EVA-TV-1200iRW, KAM-TECH, Krakow, Poland; angle lens 2.8–12 mm). Cameras were placed over pens ensuring that the bucket for feed and water for each calf was clearly visible on the video. One camera was used for recording 4 to 5 calves. Video recordings were saved on hard disk (resolution 720 × 576 pixels) with speed of 6 frames per second and watched by one person at the time of analysis. Frequency of eating SM and drinking water (no./d) and time of eating SM and drinking water (min/d) for each calf was recorded. Start of eating SM and drinking water was considered when calf put head into the bucked, and stop of eating SM and drinking water was considered when no interest with the bucked was observed for 10 s. Feeding rate of SM (g/min) was calculated by dividing SM intake (g/d) by SM eating time (min/d).

Representative samples of feeds were collected weekly and composited by month of the study. Monthly samples were than analyzed for DM, ash, CP, and ether extract content using standard analytical procedures (procedures No. 934.01, 942.05, 976.05, 2003.05 for DM, ash, CP, and ether extract, respectively; AOAC International, 2000), NDF (Van Soest et al., 1991), and ADL (Robertson and Van Soest, 1981).

Statistical Analysis

Data were analyzed with a completely randomized block design using the MIXED procedure of SAS (ver. 9.2, SAS Institute Inc., Cary, NC). Effect of treatment was included in the statistical model as a fixed effect, whereas block was used as a random term. The statistical model for repeated variables included the effect of time (day or week) and the interaction between the effect of time and treatment as fixed effects (Littell et al., 1998). Optimal covariance structure (autoregressive order one, unstructured or compound symmetry) was chosen based on Akaike's criterion. For all analyzed parameters, initial age was used as a covariate. Preplanned contrasts were used for scientific hypothesis verification (TSBM vs. TSBM/TCM and TSBM vs. TCM). Significance was declared at $P \leq 0.05$ and tendencies at $P \leq 0.10$. Data are presented as least squares means and the corresponding standard error of the mean.

Results and Discussion

One calf was removed from statistical analysis because of extremely high SM

intake and ADG and one because of extremely low ADG and poor health status, resulting in 12, 11, and 11 calves analyzed for TSBM, TSBM/TCM, and TCM, respectively. Although the SM were formulated to be similar for CP content, the SM offered to TSBM calves had slightly greater CP concentration as compared with the SM offered to TSBM/TCM and TCM calves (Table 24). Starter mixture offered to TSBM/TCM and TCM contained more NDF, ADF, and fat. The TCM calves had the least ADG from d 1 to 35 of the study ($P = 0.02$) and tended to have reduced ADG for the whole study ($P = 0.08$) as compared with TSBM calves (Table 25). Furthermore, TCM calves had the least feed efficiency, higher fecal fluidity, and greater number of days with diarrhea from d 1 to 35 of the study ($P \leq 0.03$) as well as the least feed efficiency and greater number of days with diarrhea for whole study period ($P \leq 0.03$) as compared with TSBM calves.

The ADG and feed efficiency did not differ between TSBM and TSBM/TCM calves. From d 36 to 56 of the study, fecal fluidity was higher ($P = 0.04$) for TSBM calves as compared with TCM calves and tended to ($P = 0.08$) be higher for TSBM calves as compared with TSBM/TCM calves; however, number of days with diarrhea was not different. Treatments did not differ in SM intake with the exception of a tendency ($P = 0.10$) for higher SM intake from d 1 to 35 of the study for TSBM calves as compared with TSBM/TCM calves. Frequency, time, and rate of eating the SM increased during weaning transition ($P < 0.05$; Figure 4). Simultaneously, frequency and time of drinking water increased from the period before MR step-down to weaning ($P < 0.05$; data not presented). No differences in feeding and drinking behavior were shown between treatments, with an exception for a tendency for more frequent water intake in TSBM calves (Table 26). However, it is worth noting that a tendency for a group × time interaction ($P = 0.09$) for SM eating time was observed, because of longer SM eating time on d 34 to 35 and shorter SM eating time on d 41 to 42 of the study for TSBM/TCM and TCM calves, as compared with TSBM calves.

This study confirmed a negative effect of CM use in SM on ADG and feed efficiency before weaning (Fiems et al., 1985; Khorasani et al., 1990), as well as showed a negative effect of CM on health status of calves, as indicated by higher fecal score and greater number of days with diarrhea. However, several observations arising from this study are worth discussion. First, performance of calves was negatively affected by CM use in the SM from d 1 to 35 of the study, whereas no differences in ADG, feed efficiency, or health status of calves were observed from d 36 to 56, a period covering the weaning transition. Because relatively old calves were used for this study (14.9 ± 1.6 d of age), an even more pronounced negative effect of CM use in SM on performance of the youngest calves could have been expected, based on known high susceptibility of newborn calves to presence in the diet of some anti-nutritional factors (Drackley, 2008). Second, SM intake was not different between TSBM and TCM calves in this study, suggesting that low palatability of CM is not an important factor limiting efficiency of its use for newborn calves, as suggested by the results of other studies (Fiems et al., 1985). However, the SM used in this study contained glycerin and molasses that may help mask the sensory attributes of CM. Third, up to 50% replacement of SBM with CM in SM had no negative effect on ADG of calves, feed efficiency, and number of days with diarrhea in this study, indicating that partial replacement of SBM with CM in SM for calves may be acceptable in practical conditions.

Because even small changes in chemical composition of the diet may affect

feeding behavior of cattle (Taylor and Allen, 2005), and this may itself affect the rumen environment and feed efficiency (Allen, 1997), we hypothesized that a negative effect of CM on the performance of calves may be at least partially a result of its effect on feeding behavior. This hypothesis is also supported by low palatability of CM (Fiems et al., 1985; Khorasani et al., 1990), which may have a substantial effect on feeding behavior of calves. Furthermore, because SM intake increases rapidly at weaning (Quigley et al., 2006; Laarman et al., 2012), the effect of CM use in a SM on the feeding behavior and performance of calves could be especially apparent during weaning transition. Results of this study did not confirm our hypothesis, although a tendency for a group × time interaction ($P = 0.09$) for SM eating time was observed, as a result of longer SM eating time on d 34 to 35 and shorter SM eating time on d 41 to 42 of study for TSBM/TCM and TCM calves, as compared with TSBM calves. However, feeding behavior was not analyzed during first 4 wk of the study (d 1 to 28); the time point where a negative effect of CM use in the SM on performance of calves was especially apparent. Nevertheless, SM intake in first 4 wk of the study was low (≈200 g/d), and thus, it can be speculated that the potential effect of CM on feeding behavior of calves that occurred during that period of study, if it occurred, was rather of minor importance for feed efficiency.

We conclude that the inclusion of CM in a SM does not affect feeding behavior and performance of calves during the weaning transition. However, CM inclusion as a full replacement for SBM in SM has a negative effect on ADG, feed efficiency, and fecal score of calves during the pre-weaning period.

CONCLUSIONS AND RECOMMENDATIONS

Our data clearly show that canola meal use in starter mixtures can be increased without compromising starter intake or gain. However, complete replacement of soybean meal is not recommended and attention must be made to the quality of the canola meal to ensure heated canola meal is not incorporated. At this point, we cannot determine the optimal inclusion rate; however, replacing 50% of the soybean meal had no adverse effects. In addition, the use of glycerol in starter mixtures appears to improve starter intake and may enhance ruminal fermentation and intestinal development. Moreover, microencapsulated sodium butyrate positively affects starter intake, average daily gain, and intestinal development.

Limitations of the Current Research

However, the main limitation of the current research is that a dose-titration study was never planned. Thus, while our data is promising in terms of increasing canola meal use for dairy calves prior to, at, and following weaning, we are not able to provide recommendations on the optimal level of inclusion. To address this gap, we have secured funding from the Saskatchewan Canola Development Commission to evaluate canola meal inclusion using a dose-titration.

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OUTPUTS ARISING FROM THIS BODY OF WORK

Training of Highly Qualified Personnel

- 1 PhD student – Kasia Burakowska (U of S)
- 1 M.Sc. student – Dominika Hadam (Poland)
- Technician – Gillian Gratton (U of S)

Peer-Reviewed Publications

Hadam, D., J. Kański, K. Burakowska, G.B. Penner, Z. Kowalski, and P. Gorka. 2015. Effect of canola meal use as a protein source in a starter mixture on feeding behavior and performance of calves during the weaning transition. J. Dairy Sci. 99:1247-1252.

Abstracts Presented at Scientific Conferences

Burakowska, K., P. Górka, Z. M. Kowalski, B. Laarveld, and G.B. Penner. 2016. Effect of canola meal heat treatment and glicerol inclusion in calf starter on GIT development. 5th EAAP International Symposium on Energy and Protein Metabolism and Nutrition. Krakow, Poland. September 12-15th.

K. Burakowska, M. Przybylo, G. Penner, P. Górka. 2017. Evaluating the effect of protein source and micro-encapsulated sodium butyrate in starter mixtures on gastrointestinal tract development of dairy calves. 2017 American Dairy Science Association Annual Meeting. Pittsburg, PA. June 25-28th.

Industry Presentations

Burakowska, K., P. Górka, Z. M. Kowalski, B. Laarveld, and G.B. Penner. 2016. Effect of canola meal heat treatment and glycerol inclusion in a pelleted starter for Holstein calves. Western Canadian Dairy Seminar. March 8-11th, Red Deer, AB.

K. Burakowska, G.B. Penner, and P. Gorka. 2017. Use of canola meal and micro-encapsulated sodium butyrate in starter feed for dairy calves. Western Canadian Dairy Seminar, March 7-10th, Red Deer, AB.

Industry Publications

None.

Other Media Attention

None.

APPENDICIES

Table 1. Average heat up and cool down times

Heat treatment temperature (°C)	Average heat up time (min)	SEM	Average cool down time (min)	SEM
100	26	2.1	21	2.3
110	43	4.4	24	4.1
120	67	6.7	30	0.3

Table 2. DM and CP *in situ* degradation parameters and CP intestinal digestibility.

Item	Treatment[1]					P value	
	Control	100	110	120	SEM	Linear	Quadratic
In situ DM degradation							
Kd, %/h	4.5	4.2	3.14	3.06	0.214	<0.01	0.02
Soluble fraction	25.9	21.9	19.6	18.8	0.504	<0.01	0.02
Degradable fraction	60.0	57.1	52.7	34.1	1.906	<0.01	<0.01
Undegradable fraction	14.1	21	27.7	47	2.100	<0.01	<0.01
EDDM	54.2	46.8	39.1	31.7	0.776	<0.01	<0.01
In situ CP degradation							
Kd, %/h	4.89	4.59	3.54	7.77	0.590	0.28	<0.01
Soluble fraction	16.4	11.8	8.6	12.1	2.917	0.14	0.73
Degradable fraction	79.0	70.0	59.0	22.3	3.531	<0.01	<0.01
Undegradable fraction	4.6	18.2	32.3	65.7	3.909	<0.01	<0.01
EDCP	55.5	45.3	32.7	25.4	2.652	<0.01	<0.01
Intestinal digestibility[2], %	45.9	46.1	51.0	37.2	2.550	0.48	0.03

[1]Canola meal was heated to either 100, 110 or 120 °C. The duration of time required to achieve 100, 110, and 120 were 26 (±3), 43 (±7) and 67 (±11) min respectively.

[2]Estimated intestinal digestibility was based on a 3 step procedure described by Calsamiglia and Stern (1995).

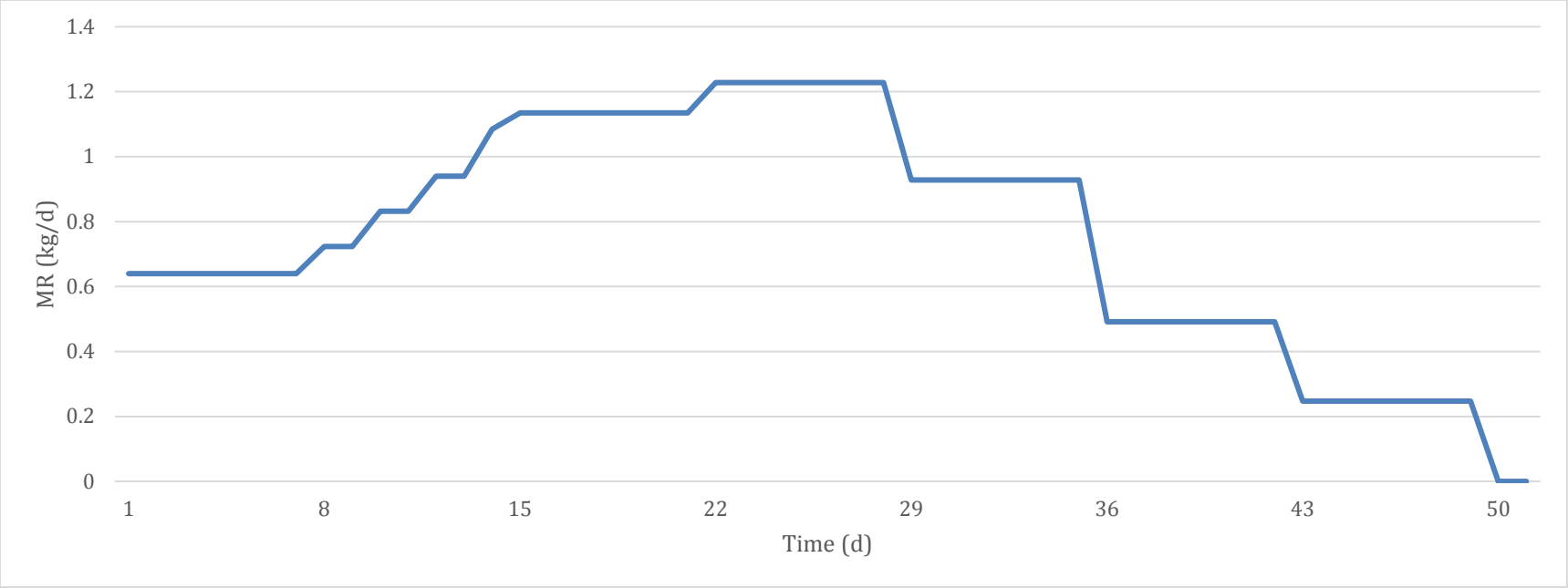

Figure 1. Approximate provision of milk replacer (kg DM/day) adjusted weekly with indicated levels of amount of milk replacer fed as percentage of BW.

Table 3. Composition of designed starter feeds, containing either non-heated or heated canola meal, with or without glycerol addition.

	Non-heated canola meal		Heated canola meal	
Ingredient, % DM	No glycerol	With glycerol	No glycerol	With glycerol
Non-heated canola meal	35	35	-	-
Heated canola meal	-	-	35	35
Barley	33	28	33	28
Corn Grain	30	30	30	30
Corn syrup	2	2	2	2
Glycerol	-	5	-	5
Chemical composition, %DM[1]				
DM	91.6	93.1	91.6	93.1
CP	19.6	19.6	19.6	19.6
NFC	50.0	51.2	50.0	51.2
ME (Mcal/kg)	2.73	2.75	2.73	2.75

[1]Values approximated from ingredient composition (NRC, 2001).

Table 4. Chemical composition of starter feeds and milk replacer used in Study 2 (on DM basis).

Variable (%DM)	Non-heated canola meal		Heated canola meal[1]		Milk replacer
	No glycerol	With glycerol	No glycerol	With glycerol	
Dry matter	96.7	96.7	96.7	96.8	94.6
Crude protein	20.4	20.6	21.2	21.2	27.3
ADF	10.2	10.1	10.3	10.7	n/a
NDF	16.1	17.1	17.0	17.0	n/a
Starch	39.2	35.9	38.1	33.9	2.6
Crude fat	3.0	3.0	3.2	3.0	17.7
Ash	8.4	8.1	8.1	8.5	7.6
Ca	1.3	1.2	1.2	1.3	1.1
P	1.0	0.9	1.0	1.0	0.9

[1]Canola meal was heated to 110°C in tumble dryer and held at the temperature for 10 min.

Table 5. Effects of heat treatment of canola meal and glycerol supplementation in intake and growth parameters.

Variable	Canola meal		Glycerol		SEM	P value		
	Heat[1]	No-Heat	With	Without		CM	GL	CM*GL
Starter intake (kg/d)[2]	0.36	0.36	0.36	0.36	0.028	0.39	0.59	0.80
MR intake (kg/d)[2]	0.72	0.72	0.72	0.72	0.013	0.22	0.21	0.47
Body weight (kg)	54.7	55.3	55.6	54.3	1.22	0.75	0.47	0.69
ADG (kg/d)	0.47	0.57	0.56	0.48	0.042	0.07	0.09	0.66

Note: Intake data were recorded daily and body weight was measured weekly.

[1]Canola meal was heated to 110°C in tumble dryer and held at the temperature for 10 min.

[2]Intakes are presented as average daily intakes on DM basis.

Table 6. Effect of heat treatment of canola meal and glycerol supplementation on short chain fatty acids (SCFA) and ammonia concentrations in rumen fluid of calves on day 51.

Variable	HEAT[1]		GLYCEROL[2]		SEM	*P*-value		
	NO	YES	NO	YES		HEAT	GLY	H×G
Rumen pH	5.14	5.22	5.31	5.05	0.10	0.47	0.04	0.07
Total SCFA (µmol/mL)	139.9	129.9	124.7	145	8.31	0.16	0.008	0.92
Acetic acid (%)	54.1	51.4	54.7	50.9	3.28	0.50	0.34	0.075
Propionic acid (%)	29.7	27.2	25.9	31.1	3.24	0.51	0.18	0.069
Iso-buyric acid (%)	0.219	0.278	0.313	0.184	0.056	0.43	0.092	0.92
Butyric acid (%)	11.2	16.5	14.7	13.1	2.23	0.100	0.62	0.70
Iso-valeric (%)	0.214	0.265	0.302	0.176	0.060	0.45	0.076	0.85
Valeric acid (%)	3.62	3.64	3.27	4	0.281	0.96	0.083	0.041
Caproic acid (%)	0.863	0.62	0.894	0.589	0.242	0.36	0.25	0.45
Ammonia (µg/mL)	26.3	28.6	27.6	27.3	4.23	0.61	0.96	0.60

[1]Canola meal was heated to 110°C in tumble dryer and held at the temperature for 10 min; [2] - Glycerol was included at 5% DM in the starter feed.

Table 7. Effects of heat treatment of canola meal and glycerol supplementation on tissue and digesta weights of the gastrointestinal tract, liver, and spleen

Variable		HEAT[1]		GLYCEROL[2]		SEM	*P*-value		
		NO	YES	NO	YES		HEAT	GLY	H×G
Rumen	Tissue (kg)	1.21	0.96	1.02	1.15	0.09	0.011	0.18	0.78
	Digesta (kg)	4.10	2.82	3.53	3.39	0.22	<0.001	0.61	0.12
Omasum	Tissue (kg)	0.206	0.213	0.192	0.227	0.019	0.77	0.14	0.42
	Digesta (kg)	0.110	0.120	0.105	0.125	0.022	0.70	0.47	0.93
Abomasum	Tissue (kg)	0.352	0.349	0.343	0.358	0.014	0.86	0.41	0.87
	Digesta (kg)	0.542	0.480	0.431	0.591	0.065	0.48	0.080	0.11
Duodenum	Tissue (kg)	0.087	0.080	0.080	0.087	0.004	0.29	0.25	0.79
	Digesta (kg)	0.019	0.017	0.016	0.021	0.002	0.38	0.058	0.12
	Length (m)	0.654	0.612	0.620	0.645	0.020	0.15	0.38	0.52
Jejunum	Tissue (kg)	1.44	1.21	1.24	1.41	0.07	0.024	0.079	0.68
	Digesta (kg)	1.027	0.880	0.772	1.135	0.078	0.14	0.001	0.17
	Length (m)	18.2	16.9	17.0	18.0	0.56	0.075	0.18	0.16
Ileum	Tissue (kg)	0.177	0.151	0.150	0.177	0.016	0.25	0.24	0.79
	Digesta (kg)	0.059	0.024	0.038	0.046	0.008	0.010	0.52	0.88
	Length (m)	1.01	0.89	0.93	0.97	0.096	0.23	0.69	0.50
Cecum	Tissue (kg)	0.077	0.071	0.073	0.075	0.005	0.38	0.82	0.26
	Digesta (kg)	0.258	0.196	0.262	0.192	0.025	0.018	0.009	0.69

	Length (m)	0.289	0.275	0.283	0.281	0.013	0.42	0.95	0.26
Colon	Tissue (kg)	0.357	0.338	0.328	0.366	0.018	0.33	0.072	0.099
	Digesta (kg)	0.378	0.297	0.299	0.376	0.042	0.13	0.14	0.73
	Length (m)	2.69	2.47	2.59	2.57	0.08	0.010	0.78	0.038
Liver	Tissue (kg)	1.41	1.30	1.30	1.41	0.045	0.024	0.030	0.45
Spleen	Tissue (kg)	0.229	0.199	0.201	0.228	0.016	0.092	0.13	0.40

Table 8. Effect of heat treatment of canola meal and glycerol supplementation on non-estrified fatty acids (NEFA), urea, glucose and insulin in plasma.

Variable	D of age	HEAT[1]		GLYCEROL[2]		SEM	*P*-value		
		NO	YES	NO	YES		HEAT	GLY	H×G
NEFA	22	146.0	137.7	144.4	139.3	6.16	0.35	0.56	0.74
(mEq/L)	43	173.0	143.6	171.9	144.7	14.08	0.072	0.092	0.11
	51	175.9	156.8	174.3	158.4	14.54	0.36	0.45	0.23
Urea	22	16.8	18.2	18.0	17.1	0.87	0.26	0.47	0.15
(mg/dL)	43	17.9	19.0	18.5	18.3	1.63	0.44	0.87	0.066
	51	19.3	22.5	20.9	20.8	1.15	0.028	0.95	0.26
Glucose	22	93.5	89.0	93.0	89.5	4.35	0.34	0.45	0.83
(mg/dL)	43	89.9	89.9	93.6	86.3	3.74	0.99	0.076	0.35
	51	68.2	63.3	65.6	66.0	2.64	0.18	0.91	0.41
Insulin	22	0.567	0.488	0.688a	0.367b	0.086	0.51	0.016	0.36
(µg/L)	43	0.316	0.397	0.480a	0.232b	0.088	0.27	0.003	0.70
	51	0.118	0.116	0.118	0.116	0.007	0.89	0.89	0.099
BHBA	22	0.450	0.373	0.390	0.433	0.045	0.096	0.33	0.26
(mmol/L)	43	0.382	0.469	0.415	0.437	0.027	0.022	0.53	0.72
	51	0.477	0.532	0.551	0.458	0.036	0.26	0.061	0.18

[1]Canola meal was heated to 110°C in tumble dryer and held at the temperature for 10 min; [2] - Glycerol was included at 5% DM in the starter feed.

Table 9. Brush border enzyme activity in different regions of the calf small intestine.

Brush border enzyme		HEAT[1]		GLYCEROL[2]		SEM	*P*-value		
		NO	YES	NO	YES		HEAT	GLY	H×G
Aminopeptidase A	Duodenum	2.65	2.41	2.62	2.44	0.204	0.42	0.54	0.19
	Middle jejunum	35.8	29.7	30.0	35.5	4.93	0.40	0.44	0.52
	Ileum	26.9	23.12	26.3	23.8	3.55	0.38	0.55	0.47
Aminopeptidase N	Duodenum	9.66	9.09	8.97	9.79	1.618	0.8	0.72	0.47
	Middle jejunum	15.5	21.9	20.1	17.4	3.41	0.18	0.57	0.91
	Ileum	26.3	23.3	28.0	21.7	3.55	0.55	0.22	0.27
Dipeptidase	Middle jejunum	4.34	3.87	3.47	4.74	0.452	0.47	0.059	0.3
	Ileum	5.09	4.97	5.61	4.45	0.654	0.89	0.18	0.94
Lactase	Duodenum	114.2	105.5	109.7	110.0	12.48	0.52	0.98	0.22
	Proximal jejunum	136.2	116.1	132.0	120.3	15.61	0.32	0.56	0.81
	Middle jejunum	63.9	70.3	63.0	71.2	8.81	0.54	0.43	0.98
	Distal jejunum	21.1	20.0	20.2	20.9	1.29	0.47	0.65	0.8
	Ileum	18.7	19.6	19.6	18.7	0.86	0.35	0.45	0.85
Maltase	Duodenum	21.5	20.3	20.1	21.7	1.58	0.35	0.21	0.18
	Proximal jejunum	29.9	30.2	33.0	27.2	3.70	0.94	0.19	0.71
	Middle jejunum	30.4	27.9	27.5	30.8	2.85	0.43	0.29	0.61
	Distal jejunum	35.5	37.9	35.7	37.8	4.55	0.61	0.66	0.34
	Ileum	19.6	19.4	19.5	19.5	2.18	0.92	0.98	0.37

[1]Canola meal was heated to 110°C in tumble dryer and held at the temperature for 10 min; [2] - Glycerol was included at 5% DM in the starter feed.

Table 10. Relative expression (fold change) of genes of interest in gastrointestinal tract of calves.

Gene of interest[1]		HEAT		GLYCEROL		SEM	*P* value		
		NO	YES	NO	YES		HEAT	GLY	H×G
CRA	MCT1	0.99	0.93	1.01	0.91	0.062	0.45	0.17	0.012
	MCT4	1.02	1.00	1.08	0.94	0.068	0.81	0.060	0.070
	UT-B	1.01	0.96	0.99	0.98	0.092	0.59	0.89	0.73
	AQP3	1.12	1.05	1.02	1.15	0.109	0.51	0.25	0.022
PROX	MCT1	1.22	1.06	0.95	1.33	0.111	0.24	0.012	0.93
	MCT4	1.17	1.07	0.98	1.25	0.085	0.40	0.030	0.84
	PEPT1	0.94	0.85	0.97	0.81	0.129	0.63	0.40	0.14
	PEPT2	1.05	1.09	1.08	1.06	0.148	0.80	0.90	0.68
	EAAC1	0.99	0.77	0.97	0.79	0.162	0.32	0.41	0.27
	ATB0	1.14	1.24	1.22	1.16	0.145	0.52	0.72	0.16
ILE	MCT1	1.05	0.96	1.05	0.96	0.121	0.19	0.21	0.37
	MCT4	0.92	0.89	0.92	0.89	0.031	0.42	0.47	0.69
	PEPT1	1.02	1.00	0.95	1.07	0.120	0.90	0.50	0.44
	PEPT2	1.40	1.28	1.26	1.42	0.194	0.50	0.35	0.23
	EAAC1	0.86	1.07	0.81	1.13	0.124	0.25	0.085	0.034
	ATB0	1.12	1.17	1.14	1.16	0.174	0.83	0.93	0.13

[1]CRA – cranial sac of the rumen, PROX – proximal jejunum, ILE – ileum.
[2]Canola meal was heated to 110°C in tumble dryer and held at the temperature for 10 min.
[3]Glycerol was included at 5% DM in the starter feed.

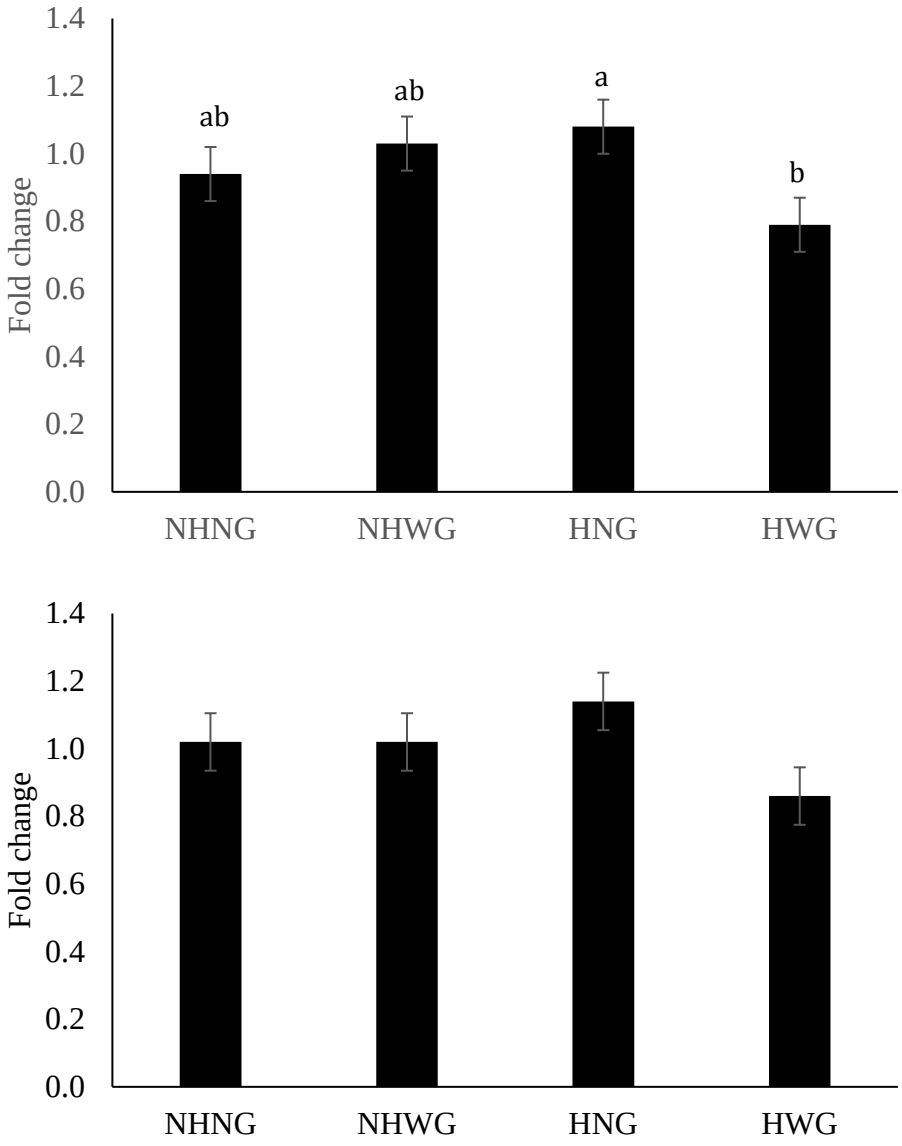

Figure 2. Interaction among heat treatment of canola meal and inclusion of glycerol on the expression of MCT1 (P = 0.012; top) and MCT4 (P = 0.07; bottom) in the rumen epithelium. Columns with different superscripts are different. NHNG = not-heated canola no glycerol, NHWG = not-heated canola with glycerol, HNG = heated canola meal no glycerol, HWG = heated canola meal with glycerol.

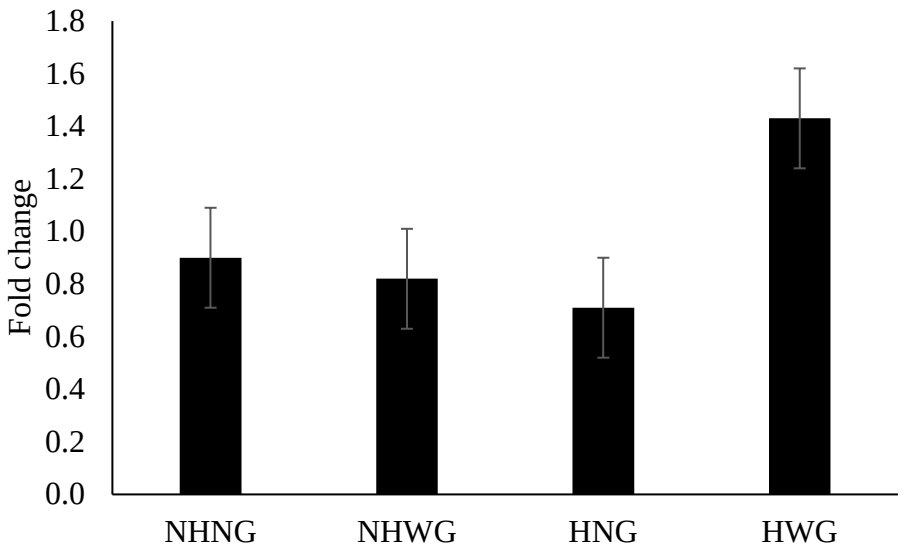

Figure 3. Interaction among heat treatment of canola meal and inclusion of glycerol on the expression of EAAC1 (P = 0.034) in the ileum. Mean separation did not identify columns that differed, although NHG and HWG tended (P = 0.06) to differ. NHNG = not-heated canola no glycerol, NHWG = not-heated canola with glycerol, HNG = heated canola meal no glycerol, HWG = heated canola meal with glycerol.

Table 11. Composition of starter mixtures for calves (% DM in the starter) – PART A

Component (% DM in starter)	Treatments[1]			
	SM	SM-MSB	CM	CM-MSB
Canola meal	-	-	35.2	35.2
Soybean Meal	24.2	24.2	-	-
Barley	28.9	28.6	18.9	18.6
Corn Grain	29.3	29.3	29.3	29.3
Wheat bran	4.8	4.8	4.8	4.8
Whey protein (dry)	2.7	2.7	2.7	2.7
Mineral-vitamin supplement	1.1	1.1	1.1	1.1
Glycerol	5	5	5	5
MSB[1]	-	0.3	-	0.3
Methionine	0.05	0.05	-	-
Monocalcium phosphate	1.1	1.1	1.1	1.1
Limestone	2.2	2.2	2.2	2.2
Salt	0.5	0.5	0.5	0.5
Chromium oxide	0.2	0.2	0.2	0.2

[1]MSB - microencapsulated sodium butyrate; SM - soybean meal without MSB; SM-MSB - soybean meal with MSB; CM - canola meal without MSB; CM - MSB - canola meal with MSB.

Table 12. Composition of starter mixtures for calves (% DM in the starter) – PART B

Component (% DM in starter)	Treatments[1]			
	SM	SM-MSB	CM	CM-MSB
Canola meal	-	-	35.2	35.2
Soybean meal	24.2	24.2	-	-
Barley	29.1	28.8	19.1	18.8
Corn grain	29.3	29.3	29.3	29.3
Wheat bran	4.8	4.8	4.8	4.8
Whey protein (dry)	2.7	2.7	2.7	2.7
Mineral-vitamin supplement	1.1	1.1	1.1	1.1
Glycerol	5	5	5	5
MSB[1]	-	0.3	-	0.3
Methionine	0.05	0.05	-	-
Monocalcium phosphate	1.1	1.1	1.1	1.1
Limestone	2.2	2.2	2.2	2.2
Salt	0.5	0.5	0.5	0.5

[1]MSB - microencapsulated sodium butyrate; SM - soybean meal without MSB; SM-MSB - soybean meal with MSB; CM - canola meal without MSB; CM-MSB - canola meal with MSB.

Table 13. Nutrient composition of starter mixtures and milk replacer – PART A

Variable	Treatment/Feed[1]				
	SM	SM-MSB	CM	CM-MSB	MR
Dry matter (DM) (%)	97.4 ± 0.2	97.4 ± 0.2	97.1 ± 0.3	96.7 ± 0.1	95.0 ± 0.2
Ash (% DM)	9.1 ± 0.2	9.1 ± 0.3	8.4 ± 0.2	8.3 ± 0.3	10.8 ± 0.4
OM (% DM)	90.9 ± 0.2	90.9 ± 0.3	91.6 ± 0.2	91.7 ± 0.3	89.2 ± 0.4
CP (% DM)	21.9 ± 0.4	21.7 ± 0.1	20.6 ± 0.5	20.2 ± 0.4	21.7 ± 0.2
Fat (% DM)	-	-	-	-	18.1 ± 0.3
NDF (% DM)	11.8 ± 0.3	14.5 ± 1.1	17.9 ± 0.6	17.7 ± 0.8	-
ADF DM)	6.1 ± 0.3	7.4 ± 0.2	10.2 ± 0.5	11.2 ± 0.4	-
Sugar (% DM)	7.8 ± 0.2	8.5 ± 0.2	8.7 ± 0.2	8.4 ± 0.2	-
Cr (g/kg DM)	1.2 ± 0.2	1.2 ± 0.2	1.4 ± 0.1	1.3 ± 0.0	-
Amino acid					
Asp (g/kg DM)	17.82 ± 0.26	18.72 ± 0.35	14.13 ± 0.09	13.21 ± 0.19	-
Thr (g/kg DM)	6.46 ± 0.09	6.91 ± 0.26	7.31 ± 0.06	6.84 ± 0.14	-
Ser (g/kg DM)	8.17 ± 0.04	9.00 ± 0.75	7.91 ± 0.09	7.58 ± 0.42	-
Glu+Gln (g/kg DM)	37.87 ± 1.08	40.24 ± 1.15	35.25 ± 0.82	32.56 ± 1.38	-
Pro (g/kg DM)	10.40 ± 0.22	11.27 ± 0.82	11.48 ± 0.64	11.65 ± 1.52	-
Gly (g/kg DM)	8.01 ± 0.23	8.58 ± 0.32	8.88 ± 0.28	8.54 ± 0.24	-
Ala (g/kg DM)	8.26 ± 0.16	8.78 ± 0.33	8.33 ± 0.20	7.99 ± 0.29	-
Val (g/kg DM)	9.54 ± 0.03	9.64 ± 0.58	9.81 ± 0.73	9.09 ± 0.63	-
Ile (g/kg DM)	7.54 ± 0.09	7.50 ± 0.25	6.69 ± 0.41	6.30 ± 0.19	-
Leu (g/kg DM)	15.01 ± 0.03	15.73 ± 0.42	14.24 ± 0.42	13.74 ± 0.33	-
Tyr (g/kg DM)	5.92 ± 0.05	5.91 ± 0.06	5.89 ± 0.03	5.80 ± 0.30	-
Phe (g/kg DM)	8.98 ± 0.24	9.49 ± 0.24	7.84 ± 0.16	7.50 ± 0.21	-
His (g/kg DM)	5.51 ± 0.12	5.89 ± 0.39	5.74 ± 0.21	5.54 ± 0.21	-
Lys (g/kg DM)	9.00 ± 0.11	9.44 ± 0.38	8.70 ± 0.26	8.50 ± 0.08	-
Arg (g/kg DM)	14.28 ± 0.28	14.98 ± 0.78	12.93 ± 0.28	12.26 ± 0.46	-
Cys (g/kg DM)	3.36 ± 0.14	3.54 ± 0.03	4.25 ± 0.15	4.43 ± 0.02	-
Met (g/kg DM)	3.87 ± 0.11	3.78 ± 0.07	3.66 ± 0.08	3.76 ± 0.05	-

[1]MSB - microencapsulated sodium butyrate; SM - soybean meal without MSB; SM-MSB - soybean meal with MSB; CM - canola meal without MSB; CM-MSB - canola meal with MSB; MR - milk replacer.

Table 14. Performance data of calves and nutrient digestibility – PART A

Variable		Treatment[1]				SEM	P-value[2]		
		SM	SM-MSB	CM	CM-MSB		PS	MSB	PS×MSB
Starter intake (kg dry matter /day)	Pre-weaning	0.246	0.267	0.219	0.238	0.019	0.012	0.064	0.96
	Weaning	1.22	1.39	1.25	1.31	0.122	0.77	0.21	0.52
	Post-weaning	1.94	1.9	2.00	2.14	0.169	0.36	0.75	0.58
	Overall	0.914	0.875	0.82	0.92	0.047	0.54	0.46	0.081
Average daily gain (kg/day)	Pre-weaning	0.652	0.646	0.594	0.614	0.044	0.30	0.87	0.76
	Weaning	0.643	0.647	0.445	0.505	0.01	0.10	0.75	0.77
	Post-weaning	0.942	0.842	0.897	0.9	0.09	0.94	0.59	0.55
	Overall	0.715	0.692	0.627	0.654	0.047	0.20	0.97	0.61
Fecal score[3]	Pre-weaning	1.86	1.95	1.88	1.75	0.061	0.27	0.79	0.17
	Weaning	1.33	1.34	1.19	1.18	0.054	0.027	0.99	0.87
	Post-weaning	1.47a	1.20ab	1.11b	1.15b	0.077	0.008	0.15	0.036
	Overall	1.67	1.63	1.55	1.50	0.061	0.025	0.41	0.91
Dry matter digestibility, %	Post-weaning	65.2	61.9	60.0	57.2	3.82	0.083	0.28	0.93

[1]SM - soybean meal without MSB; SM-MSB - soybean meal with MSB; CM - canola meal without MSB; CM-MSB - canola meal with MSB; MR - milk replacer; [2]PS - protein source; MSB - microencapsulated sodium butyrate; PS×MSB - interaction between main effects; [3]four point scale, where 1 = normal and 4 = diarrhea.

Table 15. Concentration of selected parameters in blood – PART A

Variable	Day of study	Treatment[1]				SEM	P-value[2]		
		SM	SM-MSB	CM	CM-MSB		PS	MSB	PS×MSB
Urea	1	30.4	26.1	26.0	29.4	3.48	0.87	0.88	0.23
(mg/dL)	21	14.8	14.2	16.9	15.6	0.945	0.081	0.35	0.75
	42	22.3	18.00	21.6	17.9	1.67	0.80	0.019	0.86
	63	22.8	18.7	18.8	15.6	1.98	0.059	0.061	0.81
Glucose	1	108.3	97.5	110.6	113.3	5.39	0.048	0.35	0.13
(mg/dL)	21	136.2	139.0	130.8	134.2	5.87	0.40	0.61	0.97
	42	131.3	142.6	129.5	125.3	6.30	0.12	0.58	0.22
	63	115.2	119.5	112.5	115.9	4.07	0.45	0.36	0.91
BHBA[3]	1	0.071	0.093	0.102	0.091	0.020	0.47	0.78	0.42
(mmol/L)	21	0.046	0.068	0.041	0.057	0.010	0.43	0.062	0.76
	42	0.184	0.143	0.161	0.151	0.022	0.74	0.25	0.46
	63	0.408	0.377	0.423	0.380	0.052	0.85	0.47	0.91
IGF-1	1	21.59	18.22	20.59	31.73	3.91	0.102	0.30	0.062
(ng/mL)	21	51.44	30.94	41.93	42.91	7.36	0.87	0.20	0.16
	42	54.09	44.64	39.34	40.38	9.17	0.31	0.65	0.57
	63	102.54	97.18	88.78	88.36	21.37	0.52	0.87	0.89
Insulin	1	12.41	7.16	8.35	15.39	3.63	0.49	0.76	0.051
(uIU/mL)	21	39.48	54.71	56.27	54.36	12.52	0.48	0.56	0.46
	42	33.26	40.28	53.37	62.59	15.06	0.17	0.60	0.94
	63	32.44	27.62	18.01	19.24	5.66	0.056	0.75	0.59

[1]SM - soybean meal without MSB; SM-MSB - soybean meal with MSB; CM - canola meal without MSB; CM-MSB - canola meal with MSB; MR - milk replacer; [2]PS - protein source; MSB - microencapsulated sodium butyrate; PS×MSB - interaction between main effects;[3]β-hydroxybutric acid.

Table 16. Plasma amino acids concentration – PART A

Concentration (μmol/L)	Treatments[1]				SEM	P-value[2]		
	SB	SB-MSB	CM	CM-MSB		PS	MSB	PS×MSB
Essential amino acids								
Met	15.38	15.60	20.50	18.89	2.19	0.068	0.75	0.68
Lys	81.21	99.81	84.87	75.65	8.64	0.25	0.59	0.12
Phe	61.57	62.70	54.86	48.39	4.41	0.027	0.55	0.40
Leu	124.02	125.61	111.43	106.13	10.25	0.13	0.86	0.74
Ileu	93.84	94.70	87.10	82.70	8.12	0.26	0.83	0.75
Val	205.30	237.55	207.44	192.28	16.10	0.19	0.60	0.16
Trp	30.79b	43.86a	28.29b	20.97b	2.87	0.001	0.33	0.002
Arg	148.76	155.91	142.89	121.64	14.69	0.19	0.64	0.35
His	49.00	55.02	57.44	49.72	4.98	0.76	0.87	0.18
Non-essential amino acid								
Asp	11.36	7.17	11.57	13.11	1.87	0.12	0.48	0.14
Glu	71.45	71.40	88.85	82.09	7.31	0.069	0.65	0.65
Hypro	38.50	45.22	45.76	35.98	2.76	0.70	0.57	0.006
Ser	111.71	74.24	110.29	117.64	15.38	0.19	0.34	0.16
Asn	66.93	77.86	60.91	46.42	7.89	0.028	0.82	0.12
Gly	389.62	432.70	481.78	450.82	30.88	0.066	0.83	0.20
Gln	265.62	381.81	278.69	231.46	23.36	0.008	0.16	0.002
Tau	28.83	39.45	16.24	16.14	2.63	0.001	0.053	0.041
Cit	68.31	64.42	61.65	66.09	5.23	0.64	0.96	0.44
Thr	78.37	86.82	88.95	83.93	10.49	0.72	0.87	0.53
Ala	164.01	158.93	177.04	145.09	10.49	0.97	0.088	0.20
Pro	70.25ab	78.95a	75.14ab	59.51b	4.84	0.12	0.46	0.016
1-MH	2.16	2.89	2.69	1.49	0.35	0.23	0.51	0.013
AAB	6.44ab	10.45a	6.94ab	6.37b	0.98	0.070	0.086	0.026
Tyr	51.09	55.24	51.93	48.02	5.00	0.53	0.98	0.43
Orn	78.63	88.79	76.25	70.06	7.58	0.18	0.80	0.30

[1]SM - soybean meal without MSB; SM-MSB - soybean meal with MSB; CM - canola meal without MSB; CM-MSB - canola meal with MSB; MR - milk replacer; [2]PS - protein source; MSB - microencapsulated sodium butyrate; PS×MSB - interaction between main effects.

Table 17. Rumen fermentation parameters – PART A

Variable	Treatment[1]				SEM	P-value[2]		
	SM	SM-MSB	CM	CM-MSB		PS	MSB	PS×MSB
pH	5.19	5.55	5.49	5.41	0.179	0.67	0.44	0.23
SCFA concentration (mmol/L)[3]	182.7	186.6	180.2	177.1	22.49	0.78	0.98	0.87
Acetate (%)	44.39	43.68	42.07	42.30	2.88	0.49	0.93	0.86
Propionate (%)	30.07	32.20	35.38	32.91	2.65	0.27	0.95	0.40
Iso-butyrate (%)	0.302	0.088	0.270	0.244	0.074	0.42	0.13	0.22
Butyrate (%)	19.08	17.33	18.32	20.14	2.60	0.62	0.98	0.40
Iso-valerate (%)	0.500	0.442	0.406	0.407	0.088	0.48	0.75	0.74
Valerate (%)	5.49	6.36	3.29	4.01	0.911	0.021	0.39	0.93
Ammonia (mg/dL)	18.78	19.40	13.57	14.20	2.80	0.084	0.83	0.99

[1]SM - soybean meal without MSB; SM-MSB - soybean meal with MSB; CM - canola meal without MSB; CM-MSB - canola meal with MSB; MR - milk replacer; [2]PS - protein source; MSB - microencapsulated sodium butyrate; PS×MSB - interaction between main effects;[3]Short-chain fatty acids.

Table 18. Gastrointestinal tract morphometry – PART A

Variable		Treatment[1]				SEM	P-value[2]		
		SM	SM-MSB	CM	CM-MSB		PS	MSB	PS×MSB
Reticulo-rumen	Tissue wt (kg)	1.95	1.91	2.02	1.99	0.0817	0.35	0.68	0.95
	Digesta wt (kg)	4.24	4.03	3.53	3.99	0.393	0.36	0.75	0.40
Omasum	Tissue wt (kg)	0.349	0.376	0.38	0.396	0.021	0.21	0.29	0.70
	Digesta wt (kg)	0.232	0.394	0.297	0.327	0.032	0.97	0.008	0.05
Abomasum	Tissue wt (kg)	0.371	0.382	0.418	0.419	0.021	0.063	0.79	0.81
	Digesta wt (kg)	0.406	0.328	0.468	0.418	0.084	0.35	0.43	0.85
Entire stomach	Tissue wt (kg)	2.64	2.64	2.79	2.77	0.094	0.15	0.90	0.92
	Digesta wt (kg)	4.88	4.76	4.29	4.74	0.400	0.46	0.68	0.48
Duodenum	Tissue wt (kg)	0.099	0.096	0.105	0.1	0.009	0.59	0.67	0.93
	Digesta wt (kg)	0.01	0.016	0.009	0.012	0.004	0.61	0.29	0.78
	Length (m)	0.45	0.48	0.49	0.43	0.041	0.85	0.74	0.30
Jejunum	Tissue wt (kg)	2.23	2.03	2.5	2.35	0.138	0.046	0.22	0.82
	Digesta wt (kg)	1.16	1.29	1.18	1.53	0.188	0.52	0.23	0.55
	Length (m)	20.71	20.59	23.29	21.73	0.94	0.065	0.38	0.45
Ileum	Tissue wt (kg)	0.256	0.204	0.221	0.281	0.035	0.55	0.91	0.12
	Digesta wt (kg)	0.033	0.076	0.076	0.092	0.03	0.35	0.34	0.66
	Length (m)	1.32	1.15	1.25	1.29	0.133	0.78	0.57	0.36
Total small	Tissue wt (kg)	2.59	2.33	2.81	2.69	0.15	0.069	0.22	0.62
Intestine	Digesta wt (kg)	1.22	1.37	1.5	1.3	0.15	0.45	0.85	0.22
	Length (m)	22.51	22.24	25.05	23.48	0.929	0.06	0.32	0.48

[1]SM - soybean meal without MSB; SM-MSB - soybean meal with MSB; CM - canola meal without MSB; CM-MSB - canola meal with MSB; MR - milk replacer; [2]PS - protein source; MSB - microencapsulated sodium butyrate; PS×MSB - interaction between main effects.

Table 19. Rumen epithelium development in the ventral sac of the rumen and abomasum epithelium development – PART A

Variable	Treatment[1]				SEM	P-value[2]		
	SM	SM-MSB	CM	CM-MSB		PS	MSB	PS×MSB
Rumen								
Mucosa surface (mm^2/cm^2)	1136.1	1088	1249.6	820.6	102.3	0.40	0.019	0.049
Density (number/cm^2)	140.4	158.4	181.1	129.00	15.7	0.71	0.28	0.035
Length (mm)	2.41	2.02	2.22	2.15	0.123	0.83	0.080	0.21
Width (mm)	1.61	1.63	1.56	1.49	0.067	0.14	0.71	0.47
Muscle layer (mm)	2.44	2.64	2.63	2.61	0.17	0.6	0.54	0.44
Abomasum								
Epithelium (mm)	1.01	1.10	1.13	0.99	0.051	0.94	0.58	0.038
Muscle layer (mm)	2.26	2.06	2.29	2.16	0.154	0.69	0.31	0.81

[1]SM - soybean meal without MSB; SM-MSB - soybean meal with MSB; CM - canola meal without MSB; CM-MSB - canola meal with MSB; MR - milk replacer; [2]PS - protein source; MSB - microencapsulated sodium butyrate; PS×MSB - interaction between main effects;[3]Short-chain fatty acids.

Table 20. Brush border enzymes activity in the small intestine – PART A

Variable		Treatment[1]				SEM	P-value[2]		
		SM	SM-MSB	CM	CM-MSB		PS	MSB	PS×MSB
Aminopeptidase A	Duodenum	1.35	2.88	2.01	2.76	0.66	0.64	0.066	0.50
	Prox jejunum	2.74	2.32	2.97	2.90	0.60	0.51	0.69	0.78
	Mid jejunum	18.94	13.52	22.75	20.78	4.81	0.23	0.42	0.70
	Distal jejunum	32.09	36.80	27.34	37.39	7.23	0.78	0.32	0.72
	Ileum	8.45	12.20	9.32	14.40	1.66	0.37	0.015	0.69
Aminopeptidase N	Duodenum	18.29	19.56	18.85	16.72	2.32	0.61	0.85	0.44
	Prox jejunum	19.87	19.83	19.46	19.83	1.77	0.91	0.93	0.91
	Mid jejunum	46.37	47.86	40.41	43.37	7.67	0.50	0.78	0.92
	Distal jejunum	39.31	40.61	34.81	38.73	5.15	0.54	0.62	0.80
	Ileum	33.49	34.83	29.5	42.07	3.88	0.65	0.073	0.13
Dipeptidase	Duodenum	nd[3]	nd	nd	nd	-	-	-	-
	Prox jejunum	nd	nd	nd	nd	-	-	-	-
	Mid jejunum	12.35	13.18	13.54	12.5	2.87	0.93	0.97	0.75
	Distal jejunum	11.18	13.84	13.72	13.9	2.66	0.63	0.60	0.64
	Ileum	9.36	9.07	8.74	12.85	1.44	0.29	0.20	0.14
Lactase	Duodenum	36.3	30.33	35.93	28.36	7.09	0.86	0.31	0.90
	Prox jejunum	55.89	150.8	101.42	71.03	22.64	0.46	0.17	0.012
	Mid jejunum	38.73	40.54	39.58	34.66	5.96	0.64	0.78	0.54
	Distal jejunum	2.82	2.50	2.38	2.85	0.30	0.87	0.79	0.16
	Ileum	1.55	1.48	1.41	1.53	0.12	0.71	0.83	0.44
Maltase	Duodenum	4.23	4.98	4.75	5.14	0.32	0.42	0.19	0.67
	Prox jejunum	8.9	11.11	9.63	8.62	1.33	0.52	0.66	0.24
	Mid jejunum	10.45	9.58	8.45	8.43	1.54	0.32	0.77	0.79
	Distal jejunum	8.16	8.39	7.04	7.60	0.90	0.30	0.66	0.85
	Ileum	4.13	3.86	3.59	4.66	0.30	0.64	0.17	0.028

[1]SM - soybean meal without MSB; SM-MSB - soybean meal with MSB; CM - canola meal without MSB; CM-MSB - canola meal with MSB; MR - milk replacer; [2]PS - protein source; MSB - microencapsulated sodium butyrate; PS×MSB - interaction between main effects; [3]not detected.

Table 21. Relative expression (fold change) of genes in different gastrointestinal tract regions – PART A

Gene of interest[1]		Treatment[2]				SEM	*P*-value[3]		
		SM	SM-MSB	CM	CM-MSB		PS	MSB	PS×MSB
CRA	MCT1	0.98	1.33	1.02	1.44	0.159	0.48	0.006	0.73
	MCT4	1.14	0.84	1.21	1.52	0.266	0.17	0.98	0.27
	UT-B	1.21	0.73	1.57	1.60	0.280	0.041	0.43	0.37
	AQP3	0.92	0.91	1.02	1.14	0.132	0.13	0.61	0.56
	GPR41	0.68	0.94	1.04	1.34	0.265	0.18	0.30	0.95
	GPR43	1.49	1.74	1.90	1.17	0.411	0.85	0.56	0.25
PROX	MCT1	1.10	1.12	1.23	1.33	0.150	0.27	0.68	0.79
	MCT4	1.12	0.98	1.37	1.00	0.164	0.24	0.047	0.31
	PEPT1	1.26	0.76	0.88	0.78	0.176	0.32	0.11	0.27
	PEPT2	1.21	1.01	0.65	0.66	0.193	0.029	0.61	0.60
	EAAC1	0.82	0.94	0.85	0.94	0.192	0.93	0.55	0.96
	ATB0	1.22	0.88	1.23	1.21	0.244	0.48	0.46	0.50
	GPR41	0.80	0.79	0.70	0.73	0.078	0.29	0.89	0.81
	GPR43	1.20	0.95	1.34	0.82	0.185	0.99	0.049	0.49
ILE	MCT1	1.08	1.04	1.27	1.08	0.146	0.41	0.41	0.58
	MCT4	1.10	1.19	1.12	0.09	0.114	0.21	0.52	0.16
	PEPT1	1.19	1.12	1.37	0.89	0.348	0.94	0.38	0.51
	PEPT2	1.02	0.99	1.86	1.29	0.207	0.01	0.15	0.18
	EAAC1	0.92	2.35	2.23	1.69	0.739	0.66	0.20	0.55
	ATB0	1.05	0.90	0.73	0.60	0.189	0.040	0.34	0.94
	GPR41	1.08	1.19	0.90	0.96	0.157	0.096	0.47	0.81
	GPR43	1.35	1.27	1.38	0.70	0.303	0.35	0.20	0.30

[1]CRA – cranial sac of rumen; PROX – proximal jejunum; ILE – ileum; [2]SM - soybean meal without MSB; SM-MSB - soybean meal with MSB; CM - canola meal without MSB; CM-MSB - canola meal with MSB; MR - milk replacer; [3]PS - protein source; MSB - microencapsulated sodium butyrate; PS×MSB - interaction between main effects. MCT1 = monocarboxylate transporter 1, MCT4 = monocarboxylate transporter4, UT-B = Urea transporter B, AQP3 = aquaporin 3, GPR41 =g-protein

coupled receptor 41, GPR43 = g-protein coupled receptor 43, PEPT1 = peptide transporter 1, PEPT2 = peptide transporter 2, EAAC1 = glutamate transporter, ATB0 = neutral and basic amino acid transporter.

Table 22. Nutrient composition of starter mixtures and milk replacer – PART B

Variable	Treatment/Feed[1]				
	SM	SM-MSB	CM	CM-MSB	MR
Dry matter (DM) (%)	90.8 ± 0.2	90.6 ± 0.1	90.8 ± 0.2	90.5 ± 0.1	95.3 ± 0.4
Ash (% DM)	7.5 ± 0.1	8.0 ± 0.1	7.7 ± 0.1	7.8 ± 0.2	6.7 ± 0.1
OM (% DM)	92.5 ± 0.1	92.1 ± 0.1	92.3 ± 0.1	92.2 ± 0.2	93.3 ± 0.1
CP (% DM)	19.7 ± 0.6	20.5 ± 0.4	20.4 ± 0.1	20.3 ± 0.1	23.8 ± 0.1
Fat (% DM)	-	-	-	-	17.9 ± 0.2
NDF (% DM)	13.3 ± 1.7	11.6 ± 0.6	17.8 ± 0.5	17.5 ± 0.2	-
ADF (% DM)	4.9 ± 0.1	4.8 ± 0.4	9.6 ± 0.8	11.3 ± 0.4	-
Starch (% DM)	44.9 ± 2.6	42.2 ± 0.9	37.7 ± 1.6	35.9 ± 0.7	-
Sugar (% DM)	8.1 ± 0.3	8.4 ± 0.2	9.0 ± 0.6	8.1 ± 0.2	-
Amino acid					
Asp (g/kg DM)	17.22 ± 0.85	18.44 ± 0.10	13.47 ± 0.06	12.79 ± 0.04	-
Thr (g/kg DM)	6.00 ± 0.16	6.47 ± 0.11	6.44 ± 0.07	6.88 ± 0.24	-
Ser (g/kg DM)	7.87 ± 0.61	8.56 ± 0.14	7.35 ± 0.26	7.37 ± 0.29	-
Glu (g/kg DM)	36.77 ± 0.36	37.31 ± 0.30	33.39 ± 0.45	32.59 ± 1.40	-
Pro (g/kg DM)	11.59 ± 0.26	12.75 ± 0.79	12.03 ± 0.21	12.14 ± 0.05	-
Gly+Gln (g/kg DM)	7.61 ± 0.24	7.52 ± 0.13	8.28 ± 0.26	8.23 ± 0.01	-
Ala (g/kg DM)	8.10 ± 0.22	8.17 ± 0.09	7.94 ± 0.33	7.80 ± 0.20	-
Val (g/kg DM)	9.24 ± 0.19	9.01 ± 0.16	8.90 ± 0.12	8.82 ± 0.21	-
Ile (g/kg DM)	7.09 ± 0.02	7.20 ± 0.13	6.44 ± 0.06	6.16 ± 0.08	-
Leu (g/kg DM)	14.92 ± 0.48	14.78 ± 0.18	13.79 ± 0.58	13.59 ± 0.09	-
Tyr (g/kg DM)	5.98 ± 0.41	5.95 ± 0.02	5.73 ± 0.39	5.53 ± 0.21	-
Phe (g/kg DM)	9.04 ± 0.41	8.91 ± 0.01	8.01 ± 0.06	7.22 ± 0.05	-
His (g/kg DM)	5.10 ± 0.36	5.04 ± 0.14	5.27 ± 0.17	5.21 ± 0.01	-
Lys (g/kg DM)	8.54 ± 0.39	8.63 ± 0.12	8.21 ± 0.26	8.13 ± 0.11	-
Arg (g/kg DM)	13.05 ± 0.75	13.19 ± 0.14	11.82 ± 0.64	11.60 ± 0.91	-
Cys (g/kg DM)	3.39 ± 0.14	3.40 ± 0.02	4.33 ± 0.01	4.72 ± 0.11	-
Met (g/kg DM)	3.98 ± 0.05	4.11 ± 0.11	4.22 ± 0.08	3.81 ± 0.10	-

[1]MSB - microencapsulated sodium butyrate; SM - soybean meal without MSB; SM-MSB - soybean meal with MSB; CM - canola meal without MSB; CM-MSB - canola meal with MSB; MR - milk replacer.

Table 23. Performance data of calves – PART B

Variable		Treatment[1]				SEM	P-value[2]		
		SM	SM-MSB	CM	CM-MSB		PS	MSB	PS×MSB
Starter intake (kg/day)	Overall	0.771	0.724	0.828	0.806	0.045	0.12	0.44	0.78
	Pre-weaning	0.103	0.106	0.114	0.132	0.025	0.22	0.47	0.60
	Step-down	0.943	0.848	0.971	0.923	0.087	0.51	0.36	0.76
	Post-weaning	2.307	2.174	2.396	2.44	0.119	0.14	0.10	0.46
Average daily gain (kg/day)	Overall	0.678	0.622	0.676	0.677	0.031	0.37	0.36	0.34
	Pre-weaning	0.581	0.527	0.544	0.566	0.035	0.98	0.60	0.21
	Step-down	0.60	0.56	0.62	0.64	0.077	0.46	0.93	0.61
	Post-weaning	1.003	0.926	1.072	0.999	0.071	0.32	0.30	0.98
Fecal score[3]	Overall	1.40	1.53	1.39	1.36	0.09	0.059	0.28	0.085
	Pre-weaning	1.42	1.55	1.41	1.39	0.09	0.12	0.35	0.065
No of days with diarrhea[4]	Overall	3.1	4.8	3.5	2.5	0.99	0.17	0.59	0.053
Feed efficiency (kg gain/kg of starter consumed)	Post-weaning	0.481	0.477	0.471	0.464	0.031	0.72	0.87	0.96

[1]SM - soybean meal without MSB; SM-MSB - soybean meal with MSB; CM - canola meal without MSB; CM-MSB - canola meal with MSB; MR - milk replacer; [2]PS - protein source; MSB - microencapsulated sodium butyrate; PS×MSB - interaction between main effects; [3]four point scale, where 1 = normal and 4 = diarrhea; [4]fecal score ≤ 3.

Table 24. Ingredients and chemical composition of experimental feeds

	Treatment[1]			Milk replacer
	TSBM	TSBM/TCM	TCM	
Ingredient, % in feed				
Soybean meal	24.0	12.5	–	
Canola meal	–	16.5	35.0	
Barley grain	29.0	24.5	24.5	
Corn grain	30.0	30.0	30.0	
Wheat bran	6.0	6.0	6.0	
Glycerol	3.0	3.0	3.0	
Whey	2.5	2.5	2.5	
Molasses	1.0	1.0	1.0	
Limestone	2.0	2.0	2.0	
NaCl	0.5	0.5	0.5	
Mineral-vitamin premix	1.0	1.0	1.0	
Monocalcium phosphate	1.0	0.5	–	
Chemical composition				
DM, %	90.2 ± 0.5	89.4 ± 0.3	88.9 ± 0.6	95.5 ± 0.7
CP, % DM	23.0 ± 0.4	22.2 ± 0.3	21.8 ± 0.3	27.3 ± 0.4
Fat, % DM	1.7 ± 0.3	2.0 ± 0.3	2.7 ± 0.3	17.4 ± 0.9
Ash, % DM	8.8 ± 0.1	8.6 ± 0.3	8.2 ± 0.2	8.9 ± 0.1
NDF, % DM	13.5 ± 1.2	15.9 ± 1.5	18.2 ± 0.9	–
ADF, % DM	6.0 ± 0.1	8.3 ± 0.2	11.0 ± 0.3	–
Ca, % DM[2]	1.06	1.07	1.10	–
P, % DM[2]	0.68	0.68	0.69	–

[1]Treatment: TSBM = soybean meal was used as the main source of protein in the starter mixture; TSBM/TCM = soybean meal and canola meal used as the main sources of protein in the starter mixture; TCM = canola meal used as the main source of protein in the starter mixture.
[2]Calculated from NRC.

Table 25. Least square means for performance of calves fed starter mixtures differing in protein source.

	Treatment[1]			SEM	Contrasts[2]	
	TSBM	TSBM/TCM	TCM		1	2
N	12	11	11			
Initial age, d	15.0	14.1	15.3	0.5	0.18	0.69
BW, kg						
Initial	40.6	39.8	39.7	1.7	0.64	0.59
Final	80.4	78.1	75.9	3.5	0.54	0.21
BW gain, kg	40.6	38.7	36.7	2.3	0.56	0.20
ADG, g/d						
d 1 to 28	679	622	572	32	0.21	0.02
d 29 to 56	775	736	715	81	0.64	0.47
d 1 to 56[2]	738	691	654	38	0.34	0.08
Milk replacer intake, g/d						
d 1 to 28[3]	874	859	872	6	0.06	0.78
d 29 to 42[3]	848	829	846	12	0.22	0.73
d 1 to 42[3,4]	808	797	806	5	0.12	0.83
Starter intake, g/d						
d 1 to 28[3,4]	224	186	192	23	0.26	0.32
d 29 to 56[3]	1665	1605	1643	70	0.55	0.82
d 1 to 56[3]	946	892	920	40	0.35	0.64
ADG:DM intake, g/kg						
d 1 to 28[3]	668	634	565	27	0.38	< 0.01
d 29 to 56[3]	421	427	406	32	0.83	0.59
d 1 to 56[3]	551	537	492	17	0.56	0.01
Fecal fluidity						
d 1 to 28[3]	1.02	1.13	1.24	0.05	0.14	< 0.01
d 29 to 56[3]	1.11	1.05	1.06	0.05	0.30	0.35
d 1 to 56[3]	1.07	1.09	1.14	0.04	0.69	0.12
Diarrhea, d	1.04	1.87	3.20	0.82	0.38	0.03
Medical treatments, d	0.77	1.31	0.58	0.63	0.55	0.84

Treatment: TSBM = soybean meal was used as the main source of protein in the starter mixture; TSBM/TCM = soybean meal and canola meal used as the main sources of protein in the starter mixture; TCM = canola meal used as the main source of protein in the starter mixture.
[2]1 = TSBM vs. TSBM/TCM; 2 = TSBM vs. TCM.
[3]Significant effect of time ($P < 0.01$).
[4]Significant treatment × time interaction ($P < 0.01$).

Table 26. Least square means for behavior of calves fed starter mixtures differing in protein source.

	Treatment[1]			SEM	Contrasts[2]	
	TSBM	TSBM/TCM	TCM		1	2
N	6	6	6			
Starter intake, n/d[3]	49.7	45.3	53.9	4.7	0.53	0.53
Starter intake, min/d[3]	62.3	62.0	60.1	3.8	0.95	0.67
Starter intake rate, g/min[3]	20.6	24.8	24.6	2.3	0.24	0.23
Water intake, n/d[3]	12.9	15.7	14.7	1.1	0.10	0.25
Water intake, min/d[3]	11.0	12.6	12.2	0.1	0.26	0.40

[1]Treatment: TSBM = soybean meal was used as the main source of protein in the starter mixture; TSBM/TCM = soybean meal and canola meal used as the main sources of protein in the starter mixture; TCM = canola meal used as the main source of protein in the starter mixture.
[2]1 = TSBM vs. TSBM/TCM; 2 = TSBM vs. TCM.
[3]Significant effect of time ($P < 0.01$).

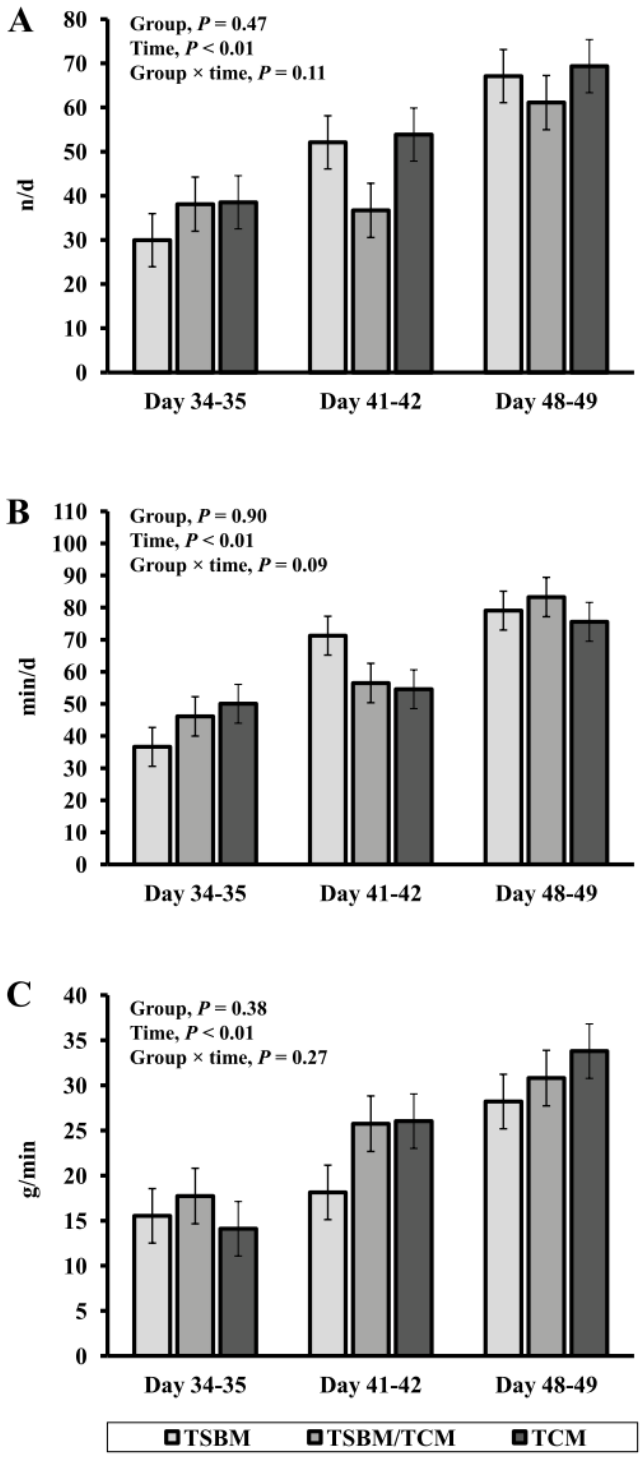

Figure 4. Effect of canola meal use as a protein source in starter mixtures on starter mixture eating frequency (A), time (B), and rate (C).