

INVESTIGATION OF METHODS TO ENHANCE THE EFFICIENCY OF CANOLA MEAL USE IN PELLETTED CALF STARTER MIXTURES AND COMPARISONS WITH OTHER HIGH-PROTEIN BY-PRODUCTS*

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Abstract

It was hypothesized that the efficiency of canola meal (CM; also known as 00 rapeseed meal) utilization in pelleted starter mixtures for calves can be increased with the use of feed additives and additional processing. Additionally, it was hypothesized that CM would yield greater feed intake, growth, and improved fecal scores compared to wheat bran and DDGS when included as a high-protein by-product in a pelleted starter mixture. In order to verify the hypotheses, four studies were conducted to determine whether lysine (Lys) inclusion, feed enzyme inclusion, and CM extrusion improve the efficiency of CM utilization in pelleted calf starter mixtures, and to compare effects of CM to that when other high-protein by-products are fed. In study 1, 45 female Holstein calves (44.7±4.2 kg, 24.2±2.8 days of age) were allocated to one of three treatments and fed a pelleted starter mixture containing: 1) soybean meal (SBM) as the main source of protein (TSBM); 2) CM as the main source of protein (TCM); or 3) CM as the main source of protein with supplemental rumen-unprotected Lys (TCML). Final body weight (BW), average daily gain (ADG), starter intake, and fecal score did not differ among treatments ($P \geq 0.20$) but the gain to feed ratio was greater for TSBM than for TCM and TCML ($P < 0.01$). No differences between TCM and TCML were found. In study 2, 100 female Holstein calves (44.3±4.8 kg, 17.7±2.1 days of age) were assigned to one of four treatments and fed pelleted starter mixtures with: 1) low CM inclusion (10%; LOW); 2) low CM inclusion supplemented with feed enzymes (xylanase, glucanase, invertase, protease, cellulase, amylase, and mannanase; LOW+); 3) high CM inclusion (32%; HIGH); and 4) high CM inclusion supplemented with the same feed enzymes (HIGH+) as for LOW+. In treatments that included feed enzymes, final BW, ADG, and starter intake were greater while the number of days with diarrhea was lower ($P \leq 0.05$). The number of days with diarrhea was also lower for high CM treatments ($P < 0.01$). In study 3, 120 female Holstein calves (42.6±4.4 kg, 17.2±2.1 days of age) were allocated to one of four treatments and fed a pelleted starter mixture: 1) containing SBM as a main source of protein (CTRL), or diets where: 2) SBM was partially replaced by wheat bran and corn DDGS (TRBP); 3) SBM was partially replaced by CM (TRCM); and 4) SBM was partially replaced by CM with feed enzymes (the same used in study 2; TRCM+). Feed intake and the gain to feed ratio did not differ among treatments. Final BW tended ($P = 0.10$) to be higher for CTRL than for TRBP and TRCM, but the CTRL group had a higher fecal score, a greater number of days with diarrhea, and more episodes of diarrhea compared to TRBP and TRCM ($P \leq 0.05$). No differences between TRCM and TRCM+ were detected. In study 4, 120 female Holstein calves (44.1±4.9 kg, 18.3±1.9 days of age) were allocated to one of four treatments and fed a pelleted starter mixture with: 1) moderate CM inclusion (24%; MC); 2) high CM inclusion (34%; HC); 3) moderate inclusion of extruded CM (MEC); and 4) high inclusion of extruded CM (HEC). Fecal score tended to be ($P = 0.10$) and the number of days with diarrhea was ($P < 0.01$) reduced when extruded CM was used in the starter mixture but BW, ADG, and the gain to feed ratio were not different among treatments. In conclusion, rumen-unprotected lysine supplementation in a pelleted starter mixture containing CM as the main source of protein does not improve the performance of calves although inclusion of enzymes may improve feed intake and ADG of calves. Extrusion of CM improves fecal score of calves but does not affect feed intake or growth. Canola meal should be considered at least as valuable source of protein as wheat bran and DDGS.

Key words: canola meal, protein source, lysine, feed enzymes, supplementation

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Although canola meal (CM; also known as 00 rapeseed meal) is not considered a preferred protein source for newborn calves due to concerns over impacts on feed intake, nutrient digestibility, and growth (Górka and Penner, 2020), results of several studies have provided evidence that it can be successfully fed to calves during the pre- and post-weaning phases. When used in a pelleted starter mixture, CM can be included at 20 to 25% of the feed or can replace 50 to 60% of soybean protein without negative impacts on the performance of calves (Burakowska et al., 2021 a; Górka and Penner, 2020; Hadam et al., 2016). Despite that, based on their common use, other high-protein by-products, such as dried distillers grains with solubles (DDGS) (Laarman et al., 2012; Suarez-Mena et al., 2011) and wheat bran (Kazemi-Bonchenari et al., 2022; Rahimi and Rafiee, 2024; Xiao et al., 2023) seem to be preferred over CM in calf starter mixtures (Górka and Penner, 2020). The preference for other high-protein by-products over CM is practiced, although the greater palatability and digestibility of those by-products has not been proven. Moreover, those by-products are sometimes used in starter diets at inclusions which may negatively affect the growth performance of calves (Kazemi-Bonchenari et al., 2022; Laarman et al., 2012), such as when inclusion rates are close to or greater than 20% (Hill et al., 2016; Suarez-Mena et al., 2011). Consequently, when the price for other high-protein ingredients is greater than the price for CM, their use in calf starter may lead to financial losses for producers.

Low palatability of CM is one of the most important factors limiting its use in calf starter formulation. Although low palatability of CM for calves was shown in several studies (Miller-Cushon et al., 2014 a, b) and that calves can sort against CM when it is included in a mash feed (Stone and Wood, 1973), selective avoidance can be easily solved by pelleting and palatability effects can be reduced through the inclusion of more palatable ingredients. By pelleting the feed and concomitant inclusion of glycerol, molasses, or flavor additives, the reduction in palatability can be diminished (Burakowska et al., 2020; Schingoethe et al., 1974). When strategies to reduce the negative consequences of CM use in calf starter mixtures were implemented, feed intake, growth performance of calves, or both were not different between starters with CM compared to those with soybean meal (SBM) (Burakowska et al., 2021 b; Hadam et al., 2016).

While the palatability issues associated with CM feeding to calves can be easily overcome, reduced nutrient digestibility and feed efficiency may be of concern (Burakowska et al., 2021 a,b; Hadam et al., 2016; Khorasani et al., 1990). Based on results of studies conducted using non-ruminant animals, the reductions in digestibility and gain to feed observed with diets containing CM can be avoided by including enzymes in those diets (Józefiak et al., 2010; Niu et al., 2022) or with CM extrusion (Ahmed et al., 2014; Heyer et al., 2021), as those two can positively affect nutrient digestibility. There are also indications that amino acid supplementation in solid feed may positively affect calf performance, particularly

when the lysine (Lys) concentration is increased (Veen et al., 1989). It is important to note that the Lys concentration of CM is less than that of SBM and Lys may be limiting for calves fed starters with high CM inclusion (Górka and Penner, 2020). Thus, the efficiency of CM use in calf starter mixtures may be improved with Lys provision and enzyme inclusion, or CM extrusion. However, the use of the previously mentioned strategies has not been tested for calves fed starter mixtures containing CM.

In this paper, results of four studies are presented that aimed to test the hypotheses that Lys and feed enzymes inclusion, and extrusion of CM will promote greater feed intake, growth, and feed efficiency for calves fed pelleted starter mixtures containing CM, and that CM is a better protein source in calf starter mixtures compared to wheat bran and DDGS. Thus, the objectives of the conducted studies were to verify whether Lys supplementation, feed enzyme supplementation, and CM extrusion can enhance feed intake, growth, and feed efficiency of calves when CM is included in pelleted starter mixtures for calves, and to compare the effect of CM use in pelleted starter mixtures with wheat bran and DDGS when used as a partial replacement for SBM.

Material and methods

All studies were conducted on a commercial dairy farm (Top Farms Głubczyce Sp. z o.o., Głubczyce, Poland). The experimental procedures followed Polish legislation requirements, which comply with the EU Directive 2010/63/EU for the protection of animals used for scientific purposes.

For the studies presented in this paper, the CM was derived from 00 rapeseed grown in Poland and the term ‘canola meal’ is used consistently in the manuscript body as ‘canola’ is synonymously used with ‘00 rapeseed’ derived from seeds low in glucosinolates and erucic acid (Górka and Penner, 2020; Martineau et al., 2013; Wheeler et al., 1980). Such an approach was used in past studies focusing on canola meal/rapeseed meal use in starter diets for calves (Burakowska et al., 2020; Burakowska et al., 2021 a, b; Górka and Penner, 2020).

Study 1

The study was conducted between September 12 and December 4, 2017. The temperature averaged $12.8 \pm 2.00^{\circ}\text{C}$ (mean $\pm$ SD) and $-1.3 \pm 1.59^{\circ}\text{C}$ during the hottest (September) and the coldest (December) months of the study, respectively. Temperatures were recorded at a research station located approximately 30 km away from the calf facility (<https://danepubliczne.imgw.pl>; Institute of Meteorology and Water Management, National Research Institute, Warsaw, Poland).

Animals, treatments, housing and feeding

Forty-five female Holstein calves (44.7 ± 4.2 kg, 24.2 ± 2.8 days of age; mean $\pm$ SD) were allocated to one

of three treatments (15 calves/treatment) and fed: 1) a pelleted starter mixture containing SBM as the main source of protein (TSBM); 2) a pelleted starter mixture containing CM as the main source of protein (TCM); or 3) a pelleted starter mixture containing CM as the main source of protein with supplemental rumen-unprotected Lys (TCML). The inclusion of Lys in the starter mixture fed to TCML was designed to increase Lys concentration and to obtain the same Lys:methionine (Met) ratio in TSBM and TCML. Feeds used for starter mixture formulation and production originated from the same batch. Starters were formulated to have similar concentrations of crude protein (CP) (Table 1).

Table 1. Ingredient and chemical composition of pelleted starter mixtures (mean±standard deviation) – study 1

	Treatments ^a		
	TSBM	TCM	TCML
1	2	3	4
Ingredient, % in feed			
soybean meal	18.00	–	–
canola meal	–	26.00	26.00
barley	32.67	25.47	25.31
corn	30.00	30.00	30.00
wheat bran	10.00	10.00	10.00
glycerin	3.00	3.00	3.00
molasses	1.00	1.00	1.00
whey	1.00	1.00	1.00
limestone	2.00	2.00	2.00
salt	0.50	0.50	0.50
monocalcium phosphate	0.80	–	–
mineral-vitamin supplement ^b	1.00	1.00	1.00
aroma	0.03	0.03	0.03
L-lysine	–	–	0.16
Chemical composition ^c			
DM, g/kg	901±0.8	897±1.2	897±1.0
ash, g/kg DM	85±1.3	78±0.8	79±2.1
crude protein, g/kg DM	206±1.9	188±2.8	196±2.9
fat, g/kg DM	28±0.4	31±0.2	30±0.9
aNDF, g/kg DM	161±0.9	190±5.4	195±2.1
ADF, g/kg DM	68±1.7	97±4.6	110±7.5
Amino acid, g/kg DM			
Ala	7.8±0.1	7.1±0.2	7.2±0.2
Arg	14.8±1.4	11.9±1.2	12.2±1.9
Asp	16.1±0.2	11.9±0.2	12.0±0.9
Cys	2.7±0.2	3.0±0.2	3.2±0.2
Glu+Gln	34.3±0.7	30.5±0.2	30.8±0.9
Gly	7.6±0.1	7.3±0.2	7.7±0.2
His	4.8±0.3	4.5±0.1	4.6±0.1
Ile	6.7±0.1	5.7±0.1	5.9±0.2
Leu	13.7±0.3	12.1±0.3	12.4±0.3
Lys	8.6±0.2	7.4±0.2	9.2±0.4

Table 1 – contd.

1	2	3	4
Met	2.5±0.1	2.6±0.1	2.9±0.1
Phe	8.3±0.1	6.7±0.2	7.0±0.1
Pro	11.3±0.2	11.3±0.1	11.8±0.2
Ser	6.8±0.2	6.1±0.5	3.1±0.1
Thr	6.0±0.2	5.9±0.3	6.1±0.2
Tyr	5.1±0.4	4.9±0.1	5.0±0.2
Val	9.4±0.2	8.8±0.2	9.1±0.2
Lys/Met	3.44	2.85	3.20

^aTSBM = starter mixture containing soybean meal as the main source of protein; TCM = starter mixture containing canola meal as the main source of protein; TCML = starter mixture containing canola meal as the main source of protein and additionally supplemented with rumen-unprotected Lys.

^bBlattin Super Premium (Blattin Polska Sp. z o.o., Izbičko, Polska): 18.3% Ca; 3% P; 8.5% Na; 5.5% Mg; 11,500 mg Zn; 5,000 mg Mn; 1,500 mg Cu; 27 mg Co; 83 mg I; 87 mg Se; 1,000,000 IU vitamin A; 160,000 IU vitamin D; 5,000 mg vitamin E; 20 mg vitamin B₁; 30 mg vitamin B₂; 2,156 mg vitamin B₃; 67 mg vitamin B₅; 100,000 µg vitamin B₆; 4 mg vitamin B₉; 25 mg vitamin B₁₂.

^cn=3.

Calves were sourced from two dairy farms belonging to Top Farms Głubczyce Sp. z o.o., both located close to the calf facility (<15 km) where calves were housed during the study. Calves were transported to the calf facility between 18 to 28 days of age in three blocks of 18, 15 and 12 calves. All calves were collected over a period of three weeks. Within each block, calves were sub-blocked by age and, within the sub-block, were randomly allocated to treatments. Treatments were balanced for the number of calves born at each of the two farms. Only healthy calves were assigned to the study, and calves born as twins or from a difficult calving were excluded.

Before study initiation, calves were treated according to a standard operating protocol adopted at the farm. Briefly, immediately after birth, calves were separated from their dam, transported to an individual hutch bedded with straw, and fed 3 L of colostrum from the first milking event within the first 3 h of life. Colostrum was fed from a bucket with a nipple. Prior to feeding to calves, colostrum quality was verified using a colostrometer and only good quality of colostrum was fed to calves (≥ 50 g/L of IgG). Colostrum feeding was continued until 48 h of life. Subsequently, calves were fed transition milk and whole milk until day 7 of life, which was followed by milk replacer (MR) feeding (Sprayfo Excellent, Trouw Nutrition, Grodzisk Mazowiecki, Poland; 22.5% CP and 18% crude fat in the MR powder; 150 g of MR powder in 1 L of MR solution) until calves were allocated to the study. Liquid feeds (including colostrum, transition milk, and MR) were fed from a bucket with a nipple in amounts equal to 5 L/day (2×2.5 L; 7:00 and 17:00 h). No solid feed was offered until calves were allocated to the study.

Beginning on the first day of the study, calves were housed in a naturally ventilated calf barn in individual pens (1.5 × 1.2 m) connected to an additional individual

outdoor area (3.0×1.2 m). Pens were bedded with straw, but the concrete outdoor area was not bedded. For the first seven days of the study, calves were fed the same MR that was offered before study initiation (Sprayfo Excellent, Trouw Nutrition; 22.5% CP and 18% crude fat in the MR powder) and then were switched to the MR for older calves (Sprayfo Yellow, Trouw Nutrition; 21.5% CP and 17.5% crude fat in the MR powder). The MR was fed from an open bucket providing 6 L/day with three equal feedings daily from day 1 to 49 of the study (7:00, 12:00 and 17:00 h; 150 g of MR powder in 1 L of MR solution), and a single feeding from day 50 to 63 of the study (at 7:00 h; 1×2.5 L; 150 g of MR powder in 1 L of MR solution). The calves remained in the study for 70 days and from day 64 to 70 only starter feed was fed to calves. The period from day 1 to 49 was considered the pre-weaning period, while the period from day 50 to 70 was considered the weaning transition period.

From the first day of the study, calves were offered experimental starter mixtures that were fed free of choice. Water was available at all times. Feeding and housing of calves after transportation to the calf barn and study initiation resembled standard procedures adapted for the calves at Top Farms Głubczyce Sp. z o.o. The only exception was the modifications made to the starter mixtures provided to the calves. Therefore, the maintenance of calves resembled typical practical conditions.

Measurements, observations and medical treatments

Calves were weighed on the first day of the study and then on days 49 and 70 by farm staff, always at the same time (11:00 h). The MR and pelleted starter mixture intakes were monitored daily by recording volume of MR refused at each feeding and weighing out starter refusals once daily, before morning feeding, individually for each animal. Fecal scores (fluidity) were recorded daily using a 4-point scale (1=normal, 4=diarrhea; Larson et al., 1977). Calves with a fecal score ≥ 2 were considered to have diarrhea and were fed MR with the addition of a feed supplement containing pectin, vitamins, minerals, and a probiotic (PECTOPRO, Over Vet, Koszalin, Poland) until the fecal score returned to normal. In cases of a persisting fecal score ≥ 3 , severe diarrhea (fecal score 4) or dehydration, medical treatment was administered on an individual basis, following the veterinarian's recommendations. Each medical treatment was recorded.

Feed samples and analysis

Samples of experimental starters were collected weekly and pooled by the month of the study. Feeds were analyzed for dry matter (DM), ash, CP, crude fat, neutral detergent fiber (aNDF; NDF assayed with a heat-stable amylase and expressed inclusive of residual ash), acid detergent fiber (ADF) as previously described by Górka et al. (2017), and amino acid concentrations as described by Burakowska et al. (2021 b).

Data processing and statistical analysis

The study was divided into two phases including day 1 to 49 of the study (pre-weaning period) and day 50 to 70 of the study (weaning transition). Feed efficiency was calculated by dividing body weight (BW) gain by DM intake (both from MR and starter) and multiplied by 1000 to express results in g of gain per 1 kg of DM intake (Quigley et al., 2006). Results of MR intake, starter intake, and fecal score were averaged by week of the study before statistical analysis. Initial age of calves, BW, average daily gain (ADG), and feed efficiency (gain to feed ratio) were analyzed as a randomized complete block design using PROC MIXED of SAS (version 9.4, SAS Institute, Cary, NC, USA). Prior to data analysis, the normality of the data and homogeneity of variance were tested using PROC UNIVARIATE. The effect of treatment was considered in the statistical model as a fixed effect, whereas the effect of block and sub-block within a block as random effects. The statistical model for repeated variables also included the effect of time and its interaction with treatment as fixed effects (Littell et al., 1998). The effect of the farm where the calf was born and its interaction with the treatment were also considered in the model as fixed effects, but were not significant ($P > 0.05$) and consequently were removed from the model. The optimal covariance structure (autoregressive order one, unstructured or compound symmetry) was chosen based on Akaike's information criterion. For BW, ADG, starter intake, and feed efficiency analysis, initial BW was included in the model as a covariate. Fecal score data were analyzed using PROC GLIMMIX of SAS using a Poisson distribution and AR(1) type covariance structure. The same effects were considered in the model as for other variables. Pre-planned contrasts were used for interpretation of results (TSBM vs. TCM and TCML, TCM vs. TCML). Significance was declared when $P \leq 0.05$, and a tendency was declared when $0.05 < P \leq 0.10$.

Study 2

The study was conducted between January 13 and April 27, 2022. The temperature averaged $6.8 \pm 3.64^\circ\text{C}$ (mean $\pm$ SD) and $0.6 \pm 1.72^\circ\text{C}$ during the hottest (April) and the coldest (January) months of the study, respectively. Temperatures were recorded at a research station located approximately 30 km away from the calf facility (<https://danepubliczne.imgw.pl>; Institute of Meteorology and Water Management, National Research Institute, Warsaw, Poland).

Animals, treatments, housing and feeding

One hundred female Holstein calves (44.3 ± 4.8 kg, 17.7 ± 2.1 days of age, 6.5 ± 2.1 g/dL of total serum protein) were allocated to one of four treatments (25 calves/treatment) in a 2×2 factorial arrangement and fed: 1) a pelleted starter mixture with low CM inclusion (10% in starter mixture; LOW); 2) a pelleted starter mixture with low CM inclusion and a mixture of feed enzymes (Superzyme-CS, CBS Bio Platforms, Canada; 1 g/kg

of feed; LOW+); 3) a pelleted starter mixture with high CM inclusion (32% in starter mixture; HIGH); or 4) a pelleted starter mixture with high CM inclusion and a mixture of feed enzymes (HIGH+). The inclusion of CM fed to LOW treatments was designed to ensure some of the protein was supplied by CM (~ 20% inclusion rate), whereas the inclusion of CM fed to HIGH treatments was based on the assumption that inclusion of CM at rates over 20–25% in the starter mixture will negatively affect growth performance of calves (Górka and Penner, 2020). The feed enzyme mixture contained: xylanase (1,300 U/g), glucanase (150 U/g), invertase (700 U/g), protease (6,000 U/g), cellulase (800 U/g), amylase (12,000 U/g), and mannanase (5 U/g) (Superzyme-CS, CBS Bio Platforms). Feeds used for starter formulation and production originated from the same batch. Starters were formulated to be similar for CP and to limit, as much as possible, differences in starch concentration among treatments. Feed enzymes were included in the starter in exchange for barley grain (Table 2).

As in study 1, calves were sourced from two dairy farms belonging to Top Farms Głubczyce Sp. z o.o. located nearby (<15 km) to the calf facility in which the study was conducted. Calves were allocated to treatments between 14 and 21 days of age and transported to the calf barn once a week (Thursday). All calves were collected over a period of seven weeks, which resulted in seven blocks of 16, 20, 12, 12, 12, 20, and 8 calves in each block. Each week, an equal number of calves were allocated to each treatment. Allocation to treatments also ensured similar initial BW, initial age, and serum total protein concentrations (measured between 2 to 4 days of age; details described below) among treatments. Treatments were also balanced for the number of calves from each of the two farms, and the number of calves born from primi- and multiparous cows. Unhealthy calves, twins or calves from difficult calvings were not allocated to the study.

Prior to the initiation of the study, the calves were kept in outdoor hutches bedded with straw and were fed colostrum and then MR according to standard procedures adopted at the farm during the period when the study was conducted. Specifically, calves were fed 3–4 L of colostrum from the first milking event within the first 2 h of life from a bucket with a nipple. Prior to feeding to calves, colostrum quality was verified using colostrometer and only good quality colostrum was fed to calves (≥ 50 g/L of IgG). Thereafter, calves were fed 5 L of MR/day (2 $\times$ 2.5 L; 7:00 and 17:00 h; Sprayfo Excellent, Trouw Nutrition, Grodzisk Mazowiecki, Poland; 22.5% of CP and 18% of crude fat in the MR powder; 150 g of MR powder in 1 L of MR solution) using a bucket with a nipple until allocated to the study. Calves had also access to the starter feed (pelleted starter mixture; Blattin Kälber Starter, Blattin Polska Sp. z o.o., Siedlec, Poland; 16% CP and 15% NDF in 1 kg of feed). Between 24 and 96 h of life, blood samples were collected by a veterinarian, and serum total protein was determined using

a refractometer (Michelsen et al., 2025; Wilm et al., 2018) as a part of the standard operating procedures adopted on the farm.

Table 2. Ingredient and chemical composition of pelleted starter mixtures (mean $\pm$ standard deviation) – study 2

	Treatments ^a			
	LOW	LOW+	HIGH	HIGH+
Ingredient (% as fed)				
canola meal	10.0	10.0	32.0	32.0
soybean meal	14.0	14.0	–	–
barley grain	25.97	25.87	10.97	10.87
corn grain	15.5	15.5	22.0	22.0
wheat	16.0	16.0	19.0	19.0
wheat bran	8.0	8.0	6.0	6.0
whey protein (dry)	2.5	2.5	2.5	2.5
mineral-vitamin supplement ^b	1.0	1.0	1.0	1.0
glycerol	3.0	3.0	3.0	3.0
molasses	1.0	1.0	1.0	1.0
monocalcium phosphate	0.5	0.5	–	–
limestone	2.0	2.0	2.0	2.0
sodium chloride	0.5	0.5	0.5	0.5
aroma	0.03	0.03	0.03	0.03
enzyme ^c	–	0.1	–	0.1
Chemical composition ^d				
DM, g/kg	897 $\pm$ 1.9	895 $\pm$ 2.8	895 $\pm$ 1.8	893 $\pm$ 2.3
ash, g/kg DM	77 $\pm$ 2.3	78 $\pm$ 2.5	78 $\pm$ 2.1	76 $\pm$ 3.8
crude protein, g/kg DM	191 $\pm$ 3.3	180 $\pm$ 1.7	184 $\pm$ 10.0	188 $\pm$ 11.0
aNDF, g/kg DM	153 $\pm$ 11.1	155 $\pm$ 9.0	184 $\pm$ 8.6	182 $\pm$ 6.0
ADF, g/kg DM	69 $\pm$ 3.0	72 $\pm$ 5.1	105 $\pm$ 2.6	106 $\pm$ 3.3
starch, g/kg DM	442 $\pm$ 0.8	441 $\pm$ 3.3	407 $\pm$ 1.1	401 $\pm$ 1.3

^aLOW = low canola meal inclusion in the starter mixture; LOW+ = low canola meal inclusion in starter mixture + enzyme; HIGH = high canola meal inclusion in the starter mixture; HIGH+ = high canola meal inclusion in starter mixture + enzyme.

^bBlattin Super Premium (Blattin Polska Sp. z o.o., Izbičko, Polska): 18.3% Ca; 3% P; 8.5% Na; 5.5% Mg; 11,500 mg Zn; 5,000 mg Mn; 1,500 mg Cu; 27 mg Co; 83 mg I; 87 mg Se; 1,000,000 IU vitamin A; 160,000 IU vitamin D; 5,000 mg vitamin E; 20 mg vitamin B₁; 30 mg vitamin B₂; 2,156 mg vitamin B₃; 67 mg vitamin B₅; 100,000 µg vitamin B₆; 4 mg vitamin B₉; 25 mg vitamin B₁₂; 250 mg vitamin B₁₂.

^cSuperzyme-CS (CBS Bio Platforms, Canada; containing: xylanase (1,300 U/g), glucanase (150 U/g), invertase (700 U/g), protease (6,000 U/g), cellulase (800 U/g), amylase (12,000 U/g) and mannanase (5 U/g)).

^dn=3.

Housing conditions during the study period were the same as in study 1. Beginning on the first day of the study, calves were fed 6 L of MR/day (2 $\times$ 3 L; Sprayfo Yellow, Trouw Nutrition; 21.5% CP and 17.5% crude fat in the MR powder; 150 g of MR powder in 1 L of MR solution). The MR was fed in equal quantities from open buckets

two times a day (7:00 and 17:00 h). On the first day of the study, 0.5 kg of the respective starter mixture was added to the feeder. Once this amount was consumed, another 0.5 kg was added, and when this amount was fully consumed over two consecutive days, the amount added to the feeder was increased to 1 kg, and subsequently to 2 kg, and 3 kg to ensure starter was available. Therefore, daily starter intake was not monitored but consumption over the whole study period (amount added to the feeder) was recorded (Górka et al., 2023). This procedure also ensured that starter mixture was fed *ad libitum*. If the feed was dirty or spoiled, it was removed and weighed. Feeders were also routinely cleaned and feed was replaced once per week (Friday). The amount of feed remaining in the feeder was weighed on the last day of the study. Water was available at all times. Calves were monitored over a period of 63 days.

Feeding and housing of calves after transportation to the calf facility followed the standard procedures used for calves in Top Farms Głubczyce Sp. z o.o. during the study period. The only exception was the intentional modifications made to the composition of the pelleted starter mixtures. Thus, the maintenance of the calves resembled typical practical conditions.

Measurements, observations and medical treatments

Calves were weighed on the first day of the study (initial body weight) and subsequently every three weeks (day 21, 42, and 63 of the study), always at the same hour of the day (11:00 h) by farm staff. The MR intake was monitored daily, and intake of starter was measured over the whole study period, as described above. The fecal scores (fluidity) were recorded daily as described in study 1. Calves with a fecal score ≥ 2 were considered to have diarrhea and had reduced MR feeding to one feeding/day. In the second feeding, those calves were offered water mixed with a feed supplement containing pectin, vitamins, minerals, and a probiotic (PECTOPRO, Over Vet, Koszalin, Poland) until the fecal score returned to normal. In cases of a persisting fecal score ≥ 3 , severe diarrhea (fecal score 4) or dehydration, medical treatment was administered on an individual basis, following the veterinarian's recommendations. Each medical treatment was recorded.

Feed samples and analysis

Samples of experimental starters were collected weekly, then pooled by month of the study, and analyzed for DM, ash, CP, crude fat, aNDF, and ADF, as described in study 1. Starch content was determined using the polarimetric method in line with the recommendation of the European Union Commission Regulation (No. 152/2009). Starters were also analyzed for xylanase activity, which served as an indicator of appropriate feed mixing and enzyme recovery. For that purpose, a pooled sample of each pelleted starter, representative for the whole study duration, was sent to CBS Bio Platforms (Calgary, Canada). The analysis of xylanase in feed was conducted using a

modified method based on the suggested Megazyme Xylanase tablets T-XAX procedure. This assay procedure measures xylanase activity by utilizing insoluble, dyed, crosslinked arabinoxylan as the substrate.

Data processing and statistical analysis

Prior to analysis, cumulative MR intake over the whole study period was calculated and results for fecal score were averaged by week of the study. Initial age of calves, total serum protein, BW, ADG, and feed efficiency (gain to feed ratio) were analyzed as a randomized complete block design using PROC MIXED of SAS (version 9.4, SAS Institute, Cary, NC, USA). Prior to data analysis, the normality of the data and homogeneity of variance were tested using PROC UNIVARIATE of SAS. The statistical model included the effect of CM inclusion in the starter mixture, feed enzyme inclusion, the interaction between those effects, and the effect of the farm, as fixed effects. The effect of the block of calves was included in the model as a random effect and initial age as a covariate. The statistical model for repeated measures also included the effect of time and its interaction with the main effects as fixed effects (Littell et al., 1998). The effect of mother status (primi- or multiparous) was also considered in the model as a fixed effect but was not significant ($P > 0.05$) and was removed from the model. The optimal covariance structure (autoregressive order one, unstructured or compound symmetry) was chosen based on Akaike's information criterion. For BW, ADG, starter intake, and feed efficiency analysis, initial BW was included in the model as a covariate. Fecal score data were analyzed using PROC GLIMMIX of SAS using Poisson distribution and AR(1) type covariance structure. The frequency and duration of diarrhea (fecal score ≥ 3 and/or ≥ 2) and frequency of medical treatments (including diarrhea and respiratory diseases) were analyzed using PROC GENMOD of SAS with a Poisson distribution. The occurrence of diarrhea (fecal score ≥ 3) and medical treatment were tested by logistic regression using a binomial distribution and PROC GLIMMIX of SAS. The same effects were considered in the model as for other variables. Significance was declared when $P \leq 0.05$, and a tendency was declared when $0.05 < P \leq 0.10$.

Study 3

The study was conducted between August 18 and December 14, 2022. The temperature averaged $20.1 \pm 2.90^\circ\text{C}$ (mean $\pm$ SD) and $-0.5 \pm 3.83^\circ\text{C}$ during the hottest (August) and the coldest (December) months of the study, respectively. Temperatures were recorded at a research station located approximately 30 km away from the calf barns (<https://danepubliczne.imgw.pl>; Institute of Meteorology and Water Management, National Research Institute, Warsaw, Poland).

Animals, treatments, housing and feeding

One hundred and twenty female Holstein calves (42.6 ± 4.4 kg, 17.2 ± 2.1 days of age, 6.6 ± 0.8 g/dL of total

serum protein) were allocated to one of four treatments (30 calves/treatment) and fed: 1) a pelleted starter mixture containing SBM as a main source of protein (CTRL); 2) a pelleted starter mixture in which SBM was partially replaced with wheat bran and corn DDGS (TRBP); 3) a pelleted starter mixture in which SBM was partially replaced by CM (TRCM); or 4) TRCM supplemented with feed enzymes (Superzyme-CS, CBS Bio Platforms, Canada; 1 g/kg; TRCM+). With these treatments, the effect of CM inclusion in the starter mixture could be compared with other high-protein by-products commonly used to partially or fully replace SBM protein. Starters were formulated to ensure that SBM inclusion in the TRBP and TRCM starters were the same to limit conclusions to the effect of the investigated SBM replacements. Furthermore, the effect of including an enzyme mixture

(xylanase (1,300 U/g), glucanase (150 U/g), invertase (700 U/g), protease (6,000 U/g), cellulase (800 U/g), amylase (12,000 U/g) and mannanase (5 U/g); Superzyme-CS, CBS Bio Platforms) in a pelleted starter containing CM was implemented to determine reproducibility of results obtained in study 2. Starters were formulated to be similar for CP and starch content (Table 3).

As described for studies 1 and 2, calves were sourced from two dairy farms belonging to Top Farms Głubczyce Sp. z o.o. Calves were allocated to treatments between 14 and 21 days of age and transported to the calf barn once a week (Thursday). All calves were collected over a period of nine weeks, which resulted in nine blocks of 12, 12, 16, 12, 8, 12, 12, 24, and 12 calves each. The allocation of calves to treatments resembled that described in study 2.

Table 3. Ingredient and chemical composition of pelleted starter mixtures (mean±standard deviation) – study 3

	Treatments ^a			
	CTRL	TRBP	TRCM	TRCM+
Ingredient (% as fed)				
soybean meal	22.0	17.0	17.0	17.0
canola meal	–	–	7.5	7.5
wheat bran	5.0	9.0	5.0	5.0
DDGS	–	9.0	–	–
barley	27.72	12.97	20.47	20.37
corn	15.0	27.0	20.0	20.0
wheat	20.0	15.0	20.0	20.0
whey	2.5	2.5	2.5	2.5
mineral-vitamin supplement ^b	1.0	1.0	1.0	1.0
glycerol	3.0	3.0	3.0	3.0
molasses	1.0	1.0	1.0	1.0
monocalcium phosphate	0.25	–	–	–
limestone	2.0	2.0	2.0	2.0
sodium chloride	0.5	0.5	0.5	0.5
aroma	0.03	0.03	0.03	0.03
enzyme ^c	–	–	–	0.1
Chemical composition ^d				
DM, g/kg	891±1.0	896±4.3	893±1.0	891±3.3
ash, g/kg DM	75±0.2	74±1.3	73±1.5	77±0.4
crude protein, g/kg DM	195±4.6	196±7.6	191±2.5	192±0.2
aNDF, g/kg DM	143±3.3	163±5.9	152±0.6	145±2.1
ADF, g/kg DM	64±0.7	66±2.5	70±2.4	67±1.6
starch, g/kg DM	451±0.4	446±2.1	443±1.7	445±6.9

^aCTRL = starter mixture containing soybean meal as a main source of protein; TRBP = starter mixture in which soybean meal was partially replaced by wheat bran and DDGS; TRCM = starter mixture in which soybean meal was partially replaced by canola meal; TRCM+ = TRCM supplemented with feed enzymes.

^bBlattin Super Premium (Blattin Polska Sp. z o.o., Izbičko, Polska): 18.3% Ca; 3% P; 8.5% Na; 5.5% Mg; 11,500 mg Zn; 5,000 mg Mn; 1,500 mg Cu; 27 mg Co; 83 mg I; 87 mg Se; 1,000,000 IU vitamin A; 160,000 IU vitamin D; 5,000 mg vitamin E; 20 mg vitamin B₁; 30 mg vitamin B₂; 2,156 mg vitamin B₃; 67 mg vitamin B₅; 100,000 µg vitamin B₆; 4 mg vitamin B₉; 25 mg vitamin B₁₂; 250 mg vitamin B₁₂.

^cSuperzyme-CS (CBS Bio Platforms, Canada; containing: xylanase (1,300 U/g), glucanase (150 U/g), invertase (700 U/g), protease (6,000 U/g), cellulase (800 U/g), amylase (12,000 U/g) and mannanase (5 U/g)).

^dn=4.

Prior to the initiation of the study, the calves were maintained and fed as described for study 2, with the exception that starter mixture was not offered as this was initiated on the first day of the study. Housing conditions during the study period were the same as in study 1 and 2, and MR feeding and starter feeding were the same as in study 2 as well as blood sampling procedure and analysis. Calves remained in the study and were monitored over a period of 63 days.

Measurements, observations and medical treatments

The BW of calves, MR intake, starter intake, and fecal score were monitored as in study 2. Calves with elevated fecal scores (>2) were treated as described in study 2. In cases of a persisting fecal score ≥ 3 , severe diarrhea (fecal score 4) or dehydration, the calves were treated according to a veterinarian's recommendations. Each medical treatment was recorded.

Feed samples and analysis

Samples of the experimental starters were collected and analyzed as described for study 2, which included analysis of xylanase activity in the experimental starters.

Data processing and statistical analysis

Data were processed and analyzed as described for study 2. The statistical model included the effect of treatment, the effect of farm of birth, and the interaction as fixed effects. The effect of block of calves was included in the model as a fixed effect, initial age as a covariate, and initial BW as a covariate for growth parameters and feed efficiency. Similar to that for study 2, the effect of parity of the dam (primi- or multiparous) was also considered as a fixed effect but was not significant ($P > 0.05$) and was removed from the model. Pre-planned contrasts were used to evaluate the effect of replacing SBM with other high-protein by-products (CTRL vs. TRBP and TRCM), to compare the use of wheat bran and corn DDGS to CM (TRBP vs. TRCM), and to evaluate whether enzyme addition improved responses (TRCM vs. TRCM+). Significance was declared when $P \leq 0.05$, and a tendency was declared when $0.05 < P \leq 0.10$.

Study 4

The study was conducted between January 22 and May 1, 2024. The temperature averaged $11.3 \pm 5.18^\circ\text{C}$ (mean $\pm$ SD) and $3.7 \pm 1.56^\circ\text{C}$ during the hottest (April) and the coldest (January) months of the study, respectively. Temperatures were recorded at a research station located approximately 30 km away from the calf barns (<https://danepubliczne.imgw.pl>; Institute of Meteorology and Water Management, National Research Institute, Warsaw, Poland).

Animals, treatments, housing and feeding

One hundred and twenty female Holstein calves (44.1 ± 4.9 kg, 18.3 ± 1.9 days of age, 6.2 ± 0.5 g/dL of total

serum protein) were allocated to one of four treatments (30 calves/treatment) and fed: 1) a pelleted starter mixture with moderate CM inclusion (24% CM in the starter mixture; MC); 2) a pelleted starter mixture with high CM inclusion (34% CM in the starter mixture; HC); 3) a pelleted starter mixture with moderate inclusion of extruded CM (MEC); or 4) a pelleted starter mixture with high inclusion of extruded CM (HEC). The inclusion of CM fed to MC and MEC treatments was based on the assumption that it would have a negative effect on the rearing of calves (Górka and Penner, 2020). Thus, MC and MEC treatments in the experimental design were to assess whether the inclusion of extruded CM in calf starter mixture results in better effects than the use of non-extruded CM. In turn, the high inclusion of CM fed to HC and HEC treatments was expected to have a negative impact on calf performance (Górka and Penner, 2020). Therefore, the presence of HC and HEC treatments in the experimental design enabled to determine whether the use of extruded CM in a starter mixture allows for high CM inclusion without adversely affecting the performance of calves.

The CM was extruded using Amandus Kahl Expander OEK 23.2 (Amandus Kahl GmbH & Co. KG, Reinbek, Germany), which exposed the CM to 40 to 60 bar of pressure, a temperature of 160 to 180°C , 15 to 20% humidity, with an extrusion time of 15 to 20 s. For all starters, CM from the same batch was used and starters were formulated to be similar in CP concentration (Table 4). Feeds used for starter formulation and production originated from the same batch.

As described for the other studies, calves were sourced from two dairy farms belonging to Top Farms Głubczyce Sp. z o.o. Calves were allocated to treatments between 14 to 21 days of age and transported to the calf barn once a week (Thursday). All calves were collected over a period of 10 weeks, which resulted in 10 blocks of 12, 12, 8, 8, 8, 20, 20, 14, 4, and 14 calves each. Allocation of calves to treatments followed that described in studies 2 and 3.

Prior to the initiation of the study, the calves were maintained and fed as described for study 3. Housing conditions during the study period were the same as in previous studies. The MR and starter feeding and blood sampling and analysis were also the same as in studies 2 and 3, except that in the first week of the study, calves were fed 4 L of MR (2×2 L). Calves were monitored over a period of 63 days.

Measurements, observations and medical treatments

The BW, MR intake, starter intake and fecal score were monitored as described for studies 2 and 3. Calves with elevated fecal scores (>2) were treated as described in studies 2 and 3. In cases of a persisting fecal score ≥ 3 , severe diarrhea (fecal score 4) or dehydration, the calves were treated according to a veterinarian's recommendations. Each medical treatment was recorded.

Feed samples and analysis

Samples of experimental starters were collected and analyzed as described for studies 2 and 3.

Data processing and statistical analysis

Data were processed and analyzed as described for studies 2 and 3. The statistical model included the effect of CM inclusion, the effect of CM processing, and the interaction as fixed effects. The effect of block of calves was included in the model as a fixed effect, initial age as a covariate, and initial BW as a covariate for growth parameters and feed efficiency. Similarly, as for study 2, the effect of parity of the dam (primi- or multiparous) was also considered in the model as a fixed effect. Parity was insignificant ($P>0.05$) and removed from the model. Also, the effect of the farm and its interaction with other fixed effects were tested in the model, but those effects were not significant ($P>0.05$) and were removed from the model. Significance was declared when $P\leq 0.05$, and a tendency was declared when $0.05<P\leq 0.10$.

Results**Study 1**

Ingredient and chemical composition of experimental pelleted starters used in study 1 are presented in Table 1. The concentration of CP was slightly less for TMC compared to other treatments and aNDF and ADF concentrations were numerically higher for TCM and TCML than for TSBM. Lys concentration was higher for TSBM than for TCM, and Lys supplementation resulted in the highest Lys concentration for TCML. However, Lys/Met ratio was less for TCML compared to TSBM, although a similar ratio of those amino acids was targeted for those 2 treatments. This was due to the highest Met concentration for TCML, which was unexpected. Of the other amino acids, Ser concentration was also unexpectedly less for TCML compared to TCM.

By accident, the observation card for one calf from block 2 was lost, and thus, one sub-block of calves was removed from the statistical analysis. Consequently, data from 14 calves/treatment were analyzed.

Table 4. Ingredient and chemical composition of pelleted starter mixtures (mean±standard deviation) – study 4

	Treatments ^a			
	MC	HC	MEC	HEC
Ingredient (% as fed)				
canola meal	24.0	34.0	–	–
extruded canola meal	–	–	24.0	34.0
soybean meal	7.0	–	7.0	–
barley grain	15.71	13.0	15.71	13.0
corn grain	22.0	22.0	22.0	22.0
wheat	20.0	20.0	20.0	20.0
wheat bran	5.0	5.0	5.0	5.0
molasses	3.0	3.0	3.0	3.0
mineral-vitamin supplement ^b	0.2	0.2	0.2	0.2
monocalcium phosphate	0.25	–	0.25	–
limestone	2.0	2.0	2.0	2.0
sodium chloride	0.7	0.7	0.7	0.7
aroma	0.1	0.1	0.1	0.1
magnesium oxide	0.4	–	0.4	–
Chemical composition ^c				
DM	875±2.8	877±1.2	878±4.6	879±4.5
ash, g/kg DM	75±5.2	71±2.8	75±1.9	75±1.1
crude protein, g/kg DM	231±3.7	225±3.7	212±7.8	230±5.9
aNDF, g/kg DM	140±6.6	169±12.2	140±15.0	151±18.5
ADF, g/kg DM	54±18.1	72±7.4	78±34.8	94±37.1
starch, g/kg DM	385±22.1	349±27.8	377±20.0	346±13.0

^aMC = moderate inclusion of canola meal in the starter mixture; HC = high inclusion of canola meal in the starter mixture; MEC = moderate inclusion of extruded canola meal in the starter mixture; HEC = high inclusion of extruded canola meal in the starter mixture.

^b45,000 mg Zn; 30,000 mg Mn; 8,000 mg Cu; 800 mg I; 200 mg Se; 40,000 mg Fe; 6,400,000 IU vitamin A; 2,200,000 IU vitamin D; 20,000 mg vitamin E; 400 mg vitamin K; 8,000 mg vitamin B₁; 1,500 mg vitamin B₂; 6,000 mg vitamin B₃; 3,000 mg vitamin B₅; 6,000 mg vitamin B₆; 40 mg vitamin B₇; 200 mg vitamin B₉; 2 mg vitamin B₁₂; 20,000 mg choline.

^cn=3.

Table 5. Initial age, BW, ADG, feed intake, feed efficiency and fecal score of calves – study 1

	Treatments ^a			SE ^b	Contrast P-value ^c	
	TSBM	TCM	TCML		1	2
n	14	14	14			
Initial age, days	24.0	24.1	24.3	0.86	0.39	0.49
BW, kg						
day ^d 1	44.4	44.2	45.6	1.49	0.70	0.38
day 49	82.2	81.0	82.1	2.48	0.82	0.73
day 70	110.5	106.6	108.6	3.23	0.43	0.63
ADG, g/day						
day 1 to 49	762	755	743	38.5	0.76	0.81
day 50 to 70	1311	1222	1257	68.3	0.36	0.70
day 1 to 70	927	896	898	41.5	0.53	0.97
Milk replacer intake, g of powder/day						
day 1 to 49	886	886	886	–	–	–
day 50 to 70	363	363	363	–	–	–
Starter intake, g DM/day						
day 1 to 49 ^e	452	525	515	57.6	0.27	0.81
day 50 to 70 ^e	2408	2412	2487	126.8	0.71	0.76
day 1 to 70 ^e	1042	1093	1105	82.2	0.29	0.89
Feed efficiency, g gain/kg DM						
day 1 to 49	583	549	548	22.7	0.06	0.95
day 50 to 70	500	460	463	14.8	0.01	0.81
day 1 to 70	543	507	508	10.8	< 0.01	0.94
Fecal score						
day 1 to 49 ^e	1.17	1.13	1.19	0.034	0.84	0.20
day 50 to 70	1.05	1.08	1.05	0.025	0.72	0.41
day 1 to 70 ^e	1.29	1.11	1.14	0.026	0.95	0.38

^aTSBM = starter mixture containing soybean meal as the main source of protein; TCM = starter mixture containing canola meal as the main source of protein; TCML = starter mixture containing canola meal as the main source of protein and additionally supplemented with rumen-unprotected Lys.

^bStandard error or standard error of the mean.

^c1 = TSBM vs. TCM and TCML; 2 = TCM vs. TCML.

^dDay of the study.

^eSignificant time effect ($P \leq 0.05$).

There were no refusals of MR, and thus, MR intake was not analyzed statistically. Moreover, the frequency and duration of diarrhea and frequency of medical treatments were not analyzed in study 1, because of low statistical power for those parameters due to the low number of calves used for the study and the few instances where calves required medical treatment.

Initial BW, BW at weaning (day 49), final BW, ADG, starter intake, and fecal score did not differ among treatments ($P \geq 0.20$; Table 5). However, feed efficiency tended ($P = 0.06$) to be greater for TSBM than for TCM and TCML pre-weaning, and was greater ($P \leq 0.01$) for TSBM than for TCM and TCML during the weaning transition and in the whole study period. No differences between TCM and TCML were detected.

Study 2

Ingredient and chemical compositions of experimental pelleted starters used in study 2 are presented in Table 2. The concentration of CP was comparable among treatments, but aNDF and ADF concentrations were higher for HIGH than for LOW treatments, and the starch

concentrations were higher for LOW than for HIGH treatments. Xylanase activities in starters fed to LOW+ and HIGH+ treatments were 1,371 and 1,459 U/kg and were comparable with the expected activity values based on enzyme inclusion $\geq 1,300$ U/kg (data not presented). In starters fed to LOW and HIGH treatments xylanase activity was ≤ 138 U/kg.

No interactions between main effects were detected ($P \geq 0.27$). Initial age, total serum protein, initial BW, cumulative MR intake, fecal score, and feed efficiency did not differ among treatments ($P \geq 0.15$; Table 6). However, ADG, final BW, and cumulative starter intake were greater for treatments with exogenous enzymes ($P \leq 0.05$). Furthermore, enzyme inclusion reduced the number of days with diarrhea, both when diarrhea was defined based on a fecal score ≥ 2 or ≥ 3 ($P \leq 0.04$). Additionally, the number of days with diarrhea (fecal score ≥ 2) was lower ($P < 0.01$) and the number of calves with diarrhea tended to be lower ($P = 0.10$) for treatments fed starter mixtures with high CM inclusion. The likelihood of developing diarrhea and medical treatment did not differ among treatments ($P \geq 0.23$; Supplementary Table S1).

Table 6. Initial age, total serum protein, BW, ADG, feed intake, feed efficiency, fecal score and health indicators of calves – study 2

	Treatments ^a				SE ^b	Main effect P-value		
	LOW	LOW+	HIGH	HIGH+		canola inclusion	enzyme	interaction
n	25	25	25	25				
Initial age, days	17.7	17.4	17.6	18.0	0.42	0.45	0.92	0.41
Total serum protein, g/dL ^c	6.61	6.52	6.46	6.37	0.158	0.26	0.50	0.96
Cumulative milk replacer intake, kg DM ^c	51.9	52.1	52.3	52.3	0.19	0.16	0.75	0.66
Cumulative starter intake, kg DM ^c	41.5	39.6	48.0	44.1	2.30	0.12	< 0.01	0.58
Initial BW, kg	44.6	44.5	44.4	44.5	1.09	0.90	0.99	0.92
Final BW, kg	97.2	99.9	96.9	99.7	1.78	0.82	0.05	0.91
ADG, g/day ^c	843	881	836	879	27.5	0.81	0.04	0.91
Feed efficiency, g gain/kg DM ^d	567	555	573	576	14.1	0.15	0.63	0.43
Fecal score ^e	1.19	1.16	1.15	1.13	0.022	0.15	0.20	0.89
Diarrhea, days ^f	1.30	1.08	1.23	0.67	0.203	0.16	0.04	0.27
Diarrhea, days ^g	10.28	8.89	8.36	7.11	0.590	< 0.01	0.02	0.90
Diarrhea, n/calf	0.78	0.70	0.67	0.36	0.158	0.10	0.16	0.32
Medical treatments, n/calf	0.72	1.04	0.96	1.16	0.200	0.33	0.17	0.67

^aLOW = low canola meal inclusion in the starter mixture; LOW+ = low canola meal inclusion in starter mixture + enzyme; HIGH = high canola meal inclusion in the starter mixture; HIGH+ = high canola meal inclusion in starter mixture + enzyme.

^bStandard error or standard error of the mean.

^cTendency to significant effect of farm (0.05 < P ≤ 0.10).

^dSignificant effect of farm (P ≤ 0.05).

^eSignificant effect of time (P < 0.01).

^fFecal score ≥ 3.

^gFecal score ≥ 2.

Table 7. Initial age, total serum protein, BW, ADG, feed intake, feed efficiency, fecal score and health indicators of calves – study 3

	Treatments ^a				SE ^b	Contrast P-value ^c		
	CTRL	TRBP	TRCM	TRCM+		1	2	3
n	30	30	30	30				
Initial age, days	17.2	17.4	17.3	17.0	0.40	0.78	0.87	0.65
Total serum protein, g/dL ^d	6.57	6.56	6.80	6.58	0.139	0.47	0.18	0.22
Cumulative milk replacer intake, kg DM	48.7	49.1	49.4	49.1	0.20	0.03	0.16	0.25
Cumulative starter intake, kg DM	42.1	40.2	39.6	40.0	2.86	0.38	0.84	0.90
Initial BW, kg	42.9	42.9	43.0	43.0	0.83	0.93	0.90	0.98
Final BW, kg	94.0	91.6	91.7	91.3	1.62	0.10	0.96	0.83
ADG, g/day ^e	815	777	779	773	26.2	0.12	0.96	0.83
Feed efficiency, g gain/kg DM	566	550	557	550	10.3	0.15	0.53	0.49
Fecal score ^e	1.31	1.26	1.23	1.25	0.234	< 0.01	0.36	0.54
Diarrhea, days ^{f,g}	1.96	1.33	1.13	1.40	0.218	< 0.01	0.48	0.32
Diarrhea, days ^h	18.0	15.2	13.5	14.8	0.73	< 0.01	0.09	0.21
Diarrhea, n/calf	1.09	0.87	0.56	0.87	0.167	0.05	0.15	0.15
Medical treatments, n/calf ⁱ	0.47	0.60	0.61	0.78	0.145	0.38	0.95	0.37

^aCTRL = starter mixture containing soybean meal as a main source of protein; TRBP = starter mixture in which soybean meal was partially replaced by wheat bran and DDGS; TRCM = starter mixture in which soybean meal was partially replaced by canola meal; TRCM+ = TRCM supplemented with feed enzymes.

^bStandard error or standard error of the mean.

^c1 = CTRL vs. TRBP and TRCM; 2 = TRBP vs. TRCM; 3 = TRCM vs. TRCM+.

^dSignificant effect of farm (P ≤ 0.05).

^eSignificant effect of time (P < 0.01).

^fFecal score ≥ 3.

^gSignificant effect of farm and treatment × farm interaction (P ≤ 0.05).

^hFecal score ≥ 2.

Table 8. Initial age, total serum protein, BW, ADG, feed intake, feed efficiency, fecal score and health indicators of calves – study 4

	Treatments ^a				SE ^b	Main effect P-value		
	MC	HC	MEC	HEC		inclusion	processing	interaction
n	29	30	30	29				
Initial age, days	18.2	18.4	18.3	18.4	0.43	0.72	0.79	0.84
Total serum protein, g/dL	6.12	6.21	6.25	6.19	0.107	0.58	0.84	0.48
Cumulative milk replacer intake, kg DM	50.6	50.5	50.6	50.8	0.17	0.63	0.35	0.24
Cumulative starter intake, kg DM	39.4	35.1	39.7	39.2	3.31	0.31	0.34	0.42
Initial BW, kg	42.4	42.5	42.3	42.6	1.04	0.82	0.98	0.89
Final BW, kg	90.9	89.7	91.9	92.0	1.79	0.62	0.23	0.63
ADG, g/day ^c	771	749	786	790	28.2	0.67	0.17	0.54
Feed efficiency, g gain/kg DM	542	553	553	557	9.46	0.37	0.32	0.63
Fecal score ^c	1.09	1.09	1.07	1.06	0.014	0.63	0.10	0.53
Diarrhea, days ^d	4.98	5.15	4.07	3.43	0.405	0.44	< 0.01	0.25
Diarrhea, days ^e	0.58	0.68	0.70	0.48	0.148	0.61	0.72	0.23
Diarrhea, n/calf	0.38	0.50	0.43	0.23	0.118	0.56	0.28	0.13
Medical treatments, n/calf	0.043	0.010	0.028	0.040	310.1	0.15	0.22	0.02

^aMC = moderate inclusion of canola meal in the starter mixture; HC = high inclusion of canola meal in the starter mixture; MEC = moderate inclusion of extruded canola meal in the starter mixture; HEC = high inclusion of extruded canola meal in the starter mixture.

^bStandard error or standard error of the mean.

^cSignificant effect of time ($P < 0.01$).

^dFecal score ≥ 3 .

^eFecal score ≥ 2 .

Study 3

Ingredient and chemical compositions of experimental pelleted starters used in study 3 are presented in Table 3. The concentration of CP and starch were comparable for all treatments, but aNDF concentration was highest for TRBP. Xylanase activity in the starter fed to TRCM+ was 1,540 U/kg and was within expected activity based on enzyme inclusion ($\geq 1,300$ U/kg; data not presented). In starters fed to other treatments xylanase activity was ≤ 146 U/kg.

Initial age, total serum protein, initial BW, feed efficiency, and the number of medical treatments did not differ among treatments ($P \geq 0.15$; Table 7). Cumulative MR intake was lower for CTRL than for TRBP and TRCM ($P = 0.03$) but did not differ between TRBP and TRCM, nor did it differ between TRCM and TRCML ($P \geq 0.16$). Final BW tended to be higher for CTRL than for TRBP and TRCM ($P = 0.10$) but did not differ between TRBP and TRRCM or between TCM and TRCML ($P \geq 0.83$); however, ADG was not different between treatments despite a numeric difference between CTRL and TRBP and TRCM ($P = 0.12$). Fecal score, number of days with diarrhea (fecal score ≥ 3), and the number of diarrhea episodes were higher for CTRL than for TRBP and TRCM ($P \leq 0.05$) but did not differ between TRBP and TRCM or between TRCM and TRCML ($P \geq 0.32$). Moreover, the number of days with diarrhea (fecal score ≥ 2) was higher for CTRL than for TRBP and TRCM ($P < 0.01$) and tended ($P = 0.09$) to be higher for TRBP than for TRCM, but did not differ between TRCM and TRCML ($P = 0.21$). The likelihood of developing diarrhea tended ($P \leq 0.08$) to

be higher for CTRL compared to TRBP and TRCM (Supplementary Table S2).

Study 4

Ingredient and chemical compositions of the pelleted starters used in study 4 are presented in Table 4. The concentration of CP was comparable for all treatments, whereas aNDF and ADF concentrations were higher, and starch concentration was lower for HC and HEC than for MC and MEC.

One MC and one HEC calf died during the first stages of the study and those calves were not replaced. Initial age, total serum protein, initial and final BW, feed intake, ADG, feed efficiency, and the number of diarrhea episodes did not differ among treatments ($P \geq 0.13$; Table 8). Fecal score tended to be reduced ($P = 0.10$) and the number of days with diarrhea (fecal score ≥ 2) was ($P < 0.01$) reduced when extruded CM was used in the starter mixture. The number of medical treatments was the lowest for HC, intermediate for MEC, and the highest for MC and HEC (interaction between main effects, $P = 0.02$). The likelihood of medical treatment was also lower for HC than for CTRL ($P = 0.02$; Supplementary Table S3).

Discussion

Although the primary objective of the conducted studies was to evaluate strategies that may allow for greater utilization of CM in pelleted starters for calves, the effect of CM inclusion warrants discussion. While

CM inclusion in pelleted calf starters at inclusion rates that are greater than 20–25% can negatively affect the performance of calves (Górka and Penner, 2020), negative impacts have not been shown in all studies. For example, Burakowska et al. (2021 b) reported the results of two studies that compared full replacement of SBM with CM in pelleted starter mixtures, which resulted in CM inclusion rates that were greater than 30%. In those studies, only one study detected a negative impact on calf growth. Similarly, of the studies in this paper that evaluated low or moderate to high inclusion rates of CM (studies 2 and 4), no negative impacts on growth performance of calves were detected. Furthermore, in the two other studies (studies 1 and 3), negative impacts were only detected for feed efficiency or a tendency for lighter final BW of calves. Of importance, CM inclusion in starter mixtures was repeatedly shown to reduce fecal score and consequently the number of days with diarrhea, and the probability of medical treatments, which are consistent with past research (Burakowska et al., 2021 b). It should be noted that there were no CM-free treatments for half of the studies in the present manuscript, which temper the conclusions regarding the efficacy of CM use. That said, these studies are interpreted to indicate that even full replacement of SBM with CM in a pelleted starter mixture may not necessarily compromise the performance of calves.

It should be acknowledged that in addition to the lack of a negative control, some of the management practices imposed may have impacted the outcomes. All studies were conducted on one commercial farm, which may not necessarily represent variation of industry practices. Furthermore, in most of the studies presented in this paper, calves had no access to solid feed during the first 2 to 3 weeks of life (i.e. prior to allocation to the study). While this practice may not be uncommon (Echeverry Munera et al., 2024; Hadam et al., 2016; Laarman et al., 2012; Urie et al., 2018), the gastrointestinal tract of calves is sensitive in first days of life (Fischer et al., 2019). Consequently, negative effects of CM intake, if any, could be expected to be more pronounced in the first weeks of calves' life (Górka and Penner, 2020; Hadam et al., 2016), and those could be overlooked as were not possible to observe in the current studies. Furthermore, it is clear that conditions on farm (e.g. hygienic conditions and exposure to pathogens) are not stable over a longer period of time, as confirmed by the significant effect of the farm from which calves were selected as well as the interaction between the effect of the farm and treatment in some but not all of the conducted studies. Consequently, results of one study conducted on the same farm may be not reproduced, as shown previously (Górka et al., 2023; Milik et al., 2023). However, consistency in the response to CM in the four studies presented in this manuscript highlight a repeatable response, despite use of a single farm for the research.

The lower concentration of Lys in solid feed when CM is the main source of protein relative to SBM can

be considered a confounding factor in many experiments evaluating CM inclusion (Górka and Penner, 2020). In the present study, we set out to test whether the provision of unprotected Lys would alter growth and starter intake for calves fed starters based on CM as the main protein source. Unprotected Lys was used to mimic availability of Lys derived with feed in the rumen as would be the partially similar to that with SBM. Results were interpreted to indicate that the addition of unprotected Lys had no effect on BW, ADG, starter intake, feed efficiency, or fecal scores. This result could be expected given the low starter intake in the first weeks of life and the fact that Lys consumed with liquid feed is the most important source of Lys flowing to the small intestine of calves (Lallés et al., 1990; Leibholz, 1978). Furthermore, as solid feed intake increases and the rumen develops, degradation of feed protein in the rumen and microbial protein flow to the small intestine increase (Lalles and Poncet, 1990; Leibholz, 1975; Quigley et al., 1985), with microbial protein flow having an amino acid composition in calves that is largely independent of the solid feed composition (Quigley et al., 1985). Moreover, with increasing age, Met, not Lys, becomes the most limiting amino acid for calves (Donahue et al., 1985), and studies in which concentrations of Lys and Met in solid feed were altered and no effects of Lys and Met on the performance of calves were reported (Hill et al., 2016). Taken together, the results of study 1 indicate that potential differences in Lys concentration for starter mixtures containing CM or SBM are not likely an important factor contributing to the potential differences in the response of calves to those protein sources. However, the unprotected Lys provided may be potentially much more rapidly degraded and degraded to a greater extent in the rumen than Lys present in feed (Robinson et al., 2006). Furthermore, some studies suggest that Lys concentration in the starter mixture may affect the growth performance of calves (Veen et al., 1989). Nevertheless, although Lys supplementation substantially increased its concentration in the starter feed, the lower than assumed Lys:Met ratio for TCML compared to TSBM was obtained, which should be taken into account while interpreting results of the study. In conclusion, the lower Lys concentration in starter mixtures containing CM relative to SBM as the main source of protein, should not be considered a factor limiting efficiency of CM use in starter diets for calves.

Feed enzymes are commonly used to increase nutrient digestibility in non-ruminant species thereby improving feed efficiency, feed intake, and growth performance (Kiarie et al., 2013; Torres-Pitarch et al., 2017). Results of study 2 provide evidence that including enzymes as part of the diet may be also justified in calf starter mixtures. In newborn calves, digestion mechanisms in the stomach (forestomach and abomasum) and intestines are not fully developed (Guilloteau et al., 2009), and thus may limit the efficiency of feed use. While hydrolytic activity in the rumen of calves increases quite rapidly in the first month of life (Rey et al., 2014), a substantial pro-

portion of nutrients consumed with solid feed passes out of the rumen (Lalles and Poncet, 1990; Leibholz, 1975). In fact, at least 2 to 3 weeks of regular solid feed intake by calves is needed to reach ruminal degradation of nutrients equivalent to what is observed in adult ruminants (Lalles and Poncet, 1990; Leibholz, 1975), but it is most likely that weaning is the most important trigger initiating the rapid increase for nutrient degradation in the rumen (Gelsinger et al., 2019; van Niekerk et al., 2021). That said, in some studies, efficiency of nutrient digestion in the rumen increased gradually over several weeks after weaning (Gelsinger et al., 2020, 2019). Of interest, Kehoe and Heinrichs (2004) reported that amylase inclusion in starter mixtures positively affected dimensions of the ruminal papillae. Moreover, Leibholz (1975) showed that, in calves, a substantial portion of ADF is digested in the hindgut and this proportion gradually decreases post-weaning, and Gelsinger et al. (2019) showed that fiber digestion in the rumen (measured using *in situ* method) increases post-weaning, indicating that enzymatic activity in the rumen of newborn calves may limit efficiency of nutrient digestion even several weeks after weaning. Furthermore, pancreatic secretion, pancreatic enzyme activity, and brush border enzyme activity are not fully developed in calves and activity of some digestive enzymes still increases post-weaning (Le Huërou-Luron et al., 1992 a, b), which inevitably may limit the efficiency of post-ruminal solid feed digestion. All the previously mentioned factors may explain the positive response of calves to feed enzyme inclusion in the current study.

Considering that based on results of available studies, high CM inclusion in feed may present a challenge for digestion in the calf gastrointestinal tract, likely due to limited secretion of digestive enzymes and limited capacity for ruminal fermentation (Górka and Penner, 2020), as already discussed, positive effects of feed enzyme inclusion were expected to be greater for the HIGH+ than the LOW+ treatment. However, this was not the case. Given the positive effect when enzymes were included, regardless of the CM inclusion rate, these data suggest nutrient digestion in calves is, in general, limited and can be supported by inclusion of enzymes in the diet. However, the response of calves may also depend on other factors, as we did not observe responses to the enzyme inclusion in study 3. It is clear that feed enzyme inclusion does not always yield positive responses (Torres-Pitarch et al., 2017). Differences in responses among studies 2 and 3 may relate to differing CM quality (e.g. protein damage due to overheating during production process) and thus digestibility as these factors are known to vary depending on its origin (i.e. manufacturer) and likely even batch (Adewole et al., 2017; Maison, 2013; Mejicanos et al., 2016). Furthermore, conditions on the farm, such as hygienic conditions or exposure to pathogens, may change dynamically within a short period of time, affecting the response of calves to feed additives over time, as shown previously (Górka et al., 2023; Milik et al., 2023). Thus, more studies are needed to determine potential factors or

interactions between various factors that may affect the response of calves to feed enzymes provision. Nevertheless, based on the results of these studies, inclusion of enzymes in starter mixtures may increase starter intake and ADG in calves.

While by-products such as DDGS and beet pulp have been tested in calf starters (Dennis et al., 2018; Suarez-Mena et al., 2011), the comparison with CM has not been evaluated. As with CM, the inclusion of other high-fiber and high-protein by-products may negatively affect the growth performance of calves (Dennis et al., 2018; Hill et al., 2016; Suarez-Mena et al., 2011). Results from study 3 provide evidence that responses from substituting SBM with wheat bran and DDGS are not different than when CM partially replaces SBM in pelleted calf starter mixtures. Consequently, CM should be considered a source of protein at least as valuable as wheat bran and DDGS, particularly given that CM use in calf starter mixtures repeatably improved firmness of feces.

Extrusion of CM in the present study reduced the number of days with diarrhea and improved the fecal score, indicating a positive impact on gastrointestinal tract function. It has been shown that CM extrusion reduces the concentration of antinutritional factors in the feed, including glucosinolates (Heyer et al., 2021). While the concentration of glucosinolates is already low in CM and 00 rapeseed meal (Górka and Penner, 2020), its further reduction, or particularly, the concomitant reduction of other antinutritional factors (Heyer et al., 2021) may be relevant for newborn calves. Furthermore, extrusion was shown to improve intestinal amino acid digestion (Heyer et al., 2021; Keady and O'Doherty, 2000), and there are indications that it may increase fiber solubility and fermentability (Cheftel, 1986), both of which may improve ruminal fiber digestion for calves. Consequently, the amount of undigested nutrients entering the hindgut may be reduced (Heyer et al., 2021), resulting in improved firmness of feces. That said, it is unclear why the observed responses for extruded CM did not translate into greater feed intake, ADG or feed efficiency as past research evaluating the extrusion of SBM reported an improvement in the growth performance of calves (Berenti et al., 2021). Future research on the use of extrusion for CM and particularly when used for calves is needed as it could be expected that extrusion may have increased the rumen undegradable protein concentration, which may not have a positive impact on the performance of young calves (Burakowska et al., 2020; Kazemi-Bonchenari et al., 2016). Overall, based on results of the conducted study, extrusion of CM can be used to reduce fecal score of calves.

In conclusion, rumen-unprotected Lys supplementation in a pelleted starter mixture containing CM as the main source of protein did not improve the performance of calves indicating that the lesser concentration of Lys in starters containing CM as main source of protein relative to those containing SBM should not be considered as a factor limiting growth performance of calves. The in-

clusion of enzymes in the starter mixture increased both starter intake and ADG in calves, independent of CM inclusion, although this effect was not consistent across the two studies. The effects of partial replacement of SBM with CM in calf starter mixtures or the replacement of SBM with a combination of wheat bran and DDGS were comparable. These data are interpreted to indicate that CM should be considered a source of protein at least as valuable as wheat bran and DDGS, particularly given that CM use and increased inclusion in calf starter mixtures repeatably improved firmness of feces. Extrusion of CM reduced fecal score during the study period.

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