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Can Solvent-or Mechanically-Extracted Carinata Meal Be Used as an Effective Source of Supplemental Protein to Cows Fed Poor Quality Forages?

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CAN SOLVENT- OR MECHANICALLY-EXTRACTED CARINATA MEAL BE USED AS AN EFFECTIVE SOURCE OF SUPPLEMENTAL PROTEIN TO COWS FED POOR QUALITY FORAGES?

BY

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This thesis is approved as a creditable and independent investigation by a candidate for the Master of Science in Animal Science degree and is acceptable for meeting the thesis requirements for this degree. Acceptance of this does not imply that the conclusion reached by the candidate are necessarily the conclusions of the major department.

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CAN SOLVENT- OR MECHANICALLY-EXTRACTED CARINATA MEAL BE USED AS AN EFFECTIVE SOURCE OF SUPPLEMENTAL PROTEIN TO COWS FED POOR QUALITY FORAGES?

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Brassica *carinata* is an oilseed crop that requires a relatively short growing season and produces high crop yields. It is a great source for aviation biofuel, however it produces a large amount of waste that may be used as a protein supplement for cattle. We tested the effects of solvent- or mechanically-extracted carinata meal as a protein supplement to ad libitum ground or long-stem corn residue (i.e., corn plant left over from grain harvest) had on cow performance and digestibility. Fifty-six non-pregnant cows in 8 pens had ad libitum access to either long-stem or ground corn residue in addition to mechanically- or solvent-extracted carinata meal, canola meal (positive control), or no supplemental protein (negative control). Overall, change in body condition score (ΔBCS) was -0.6 ± 0.06 and was not affected by forage length or supplemental protein ($P = 0.37$). However, change in body weight (ΔBW) was greatest ($P < 0.01$) among cows fed canola meal, least among cows fed supplemental mechanically-extracted carinata meal or no protein, and intermediate among cows fed solvent-extracted carinata meal. Cows fed ground corn residue had less ΔBW ($P < 0.02$) cows fed long-stem corn residue. Carinata meal has a high concentration of glucosinolates, however the value of these chemical compounds can vary from processing methods. Due to the high level of glucosinolate concentration in mechanically- and solvent-extracted carinata meal (72.34 μmol/g DM

and 16.51 µmol/g DM, respectively), which can impair thyroid function and cause other negative effects, levels of T3 and T4 were tested in cows. At d 56, T3 was greater (P = 0.05) in cows fed canola meal compared to negative control cows and carinata meal was different. Protein supplement type had no effect on T4, but T3 and T4 were less (P < 0.04) at d 28 and d 56 among cows fed ground corn residue compared to cows fed long-stem corn residue. Total DMI was less among cows fed no supplemental protein, intermediate for cows fed mechanically- or solvent-extracted carinata meal, and a tended (P = 0.10) to be greatest among cows fed canola (positive control) meal. Furthermore, forage intake was greater (P < 0.01) in cows fed long-stem corn residue compared to cows fed ground corn residue. However, measures of total-tract DM, OM, NDF, and ADF digestibility were increased (P < 0.01) by more than 23% among cows fed long-stem compared to ground corn residue. Carinata meal could potentially serve as a source of supplemental protein for cows as a solvent-extracted form, but apparently not mechanically-extracted.

Chapter 1:

LITERATURE REVIEW

LITERATURE REVIEW

Brassicaceae (Mustard Family)

The Brassicaceae family (formerly known as Cruciferae), is commonly known as the mustard family and contains over 3700 species in about 300 different genera (Warwick et al., 2006). The Brassicaceae family includes cauliflower, cabbage, cameline, turnips, and canola. Brassicaceae have been commonly used as a world-wide source of oil, vegetables, and forage for livestock even though Brassicaceae typically thrive in semi-arid environments and poor fertility soils (Warwick et al., 2009). The greatest abundance of Brassicaceae are found throughout the Mediterranean and Asia (Hedge, 1976; Holm et al., 1977; Warwick et al., 2009; Al-Shehbaz et al., 2006). Many species in the Brassicaceae family are tolerant to high levels of salts and heavy metals (Megdiche et al., 2007; Przedpelska and Wierzbicka, 2007; Rascioa and Navari-Izzo, 2011). Recently, the Brassicaceae family has attracted interest as a source of biofuel due to the chemical properties of the seeds. Additionally, modest agronomic production input costs, high levels of erucic acid, and resistance to disease and pests in comparison to corn and soybeans.

Brassica carinata

Brassica *carinata* is commonly known as Ethiopian mustard and is a natural cross between *B. nigra* and *B. oleracea* in East Africa (Tsunoda, 1980; Gomez-Campo and Prakash, 1999; Rakow and Getinet, 1998). Compared to other Brassica species, *Brassica carinata* has a relatively large seed size (Getinet et al., 1997), is heat and drought tolerant (Schreiner et al., 2009), has a wide range of adaptability and has been cultivated in Europe (Mazzoncini et al., 1993; Velasco et al., 1999), Asia (Lekh et al., 1998) and North

America (Rakow and Getinet, 1998). Additionally, Ethiopian mustard has good seed yield (2.5-3.6 t ha^{-1}) and is highly tolerant to pests, disease (Bayeh and Gebre Medhin, 1992; Gugel et al., 1990; Monti et al., 2009).

Currently there is little commercial production of Brassica *carinata* and in the United States, it is most commonly grown in the Southeast region (Troy, 2018). However, there are some data on commercial production lines which have yielded 84 bushels per acre with little or no damage from disease or frost, and had average seed prices of $8/lb, inputs of $275/acre, and a net profit of $397/acre (Seepaul et al., 2016). Its potential to be used as biofuel in jet engines has been widely acknowledged ever since the Falcon20 completed a full flight powered entirely from 100% unblended bio-fuel from B. carinata oilseed (Fougeres, 2012). Not only can B. carinata be used as a source of biofuel, the oil can also be used for lubricants, waxes, plasticizers, detergents, and cosmetics (Cardone et al., 2003; Taylor et al., 2010; Warwick et al., 2006).

Brassica *carinata's* potential to be used as biofuel in jet engines has been widely acknowledged and aviation biofuel synthesized from Brassica *carinata* have successfully powered flights in jet powered aircraft (Fougeres, 2012). Additionally, Brassica *carinata* can also be useful toward synthesis of lubricants, waxes, plasticizers, detergents, and cosmetics (Cardone et al., 2003; Taylor et al., 2010; Warwick et al., 2006). However, commercial production of Brassica *carinata* in the United States has been small and most commonly grown in the Southeast region (Troy, 2018). Typically, Brassica *carinata* yields nearly 60 bushels per acre when grown in the southeastern United States (Troy, 2018) and at least one cultivator of Brassica *carinata* has yielded 84 bushels per acre with little or no damage from disease or frost, and garnered seed prices of $8/lb, inputs of

$275/acre, and a net profit of $397/acre (Seepaul et al., 2016). Further, Brassica *carinata* has also been found to yield benefits as a winter cover crop, and can be useful in mitigating soil erosion and nutrient leaching, and increases soil organic matter and moisture (Troy, 2018).

In general, Brassica carinata contains relatively high levels of glucosinolates and erucic acid compared to other oilseed crops. However, low glucosinolate cultivators have been developed, which may increase the potential to use the meal from Brassica carinata as a source of livestock feed (Getinet et al., 1997). In addition, cultivators of Brassica carinata with reduced glucosinolate content also have reduced amounts of 2-propenyl glucosinolate (a flavonoid compound that imparts the bitter taste associated with Brassica carinata meal; Getinet et al., 1996b; Getinet et al., 1997).

Oil content of Brassica *carinata* seeds can range between 37-51% (Getinet et al., 1996a; Mosca, 1998; Ripley et al., 2006). Seed oil is high in unsaturated fatty acid, with a negative correlation between erucic acid and eicosenoic acids and linoleic acids. The meal that remains after oil extraction from seeds is protein rich containing 30-45% protein (Nigussie, 1999). Furthermore, a bioactive peptide sequence with lipid and cholesterol-lowering properties has also been identified in carinata (Pedroche et al., 2007), which could be a benefit to humans in controlling high blood pressure.

Canola

Another oilseed crop that is more commonly used as a protein supplement for cattle is canola meal. Canola stands for Canada oil-low acid and has been referred to as "the new and improved rapeseed" (Nelson and Landblom 1990). Canola meal is similar to linseed meal and soybean oil meal. It is considered to be one of the world's healthiest

(Nelson and Landblom 1990) vegetable oils for human foods with seed oil content averaging above 44%. Canola meal contains high (40%) protein (NASEM, 2016). In addition, canola meal contains high levels of lysine and arginine, and is rich in vitamins and essential minerals. It is the second most used protein source in animal diets (King et al., 2001; Arntfield and Hickling, 2011).

Current canola varieties contain more fiber than desired in ruminant diet formulation, however new varieties of black-seeded canola (*Brassica napus*) are being found with increased concentration of protein and a reduced concentration of fiber (Berrocoso et al., 2015; Liu et al., 2016).

Processing methods

Processing methods of extracting oil from seeds is constantly changing and being researched. However, solvent extraction, also known as hexane extraction, is one of the most popular methods used, along with mechanical extraction via a screw press technique. After these extraction processes, there is a residual waste product, called meal. This meal is often high in protein and can be used as part of livestock feed. Digestibility and overall nutritional quality of oilseed extracted meals for ruminant livestock may be affected by the processing method used.

Solvent extraction

The most common oilseed processing method by far is solvent-extraction. It is also known as liquid extraction and is used by separating compounds based on their relative solubilities in two different immiscible liquids, typically water and an organic solvent. According to SRS Biodiesel, (2013), hexane is the most commonly used solvent for extraction as it has a boiling point of 69°C meaning it can retain liquid state at all

atmospheric conditions other than extreme climates. Furthermore, hexane uses less energy and has greater efficiency to extract oil compared to other solvents such as petroleum ether or ethyl acetate.

The 3 major steps in solvent extraction include oil extraction, solvent recovery, and meal toasting. During the oil extraction process, about 80% of oil is removed from the seeds. Following the oil extraction step, the solvent solution (most commonly hexane) is added which allows the solvent to bond with the remaining oil left in the meal. Lastly, the solvent in the meal is then removed by a desolventizer-toaster that heats the meal to evaporate solvent (Sackey, 2015).

Mechanical extraction

Mechanical extraction consists of two steps which include seed preparation based on processing type and then the actual extraction of oil from the seeds. Mechanical extraction of oil is accomplished by a sufficient force on seeds through a screw press. This process is performed without any supplemental heating and is sometimes known as cold-pressed extraction. If any heat is to occur during processing, it is likely caused from friction of the screw press and is not added into the method intentionally (Herkes et al., 2015). There is a concern for maillard reactions from oil extraction processes, so it is worth considering the effects of temperature on protein/polyphenolic interactions and protein/protein interactions as these can decrease the quality of the meal (Sackey, 2015).

Glucosinolates

Unlike canola meal, Brassica *carinata* contains higher levels of plant metabolites known as glucosinolates. Glucosinolates are β-thioglucoside β-hydroxysulfates esters with a side chain and sulfur linked β-D-glucopyranose found chiefly in the plant order

Brassicales, belonging to the family Brassicaceae (Tian et al., 2005; Winde and Wittstock, 2011). Glucosinolates are synthesized from select protein amino acids and have side-chains that are highly variable that, together with chain-elongated amino acid homologues, are responsible for the chemical diversity that constitute more than 200 reported structures (Clarke, 2010). Despite the large number of glucosinolates compounds in this group all share the same chemical skeleton (Fig. 1) and can be grouped into aliphatic, indole, and aromatic depending on the amino acid precursor (Padilla et al., 2007; Van Eylen et al., 2009; Hanschen et al., 2014). Glucosinolates are most commonly aliphatic (>50%) in nature and can be further subdivided into straight or branch chain alkenyl glucosinolates with or without a hydroxyl group (Hanschen et al., 2014).

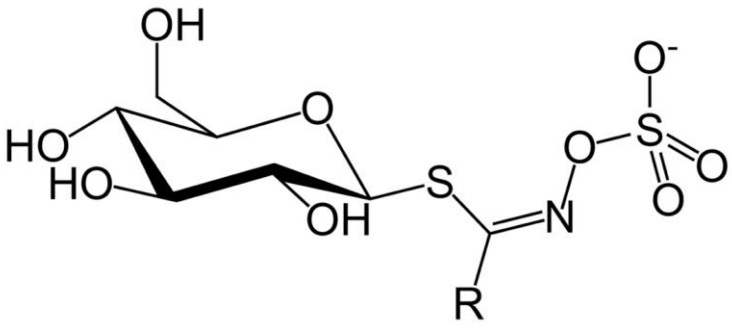

Figure 1. Glucosinolate structure; side group R will vary.

Even within tissues of the same plant species, variations in concentration and composition of glucosinolates may occur with influence from genetic makeup (Bellostas et al., 2007). For example, in *B. carinata*, glucosinolates may include but-3-enylglucosinolate, 4-hydroxyindol-3-ylmethyl, phenethyl, and 2-hydroxybut-3-enylglucosinolate (Bellostas et al., 2007; Fahey et al., 2001). However, in *Camelina sativa*, another member of the Brassica family, gluconsinolates may include 9-methyl-

sulfinyl-nonyl-glucosinolate, 10-methylsulfinyl-decyl-glucosinolate and 11-methyl-sulfinyl-undecyl-glucosinolate (Shuster and Friedt, 1998; Matthäusa and Zubr, 2000). Glucosinolate concentration in *B. carinata* can contain amounts up to 116 µmol g/DM (Bellostas et al., 2007). However, *C. sativa* of the same family, contains a much lower concentration with a range from 9 to 36.2 µmol of glucosinolates/g of dry seed (Lange et al., 1995; Schuster and Friedt, 1998; Matthäusa and Zubr, 2000). Not only do concentrations vary with plant species, but they can also fluctuate with age of the plant and can very likely be influenced by external factors such as nutrient availability, soil type, and growing season.

Myrosinase is present in the plant and seed or produced by intestinal microflora and upon tissue distribution or animal ingestion, it can cause hydrolysis to glucosinolates (Larsen 1981; Mawson et al., 1993). Myrosinase breaks down the thioglucosidic bond found in glucosinolates which produces glucose and an unstable aglycone, the thiohydroximate-O-sulfonate. Depending on the pH and temperature of the surrounding environment- or if there is a presence of Fe^{2+}, the thiohydroximate-O-sulfonate can undergo rearrangement to form a number of derivatives that include isothiocyantes, nitriles, thiocyanates, epithionitriles and oxazolidinethiones (Fig. 2; Foo et al., 2000; Ludikhuyze, 2000; Bennett et al., 2004; Cheng et al., 2004; Fahey et al., 2001; Rask et al., 2000).

Other breakdown products such as glucoraphanin, have pharmacological importance and may be a cancer chemopreventative (Fahey et al., 2003). However, other breakdown products may have adverse effects such as isothiocyantes, which are mutagenic, carcinogenic, and responsible for the bitterness of many oilseed meals

(Fenwick et al., 1983; Hill 1992; Mithen et al., 2000). These concerns may lead to a guarded interest in the potential of *B. carinata* meal being recommended as livestock feed.

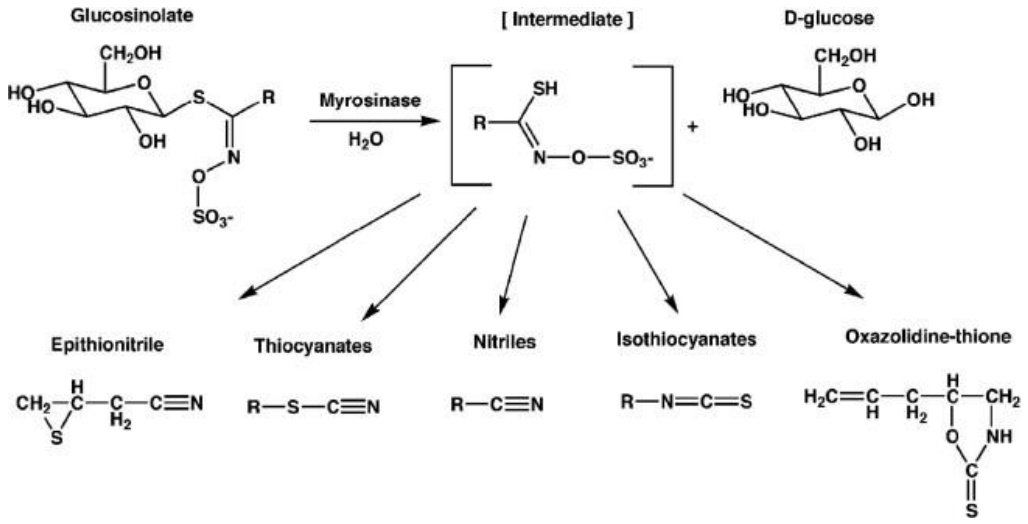

Figure 2. The general structure of glucosinolates and their enzymatic degradation products. Adapted from Rask et al. (2000).

Thyroid function

Functions of the thyroid gland include producing hormones with involvement in the metabolic response of animals to certain nutritional, environmental, and/or disease-related challenges in ruminants (Huszenicza et al., 2002). Additionally, thyroid hormones may be used as markers for the selection of high genetic merit breeds/lines. The predominant product of the thyroid gland is thyroxine (T4) in addition to trace amounts of triiodothyronine (T3; Huszenicza et al., 2002).

Impaired thyroid function can occur from consuming glucosinolates through thiocyanate and oxazolidinethione toxicity because of depressed iodine uptake (Walling et al., 2002), which results in hypertrophy of the thyroid (Griffiths et al., 1998; Halkier and Gershenzon, 2006). Because of these chemical properties, studies have shown

reduced feed intake and feed conversion ratios, impaired growth rate and ultimately decreased productivity of domestic livestock (Mawson et al., 19947a,b; Mithen et al., 2000; Burel et al., 2001; Conaway et al., 2002). Additionally, alterations in plasma T4 levels have been associated with the energy balance and metabolism (Riis and Madsen, 1985). Thus, when thyroid function is decreased, cow performance will decrease. The glucosinolate related toxicity has been documented in cattle (Virtanen et al., 1958; Vincent et al., 2988; Tripathi et al., 2001, Alexander et al., 2007), sheep (Mandiki et al., 2002), pigs (Eggum et al., 1985; Bourdon and Aumaitre, 1990), poultry (Akiba and Matsumoto, 1977), and fish (Burel et al., 2001).

Studies show that ruminant animals are more tolerant of glucosinolates than non-ruminants, but this depends on the breakdown products and composition of the included glucosinolates (Tripathi and Mishra, 2007). Results from a study by Ahlin et al., (1994), demonstrated that dairy cows had depressed fertility with inclusion of higher amounts of low glucosinolate rapeseed meal; however, calf performance was not affected at levels up to 7.7 µmol g^{-1} (Mowson et al., 1994a). Signs of toxicity and depressed thyroid function along with depressed fertility occurred when cows had a daily glucosinolate intake of 44 mmol/day (equivalent to 31 mmol/kg DM) (Ahlin et al., 1994).

Corn Residues

In the United States, there are over 303.4 billion kg DM of non-grain corn residues (i.e., leaves, husks, and stalks) from grain production each year (Schmer, 2017). This corn residue left over after harvest can be a great source of plentiful and inexpensive forage for beef cattle. According to Gallagher and Baumes, (2012), corn residues account for 45-55% of the total biomass of senesced corn plants. It was also found that amounts

of corn residue was linear related to the amounts of corn grain produced. Biomass of corn residues has yields similar to the amounts of grain harvested (Owen, 1976).

Currently, there is no research done on the energy value retained from feeding leaf and husk corn residue to cattle. However, the 2016 NRC for Beef Cattle estimate cornstalks having an ME value of 1.90 Mcal/kg (NASEM, 2016). Crude protein (CP) content of corn residue is relatively low (4.5%, Leask and Daynard, 1973), so generally cattle fed cornstalks as a source of forage would require protein supplementation. CP content of leaf residue is the greatest of all botanical corn parts at 6.5% (Stalker et al., 2015), husk contains lower (4.0%) CP and stalk contains the lowest CP (3.0%; Gutierrez-Ornelas and Klopfenstein, 1991). Research that has investigated ruminant animal digestibility of corn residue shows each botanical corn part to differ. Corn husk is the most digestible botanical part (64%) in-vitro, while stalk is lower (44%) in in-vitro organic matter digestibility (Stalker et al., 2015). Additionally, Gardine et al. (2016), found husk to be the most digestible (55.6%) while leaf and stalk were less digestible (40.7% and 386% respectively). There is more research that needs to be done in order to understand the retained energy value of these botanical parts.

Sorting and intake

When cattle are grazing long-stem corn residue, any other longer-stem forage, or larger particle size it allows for sorting and thus diet selection usually resulting in a diet with the higher nutritive value. When cattle are selective of consuming these botanical parts it is based off of digestibility and optimal N conditions that follow the bulk fill mechanism where cattle consume diets that contribute less to ruminal fill (Mertens, 1986; Church, 1988). Thus, cattle fed a ground forage lose the ability to sort out various

botanical parts. Therefore, cattle fed a ground forage versus long-stem forage result in a less digestibility of that forage but greater intake due to a faster passage rate, or decreased rumen retention time (Rasby, 2015).

Ruminally available nitrogen

Due to the large amount of lignin that corn plants contain, ruminal fermentation of corn residues are often limited (Chesson, 1984). Ruminally available N differs across botanical parts (i.e., leaves, husks, and stalks) of corn residue, but the amounts are likely insufficient to provide the amount of N needed for optimal microbial growth and rumen efficiency. Thus, protein supplementation may be needed for optimal fermentation of corn residue. Cattle fed low-quality forage such as corn residue, elicit dramatic increases in forage intake when DIP supplementation occurred (Koster et al., 1996). Similarly, other studies showed increases in low-quality forage intake in response to increasing quantities of protein supplements (Guthrie and Wagner, 1988; Stokes et al., 1988; Scott and Hibberd, 1990). Owens et al. (1991), suggested that increased dry organic matter intake (DOMI) and improved efficiency of ME use had resulted from protein supplementation. Ellis (1978) and McCollum and Gaylean (1985) suggested that improvements in voluntary intake of low-quality forages as a result of N supplementation are often associated with increases in rate of passage as well as forage digestion. Additionally, studies have shown that increased digestibility occurs when N was supplemented to beef cattle consuming low-quality forage (Del Curto et al., 1990; Scott and Hibberd, 1990; Hannah et al., 1991). When cattle are not supplemented adequate DIP, negative ruminal N digestibilities are observed (Church and Santos, 1981; Hannah et al., 1991) and are largely the result of N recycling (Bunting et al., 1989).

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CHAPTER 2:

CAN SOLVENT- OR MECHANICALLY-EXTRACTED CARINATA MEAL BE USED AS AN EFFECTIVE SOURCE OF SUPPLEMENTAL PROTEIN TO COWS FED POOR QUALITY FORAGES?

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ABSTRACT

Oils from Brassica *carinata* seeds may be useful to the synthesis of aviation-based biofuels. Carinata meal is a coproduct derived from lipid extraction of Brassica *carinata* seeds and may be used as a source of supplemental protein to ruminants; however, few data are available on the value of carinata meal to cattle. We evaluated the effects of solvent- or mechanically-extracted carinata meal on performance and apparent total-tract digestibility among cows fed ad libitum amounts of ground or long-stem corn residue. Overall, change in body condition score (ΔBCS) was -0.6 ± 0.06 with no interaction between forage length and supplemental protein ($P > 0.37$). Nonetheless, ΔBW was greatest ($P < 0.01$) among cows fed canola meal (12.35 kg ΔBW), least among cows fed mechanically-extracted carinata meal (-2.72 kg ΔBW) or no supplemental protein (-14.03 ΔBW), and intermediate (3.32 ΔBW) among cows fed solvent-extracted carinata meal. Cows fed ground corn residue had less ΔBW than cows fed long-stem corn residue. Mechanically-extracted carinata meal had greater levels of glucosinolates (72.34 mmol/g), compared to solvent-extracted carinata meal (16.51 mmol/g) or canola meal (1.57 mmol/g). After 56d of receiving supplemental protein, triiodothyronine (T3) was greater ($P = 0.05$) among cows fed canola meal compared to cows that received no supplemental protein; cows provided either mechanically- or solvent-extracted carinata meal had intermediate amounts of T3. Protein supplement had no effect on T4, but cows fed ground corn residue has less ($P < 0.04$) T3 and thyroxine (T4) at d 28 and d 56 compared to cows fed long-stem corn residue. Total DMI was less among cows fed no supplemental protein, intermediate for cows fed mechanically- or solvent-extracted carinata meal, and greatest among cows fed canola (positive control) meal. Furthermore, forage intake was greater ($P < 0.01$) in cows fed long-stem corn residue compared to

cows fed ground corn residue. Measures of total-tract DM, OM, NDF, and ADF digestibility were increased ($P < 0.01$) more than 23% among cows fed long-stem compared to ground corn residue. These data suggest that carinata meal may be a useful source of supplemental protein to cattle fed low-quality forage; however, processing methods used in manufacturing of carinata meal affect feeding value of carinata meal to cattle.

KEY WORDS: cattle, corn residues, protein supplement, canola meal, carinata meal, performance, digestibility

INTRODUCTION

Biofuels can mitigate environmental impacts of combustion engines and grow agricultural economies. The United States Federal Aviation Administration has set a goal that at least 3,800,000,000 L of renewable jet fuel be used annually by United States aircraft (FAA, 2018). However, benefits are limited when biofuels are manufactured from seedstocks (e.g., corn, soybeans) that require relatively large agricultural inputs (e.g., fertilizer, energy), land with large agricultural value, and high-energy inputs in refining (Hill et al., 2006). Brassica *carinata* (also referred to as Ethiopian mustard, African cabbage, or Ethiopian kale) is a leafy mustard plant that produces large yields and has a relatively short growing season. Additionally, Brassica *carinata* is tolerant to heat, drought, pests, disease, and does not require large agricultural inputs (Teklewold and Becher, 2006). Brassica *carinata* contains relatively large concentrations of erucic acid (22:1ω9) which allows for efficient conversion of plant lipid to aviation fuel (Jadhav et al., 2005).

Some byproducts from some biofuel production processes have nutritional value to livestock (e.g., distillers' grains, glycerol) and can be important toward improving the economic feasibility of biofuel production (Coyle, 2007; Hofstrand, 2018). Oil yields from Brassica *carinata* for production of aviation of fuel are typically around 33%. Thus, carinata meal represent nearly 67% of the mass of all Brassica *carinata* used for biofuel production. Carinata meal generally has relatively large amounts of crude protein (CP), but small amounts of Neutral Detergent Fiber (NDF) (Anderson et al., 2015). Further, carinata meal contains relatively large amounts of glucosinolates and, like all other mustard meals, the United States Food and Drug Administration (FDA) limits amount of

carinata meal fed livestock due to concerns related to increased intake of erucic acid, which can contribute to myocardial lipidosis in mammals (FDA, 2018). Glucosinolates are secondary plant metabolites that impart a bitter taste to Brassica vegetables and when consumed in large quantities glucosinolates can interfere with normal thyroid function (Lardy and Kerley, 1994). It is possible that carinata meal could be used as a source of supplemental protein to cattle; however, it is unclear if the feeding value of carinata meal is limited by inherent amounts of glucosinolates.

Typically, oil is extracted from oilseed either by pressing (mechanically-extracted) or extraction with lipid-soluble solvents (e.g., hexane). Solvent-extraction removes greater amounts of oil in comparison to mechanical extraction, but the solvent extraction processes generally use higher extraction temperatures in comparison to mechanical extraction techniques. Currently, there is a paucity of data about the feeding value of carinata meal to cattle. Large quantities of carinata meal are likely to be manufactured from either solvent- or mechanical-extraction techniques, and we are unaware of any data related to the feeding value of either solvent- or mechanically-extracted carinata meal to beef cattle.

MATERIALS AND METHODS

Animal Husbandry and Sample Collection

All protocols that involved the use of animals in this study were approved by the South Dakota State Institutional Animal Care and Use Committee (protocol approval No. 16-031A). Fifty-six non-pregnant non-lactating black commercial cows were blocked by initial BW (average BW = 482.4 ± 9.5 kg) and placed in a split-plot design to evaluate

effects of different sources of supplemental protein to cows fed long-stem or ground corn residues dry matter (DM) = 87.4 ± 0.03%, crude protein (CP) = 4.1 ±0.01%, NDF = 76.1 ± 0.8%). Forage length was the whole plot factor and cows were randomly assigned across pens within each BW block as a randomized incomplete block design. Source of supplemental protein was the subplot factor. Each 7 cows within each BW block (n = 14 cows per BW block) were randomly placed in a pen (38.4 m × 30.8 m) and randomly assigned to 1 of 4 supplemental protein treatments (Table 1). Cows were provided ad libitum access to corn residue, water and a pressed vitamin and mineral block (Prairie Pride 4% Mineral Block, Ridley Inc., Mankato, MN; 20% Ca, 12% NaCl, 4% P, 1,000 parts per million (ppm) Zn, 100 ppm Cu, 36 ppm I, 36 ppm Se, 143,300 IU/kg vitamin A, and 35,932 IU/kg vitamin D_3). Two cows within each pen received either 878 g/d of mechanically-extracted carinata meal, 821 g/d of solvent-extracted carinata meal, or 1004 g/d solvent-extracted canola meal (positive control). Each source of supplemental protein was provided in amounts designed to meet requirements for ruminally available N (NRC, 2000). Amounts of ruminally available N for canola meal were based on tabular values (NRC, 2000). Amount of ruminally available N from each source of carinata meal were estimated from *in situ* measures of ruminal N disappearance (Sackey, 2015) and an estimated total mean retention time of 48 h. The remaining cow in each pen received no supplemental protein and served as a negative control. Prior to d 0 of the feeding trial, cows had access to grazing while on pasture with no adaptation period.

Supplemental protein was fed to cows daily (0900 h) by placing cows in individual pens (3.0 m × 1.5 m) located in an enclosed building immediately adjacent to the pens in which cattle were housed. Cows were allowed 15 minutes to consume

supplemental protein and then returned to their pens. If any feed remained, orts were collected and composited (50 g/d; subsampled) by cow for analyses of DM, OM, ADF, ADIA, and NDF. Cows assigned to the negative control were placed in stalls daily but were provided no supplemental protein. Samples of long-stem corn residues were obtained by removing triplicate cores (Nasco Forage Sampler, 18" Round Shank, C06541N, Pennsylvania State University) taken from separate locations of each bale of corn residues. Cores of every bale were composited and analyzed for DM, ash, ADF, ADIA, and NDF. Samples of ground corn residues were collected by collecting triplicate spot samples immediately prior to feeding ground corn residues. Corn residue samples were composited from all cores by forage length (long-stem or ground) and analyzed for DM, OM, ADF, ADIA, and NDF.

Cow BCS was evaluated immediately before and BW was measured immediately after cows were offered supplemental protein on d 1, 14, 28, 42, and 56. Water was not withheld from cows prior to any measurements of BW or BCS. Measures of BCS (1 to 9 scale; Cantrell et al., 1982; Wagner, 1984; Selk et al., 1988; Whitman, 1975) were determined by a panel of 3 trained technicians, and reported values represent an average score. Blood was collected by jugular venipuncture (10 mL; BD Vacutainer; Franklin Lakes, NJ, USA) after cows were offered supplemental protein on d 1, 42, and 56. Subsequently, plasma was harvested (1,500 × g for 15 min at 4°C) and frozen prior to analysis of triiodothyronine (T3) and thyroxine (T4). For T3 concentrations, serum concentrations were determined in duplicate by free RIA with the T3 Solid Phase RIA System (06B-254215, MP Biomedical, Solon, OH, USA) according to the manufacture's instructions. Intra-and inter-assay CV were 6.0% and 6.4%, respectively. Sensitivity of

the assay was 4.8 ng/dL. For T4 concentrations, serum was determined in duplicate by free RIA with the 4 Monoclonal RIA System (06B-254011, MP Biomedical, Solon, OH, USA) according to the manufacture's instructions. Intra- and inter-assay CV were 7.6% and 8.2%, respectively. Sensitivity of the assay was 0.51 µg/dL.

Cows were provided chromic oxide (Cr_2O_3; 10 g/d) orally in a gelatin capsule using a bolus gun (WI-0000851, TORPAC, Fairfield, NJ) immediately after offering supplemental protein each day from d 25 to 34. Spot samples of feces weighing (200 g/d) were collected six times per day from d 30 to 34 as cows were run through the chute. Samples of feces were collected each 4-h beginning at 0900 h and sampling time was delayed by 1 h daily so that composite feces reflected every h in a 24 h period. Feces was composited by cow and frozen (-20°C) prior to analyses of DM, ash, ADF, ADIA, NDF, and Cr_2O_3 concentration after each sampling period.

Following measures of performance and digestibility, cows were administered Gonadotropin-releasing Hormone (GnRH) on d 56 (100 µg as 2 mL of Factrel i.m.; Zoetis, Parsippany, NJ) and CIDRs (Zoetis, Parsippany, NJ) were inserted intravaginally immediately following injection of GnRH. On d 63, PGF2α was administered (25 mg as 5 mL of Lutalyse i.m.; Zoetis, Parsippany, NJ), and CIDRs were removed. Cows were artificially inseminated with semen from one of two sires equally allotted between treatments at 60 to 66 h after CIDR removal and were administered an injection of GnRH (2 mL Factrel i.m.) at the time of insemination. Following insemination all cows were moved to a common pasture and managed as a single group until pregnancy was determined by transrectal ultrasonography 30d following breeding on d65.

Prior to laboratory analyses samples of feces were weighed and partially dried (55°C for 36 h). Corn residue, each supplemental protein, orts and feces were then ground to pass a 1-mm screen (Thomas Wiley Mill Model 4; Thomas Scientific Swedesboro, New Jersey, USA) and analyzed for DM, OM, NDF, ADF, and ADIA. Dry matter was measured by drying at 105°C for 16 h, and OM was determined by combustion (500°C for 16 h). Nitrogen content was analyzed by the Dumas procedure (method no. 968.06; AOAC, 2016; rapid Max N exceed; Elementar, Mt. Laurel, NJ). Neutral detergent fiber was measured as described by Van Soest et al. (1991) and included additions of □-amylase and sodium sulfite. Acid detergent fiber was measured nonsequential to NDF (Van Soest et al., 1991), and ADIA was calculated by combustion (500°C for 16 h) of ADF residue. Measures of NDF and ADF were corrected for ash content which was measured by combustion (500°C for 8 h). Chromic oxide concentration was measured by atomic absorption after acid digestion (Potassium bromate and manganese sulfate) of feces (Williams et al., 1962).

Calculations

Dry matter was calculated as partial DM (55°C for 36 h) multiplied by DM measured after drying at 105°C for 16 h. Fecal output was calculated as the quotient of Cr_2O_3 intake (10g/d) and fecal Cr_2O_3 concentration. Fecal excretion of N, OM, NDF, ADF, and ADIA was calculated by multiplying daily fecal output by fecal concentration of N, OM, NDF, ADF, and ADIA, respectively. Subsequently, DMI was estimated as described by (Merchen, 1988):

$$\text{DMI} = \text{fecal output} \times (100/\text{percent indigestibility of DM})$$

Intake of supplemental protein was measured gravimetrically, corrected for DM content and intake of OM, N, NDF, ADF, and ADIA from supplemental protein was calculated as the product of supplemental protein intake and the concentration of each nutrient in each source of supplemental protein. Forage DMI was calculated from the concentration of ADIA in forage and amounts of ADIA excreted in feces after subtracting intake of ADIA from supplemental protein (DM-basis). Subsequently, intake of OM, NDF, and ADF, from corn residue was calculated as the product of forage DMI and OM, NDF, or ADF, in corn residue (Table 2). Total intake of OM, CP, NDF, and ADF were calculated as the sum of intake from forage and supplemental protein. Glucosinolate concentration in the protein supplements was measured through the University of Washington. Mechanically-extracted carinata meal contained 72.34 µmol/g DM, solvent-extracted carinata meal contained 16.51 µmol/g DM, and canola meal (positive control) contained 1.57 µmol/g DM. Thus, cows in the mechanically-extracted carinata treatment were consuming amounts up to 63 mmol/d DM.

Statistical Analyses

Two cows were removed (studentized residual equal to -6.4705 and -5.1376, respectively) from all data analyses using INFLUENCE diagnostics due to minimal intake of supplemental protein (13.2% and 25.3% total DM offered, respectively). Both cows were in the different pens receiving canola meal (positive control), but were receiving different protein treatments. A third cow died on d 44 during the experiment and necropsy results revealed circumstances were not related to the study. Data from this cow was kept in the statistical analyses up until the cow was deceased, then further recorded as a missing observation.

Data were analyzed as a randomized split-plot design using the MIXED procedure of SAS 9.3 (SAS Inst. Inc., Cary, NC), and forage length was the whole plot factor. Animal was considered the experimental unit because cattle were fed supplemental protein and chromic oxide boluses individually, thus individual intake and performance was determined. For measures of DMI, nutrient flow to feces and total-tract digestibility (i.e. non-repeated measures), terms in the model included source of supplemental protein, forage length, and their interaction, as well as the effects of block and block × forage length. Conception data were analyzed using a GLIMMIX procedure of SAS. Fixed effects were supplemental protein type, forage length, and interaction of supplemental type and forage length. Pen was used as the random effect. All data are reported as LSmeans ± SE of the mean. Mean separation was performed using least significant differences (pdiff function in SAS), and differences were considered to be significant when $P \leq 0.05$ and a tendency when $P > 0.05$ but $P \leq 0.10$.

Performance data and measures of circulating amount of T3 and T4 were analyzed as repeated measures using the MIXED procedure of SAS. The respective best fit model was used for each analysis. The statistical model considered forage length, protein source, day, and all 2 and 3-way interactions. Day was used as the repeated term. Linear and quadratic differences were used to determine effects of day on ΔBW, ΔBCS, and circulating amounts of T3 and T4. All data are reported as LSmeans ± SE of the mean. When a significant effect ($P \leq 0.05$) or tendency ($P \leq 0.10$) was detected the pairwise comparisons from the analysis were used to determine level of significance. Pairwise mean separation was performed using least significant differences (pdiff function in SAS).

RESULTS

Effects of supplemental protein and forage length on dry matter intake (DMI), fecal output and total-tract nutrient digestion supplement are reported in Table 2. There were differences in intake ($P \leq 0.01$) between sources of supplemental protein. Cows were provided amounts of mechanically-extracted and solvent-extracted carinata meal or canola meal designed to meet needs for ruminally available N. As expected, cows fed canola meal (positive control) had the greatest amount of protein intake ($P \leq 0.01$). Cows fed solvent-extracted carinata meal had slightly greater amount of supplemental protein intake compared to cows fed mechanically-extracted carinata meal ($P \leq 0.01$). Interestingly, voluntary intake of mechanically-extracted carinata meal was small (14.3% of total DM offered). Estimates of corn residue intake ($P \leq 0.01$) were 44.5% greater among cows fed long-stem corn residue in comparison to cows fed ground corn residue. There was no interaction of forage length and supplemental protein on estimates of corn residue intake or intake of supplemental protein ($P \geq 0.13$). Cows fed either solvent-extracted carinata or canola meal (positive control) had greater ($P \leq 0.01$) amounts of corn residue intake than cows fed mechanically-extracted carinata meal or no supplemental protein (negative control). There was no interaction of forage length on supplemental protein intake ($P = 0.32$) and no interaction of source of supplemental protein and forage length on supplement intake ($P = 0.26$).

Estimates of DM (60.4 ± 1.36), OM (65.8 ± 1.40), NDF (73.6 ± 1.41), or ADF (64.2 ± 1.37) total-tract digestibility were not affected by supplemental protein. However, estimates of DM, OM, NDF, and ADF were increased ($P \leq 0.01$) more than 22% among cows fed long-stem corn residue compared to ground corn residue. There

was; however, no interaction between forage length and supplemental protein on estimates of total-tract DM, OM, NDF, and ADF digestibility ($P \geq 0.06$).

Effects of supplemental protein and forage length on ΔBCS and ΔBW are reported in Table 3. There was no protein × length × time interaction ($P = 0.22$) between supplemental protein, length of forage or time on ΔBCS or ΔBW. Cows provided no supplemental protein (negative control) lost 14.0 kg in BW and 0.5 BCS overall, which suggests that energy derived from fermentation of corn residue alone did not provide adequate amounts of energy to meet the maintenance requirements of cows. Alternatively, cows provided canola meal (positive control) had a smaller decrease ($P < 0.01$) in overall ΔBCS and greater overall ΔBW in comparison to cows provided no supplemental protein (negative control). Similarly, cows provided solvent-extracted carinata meal had a smaller decrease ($P < 0.01$) overall in overall ΔBCS than cows provided no supplemental protein, but overall ΔBCS was not different ($P = 0.23$) between cow provided solvent-extracted carinata meal or canola meal (positive control). Yet, cows provided mechanically-extracted carinata meal had a greater ($P < 0.01$) loss of BCS than cows provided solvent-extracted carinata meal or canola meal (positive control), but losses in BCS among cows fed mechanically-extracted carinata meal tended ($P = 0.07$) to be greater than losses in BCS among cows provided no supplemental protein (negative control). As expected, ΔBW were largest ($P \leq 0.01$) among cows provided the positive control (canola meal) and least among cows provided no supplemental protein (negative control). Nonetheless, ΔBW were not different ($P = 0.16$) between cows provided mechanically-extracted or solvent-extracted carinata meal and were intermediate to ΔBW among cows provided canola

meal (positive control) and cows provided no supplemental protein (negative control). There was no effect ($P = 0.22$) of forage length on ΔBCS. However, cows fed ground corn residue lost ($P = 0.02$) 8.4 kg BW compared to a 10.7 kg increase in BW among cows fed long-stem corn residue.

Effects of supplemental protein and forage length on T3 and T4 are reported in Table 4. There was no interaction among protein, forage length, or time on circulating concentrations of T3 ($P = 0.87$) or T4 ($P = 0.66$) in jugular blood. Cows provided supplemental canola meal (positive control) had the greatest ($P \leq 0.01$) amount of circulating T3, and cows provided no supplemental protein (negative control) had the least. Similar to the negative control, cows supplemented with mechanically-extracted carinata meal had less ($P \leq 0.01$) circulating amounts of T3 than cows supplemented with canola meal. Additionally, cows fed solvent-extracted carinata meal tended ($P = 0.07$) to have less circulating T3 than cows fed canola meal. Amounts of circulating T3 were not different ($P = 0.41$) between cows fed mechanically- or solvent-extracted carinata meal. We did not observe any difference in circulating amounts of T4 in response to supplemental protein; however, circulating amounts of T3 and T4 decreased ($P < 0.01$) as days on feed increased. Additionally, cows fed long-stem corn residue had ($P \leq 0.01$) nearly 23% more circulating T3 and 28% more T4 in comparison to cows fed ground corn residue.

There was no interaction of main effects of forage length ($P = 0.23$), protein source ($P = 0.53$), or forage × protein interaction ($P = 0.27$) on conception rates. This could be in part due to a lack of an adequate number of observations ($n = 54$) to account for amounts of variance typically inherent to measures of conception rate in cattle. This

is supported by the fact that with 54 animals a power test indicated we were only able to detect a 0.382 or greater difference in conception rates.

DISCUSSION

Ruminal fermentation is often limited when cattle are fed forages with small amounts of ruminally available N (e.g., corn residues, prairie hay, citrus pulp). Limitations in ruminal fermentation of fiber in response to small amounts of ruminally available N also limit intake of forage (Koster et al., 1996). Overall, limitations in DMI and amounts of fermentation end products produced limit amounts of energy available for physiologically productive purposes when cattle are fed forage-based diets with small amounts of ruminally available nitrogen. Therefore, when cattle fed forages with limited amounts of N are realimented by feeding supplemental protein ruminal fermentation of fiber, intake and net energy derived from the diet are increased (Owens et. al., 1991). Generally, amounts of ruminally available N in corn residues are inadequate to support optimal ruminal fermentation of fiber (NASEM, 2016). In this experiment, cattle fed corn residue without any supplemental protein had decreased body weight and decreased BCS. Amounts of supplemental protein offered to cows in this study were designed to meet the needs for ruminally available nitrogen (Burroughs et al., 1978). Cows fed no supplemental protein (negative control) had the smallest DMI, ΔBW, and ΔBCS and tended to have the lowest estimates of forage intake. Alternatively, cows fed canola meal (i.e., the positive control) had the greatest DMI, ΔBW, and ΔBCS and tended to have the greatest estimates of forage intake. Together, differences between the positive control and negative controls for protein

supplementation provide strong evidence that amounts of ruminally available N provided from corn residues alone were inadequate to optimize ruminal fermentation of fiber. Measures of DMI, BW, and BCS among cows provided carinata meal were intermediate to the positive and negative control. Interestingly, measures of DMI, ΔBW, and ΔBCS among cows provided mechanically-extracted carinata meal were similar to cows fed no supplemental protein, but cows fed solvent-extracted carinata meal were intermediate to the negative and positive control. Cows fed cold-pressed carinata meal only consumed 14.3% of total DM offered as supplemental protein. Thus, it is likely that the aversion to mechanically-extracted carinata meal by cows in this study limited benefits to performance, intake and digestion rather than limits in ruminal N availability within mechanically-extracted carinata meal, per se. Ban et al., (2017) reported that *in situ* protein degradability was less in solvent-extracted carinata meal in comparison to *in situ* protein disappearance from cold-pressed carinata meal. Diet metabolizable energy contents can be reduced when cattle are fed rapeseed meal (a protein with amounts of glucosinolates similar to carinata meal) in comparison to other protein meals (e.g., soybean meal) that do not contain glucosinolates (Bell, 1983).

Cows fed long-stem corn residue had greater amounts of DM, OM, NDF, and ADF digestibility compared to cows fed ground corn residue. We did not measure daily refusals of corn residue in this study; however, it seems likely that the ability of cattle to select different botanical parts from baled corn residues was reduced among cows fed ground corn residues compared to cows fed long-stem corn residue. Typically, cattle select diets with greater digestibility than the apparent digestibility of the overall biomass (Miller-Cushon et. al., 2016). It is likely that DMI is also limited when the

ability of cattle to select more digestible botanical parts is restricted. It is possible that cattle fed long-stem corn residue selected botanical parts with greater amounts of ruminally available N. Nonetheless, we did not observe any interaction between supplementation of protein and forage length on measures of DMI or total-tract digestion of nutrients. This suggests that even when cattle were better able to sort different botanical parts of corn residue amount of ruminally available N still limited DMI, but to a lesser extent than cattle fed ground corn residue.

Glucosinolates are a sulfur-containing compound that can affect liver function and hinder normal thyroid hormone production through the breakdown activities of thiocyanate, isothiocyanate, oxazolidinethione (goitrin) and nitriles. Glucosinolates impart the characteristic bitter taste to brassicas (Fenwick et al., 1983; Hill 1991; Mithen et al., 2000). However, we are unaware of any measures on the ability of cattle to taste glucosinolates specifically. Cattle in this study refused to consume a large amount of supplemental protein from mechanically-extracted carinata meal, which also had the greatest concentration of glucosinolates. Alternatively, cattle consumed nearly all amounts of solvent-extracted carinata meal offered, which had only small amounts of glucosinolates. It is possible that intake of mechanically-extracted carinata meal was reduced because of greater concentration of glucosinolates in this meal.

Glucosinolate toxicity may affect thyroid function. Circulating T3 and T4 amounts in livestock are reflective of energy status and iodine uptake. Ahlin et al., (1994) reported that thyroid function can be reduced when cattle are fed diets with increased amounts of glucosinolates (31 mmol/kg DM) in the feed daily. Circulating amounts of T3 were not different between cows provided mechanically- or solvent-extracted carinata

meal in this study and supplemental protein had little impact on circulating amounts of T4 even though amounts of T3 and T4 were reduced among cows fed ground versus long-stem corn residues. Furthermore, intake of mechanically-extracted carinata meal was small. It seems unlikely that intake of glucosinolates directly impacted thyroid function among cows in this experiment, and differences in circulating amounts of T3 and T4 are likely reflective of differences in energy balance.

Apparently, Brassica *carinata* meal may be a beneficial source of supplemental protein to cattle; however, processing methods affect the extent to which cows utilize it. There was a lack of evidence to prove that the glucosinolate concentration in mechanically- or solvent-extracted carinata meal was high enough to directly impact thyroid function,and subsequently, cow performance.

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Table 1. Chemical composition of mechanically-extracted (MECM) or solvent-extracted (SECM) carinata meal and canola meal supplemented to cows.

Chemical component	MECM	SECM	Canola
DM, %	91.1 ± 0.01	92.32 ± 0.02	91.90 ± 0.02
OM, %	93.47 ± 0.01	91.28 ± 0.01	91.07 ± 0.01
CP, %	40.07 ± 0.02	49.56 ± 0.01	39.27 ± 0.01
NDF, %	23.78 ± 0.	31.19 ± 1.64	33.26 ± 0.56
ADF, %	16.17 ± 0.61	16.20 ± 0.72	23.83 ± 0.21
ADIA, %	-0.32 ± 0.07	0.49 ± 0.07	0.37 ± 0.07
Glucosinolates, µmol/gDM			
Progoitrin	0.62 ± 0.07	0.19 ± 0.01	0.39 ± 0.06
Glucoraphanine	0.00 ± 0.00	0.00 ± 0.00	0.04 ± 0.02
Sinigrin	65.19 ± 7.82	14.92 ± 0.04	0.10 ± 0.04
Glucoalyssin	0.03 ± 0.02	0.02 ± 0.02	0.02 ± 0.02
Sinalbin	0.10 ± 0.02	0.83 ± 0.27	0.02 ± 0.01
Gluconapin	0.63 ± 0.06	0.14 ± 0.01	0.28 ± 0.04
t-Butyl	0.09 ± 0.01	0.04 ± 0.01	0.04 ± 0.02
Glucoiberverin	2.87 ± 0.57	0.02 ± 0.00	0.46 ± 0.04
4-Hydroxyglucobrassicin	1.15 ± 0.20	0.06 ± 0.01	0.16 ± 0.01
Glucotrapaeolin	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.01
Glucobrassicin	0.17 ± 0.03	0.03 ± 0.01	0.05 ± 0.01
Gluconasturtiin	0.72 ± 0.10	0.13 ± 0.01	0.00 ± 0.00
Neoglucobrassicin	0.77 ± 0.10	0.13 ± 0.01	0.00 ± 0.00

Table 2. Effects of ground or long-stem corn residue and supplemental protein from mechanically- (MECM), or solvent-extracted (SECM) carinata meal or canola meal on estimates of nutrient intake, fecal nutrient flows and apparent total-tract digestion in cows[1]

	Long-stem corn residue				Ground corn residue				*P*		
	None	MECM	SECM	Canola	None	MECM	SECM	Canola	*Forage*	*Protein*	*Forage* × *protein*
DMI, kg/d.	13.9	13.1	16.8	16.6	9.2	10.4	10.0	13.8	<0.01	<0.01	0.13
Corn residue intake	13.9	13.0	16.0	15.5	9.2	10.2	9.2	11.8	<0.01	0.10	0.13
Supplement intake[2]	0.0	0.1	0.8	1.0	0.0	0.2	0.8	1.0	0.32	<0.01	0.26
Nutrient flow to feces, kg/d											
DM[3]	4.4	4.3	5.0	4.9	3.86	4.25	4.67	5.38	0.44	<0.01	0.26
OM[3]	3.3	3.2	3.7	3.6	3.07	3.40	3.83	4.21	0.18	<0.01	0.34
NDF[4]	1.9	1.9	2.2	2.2	2.16	2.14	2.64	3.09	<0.01	<0.01	0.16
ADF[3]	2.2	2.2	2.6	2.6	2.17	2.30	2.58	3.00	0.25	<0.01	0.28
Total tract digestion, %											
DM	67.7	66.3	68.2	60.1	56.5	57.3	47.4	53.7	0.01	0.28	0.09
OM	73.4	72.1	74.0	74.0	61.3	61.9	51.9	59.7	0.01	0.22	0.07
NDF	81.5	81.2	81.8	81.6	67.7	71.5	60.6	65.2	<0.01	0.30	0.09
ADF	72.5	71.0	72.4	72.3	59.0	61.0	50.9	56.5	<0.01	0.18	0.06

[1]Supplemental protein was provided to meet daily degradable intake protein (DIP) requirements from either mechanically-extracted (MECM) or solvent-extracted (SECM) carinata meal or from solvent-extracted canola meal.

[2]Canola > SECM > MECM > None.

[3]Canola and SECM > MECM and None.

[4]Canola and SECM > MECM; Canola > None; SECM and None did no differ ($P = 0.06$).

Table 3. Effects of ground or long-stem forage and supplemental protein from no supplemental protein (none), mechanically- (MECM), or solvent-extracted (SECM) carinata meal or canola meal on changes in body weight and body condition score in cows[1]

	Long-stem corn residue				Ground corn residue					*P*			Day	
	None	MECM	SECM	Canola	None	MECM	SECM	Canola	SEM	*Forage*	*Protein*	*Forage* × *protein*	*Linear*	*Quadratic*
ΔBW[2]									12.43	0.02	<0.01	0.79	0.34	0.20
d1	482.2	478.7	485.8	482.6	497.0	480.7	480.2	494.5						
d14	-0.7	-1.3	6.5	13.2	-9.6	4.4	2.3	3.6						
d28	-1.1	31.2	6.4	22.9	-21.8	-12.0	3.3	1.9						
d42	-16.3	-12.5	5.9	18.3	-40.5	-18.5	-8.3	1.5						
d56	0.1	1.3	14.5	30.3	-22.2	-14.3	-4.1	7.1						
ΔBCS[3]									0.16	0.22	<0.01	0.62	<0.01	<0.01
d1	4.98	5.18	4.82	4.95	4.89	4.99	4.82	4.96						
d14	-0.06	-0.24	0.01	0.04	-0.31	-0.15	0.09	0.10						
d28	-0.48	-0.22	-0.10	0.06	-0.48	-0.35	-0.07	-0.07						
d42	-0.23	-0.24	0.09	0.09	-0.65	-0.38	-0.26	0.01						
d56	-0.85	-0.58	-0.32	-0.37	-0.98	-0.92	-0.45	-0.33						

[1]The interaction of *forage* × *protein* × *day* was not significant ($P = 0.69$)

[2]Canola > MECM and SECM > None; MECM and SECM did not differ ($P = 0.16$).

[3]Canola and SECM > MECM > None; Canola and SECM did not differ ($P = 0.23$).

Table 4. Effects of ground or long-stem corn residue and supplemental protein from no supplemental protein (none), mechanically- (MECM), or solvent-extracted (SECM) carinata meal or canola meal (can) on circulating amounts of triiodothyronine (T3) or thyroxin (T4)[1]

	Long-stem corn residue				Ground corn residue					*P*				
													Day	
	None	MECM	SECM	Can	None	MECM	SECM	Can	SEM	*Forage*	*Protein*	*Forage* × *protein*	*Linear*	*Quadratic*
T3[2,3]									12.4	<0.01	<0.01	0.07	<0.01	<0.01
d1	91.5	87.8	92.5	94.3	65.0	78.5	87.4	80.3						
d28	103.5	90.8	91.7	107.7	53.4	74.4	79.1	86.3						
d56	62.3	70.9	67.8	78.9	39.2	51.7	53.8	65.5						
T4[2]									0.16	<0.01	0.16	0.23	<0.01	<0.01
d1	5.01	4.35	4.68	4.71	3.41	4.42	4.79	4.00						
d28	5.63	5.90	6.32	5.69	3.25	4.01	4.47	4.53						
d56	4.48	4.54	4.17	4.71	2.17	2.21	2.82	3.18						

[1]The interaction of *forage* × *protein* × *day* was not significant ($P \geq 0.66$)

[2]T3 and T4 values are represented as ng/dL.

[3]Canola > MECM and None; SECM > None; Canola and SECM did not differ ($P = 0.07$); SECM and MECM did not differ ($P = 0.41$).

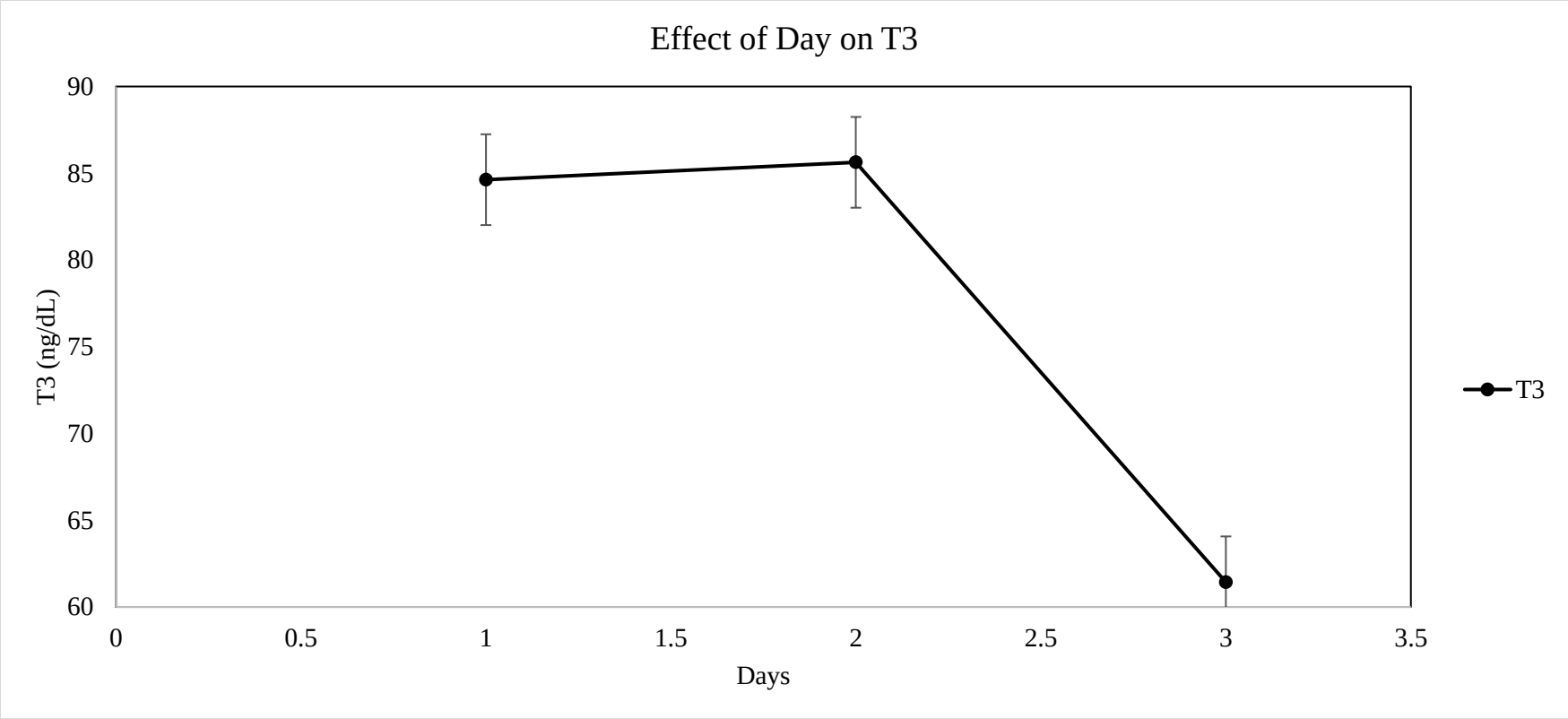


Figure 1. The effect of *Day* on triiodothyronine (T3) levels (ng/dL).

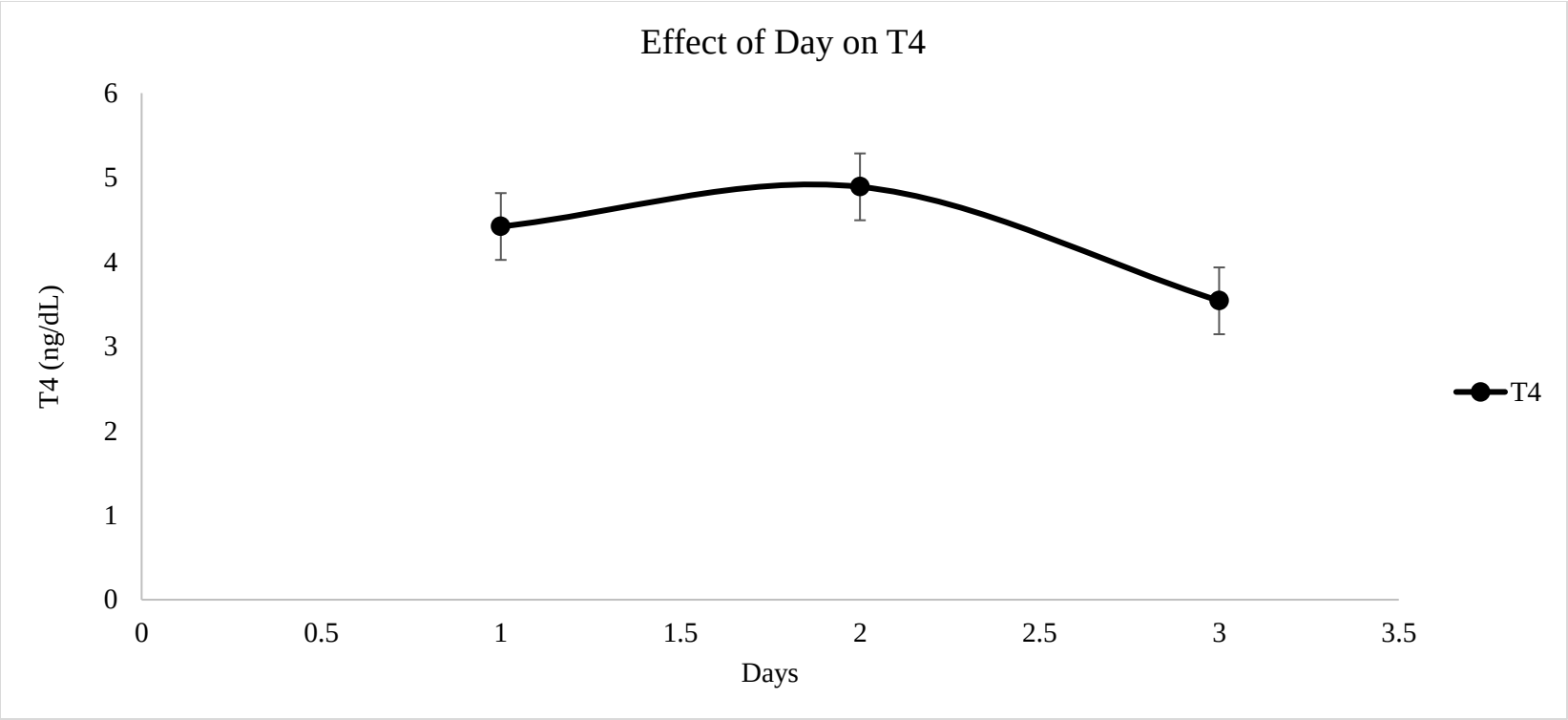


Figure 2. The effect of *Day* on thyroxine (T4) levels (ng/dL).

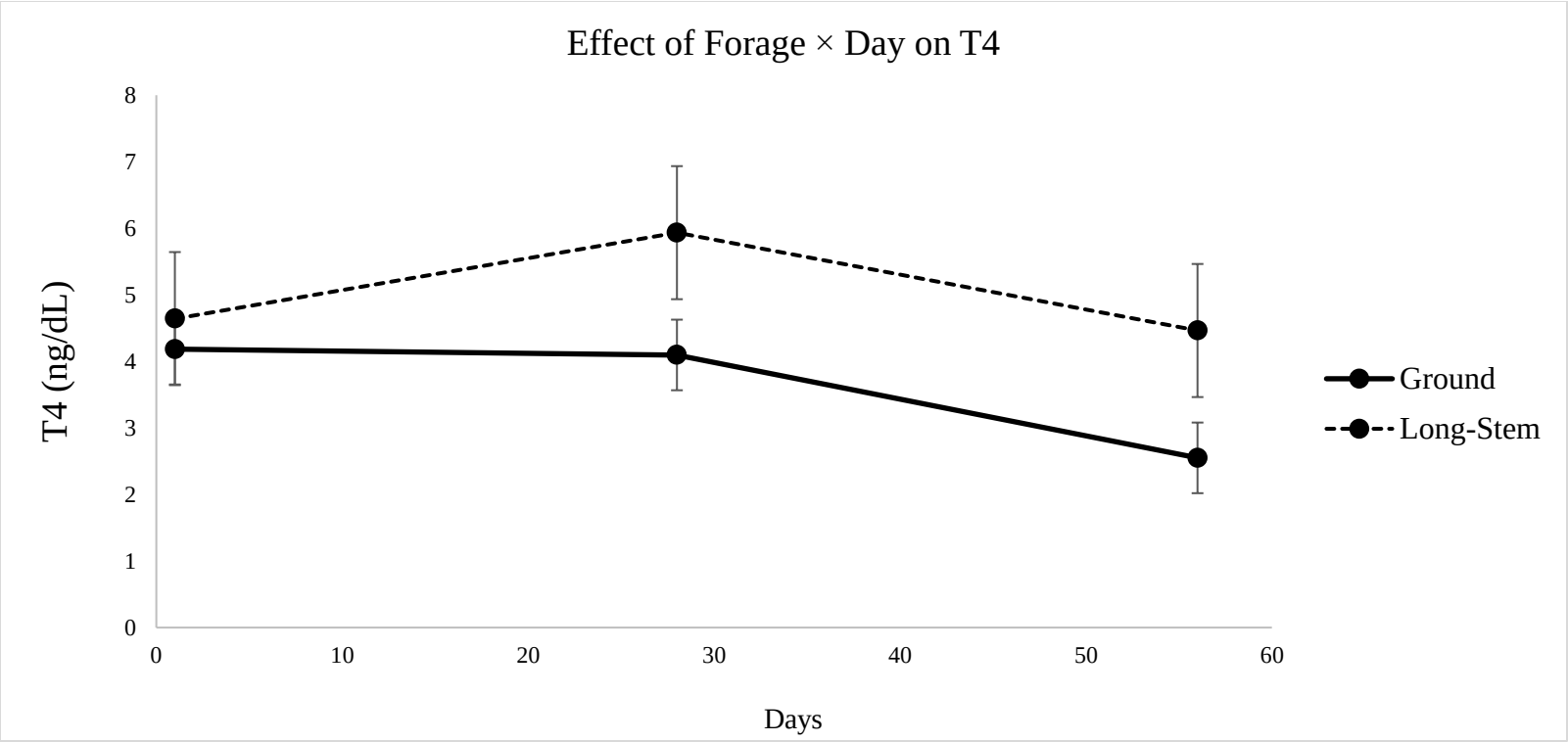


Figure 3. The effect of *Forage* × *Day* on thyroxine (T4) levels (ng/dL).

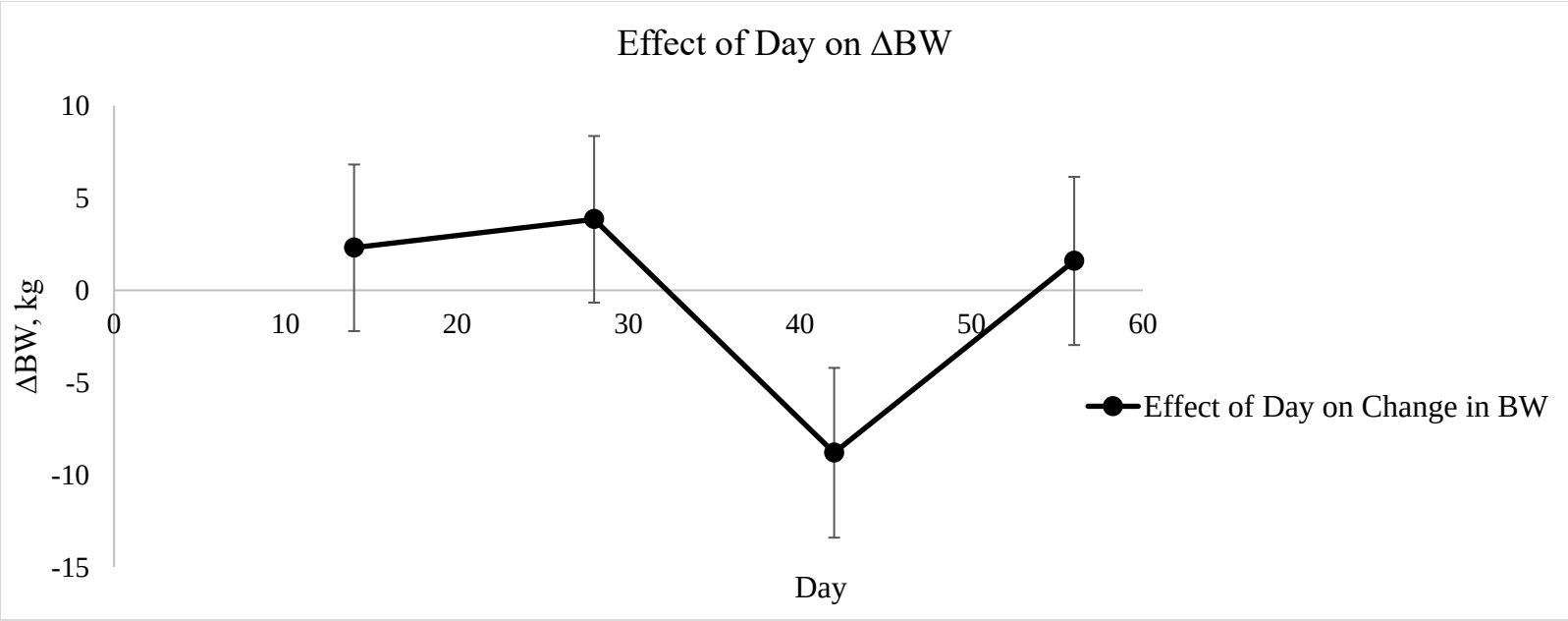
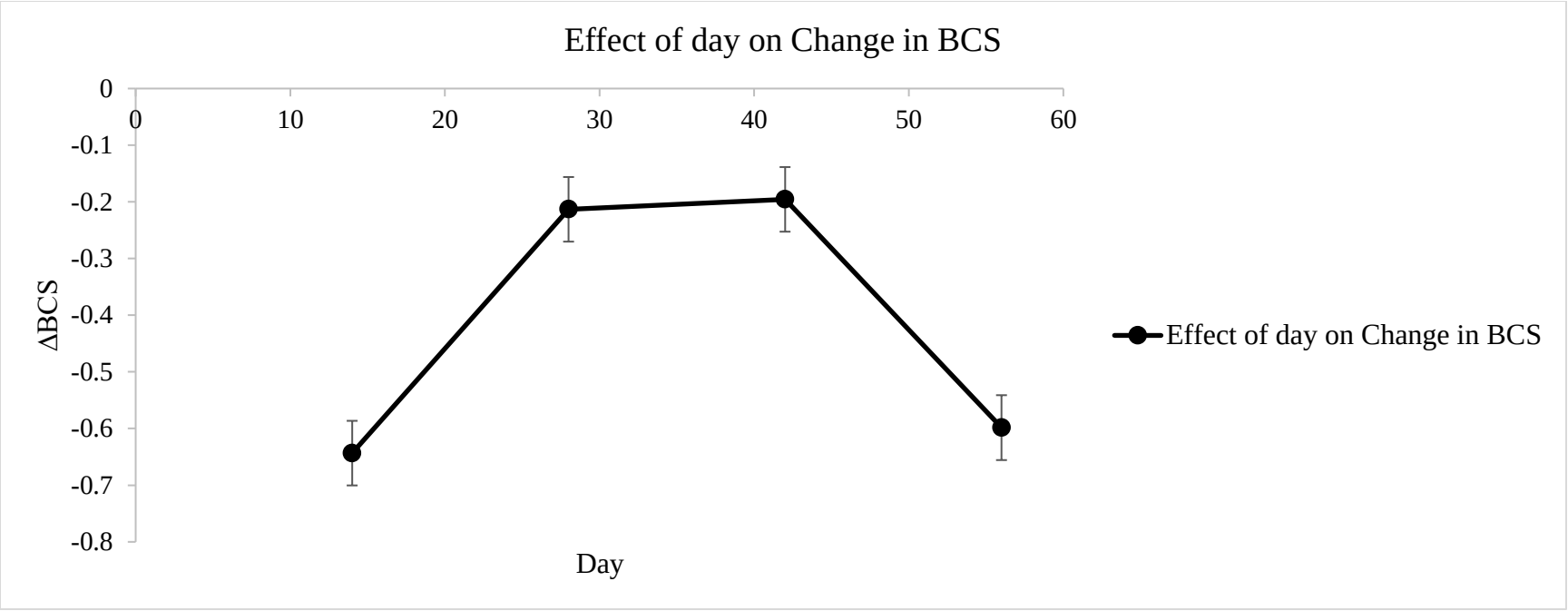


Figure 4. The effect of *Day* on change in body weight (ΔBW).



` Figure 5. The effect of *Day* on change in body condition score (ΔBCS).