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Abstract

The research aimed to assess the response in growth performance, carcass characteristics, physicochemical properties, and fatty acid composition of Dohne Merino rams (DMRs) when fed inclusion levels of canola meal (CM). Forty DMRs, weighing 24 ± 2.63 kg and aged 8-9 months, were individually housed and randomly assigned to one of four isonitrogenous and isoenergetic diets. The experimental diets contained 5% oil cake meal, soya bean meal (SBM) or CM, CM replaced SBM at 0% (T1), 50% (T2), 75% (T3) and 100% (T4). The results revealed a quadratic increase in average daily feed intake as CM levels increase. Average daily gain and feed conversion ratio decreased. Blood urea nitrogen and total cholesterol showed a significant linear decline with increasing CM levels, while glucose-fasting, total protein, and albumin did not exhibit significant relationships. The carcass traits such as warm and cold dressing percentages, pH and temperature measurements, demonstrated a quadratic decrease with increasing CM inclusion levels. The physicochemical properties of the meat did not show significant relations, except for fat-free dry matter, which decreased quadratically. Fatty acids like Capric, Oleic, and Eicosapentaenoic acids decreased significantly with CM levels, while Margaric acid decreased linearly, and Alpha-linolenic acid increased linearly. These findings suggest that restricting CM inclusion in sheep diets to below 5% could help mitigate adverse effects on growth performance. The possible antagonistic interaction between SBM and CM highlights the recommendation against combining CM with SBM in rations.

Keywords: Sheep, soybean meal, rapeseed meal, growth rate, meat quality, blood chemistry

Introduction

Small ruminant farming, specifically sheep farming, is a crucial enterprise in the agricultural sector that has the potential to enhance the socio-economic well-being of rural communities worldwide (Elmaz et al., 2014; de Miguel et al., 2022). For numerous years, sheep production has been approved as a fundamental component of agriculture, serving as a cornerstone in

sustaining the livelihoods of individuals (Scortichini et al., 2016). The significance of sheep farming within the agricultural sector, especially in its role in advancing food security and rural development on a global scale, is paramount. Nonetheless, sheep producers frequently encounter numerous obstacles that impede their economic viability and long-term sustainability. A substantial challenge in sheep production, particularly among smallholder farmers, is the utilization of inferior-quality feeds (Simões et al., 2021). Sheep necessitate a balanced diet to preserve their health and generate high-quality products, which directly impact their growth, weight gain, and productivity (Prache et al., 2022). Inadequate nutrition, stemming from the consumption of low-quality feed, may result in suboptimal growth and development, leading to diminished performance and inferior-quality products (Balehegn et al., 2020; Gabr et al., 2023). Consequently, this situation may negatively affect farm revenue.

Supplementary feeds have the potential to offer superior nutritional benefits and enhance the performance of sheep; however, they may impose a substantial financial burden on livestock farmers, often exceeding 70% of their total operational costs (Delgado et al., 2001; Obeidat et al., 2017). Several studies have emphasized the widespread use of SBM as a primary protein supplement in sheep husbandry for many decades, owing to its high protein content and the inclusion of advantageous nutrients such as carbohydrates, fats, fiber, essential minerals, and vitamins (Lokuruka, 2010; Delele, 2021; Kalogianni et al., 2022). Although the nutritional value of SBM in livestock feeding regimens is widely recognized, the urgency to either partially or fully substitute this protein supplement has become increasingly apparent, primarily due to logistical, economic, and environmental considerations (Van der Poel et al., 2020). It is of importance to highlight that in excess of 40% of the global SBM supply is directed towards European countries, primarily due to their limited self-reliance, consequently playing a role in shaping an economically and environmentally unfavourable transatlantic trade framework (Hensele et al., 2013). The predominance of SBM exports to European nations poses challenges

for small-scale sheep producers in developing regions like South Africa. These challenges encompass price fluctuations, restricted access to competitive pricing, resource competition, environmental repercussions, supply chain susceptibilities, and market distortions. Therefore, addressing these issues may require a multifaceted strategy involving improving resource and market access, embracing sustainable agricultural practices, and investigating cost-efficient, domestically sourced alternative protein options for livestock to lessen dependence on imported SBM.

Canola meal (CM) is derived as a residue from the oil extraction process of canola seeds and is widely utilized as a constituent in animal feed formulations (Hussain et al., 2022). Canola, scientifically known as *Brassica napus*, belong to the *Brassicaceae* family (USDA, 2021). It is positioned as the world's second-largest protein meal after SBM (Aachary et al., 2014). Canola meal (CM) is characterized by a protein content ranging from approximately 38% to 43% and demonstrates a balanced profile of essential amino acids, including methionine, cysteine, threonine, and tryptophan, resembling those present in SBM (Newkirk et al., 2003; CCC, 2019). Furthermore, CM exhibits a higher fat content compared to SBM, primarily due to the presence of gums like phospholipids, glycolipids, triglycerides, and free fatty acids, which are commonly reintroduced into the meal post oil refining (Broderick et al., 2015). It serves as a valuable source of readily available calcium, iron, manganese, selenium, and various B vitamins (Wickramasuriya et al., 2015). Additionally, CM emerges as a prominent phosphorus source, containing 0.38% as opposed to 0.28%, 0.23%, 0.09%, 0.26%, 0.07%, and 0.13% in SBM, cottonseed meal, wheat, wheat bran, corn, and barley, respectively (Khajali and Slominski, 2012). Khajali and Slominski (2012) also noted that CM surpasses SBM in crude fiber (CF), acid detergent fiber (ADF), neutral detergent fiber (NDF), and total dietary fiber content due to its notably higher lignin content and associated polyphenols.

Research has demonstrated that CM can serve as a valuable protein supplement in livestock diets, including those for sheep, owing to its high protein content and balanced amino acid profile (Lourenco et al., 2017). This suitability extends to animals with elevated requirements for methionine, cystine, and histidine, such as dairy cattle and laying chickens (Newkirk, 2009; Khajali and Slominski, 2012), aligning with the nutritional needs of sheep. Furthermore, CM has been identified as a cost-effective and sustainable substitute for soybean meal (SBM) in ruminant diets, providing a protein source abundant in essential amino acids like methionine and cysteine (Holtshausen et al., 2021). Studies have shown that CM can effectively replace SBM in finishing diets for Meatmaster lambs without compromising growth performance, presenting a viable and economical option for sheep nutrition (Brand et al., 2023). Additionally, findings from Sekali et al. (2016) indicate that incorporating CM does not adversely impact the meat quality of Mutton Merino lambs, suggesting its inclusion in lamb diets without compromising performance. Therefore, considering its protein content, amino acid balance, and cost-effectiveness relative to SBM, CM can be utilized as a substitute for SBM in sheep husbandry amongst smallholder farmers.

The Western Cape Province of South Africa annually produces approximately 1.5 million tons of canola grain, predominantly cultivated across 75,000 to 85,000 hectares of land (Shoko, 2021). With the expansion of canola cultivation and increasing yields in South Africa, it has emerged as a readily available protein supplement for livestock feed (Harker et al., 2012). Depending on the geographical region, CM may be more accessible and economically feasible for smallholder farmers compared to SBM, thereby potentially reducing transportation costs and logistical complexities. The cost-benefit analysis of using CM as a feed ingredient compared to traditional SBM, several factors come into play. For example, CM availability and local production can lead to cost savings due to reduced transportation and import costs associated with SBM. Moreover, the environmental benefits of using locally sourced CM, such as lower carbon footprint and

reduced reliance on imported feed ingredients, can add value to the overall cost-benefit analysis. To the best of our knowledge, limited research has explored the effects of dietary incorporation of CM on growth performance, carcass traits, physicochemical characteristics, fatty acid profiles, and serum biochemistry of DMRs, a breed indigenous to South Africa commonly employed by smallholder farmers. parameters in DMRs. The primary objective of this study was to determine the response in growth performance, carcass traits, physicochemical properties and fatty acid profiles of DMRs fed different levels of CM.

Materials and Methods

Study site ethical approval

The study was conducted at the Carnarvon Estate Farm, located approximately 50 km from Sterkstroom, within a 20 km radius, along the 344 to Dordrecht and Queenstown in the Eastern Cape Province of South Africa. The study period was during the winter months of June and July. The farm is located at latitude 31°35'54.8" South and longitude 26°42'09.7" East, with an altitude located at an elevation of 1,076 meters above sea level. The region experiences a Mid-latitude steppe climate (classified as BSk). Its annual temperature averages 18.29℃ (64.92℉), which is 2.93% lower than the national average in South Africa, typically receives approximately 90.83 millimeters (3.58 inches) of precipitation annually, with rainy days occurring 134.89 times per year, accounting for 36.96% of the time. The use of animals in this study was authorized by the Animal Research Ethics Committee (AREC) at the University of Fort Hare, South Africa (Ethical clearance: MBA011SMBA01).

Diets, animals, and experimental design

The ingredients used in the research were procured from Swellendam, Western Cape, South Africa, which included CM. Formulation of diets adhered to NRC (2007) guidelines to fulfil the nutritional needs of ruminants. Forty rams, aged 8 - 9 months with an initial weight of 24 ± 2.63 kg, were selected for the study. These rams were individually housed and randomly allocated to

one of four experimental diets. In this study, experimental rations contained 5% oil cake meal, SBM or CM, CM replaced SBM at 0% (T1), 50% (T2), 75% (T3) and 100% (T4) respectively and were fed to rams *ad libitum* with fresh water provided. All rams were obtained from Carnarvon Estate Farm, Eastern Cape Province of South Africa. Prior to the experiment, the rams were dosed with Maxicare to prevent internal parasites and vaccinated with Pulpvax immunization against Pulpy kidney. The animals received acaricide baths as a preventive measure against external parasites. Feeding occurred twice daily at 08:00 AM and 16:00 PM, with feed adjustments made at least twice daily to minimize wastage. Furthermore, the animals had unrestricted access to water. The study lasted seventy days, with a one-week adaptation period included. The composition of the experimental diets is detailed in Table 1.

Chemical composition of experimental diets

The study involved the analysis of crude protein (CP), ether extract (EE), neutral detergent fiber (NDF), acid detergent fiber (ADF), Ash content and metabolizable energy (ME). These analyses were conducted at the Soil Fertility and Analytical Services Division of the KwaZulu-Natal Department of Agriculture and Rural Development, South Africa (KZNDARD). The total nitrogen (N) content was ascertained utilizing the standard macro Kjeldahl method and then converted to CP using a multiplication factor of 6.25 (AOAC, 2005). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined following the methodology outlined by Van Soest et al. (1991). Ether extract (EE) was assessed using the Soxhlet method (AOAC, 2005), while ME was determined in accordance with the procedure outlined by Godfrey et al. (1993).

Determination of growth performance

Before commencing the trial, the rams underwent initial weighing to determine their baseline body weight. Subsequently, they were weighed weekly on Monday mornings at 08:00 AM to monitor changes in body weight (BW). Average daily feed intake (ADFI) was calculated weekly

by subtracting feed refusals from the total feed offered. Average daily gain (ADG) was determined by dividing the difference between final and initial weights by seven. Feed conversion ratio (FCR) was subsequently calculated by dividing ADFI by ADG.

Serum biochemistry

On the final day of the experiment (Day 70), following to a fasting period, a minimum of 8 ml of blood samples per animal were procured to assess blood chemistry parameters. These samples were acquired three hours post-morning feeding via vein puncture into tubes devoid of anticoagulants. Following this, they underwent centrifugation at 3,220 × g for 8 minutes at 4 ℃. After centrifugation, serum was extracted and preserved at – 20 ℃ for subsequent analysis of GCF, TTP, TTC, ABL, and BUN levels. The analysis of GCF adhered to the protocol delineated by Barham and Trinder (1972), while the determination of TTP followed the method expounded by Weichselbaum (1946). Total cholesterol (TTC) levels were determined utilizing the technique devised by Morgan (1998), and ABL albumin levels were determined in accordance with the procedure outlined by Doumas and Biggs (1972).

Slaughtering procedure and carcass evaluation

Before slaughter, all rams were weighed to determine the final weight (FW) and were subsequently transported to a commercial abattoir situated in Queenstown, Eastern Cape Province, South Africa. The slaughter activities adhered to the stipulations outlined in the Meat Safety Act (Act No. 40 of 2000), which governs abattoir operations in South Africa. Subsequently, carcass traits such as hot carcass weight (HCW), cold carcass weight (CCW), hot dressing percentage (HDP), and cold dressing percentage (CDP) were determined. The warm carcass weight (WCW) was measured immediately after the animals were slaughtered, while the carcasses were still warm. Subsequently, the carcasses were placed in a chilled cold room overnight and then re-weighed to determine the cold carcass weight (CCW). The hot dressing percentage (HDP) and cold dressing percentage (CDP) were then calculated using the formulas:

HDP% = HCW/FW x 100

CDP% = CCW/FW x 100

Determination of pH, temperature and colour

The pH and temperature (T) of the meat were assessed at specific time intervals: 45 minutes, 1 hour, 3 hours, 6 hours, and 24 hours, labelled as pH-45min, pH-1hr, pH-3hr, pH-6hr, pH-24hr, and T45-min, T-1hr, T-3hr, T-6hr, T-24hr, respectively. Analysis was conducted on the *longissimus thoracis et lumborum* (LTL) muscle from the left side of each carcass while it was hanging. The meat was determined using a pH meter (CRISON pH 25 Instruments, Spain) and the temperature by thermometer between the 12^{th} and 13^{th} ribs. Following that, meat samples were collected on the same position and vacuum-packed and stored at -20℃ until analysis. In this study, Meat colour was determined using a Minolta colour-guide 45/0 BYK-Gardener GmbH machine with a 20 mm diameter measurement area and illuminant D65-day light, 10° observation angle after 30 minutes blooming time and colour variables included L* (lightness), a* (redness), and b* (yellowness). Before taking measurements, the machine was calibrated using the green, black, and white standard colour samples and the average values for colour was obtained by rotating the colour guide at 90° between measurements (Commision International De L' Eclairage, 1976). The psychometric hue angle, which indicates the angle at which a vector radiates into the red-yellow quadrant, was calculated as Hue angle = [tan-1 (b*) / (a*)]. Furthermore, the psychometric chroma, a metric assessing colour saturation, was calculated as the square root of a*2 + b*2, the vividness (MacDougall, 1982). The samples were then vacuum-packed and stored at 0 - 4° C in the refrigerator pending analyses of thawing loss, cooking loss, and shear force.

Determination of thawing loss and cooking loss

The samples underwent thawing, followed by placement in a plastic bag and cooking in a laboratory water bath maintained at 85 ℃ for 45 minutes as per the methodology outlined by

Kim et al. (2013). Subsequently, the samples were cooled and reweighed to ascertain the cooking loss. Thawing loss and cooking loss were then computed utilizing the subsequent formulas.:

Thawing loss (TL) % = ([(weight before thaw-weight after thaw)])/ (weight before thaw) ×100

Cooking loss (CL) % = ([(weight of raw steak after thawing-weight of cooked steak)])/ (weight of raw steak after thawing) × 100

Determination of shear force

After measuring thawing and cooking loss, three sub-samples were taken from the cooked samples, cut parallel to the direction of muscle fibers, with a 10 mm core diameter. Each core underwent a single shearing event along its central axis, perpendicular to the orientation of the fibres, using a Warner-Bratzler shear device affixed to a Universal Instron apparatus (Model 4301, Instron Ltd, Buckinghamshire, UK) operating at a crosshead speed of = 400 mm/min. The Warner-Bratzler Shear Force (WBSF) was quantified as the maximum force in Newtons (kg/m) averaged across three cores per specimen following the procedure described by Webb et al. 2018).

Determination of proximate analysis and fatty acid profile

Muscle samples underwent quantitative lipid extraction following the methodology described by Folch et al. (1957), utilizing a 2:1 ratio of chloroform and methanol. The antioxidant, butylated hydroxytoluene (BHT), was combined at a concentration of 0.001% into the chloroform: methanol mixture to prevent oxidation during the extraction process. The fat extracts were then dried using a rotary evaporator under vacuum and further desiccated overnight in a vacuum oven at 50℃ with phosphorus pentoxide as a desiccant. The total extractable intramuscular fat content was determined by weight from the extracted fat and expressed as a percentage (w/w) per 100 g of tissue. The fat-free dry matter (FFDM) content was determined by weighing the residue, which had undergone the Folch extraction process, on a pre-weighed filter paper following the drying stage. The FFDM was then calculated as a percentage of the initial sample weight,

expressed as % FFDM (w/w) per 100 g of tissue, by subtracting the weight of the residue from the initial weight of the sample. The moisture content of the muscle and subcutaneous fat was calculated by subtraction (100% - % lipid - % FFDM) and expressed as % moisture (w/w) per 100 g of tissue. The extracted fat was preserved in a polytop (glass vial with push-in top) under a nitrogen blanket and frozen at –20℃ pending fatty acid analyses. A lipid aliquot (approximately 30 mg) of muscle lipid was converted to methyl esters through base-catalyzed transesterification to prevent conjugated Linoleic Acid (CLA) isomerization, using sodium methoxide (0.5 M solution in anhydrous methanol) for 2 hours at 30℃ (Park et al., 2001; Kramer et al., 2002; Alfaia et al., 2007). Fatty acid methyl esters (FAMEs) from feed, muscle, and subcutaneous fat were quantified using a Varian 430 flame ionization gas chromatograph (GC) (Middelburg, The Netherlands) equipped with a fused silica capillary column, Chrompack CPSIL 88 (100 m length, 0.25 mm ID, 0.2 μm film thickness) Chemetrix, Johannesburg, South Africa). The analysis involved an initial isothermic period at 40℃ for 2 minutes, followed by a temperature increase at a rate of 4℃/minute up to 230℃. Subsequently, an isothermic period at 230℃ for 10 minutes ensued. Fatty acid methyl esters (FAMEs) - n-hexane (1 μl) were injected into the column using a Varian CP 8400 Autosampler (Middelburg, The Netherlands). The injection port and detector were maintained at 250℃. Hydrogen at 45 psi served as the carrier gas, with nitrogen as the makeup gas. The Galaxy Chromatography Data System Software (Middelburg, The Netherlands) recorded the chromatograms. The identification of fatty acid methyl ester (FAME) samples was achieved by comparing the retention times of FAME peaks from the samples with standards from Supelco (Supelco 37 Component FAME Mix 47885-U, Sigma-Aldrich, Aston Manor, Pretoria, South Africa). Conjugated linoleic acid (CLA) standards were obtained from Matreya Inc. (Pleasant Gap, United States), including cis-9, trans-11, and trans-10, cis-12-18:2 isomers. The fatty acids were expressed as a percentage of each individual fatty acid relative to the total fatty acids present in the sample. The fatty acid data were used to calculate

various fatty acid ratios, such as the total saturated fatty acids (SFAs), total monounsaturated fatty acids (MUFAs), total polyunsaturated fatty acids (PUFAs), PUFA/SFA ratio, Δ9 desaturase index (C18:1c9/C18:0), total omega-6, total omega-3, and the ratio of omega-6 to omega-3 (n-6)/(n-3) fatty acids.

Statistical analysis

The General Linear Model (PROC GLM) procedure of SAS (2009) was used to determine the effect of dietary inclusion levels of CM on growth performance, serum biochemical traits, physico-chemical traits, and fatty acid profile of DMRs and thereafter, the polynomial regression (PROC REG) procedure SAS, (2009) was used to determine the response in growth performance, serum biochemistry carcass traits, physicochemical properties, and fatty acid composition of DMRs. The significant difference was considered at level of $p < 0.05$. The polynomial regression model used was as follows:

$$Y = \beta 0 + \beta T1 + \beta 2T2 + E$$

Where: Y = is the response variables (growth performance, serum biochemistry carcass traits, physicochemical properties and fatty acid profile).

$\beta 0 + \beta 1 + \beta 2$ = are the regression coefficients.

T = is the dietary levels CM.

E = is the residual error.

Results

The response in growth performance of DMRs fed CM inclusion levels are reported in Table 3. There was a significant ($p < 0.0$) quadratic increase in ADFI with increasing CM inclusion levels. A notable ($p < 0.05$) decrease in ADG was observed with increasing CM inclusion levels. Moreover, increasing CM inclusion levels led to an increase in FCR in this study, revealing significant differences ($p < 0.05$) between treatments T1 and T4 as opposed to T2 and T3. According to Table 4 blood urea nitrogen (BUN) significantly ($p < 0.05$) displayed a linear

response to the increasing CM inclusion, wherein treatment T1 exhibited higher levels compared to the other treatments. Furthermore, a significant ($p < 0.05$) linear increase in total cholesterol was observed with the increasing CM inclusion. The glucose-fasting, total protein and albumin did not exhibit significant ($p > 0.05$) relationships with the increasing CM inclusion. The effects of different CM inclusion levels on carcass traits of DMRs are reported in Table 5. The CM inclusion levels did not have a significant effect ($p > 0.05$) on warm carcass WCW, CCW, temperature at 1 hour (T-1hr), and temperature at 3 hours (T-3hr) of the meat in DMRs. The study found that as the inclusion levels of CM increased, there was a quadratic decrease ($p < 0.05$) in the following carcass traits: warm dressing percentage (WD%), cold dressing percentage (CD%), pH at 45 minutes (pH-45 min), and pH at 1 hour (pH-1 hr) (Table 5). The response in physicochemical traits of DMRs fed different levels of CM are reported in Table 6. The physicochemical properties of the meat did not show significant relations, except for fat-free dry matter (FFDM), which decreased quadratically. The response in fatty acid profile of DMRs CM inclusion levels are reported in Table 7. The research findings noted that most of the tested fatty acids (FAs) did not show a statistically significant ($p > 0.05$) as CM inclusion levels increase. Nonetheless, FAs like Capric, Oleic, and Eicosapentaenoic acids decreased significantly with CM levels, while Margaric acid decreased linearly, and Alpha-linolenic acid increased linearly.

Discussion

Identifying alternative feed ingredients such as CM is essential for optimizing sheep production, considering the logistical, economic, and environmental challenges of SBM as stipulated by Van der Poel et al. (2020). The observed quadratic increase in ADFI due to increasing levels of CM in the present study could possibly be attributed to either feed palatability or the animal's reaction to the protein content. Pond et al. (2007) reported that animals generally exhibit a natural tendency to increase their feed intake when they recognise a rise in the protein content of their diet. This phenomenon is commonly* referred to as "protein

appetite," and it often translates into animals consuming higher quantities of food in order to fulfil their protein needs, ultimately contributing to enhanced growth and performance (Sclafani and Ackroff, 2012; Raubenheimer and Simpson, 2019). The decrease in ADFI noted in treatment T4, characterized by the highest CM inclusion, may be linked to the presence of potential anti-nutritional factors in CM, which could detrimentally impact palatability and diminish overall feed consumption (Montagne et al., 2003). These factors can manifest symptoms such as anorexia, lethargy, gastrointestinal disturbances, and weight loss in sheep (Agwa et al., 2023). Furthermore, compounds like tannins and phytates, recognized as anti-nutritional elements, have been shown to influence the nutritional quality of feed ingredients and decrease feed efficiency in animals, as previously documented (Stanford et al., 2000; Woyengo and Nyachoti, 2013).

Although there were no significant relationships observed between ADG, FCR and FW concerning the inclusion of CM in the diet, treatments T4, followed by T1, T3, and T2, exhibited comparatively lower FCR alongside higher ADG and FW in sequential order. This pattern suggests that FCR rates correspond to increased FW gain in sheep, while elevated ADG also contributes to higher FW. The results demonstrate a clear pattern where the combination of SBM with CM results in an increase in FCR (significant differences observed between T1 and T4 compared to T2, with no significant difference noted with T3) and a decrease in ADG (significant differences noted between T1 and T4 compared to T2 and T3). These outcomes strongly suggest the presence of an antagonistic effect between SBM and CM, indicating that incorporating CM into diets containing SBM should be avoided. The fact that treatments T1 and T4 did not show significant differences in terms of ADG and FCR in our study supports the potential for CM to effectively substitute SBM. Furthermore, the higher ADG and FW observed in our study imply that including CM may promote accelerated growth and improved final weights at slaughter, potentially enhancing economic returns for sheep producers. It is worth

noting that the ADG values observed across all treatment groups in our study align with those documented in other research studies (Sekali et al., 2016; Ata et al., 2020).

The linear decrease in BUN levels was evident with increasing CM levels in the study. Blood urea nitrogen (BUN) serves as a vital marker of protein metabolism and renal function, with its fluctuations potentially influenced by the composition of experimental diets (Kohn et al., 2005; Krstic et al., 2016). Notably, the variations in dietary composition, including EE content representing dietary fat, ash content, and fiber components such as NDF and ADF, may interplay with BUN levels. Furthermore, the presence of anti-nutritional factors in CM, like glucosinolates and phytic acid, could also impact nitrogen metabolism and renal function (Gilani et al., 2012), potentially contributing to the observed linear decrease in BUN levels. Further studies are warranted to elucidate the involved mechanisms linking dietary composition, including anti-nutritional factors in CM, and BUN levels. The observed linear decrease in total cholesterol with increasing CM inclusion may be attributed to the differences in CP content among the experimental diets. This relationship is supported by research indicating that CP levels can impact cholesterol metabolism, with higher CP levels potentially linked to enhanced hepatic cholesterol synthesis (Xia et al., 2018). The inverse relationship between EE and cholesterol levels suggests a potential role of dietary fat in cholesterol metabolism, with diets rich in EE possibly enhancing cholesterol excretion (Gautam et al., 2016). The increasing levels of NDF and ADF, indicative of higher fiber content, may impact cholesterol absorption and metabolism by binding cholesterol in the gut, limiting its absorption, and promoting excretion (Chuang et al., 2021;). These interactions with dietary components, including potential anti-nutritional factors in CM, highlight the complexity of the relationship between diet composition and cholesterol levels, warranting further exploration.

The quadratic decreases observed in WD% and CD% as CM inclusion levels increased suggest that dressing percentages may be impacted by the nutritional balance within the diet,

encompassing protein, energy, and other essential nutrients. Thacker and Widyaratne (2012) have highlighted the elevated fat and fiber content in CM, a composition that could potentially lead to suboptimal growth and muscle development, consequently resulting in diminished dressing percentages, as noted by Smit et al. (2014). The decrease in carcass weights in sheep fed CM in this study may be associated with the presence of anti-nutritional factors in CM. Anti-nutritional components such as glucosinolates, sinapine, sinapic acid, tannin, phytic acid, and crude fiber present in CM can exert detrimental effects on animal health and performance (Feng and Zuo, 2007; Mejicanos et al., 2016). Hence, the existence of anti-nutritional factors in CM could potentially contribute to any observed reductions in carcass weights when administered to sheep. The anti-nutritional factors in the study were not determine, hence warrants further investigations.

Muscle pH and temperature quadratically decreased with inclusion levels of CM when measured at various time intervals. This outcome was expected due to the fact that numerous physiological and biochemical transformations occur in animals after they are slaughtered, encompassing two fundamental phases, the maturation of the meat and the onset of rigor mortis (Lawrie and Ledward, 2006). A study conducted by Terlouw et al. (2021) highlighted that in the early moments following animal slaughter, there tends to be a relatively rapid decrease in both muscle pH and temperature. This initial decline is often associated with the phase known as "cold shortening," where the rapid cooling of the muscle fibers leads to a more immediate onset of rigor mortis (Hwang et al., 2004). The findings regarding the ultimate pH (pH-24 hr) in this investigation, a critical indicator of meat quality, align with previous studies as reported by various researchers (Ciurescu et al., 2017; Sekali et al., 2016). It is remarkable that the ultimate pH values observed across the different experimental groups fell within the established range of 5.4 to 5.8, indicative of high-quality meat (Miranda-de la Lama et al., 2012). This consistency in

pH levels suggests the conversion of glycogen into lactic acid during the post-mortem transition from muscle to meat, underscoring the quality attributes of the meat samples analysed.

One potential explanation for the lack of significant changes in the physicochemical properties of DMRs could be attributed to a potential adaptation of these animals to varying dietary compositions, CM inclusion levels. Research has indicated that ruminants possess the capacity to adjust their digestive processes to efficiently utilize diverse feed types, a factor that may contribute to the maintenance of consistent meat quality despite dietary variations (Ikusika et al., 2019). Furthermore, the duration of the study may not have been sufficiently prolonged to detect significant shifts in meat quality attributes. This view is in line with Ö NENÇ and Ö ZDOĞAN, (2022) who stated that certain dietary impacts on meat quality may require an extended period to become apparent, particularly when they involve gradual modifications in muscle composition or metabolism.

Ruminants, in contrast to non-ruminants, exhibit a relatively low polyunsaturated fatty acid (PUFA) content due to microbial biohydrogenation in the rumen, resulting in a fatty acid (FA) profile that closely reflects their dietary intake (Wood et al., 2004). The study noted a quadratic reduction in capric acid as CM inclusion levels increased in DMRs. The observed quadratic decreases in Capric, Oleic, and Eicosatetraenoic acids with increasing levels of CM in DMRs can be linked to alterations in dietary composition influenced by the inclusion of CM. It is essential to highlight that CM, derived from canola seed processing, possesses a distinctive FA composition (He et al., 2013). However, limited research has explored the potential impact of CM utilization on the FA profile of sheep meat, indicating the need for further investigation. A study by Gao et al. (2021) indicated that the striking antinutritional factors in CM may also affect the composition and activity of rumen microbial populations (Gao et al., 2021). Certain fatty acids, including Capric and Oleic are products of ruminal microbial fermentation and biohydrogenation processes as articulated by Lourenco et al. (2010). Therefore, changes in

microbial populations due to antinutritional factors in CM could change the production and biohydrogenation of these fatty acids (FAs) in the rumen. The study also noted a linear reduction in Margaric acid as CM inclusion levels increased in DMRs. Margaric acid, a SFA, may contribute to elevated levels of low-density lipoprotein (LDL), and cholesterol, commonly referred to as "bad" cholesterol in humans (Sinatra et al., 2014; Islam et al., 2019). Contrariwise, a linear increase in alpha-linolenic acid (ALA) was observed with higher CM inclusion levels in DMRs. This increase is likely influenced by the composition of CM, which is rich in this essential omega-3 fatty acid (MacIntosh et al., 2021), suggesting direct incorporation of CM FAs into the animal's tissues with minimal modification or metabolism. This observation aligns with the principle that the FA composition of an animal's tissues reflects its dietary composition (Scollan et al., 2001). The elevation in ALA is remarkable due to its status as an essential FA with potential health benefits for both animals and humans (Barceló-Coblijn et al., 2009). Alpha-linolenic acid (ALA) serves as a precursor to longer-chain omega-3 fatty acids like eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), known for their positive effects on cardiovascular health and various physiological functions (Nguemeni et al., 2013; Sain et al., 2018). A quadratic decrease in increase in eicosapentaenoic acid (EPA) was observed with higher CM inclusion levels in DMRs.

Conclusion

The results of the present study indicate that incorporating CM into the diet of DMRs does not have detrimental effects on their carcass traits, physicochemical properties, or fatty acid composition. However, a decrease in ADFI and ADG was observed at a dietary level of 5% CM (T4). Furthermore, an increase in FCR was evident in treatment groups T2 and T3. Notably, there were no significant differences in ADG and FCR between T1 and T4, suggesting the potential for CM to replace SBM effectively. These results imply a possible antagonistic

interaction between SBM and CM, emphasizing the recommendation against incorporating CM into diets containing SBM.

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Table 1: The composition of the experimental diets (% dry matter)

Ingredients	T1	T2	T3	T4
Lucerne	8	8	8	8
Grass hay	8	8	8	8
Yellow maize	56.7	53.7	54.72	59.72
Wheat brand	10	13	12	7
Molasses meal	10	9.95	9.85	9.7
Canola meal	0	2.5	3.75	5
Soybean meal	5	2.5	1.25	0
Feed grade urea	0.8	0.85	0.93	1.08
Salt	0.5	0.5	0.5	0.5
Feed limestone	1	1	1	1
Vitamin mineral premix	0.15	0.15	0.15	0.15

*Canola meal (CM) replaced soybean meal (SBM) at 0% (T1), 50% (T2), 75% (T3) and 100% (T4).

Table 2: Analysed chemical composition of experimental diets

Traits	T1	T2	T3	T4
Crude protein (g/kg)	156.9	156.2	156.5	156.7
Ether extract (g/kg)	29.2	29.9	27.2	29.9
Neutral detergent fibre (g/kg))	216.1	218.9	219.6	222.3
Acid detergent fibre (g/kg))	10.74	12.51	14.36	18.73
Ash (g/kg)	71.5	69.1	65.0	61.0
Metabolism energy (MJ/kg)	11.85	12.07	11.94	12.14

*Canola meal (CM) replaced soybean meal (SBM) at 0% (T1), 50% (T2), 75% (T3) and 100% (T4).

Table 3 . Response in growth performance of DMRs to CM inclusion levels

Traits	CM inclusion levels				S. E	Significance levels	
	T1	T2	T3	T4		Linear	Quadratic
Initial weight (kg)	25.10	25.70	26.00	27.70	1.21	NS	NS
Average daily feed intake (kg/day)	1.20	1.20	1.22	1.11	0.04	NS	*
Average daily gain (kg/day)	0.23[a]	0.17[b]	0.17[b]	0.24[a]	0.05	NS	NS
Feed conversion ration	5.54[b]	7.38[a]	6.40[ab]	4.85[b]	0.53	NS	NS
Final weight gain (kg)	41.20[ab]	37.50[b]	38.00[b]	43.50[a]	1.34	NS	NS

[ab] Least square means in the same row with different superscript letters differ significantly ($p < 0.05$); NS, not significant ($p > 0.05$); S. E, standard error of means.

Table 4. Response in serum biochemistry of DMRs to CM inclusion levels

Traits	CM inclusion levels				S. E	Significance levels	
	T1	T2	T3	T4		Linear	Quadratic
Blood urea nitrogen (mmol/L)	8.02[a]	5.55[b]	4.40[c]	4.02[c]	0.40	NS	NS
Glucose-fasting (mmol/L)	3.66	3.85	3.72	3.74	0.16	NS	*
Total protein (g/L)	55.60	63.40	55.70	63.00	2.64	NS	NS
Albumin (g/L)	16.70	14.80	16.30	15.80	0.54	NS	NS
Total cholesterol (mmol/l.)	1.20[a]	1.50[b]	1.38[b]	1.48[a]	0.06	NS	NS

[ab] Least square means in the same row with different superscript letters differ significantly ($p < 0.05$); NS, not significant ($p > 0.05$); S. E, standard error of means.

Table 5: Response in carcass traits of DMRs to CM inclusion levels

Traits	CM inclusion levels				S. E	Significance levels	
	T1	T2	T3	T4		Linear	Quadratic
Warm carcass weight (kg)	21.36	20.96	20.53	23.23	0.80	NS	NS
Cold carcass weight (kg)	20.80	20.49	19.95	22.24	0.83	NS	NS
Warm dressing (%)	52.09	55.95	53.96	53.47	1.27	NS	*
Cold dressing percentage (%)	50.68	54.70	52.52	51.11	1.26	NS	**
pH-45 min	6.99	6.94	6.92	6.91	0.13	NS	***
pH-1 hr	6.86	6.88	6.87	6.86	0.12	NS	**
pH-3 hr	6.76	6.79	6.78	6.77	0.12	NS	**
pH-6 hr	6.20	6.55	6.64	6.63	0.13	NS	*
pH-24 hr	5.52	5.45	5.54	5.53	0.13	NS	*
Temperature (℃) at 45 min	14.95	15.94	14.13	14.13	0.91	NS	**
Temperature (℃) -1hr	11.04	11.53	11.45	11.66	0.32	NS	NS
Temperature (℃) -3hr	7.60	8.19	7.41	7.33	0.34	NS	NS
Temperature (℃) -6hr	7.64[ab]	7.07[b]	8.11[a]	6.54[c]	0.22	*	*
Temperature (℃) -24hr	3.73	3.63	3.65	3.58	0.17	NS	*

[ab] Least square means in the same row with different superscript letters differ significantly ($p < 0.05$); NS, not significant ($p > 0.05$); S. E, standard error of means.

Table 6: Response in physicochemical traits of DMRs to CM inclusion levels

Traits (%)	CM inclusion levels				S. E	Significance levels	
	T1	T2	T3	T4		Linear	Quadratic
Lightness (L*)	30.13	30.56	29.32	34.60	2.24	NS	NS
Redness (a*)	17.98	17.28	19.78	16.97	1.44	NS	NS
Yellowness (b*)	9.83	9.11	9.54	11.65	0.94	NS	NS
Chroma (C)	20.67	19.59	22.14	20.73	1.01	NS	NS
Hue angle (H)	28.32	27.80	25.49	35.59	3.02	NS	NS
Thawing loss (TL)	1.34	1.84	1.20	0.86	0.40	NS	NS
Cooking loss (CL)	35.21	34.77	36.44	34.67	1.83	NS	NS
Shear force (F)	18.29	19.06	21.68	21.34	2.77	NS	NS
Fat	4.26	3.53	3.85	4.03	0.31	NS	NS
Fat free dry matter (FFDM)	21.53	21.89	21.97	22.34	0.31	*	*
Moisture	74.21	74.59	74.18	73.63	0.38	NS	NS

[ab] Least square means in the same row with different superscript letters differ significantly ($p < 0.05$); NS, not significant ($p > 0.05$); S. E, standard error of means.

Table 7. Response fatty acid profile of DMRs to CM inclusion levels

Traits (%)	CM inclusion levels				S. E	Significance levels	
	T1	T2	T3	T4		Linear	Quadratic
Capric acid	0.06	0.06	0.08	0.03	0.02	NS	*
Lauric acid	0.05	0.02	0.03	0.04	0.02	NS	NS
Myristic acid	2.44	2.00	2.24	2.28	0.19	NS	NS
Myristoleic acid	0.02	0.01	0.01	0.01	0.01	NS	NS
Pentadecylic acid	0.21	0.16	0.18	0.18	0.02	NS	NS
Palmitic acid	28.67	26.66	27.38	27.47	0.69	NS	NS
Palmitoleic acid	1.78	1.61	1.61	1.54	0.12	NS	NS
Margaric acid	0.88	0.76	0.75	0.78	0.04	*	NS
Stearic acid	17.26	17.75	17.41	17.01	0.69	NS	NS
Elaidic acid	0.19	0.21	0.16	0.19	0.01	NS	NS
Oleic acid	41.37	41.97	42.08	41.60	0.84	NS	*
Vaccenic acid	0.34	0.70	1.03	1.02	1.70	NS	NS
Nonoadecanoic acid	0.02	0.01	0.01	0.02	0.01	NS	NS
Linolelaidic acid	0.17	0.22	0.19	0.17	0.05	NS	NS
Linoleic acid	3.74	4.68	4.34	4.43	0.35	NS	NS
Arachidic acid	0.05	0.04	0.05	0.03	0.01	NS	NS
Alpha-linolenic acid	0.06	0.62	0.62	0.67	0.04	*****	NS
Conjugated linoleic acid	0.27	0.26	0.21	0.27	0.05	NS	NS
Erucic acid	0.07	0.12	0.10	0.07	0.02	NS	NS
Arachidonic acid	1.02	1.42	1.28	1.32	0.19	NS	NS
Eicosopentaenoic acid	0.23	0.31	0.26	0.26	0.04	NS	*

Docosapentaenoic acid	0.36	0.37	0.34	0.30	0.04	NS	NS
Docosahexanoic acid	0.01	0.04	0.02	0.04	0.02	NS	NS
SFAs	49.88	47.46	48.13	47.82	0.10	NS	NS
MUFAs	43.75	44.60	44.68	44.74	0.93	NS	NS
PUFAs	6.36	7.93	7.18	7.44	0.60	NS	NS
Omega - 6 fatty	5.21	6.60	6.02	6.17	0.52	NS	NS
Omega - 3 fatty acids	1.15	1.34	1.16	1.27	0.11	NS	NS
PUFA: SFA	0.13	0.17	0.15	0.16	0.01	NS	NS
PUFA: MUFA	4.57	4.85	5.25	4.90	0.056	NS	NS
n-6/n-3	4.57	5.03	5.25	4.90	0.59	NS	NS

ab Least square means in the same row with different superscript letters differ significantly ($p < 0.05$); NS, not significant ($p > 0.05$); S. E, standard error of means. SFAs, total saturated fatty acids; MUFAs, monounsaturated fatty acids; PUFAs, total polyunsaturated fatty acids; n-6/n-3, the ratio of omega-6 to omega-3 (n-6)/(n-3) fatty acids.