**Title**

# Composition of a low erucic acid, low fiber field pennycress (*Thlaspi arvense* L) grain referred to as CoverCress™ developed through breeding and gene editing

**Author List**

Gary Hartnell[1*], Shawna Lemke[2], and Chris Aulbach[3]

*Corresponding author (email): gary.hartnell@gmail.com

**Author Affiliation**

[1]President/Owner Hartnell International Consulting, LLC (St. Peters, MO)

[2]Principal/Owner SLL Consulting & Services, LLC (St. Louis, MO)

[3]Vice President Agronomy, CoverCress Inc. (St. Louis, MO)

**Abstract**

Field pennycress (*Thlaspi arvense* L.) can be domesticated and cultivated as an annual in a corn / pennycress / soybean rotation where pennycress is sown and harvested as a cash cover crop. To improve the nutritional profile, pennycress was modified in two ways to achieve the same alterations in characteristics: 1) through selection of mutants and 2) through gene editing. These alterations resulted in a low erucic acid, lower fiber phenotype and the resulting products from these combinations are referred to as CoverCress CCWG and CoverCress B3WG, respectively. CCWG and B3WG were planted as cover crops in five U.S. locations in the fall and the grain was harvested in the subsequent June. The grain was treated with 83 mM copper sulfate solution as a potential palatability agent for the naturally high glucosinolate levels, and was subsequently analyzed for nutrient (proximates, minerals, fatty acids, amino acids, and vitamins) and anti-nutrient (sinapine, glucosinolates, mold) content. The low erucic acid, lower fiber phenotype was consistently achieved across five lots. Generally, the nutrient content for both CCWG and B3WG were similar to canola grain. Canola grain contains the anti-nutrient sinapine but is

significantly reduced to below the level of detection in CoverCress grain. As expected, CoverCress grain contains about 10 times more glucosinolates than canola. Based on the composition of CoverCress grain, it may provide a source of energy and amino acids to animals with restricted inclusion based on the glucosinolate content.

**Keywords**

Pennycress, CoverCress, low-erucic acid, cover crop, gene editing

## Introduction

Field pennycress (*Thlaspi arvense L.*) is an annual weed that grows over the winter in Eurasia and North America. It is a member of the Brassicacae family, commonly known as the mustard family. Field pennycress contains high levels of oil (~25-30%) that makes it a desirable ultra-low carbon fuel feedstock. The potential commercial value of field pennycress production has been researched extensively in a variety of leading laboratories including the National Center for Agricultural Innovation, USDA ARS, Peoria, IL, Western Illinois University, Illinois State University and the University of Minnesota. The cumulative literature and experience establish that field pennycress can be domesticated and cultivated as a winter annual in a corn / pennycress / soybean rotation where pennycress is sown and harvested as a cash winter cover crop as (Phipphen and Phippen, 2012; Sedbrook *et al*., 2014).

In addition to this primary value for fuel, the seed could provide an energy source for animal feeds such as chicken feed, however, the oil typically contains >35% erucic acid. Erucic acid is a 22-carbon monounsaturated acid that is absorbed, distributed and metabolized like other fatty acids involving primarily metabolism via mitochondrial beta-oxidation and, to a lesser extent, peroxisomal beta-oxidation. Like other longer-chain fatty acids, the rate of mitochondrial beta oxidation is comparatively lower for erucic acid; however, elevated erucic acid levels induce liver peroxisomal oxidation pathways as a mechanism of compensation. Interest in the safety of erucic acid occurred when results of studies in rats associated the dietary intake of high doses of erucic acid with myocardial lipidosis and heart lesions (Bremer and Norum, 1982). Oilseed rape conventionally contains similarly high levels of erucic acid. Low erucic acid varieties were identified and marketed as canola, which have been shown to be safe to broiler chickens when incorporated in diets up to 25% inclusion. Reduction in erucic acid is achieved through disruption of Fatty Acid Elongation 1 (FAE1), resulting in higher levels of oleic (18:1). It is through this same mechanism that erucic acid levels has been lowered in pennycress (McGinn et al., 2019).

Field pennycress is also high in fiber, which can impact digestibility as a feed ingredient. The production of seed coat fiber was first characterized in the model plant *Arabidopsis*. *Arabidopsis* seed coats derive their brown color from the accumulation of proanthocyanidins (PAs), a class of flavonoid chemicals (polymerized flavan-3-ols, or condensed tannins) that protect against a variety of biotic and abiotic stresses and help maintain seed dormancy and

viability (Debeaujon *et al*., 2003). PAs start out as colorless epicatechin compounds until they are transported to the vacuole where they are polymerized and oxidized as the seed desiccates. In *Arabidopsis,* PAs are only produced in a narrowly defined cell layer in the endothelium of the seed, and the genes TTG1, TT8/bHLH042, and TT2/MYB123 and have been demonstrated as being the three main regulators of PA biosynthesis in seed coat (Baudry *et al*., 2004; Lepiniec *et al*., 2006). Gonzalez *et al*. (2009) described how the TTG1 works in a complex with a particular combination of MYB class and bHLH class transcription factors to regulate epidermal development of the seed coat. Loss-of-function mutants in these genes exhibit the transparent "testa" phenotype as a result of low levels of oxidized PAs in the seed coat. Similarly, the transparent testa phenotype was observed with loss-of-function mutations in orthologs of these genes in pennycress (Chopra *et al*, 2018), resulting in reduced fiber content. This phenomenon has been observed in other brassicas such as canola and is characterized by yellow seeds that have more oil because of the resulting thinner seed coat and larger embryo (Abraham & Bhatia, 1986). Meal from these brassicas have also been shown to be useful in animal feed because of the relatively lower fiber and higher metabolizable energy (Slominski *et al*., 1994, Slominski, 2015;).

Reduction of erucic acid and fiber levels in pennycress has been achieved through conventional breeding and gene editing for loss of function in the associated gene pathways. A low-erucic acid, lower-fiber pennycress is being developed under the trade name of CoverCress™ .

CoverCress[1] represents a clear opportunity for sustainable optimization of certain agricultural systems. It serves as an important winter cover, working within the no/low-till cropping systems to prevent soil erosion from fallow fields and improve soil nitrogen management. As part of the safety assessment of this new grain for animal feed, a comprehensive compositional study was conducted on both the mutant breeding line and gene edited lines.

[1] For simplicity, CoverCress is not marked with ™ for the remainder of the document.

## Materials and Methods

### *CoverCress Line Development*

Two studies were conducted to assess the composition of CoverCress grain. The first study utilized grain from a low-erucic acid, lower-fiber variety of CoverCress developed through breeding by identification of mutants. One parent line, referred to as MN106-V300, was developed via ethyl methanesulfonate (EMS) mutation breeding. This line contains a mutation to the FAE1 gene (specifically, a cytosine to thymine change at base position 1018bp), resulting in a premature stop codon and complete loss of function of the gene, causing a reduction in erucic acid level (Chopra *et al*., 2020). The second parent line, referred to as Y1126, was isolated from a cultivated field in Grantfork IL and was identified as a lower-fiber phenotype with a yellow seed coat. This line contains a naturally-occurring deletion of 21bp in the TRANSPARENT TESTA GLABRA 1 (TTG1) gene. This mutation results in a deletion of 7 amino acids in the conserved area of TTG1 protein, leading to a complete loss of function. The MN106-V300 and Y1126 lines were crossed in fall 2017, and F2 plants carrying homozygous mutant alleles for these genes were further propagated in bulk, first in local greenhouses in the spring of 2018 and then in the field in an 0.3-acre increase planted at Sigel, IL in the fall of 2018. Seeds from this increase were harvested in bulk in the spring of 2019, and this seed formed the foundation source. It should be noted that this source is segregating for all background genetics that differ between Y1126 and MN106-V300 and fixed only for FAE1 and TTG1. This line is referred to as CoverCress Whole Grain (CCWG).

The second study used a line that was generated through application of gene editing techniques to obtain lines of low-erucic acid, lower-fiber pennycress and is referred to as B3WG. To generate this line, mutations were introduced into pennycress cultivar B3 using a CRISPR/SpCas9 DNA construct designed to target genomic edits to the FAE1 and TT8 genes. This transgene construct was delivered to B3 using a disarmed Agrobacterium tumefaciens strain (GV3101) and a standard floral dip transformation method. Presence of the edits in T1 plants was confirmed through multiple methods including confirmatory PCR screening of a fragment of the T-DNA. Seed from the progeny T2 generation was screened for segregants that did not have the transgene.

132 Resulting progeny in the T3 generation were screened again for negative presence of
133 DsRED and Cas as well as homozygous edits to TT8 and FAE1. Three types of plants were
134 identified. B3 [WG].1 harbors a single G insertion in the TT8 gene and a single A insertion in the
135 FAE1 gene. B3 [WG].2 harbors a single base deletion in the TT8 gene and a single A insertion in
136 the FAE1 gene. In both iterations, B3WG plants produced yellow seed (a marker for low fiber)
137 and low accumulation of erucic acid in seeds. The B3WG.1 and B3WG.2 plants were taken
138 forward for subsequent characterization and seed bulk up.
139
140 ***Grain production***
141 Two studies were conducted to measure the nutrient and antinutrient content of
142 CoverCress grain. In Study A, CoverCress seed developed through breeding (CCWG) and
143 grown in five locations (Venedy, IL; Sigel, IL; Havana, IL; and two locations in Mt. Pulaski, IL)
144 from September 2019 and harvested in June of 2020. In Study B, CoverCress seed was
145 developed through gene editing (B3WG) and grown in five locations (Havana, IL; Arenzville,
146 IL; Mt. Pulaski, IL: and two locations in Martinsville, IL) from September 2020 and harvested in
147 June of 2021. Seeds were transported to CoverCress Inc. (St. Louis) and cleaned.
148
149 ***Treatment of grain with copper sulfate***
150 Native pennycress and CoverCress grain contain high levels of glucosinolates in the form
151 of aliphatic sinigrin (approximately 35-110 μ moles/g meal). Glucosinolates are considered an
152 antinutrient and can prevent the absorption of iodine and may affect thyroid function in target
153 species (Schöne et al., 1993). Glucosinolates can also impart a bitter flavor which may affect
154 feed consumption (Bischoff, 2021). Copper sulfate treatment has been suggested to overcome the
155 deleterious effects of glucosinolates for other high glucosinolate grains (Schöne *et al*., 1993;
156 Singhal & Sinha 2000; Payvastagan, 2013). It was theorized that copper may reduce or mask the
157 bitter taste of glucosinolates, improving palatability. Therefore, both the CCWG and B3WG
158 grains were pre-treated with copper sulfate prior to characterization of the grain composition and
159 use in subsequent broiler feeding studies.
160 Three hundred grams of CCWG grain from each of the five locations was blended with
161 45 ml of 83.3 mM copper sulfate pentahydrate solution. The samples were labelled CCWG-15-
162 32-A, CCWG-15-32-B, CCWG-15-32-C, CCWG-15-32-D, and CCWG-15-32-E. Four hundred

163 and sixty grams of CCWG and B3WG grain from each of the five locations were blended with
164 60 ml of 83.3 mM copper sulfate pentahydrate solution. The samples were labelled, B3WG-15-
165 32-A, B3WG-15-32-B, B3WG-15-32-C, B3WG-15-32-D, and B3WG-15-32-E. Samples were
166 stored at 2 to 8ºC.
167
168 ***Methods of Analysis***

169 The five lots of CCWWG-15-32 and B3WG-15-32 were analyzed duplicate by EPL Bio
170 Analytical Services (Niantic, IL) following Good Laboratory Practices (GLP) and by Dairyland
171 Laboratories (Arcadia, WI) a commercial laboratory that uses methods on the latest research
172 findings and technical methodologies available to the feed industry. Dairyland Laboratories
173 participates (DLL) in the National Forage Testing Association, North American Proficiency
174 Testing Program (NAPT), and American Association of Feed Control Officials (AAFCO)
175 Proficiency Testing Program. These programs help laboratories generate accurate and precise
176 analyses.

177 The list of analytes and the respective Standard Operating Procedure (SOP) used by each
178 lab are shown in Table 1. The details of the specific analytical methodology used can be
179 obtained from the respective laboratories.

180 Table 1. List of analytes measured by EPL Bio Analytical Services (EPL) and Dairyland
181 Laboratories and Standard Operating Procedures (SOP).

| | EPL | Dairyland Laboratories |
|---|---|---|
| Analyte | SOP | SOP |
| Moisture/Dry Matter | GSOP-NC-4 | DLARC.SOP.FEED.WCFEED.Lab Dry Matter 105 3hr.001 |
| Crude Protein | GSOP-NC-20 | DLARC.SOP.FEED.WCFEED.Leco528.008 |
| Ash | GSOP-NC-2 | DLARC.SOP.FEED.WCFEED.Ash.003 |
| Crude Fiber | GSOP-NC-5 | DLARC.SOP.FEED.WCFEED.Crude Fiber.006 |
| Acid Detergent Fiber (ADF) | GSOP-NC-3 | DLARC.SOP.FEED.WCFEED.Sequential NDF/ADF/Lignin Analysis.000 |
| Neutral Detergent Fiber (NDF) | GSOP-NC-9 | DLARC.SOP.FEED.WCFEED.Sequential NDF/ADF/Lignin Analysis.000 |
| Total Dietary Fiber (TDF) | GSOP-NC-359 | Not measured |
| Crude Fat (Acid hydrolysis) | GSOP-NC-141 | DLARC.SOP.FEED.WCFEED.AH Fat.002 |
| Minerals (Ca, P, Mg, K, Na, S, Cu, Mn, Fe, Zn) | GSOP-NC-60 | DLARC.SOP.FEED.WCFEED.ICP Analysis.006 |
| Chloride | GSOP-NC-328 | DLARC.SOP.FEED.WCFEED.Salt.006 |
| Carbohydrates | GSOP-NC-494 | Not Measured |

| | | |
|---|---|---|
| Cysteine and Methionine | GSOP-NC-279 | DLARC.SOP.FEED.WCFEED.Cysteine and Methionine.000 |
| Tryptophan | GSOP-NC-22 | DLARC.SOP.FEED.WCFEED.Tryptophan Analysis.001 |
| 15 Amino Acids | GSOP-NC-58 | DLARC.SOP.FEED.WCFEED. Amino Acid Analysis.000 |
| Fatty Acids | GSOP-NC-319 | DLARC.SOP.FEED.WCFEED.Fatty Acid Analysis.004 |
| Vitamin B1 (Thiamine) | GSOP-NC-262 | Not Measured |
| Vitamin B2 (Riboflavin) | GSOP-NC-46 | Not Measured |
| Vitamin B6 (Pyridoxine) | GSOP-NC-220 | Not Measured |
| Folic Acid | GSOP-NC-26 | Not Measured |
| Niacin | GSOP-NC-28 | Not Measured |
| Tocopherols (Alpha, beta, gamma, delta) | GSOP-NC-341 | Not Measured |
| Fumonisins (B1, B2, B3) | GSOP-NC-336 | Not Measured |
| Aflatoxin (B1, B2, G1, G2), T-2 Toxin, Ochratoxin-A | GSOP-NC-337 | Not Measured |
| Deoxynivalenol, 3/15-acetyl-deoxynivalenol, Zearalenone | GSOP-NC-338 | Not Measured |
| Ergosine, Ergotamine, Ergoocornine, Ergocryptine, Ergocristine | GSOP-NC-456 | Not Measured |
| | | Not Measured |
| Sinapine | GSOP-NC-208 | Not Measured |
| Sinigrin | GSOP-NC-650 | Not Measured |

***Data Handling***

The data for each study consisted of five samples each from one of five locations grown in Illinois with duplicate values for each sample. The duplicates were averaged to give one value for each of the five samples.

For the data generated by EPL, Statistical Consultants Plus, LLC (Fenton, MO) calculated measures of data dispersion (variability/standard deviation and data ranges) and measures of central tendency (mean and median) for each analyte using the analytical data from the five test substances each representing five different field locations using SAS, version 9.4. For the data generated by Dairyland Laboratories, Hartnell International Consulting LLC (St. Peters, MO) calculated measures of data dispersion (variability/standard deviation and data ranges) and measures of central tendency (mean and median) for each analyte. Measures were calculated using Microsoft Excel.

**Results and Discussion**

**Study A – CCWG-15-32**

<u>Proximates</u>

Table 2 contains the results of proximate analyses. In general, data were in agreement between laboratories except for acid detergent fiber (ADF), neutral detergent fiber (NDF) and crude fat. The two labs used different fat extraction methods. Methodology was also different for the analysis of ADF and NDF, however, in both cases the ADF and NDF analyses were conducted on the residue after fat extraction. It is postulated that Dairyland Laboratories's method extracted more fat from the product than EPL's method and that EPL's assessment of ADF and NDF may be higher because of residual fat, which inflated the ADF and NDF values. In addition, there was greater variation noted with EPL's fat values than with Dairyland Labs. The Fiber Best Practices Working Group under AAFCO's Laboratory and Services Committee provides a detailed discussion on the critical factors in determining fiber in feed and forages (AAFCO, 2017). As expected, mean moisture levels were 15.6 -17.1% because of the addition of the copper sulfate solution to the CoverCress grain. Mean and median values are similar for all parameters, indicating that the values from the five lots are evenly distributed, i.e. the data are symmetrical in distribution.

Table 2. CCWG-15-32 Proximates (100% dry matter basis)

| Analytical Lab[1] | Variable | Units | Mean | Standard Deviation | Median | Range Minimum | Range Maximum |
|---|---|---|---|---|---|---|---|
| EPL | Moisture | % | 17.1 | 0.23 | 17.0 | 16.9 | 17.4 |
| DLL | Moisture | % | 15.6 | 0.34 | 15.5 | 15.1 | 16.1 |
| EPL | Dry Matter | % | 82.9 | 0.23 | 83.1 | 82.7 | 83.1 |
| DLL | Dry Matter | % | 84.4 | 0.34 | 84.5 | 83.9 | 84.9 |
| EPL | Crude Fiber | % | 23.1 | 1.28 | 22.8 | 21.5 | 25.0 |
| DLL | Crude Fiber | % | 23.0 | 1.72 | 22.7 | 20.9 | 24.9 |
| EPL | Acid Detergent Fiber | % | 30.1 | 1.54 | 29.9 | 28.0 | 32.1 |
| DLL | Acid Detergent Fiber | % | 13.9 | 1.58 | 13.5 | 12.1 | 15.8 |
| EPL | Neutral Detergent Fiber | % | 29.5 | 1.55 | 29.2 | 27.7 | 32.0 |
| DLL | Neutral Detergent Fiber | % | 20.3 | 2.76 | 19.3 | 17.3 | 24.5 |
| EPL | Total Dietary Fiber | % | 30.0 | 0.97 | 29.8 | 28.9 | 31.5 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| EPL | Crude Protein | % | 25.1 | 1.53 | 25.5 | 22.6 | 26.7 |
| DLL | Crude Protein | % | 25.5 | 1.63 | 25.6 | 22.7 | 26.7 |
| EPL | Crude Fat | % | 32.7 | 1.26 | 33.1 | 30.6 | 33.7 |
| DLL | Crude Fat | % | 36.3 | 0.52 | 36.5 | 35.5 | 36.7 |
| EPL | Carbohydrates | % | 36.9 | 1.61 | 36.9 | 34.6 | 38.5 |
| EPL | Ash | % | 5.3 | 0.39 | 5.5 | 4.9 | 5.7 |
| DLL | Ash | % | 5.8 | 0.40 | 5.9 | 5.3 | 6.2 |

[1] Analytical Lab: EPL – EPL Bio Analytical Services; DLL – Dairyland Laboratories

Amino Acid analysis

Table 3 contains the amino acid profile of CCWG-15-32 when expressed on a 100% dry matter basis and Table 4 contains the amino acid profile of CCWG-15-32 when expressed on a % of total protein basis. The amino acid values from the five lots showed a symmetrical distribution with the means and median values being similar. EPL consistently had lower variability (smaller standard deviations) than Dairyland Laboratories. Dairyland reported numerically higher values for most amino acids whether expressed as a percent of dry matter or as a percent of protein. Dairyland had a higher percentage of total amino acids when expressed as total amino acids as a percent of crude protein (97% versus 89.4%).

Table 3. CCWG-15-32 Amino Acids (100% dry matter basis)

| Analytical | | | | Standard | | Range | |
|---|---|---|---|---|---|---|---|
| **Lab[1]** | **Variable** | **Units** | **Mean** | **Deviation** | **Median** | **Minimum** | **Maximum** |
| EPL | Alanine | % | 1.18 | 0.074 | 1.18 | 1.08 | 1.28 |
| DLL | Alanine | % | 1.20 | 0.107 | 1.20 | 1.09 | 1.38 |
| EPL | Arginine | % | 1.53 | 0.071 | 1.55 | 1.42 | 1.60 |
| DLL | Arginine | % | 1.82 | 0.159 | 1.86 | 1.58 | 1.98 |
| EPL | Aspartic Acid | % | 2.03 | 0.245 | 1.99 | 1.74 | 2.42 |
| DLL | Aspartic Acid | % | 2.34 | 0.335 | 2.30 | 2.01 | 2.89 |
| EPL | Cystine | % | 0.41 | 0.053 | 0.44 | 0.32 | 0.45 |
| DLL | Cysteine | % | 0.41 | 0.058 | 0.42 | 0.31 | 0.47 |
| EPL | Glutamic Acid | % | 3.79 | 0.289 | 3.82 | 3.34 | 4.13 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| DLL | Glutamic Acid | % | 4.16 | 0.407 | 4.19 | 3.65 | 4.77 |
| EPL | Glycine | % | 1.56 | 0.087 | 1.57 | 1.42 | 1.66 |
| DLL | Glycine | % | 1.69 | 0.159 | 1.68 | 1.52 | 1.95 |
| EPL | Histidine | % | 0.69 | 0.026 | 0.70 | 0.64 | 0.71 |
| DLL | Histidine | % | 0.59 | 0.058 | 0.59 | 0.51 | 0.65 |
| EPL | Isoleucine | % | 1.01 | 0.050 | 1.03 | 0.94 | 1.07 |
| DLL | Isoleucine | % | 0.93 | 0.111 | 0.92 | 0.77 | 1.08 |
| EPL | Leucine | % | 1.72 | 0.125 | 1.71 | 1.54 | 1.89 |
| DLL | Leucine | % | 1.91 | 0.220 | 1.90 | 1.65 | 2.26 |
| EPL | Lysine | % | 1.28 | 0.050 | 1.29 | 1.20 | 1.33 |
| DLL | Lysine | % | 1.40 | 0.077 | 1.43 | 1.29 | 1.48 |
| EPL | Methionine | % | 0.41 | 0.026 | 0.42 | 0.36 | 0.43 |
| DLL | Methionine | % | 0.48 | 0.047 | 0.47 | 0.42 | 0.53 |
| EPL | Phenylalanine | % | 1.13 | 0.060 | 1.15 | 1.03 | 1.18 |
| DLL | Phenylalanine | % | 1.20 | 0.135 | 1.20 | 0.99 | 1.36 |
| EPL | Proline | % | 1.25 | 0.060 | 1.27 | 1.17 | 1.32 |
| DLL | Proline | % | 1.38 | 0.079 | 1.20 | 1.27 | 1.48 |
| EPL | Serine | % | 1.01 | 0.072 | 1.02 | 0.92 | 1.12 |
| DLL | Serine | % | 1.13 | 0.116 | 1.12 | 1.03 | 1.32 |
| EPL | Threonine | % | 1.14 | 0.053 | 1.15 | 1.06 | 1.21 |
| DLL | Threonine | % | 1.25 | 0.106 | 1.25 | 1.14 | 1.42 |
| EPL | Tryptophan | % | 0.35 | 0.011 | 0.35 | 0.34 | 0.37 |
| DLL | Tryptophan | % | 0.52 | 0.035 | 0.52 | 0.48 | 0.57 |
| EPL | Tyrosine | % | 0.64 | 0.026 | 0.63 | 0.61 | 0.67 |
| DLL | Tyrosine | % | 0.88 | 0.102 | 0.86 | 0.74 | 1.02 |
| EPL | Valine | % | 1.30 | 0.063 | 1.32 | 1.21 | 1.37 |
| DLL | Valine | % | 1.45 | 0.148 | 1.47 | 1.24 | 1.65 |

[1] Analytical Lab: EPL – EPL Bio Analytical Services; DLL – Dairyland Laboratories

234 Table 4. CCWG-15-32 Amino Acids (% of protein basis)

| Analytical Lab[1] | Variable | Units | Mean | Standard Deviation | Median | Range Minimum | Range Maximum |
|---|---|---|---|---|---|---|---|
| EPL | Alanine | % | 4.70 | 0.082 | 4.75 | 4.59 | 4.78 |
| DLL | Alanine | % | 4.73 | 0.251 | 4.68 | 4.46 | 5.11 |
| EPL | Arginine | % | 6.11 | 0.232 | 6.21 | 5.71 | 6.26 |
| DLL | Arginine | % | 7.12 | 0.241 | 7.22 | 6.79 | 7.36 |
| EPL | Aspartic Acid | % | 8.09 | 0.561 | 7.82 | 7.68 | 9.06 |
| DLL | Aspartic Acid | % | 9.15 | 0.914 | 8.89 | 8.38 | 10.73 |
| EPL | Cystine | % | 1.65 | 0.245 | 1.75 | 1.21 | 1.78 |
| DLL | Cysteine | % | 1.6 | 0.268 | 1.65 | 1.16 | 1.83 |
| EPL | Glutamic Acid | % | 15.10 | 0.292 | 15.00 | 14.75 | 15.45 |
| DLL | Glutamic Acid | % | 16.32 | 0.822 | 16.11 | 15.50 | 17.69 |
| EPL | Glycine | % | 6.20 | 0.062 | 6.21 | 6.13 | 6.29 |
| DLL | Glycine | % | 6.63 | 0.366 | 6.56 | 6.27 | 7.22 |
| EPL | Histidine | % | 2.75 | 0.113 | 2.78 | 2.56 | 2.86 |
| DLL | Histidine | % | 2.32 | 0.122 | 2.30 | 2.16 | 2.48 |
| EPL | Isoleucine | % | 4.03 | 0.077 | 4.02 | 3.93 | 4.14 |
| DLL | Isoleucine | % | 3.62 | 0.233 | 3.57 | 3.40 | 4.02 |
| EPL | Leucine | % | 6.84 | 0.149 | 6.82 | 6.72 | 7.08 |
| DLL | Leucine | % | 7.47 | 0.512 | 7.29 | 7.08 | 8.37 |
| EPL | Lysine | % | 5.10 | 0.171 | 5.07 | 4.85 | 5.28 |
| DLL | Lysine | % | 5.51 | 0.139 | 5.50 | 5.30 | 5.68 |
| EPL | Methionine | % | 1.62 | 0.014 | 1.62 | 1.61 | 1.64 |
| DLL | Methionine | % | 1.88 | 0.121 | 1.86 | 1.74 | 2.03 |
| EPL | Phenylalanine | % | 4.50 | 0.051 | 4.52 | 4.42 | 4.54 |
| DLL | Phenylalanine | % | 4.69 | 0.249 | 4.64 | 4.37 | 5.06 |
| EPL | Proline | % | 4.98 | 0.254 | 5.06 | 4.55 | 5.17 |
| DLL | Proline | % | 5.42 | 0.399 | 5.34 | 4.93 | 5.90 |
| EPL | Serine | % | 4.04 | 0.103 | 4.03 | 3.91 | 4.19 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| DLL | Serine | % | 4.42 | 0.313 | 4.35 | 4.05 | 4.89 |
| EPL | Threonine | % | 4.55 | 0.076 | 4.54 | 4.48 | 4.68 |
| DLL | Threonine | % | 4.91 | 0.243 | 4.85 | 4.62 | 5.26 |
| EPL | Tryptophan | % | 1.41 | 0.052 | 1.39 | 1.37 | 1.50 |
| DLL | Tryptophan | % | 2.05 | 0.066 | 2.06 | 1.96 | 2.12 |
| EPL | Tyrosine | % | 2.55 | 0.139 | 2.49 | 2.41 | 2.75 |
| DLL | Tyrosine | % | 3.43 | 0.215 | 3.34 | 3.27 | 3.80 |
| EPL | Valine | % | 5.20 | 0.102 | 5.20 | 5.08 | 5.34 |
| DLL | Valine | % | 5.69 | 0.267 | 5.61 | 5.48 | 6.14 |

[1] Analytical Lab: EPL – EPL Bio Analytical Services; DLL – Dairyland Laboratories

Fatty Acid Characterization

Tables 5 and 6 contain the fatty acid profile of CCWG-15-32 when expressed on a 100% dry matter basis and percent of total fatty acids, respectively. In general, there was good agreement between laboratories. The fatty acid values from the five lots showed a symmetrical distribution with the means and median values being similar. The major fatty acids are oleic (40% of the fatty acids) followed by linoleic (34.5% of the fatty acids) and linolenic (~18% of fatty acids). These three fatty acids comprise over 90% of the fatty acids in CCWG-15-32. No long chain polyunsaturated long chain fatty acids such as EPA and DHA were detected. Results confirm that CCWG-15-32 contains negligible levels of erucic acid. Dairyland Laboratories did not detect it and EPL reported erucic acid to be less than 0.1% of the total fatty acids.

Table 5. CCWG-15-32 Fatty Acids (100% dry matter basis)

| Analytical | | | | Standard | | Range | |
|---|---|---|---|---|---|---|---|
| **Lab[1]** | **Variable** | **Units** | **Mean** | **Deviation** | **Median** | **Minimum** | **Maximum** |
| EPL | Myristic (C14:0) | % | 0.02 | 0.001 | 0.02 | 0.02 | 0.02 |
| DLL | Myristic (C14:0) | % | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| EPL | Palmitic (C16:0) | % | 0.79 | 0.085 | 0.82 | 0.67 | 0.88 |
| DLL | Palmitic (C16:0) | % | 0.75 | 0.063 | 0.74 | 0.67 | 0.82 |
| EPL | Palmitoleic (C16:1) | % | 0.03 | 0.005 | 0.03 | 0.02 | 0.03 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| DLL | Palmitoleic (C16:1) | % | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| EPL | Heptadecanoic (C17:0) | % | 0.01 | 0.001 | 0.01 | 0.01 | 0.01 |
| DLL | Heptadecanoic (C17:0) | % | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| EPL | Heptadecenoic (C17:1) | % | 0.01 | 0.001 | 0.01 | 0.01 | 0.02 |
| DLL | Heptadecenoic (C17:1) | % | NA[2] | NA | NA | NA | NA |
| EPL | Stearic (C18:0) | % | 0.20 | 0.024 | 0.19 | 0.17 | 0.23 |
| DLL | Stearic (C18:0) | % | 0.18 | 0.020 | 0.18 | 0.16 | 0.21 |
| EPL | Oleic (C18:1) | % | 8.04 | 0.962 | 8.04 | 6.73 | 9.19 |
| DLL | Oleic (C18:1) | % | 7.45 | 0.815 | 7.35 | 6.36 | 8.28 |
| EPL | Linoleic (C18:2) | % | 6.87 | 0.605 | 7.19 | 6.08 | 7.46 |
| DLL | Linoleic (C18:2) | % | 6.40 | 0.570 | 6.35 | 5.55 | 6.93 |
| EPL | Linolenic (C18:3) | % | 3.62 | 0.241 | 3.68 | 3.34 | 3.87 |
| DLL | Linolenic (C18:3) | % | 3.07 | 0.196 | 2.98 | 2.82 | 3.27 |
| EPL | Nonadecenoic (C19:1) | % | 0.01 | 0.001 | 0.01 | 0.01 | 0.01 |
| DLL | Nonadecenoic (C19:1) | % | NA | NA | NA | NA | NA |
| EPL | Arachidic (C20:0) | % | 0.03 | 0.004 | 0.03 | 0.03 | 0.04 |
| DLL | Arachidic (C20:0) | % | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| EPL | Eicosenoic (C20:1) | % | 0.16 | 0.011 | 0.16 | 0.15 | 0.17 |
| DLL | Eicosenoic (C20:1) | % | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| EPL | Eicosadienoic (C20:2) | % | 0.03 | 0.002 | 0.03 | 0.03 | 0.03 |
| DLL | Eicosadienoic (C20:2), Eicosatrienoic(C20:3) | % | 0.45 | 0.179 | 0.51 | 0.17 | 0.64 |
| EPL | Behenic (C22:0) | % | 0.02 | 0.002 | 0.02 | 0.02 | 0.02 |
| DLL | Behenic (C22:0) | % | NA | NA | NA | NA | NA |
| EPL | Erucic (C22:1) | % | 0.06 | 0.013 | 0.07 | 0.04 | 0.08 |
| DLL | Erucic (C22:1) | % | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| EPL | Lignoceric (C24:0) | % | 0.02 | 0.002 | 0.02 | 0.01 | 0.02 |
| DLL | Lignoceric (C24:0) | % | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| EPL | Nervonic (C24:1) | % | 0.08 | 0.007 | 0.07 | 0.07 | 0.08 |
| DLL | Nervonic (C24:1) | % | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |

250 [1] Analytical Lab: EPL – EPL Bio Analytical Services; DLL – Dairyland Laboratories

[2] NA: Not Analyzed
The following fatty acids were below the level of detection for Lab EPL: Arachidonic (C20:4), Capric (C10:0), Caproic (C6:0), Caprylic (C8:0), Docosadienoic (C22:2), Eicosatrienoic (C20:3), Gamma Linolenic (C18:3), Heneicosanoic (C21:0), Lauric (C12:0), Myristoleic (C14:1), Nonadecanoic (C19:0), Pentadecanoic (C15:0), Pentadecenoic (C15:1), Tricosanoic (C23:0); for Lab DLL: Arachidonic (C20:4), Docosahexanoic acid (C22:6); Eicosapentaenoic acid (C20:5).

Table 6. CCWG-15-32 Fatty Acids as a Percent of Total Fatty Acids

| Analytical Lab[1] | Variable | Units | Mean | Standard Deviation | Median | Range Minimum | Range Maximum |
|---|---|---|---|---|---|---|---|
| EPL | Myristic (C14:0) | % | 0.09 | 0.006 | 0.09 | 0.09 | 0.10 |
| DLL | Myristic (C14:0) | % | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| EPL | Palmitic (C16:0) | % | 3.98 | 0.066 | 4.00 | 3.86 | 4.02 |
| DLL | Palmitic (C16:0) | % | 4.06 | 0.076 | 4.04 | 4.00 | 4.20 |
| EPL | Palmitoleic (C16:1) | % | 0.15 | 0.012 | 0.16 | 0.13 | 0.16 |
| DLL | Palmitoleic (C16:1) | % | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| EPL | Heptadecanoic (C17:0) | % | 0.03 | 0.018 | 0.03 | 0.00 | 0.05 |
| DLL | Heptadecanoic (C17:0) | % | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| EPL | Heptadecenoic (C17:1) | % | 0.07 | 0.011 | 0.08 | 0.06 | 0.09 |
| DLL | Heptadecenoic (C17:1) | % | NA[2] | NA | NA | NA | NA |
| EPL | Stearic (C18:0) | % | 1.00 | 0.044 | 1.02 | 0.94 | 1.04 |
| DLL | Stearic (C18:0) | % | 0.99 | 0.050 | 1.01 | 0.91 | 1.03 |
| EPL | Oleic (C18:1) | % | 40.14 | 1.223 | 40.55 | 38.50 | 41.55 |
| DLL | Oleic (C18:1) | % | 40.07 | 0.943 | 40.19 | 38.61 | 41.06 |
| EPL | Linoleic (C18:2) | % | 34.41 | 0.611 | 34.20 | 33.80 | 35.30 |
| DLL | Linoleic (C18:2) | % | 34.48 | 0.337 | 34.39 | 34.17 | 34.94 |
| EPL | Linolenic (C18:3) | % | 18.15 | 0.722 | 17.90 | 17.55 | 19.40 |
| DLL | Linolenic (C18:3) | % | 16.55 | 0.632 | 16.32 | 16.18 | 17.67 |
| EPL | Nonadecenoic (C19:1) | % | 0.03 | 0.018 | 0.03 | 0.00 | 0.05 |
| DLL | Nonadecenoic (C19:1) | % | NA | NA | NA | NA | NA |
| EPL | Arachidic (C20:0) | % | 0.03 | 0.004 | 0.03 | 0.03 | 0.04 |
| DLL | Arachidic (C20:0) | % | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| EPL | Eicosenoic (C20:1) | % | 0.16 | 0.011 | 0.16 | 0.15 | 0.17 |
| DLL | Eicosenoic (C20:1) | % | 1.47 | 0.088 | 1.44 | 1.38 | 1.60 |
| EPL | Eicosadienoic (C20:2) | % | 0.03 | 0.002 | 0.03 | 0.03 | 0.03 |
| DLL | Eicosadienoic (C20:2), Eicosatrienoic(C20:3) | % | 2.38 | 0.939 | 2.54 | 1.09 | 3.54 |
| EPL | Behenic (C22:0) | % | 0.02 | 0.002 | 0.02 | 0.02 | 0.02 |
| DLL | Behenic (C22:0) | % | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| EPL | Erucic (C22:1) | % | 0.06 | 0.013 | 0.07 | 0.04 | 0.08 |
| DLL | Erucic (C22:1) | % | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| EPL | Lignoceric (C24:0) | % | 0.07 | 0.021 | 0.08 | 0.04 | 0.09 |
| DLL | Lignoceric (C24:0) | % | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| EPL | Nervonic (C24:1) | % | 0.37 | 0.024 | 0.37 | 0.34 | 0.40 |
| DLL | Nervonic (C24:1) | % | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |

[1] Analytical Lab: EPL – EPL Bio Analytical Services; DLL – Dairyland Laboratories
[2] NA: Not Analyzed
The following fatty acids were below the level of detection for Lab A: Arachidonic (C20:4), Capric (C10:0), Caproic (C6:0), Caprylic (C8:0), Docosadienoic (C22:2), Eicosatrienoic (C20:3), Gamma Linolenic (C18:3), Heneicosanoic (C21:0), Lauric (C12:0), Myristoleic (C14:1), Nonadecanoic (C19:0), Pentadecanoic (C15:0), Pentadecenoic (C15:1), Tricosanoic (C23:0); for Lab B: Arachidonic (C20:4), Docosahexanoic acid (C22:6); Eicosapentaenoic acid (C20:5).

## Glucosinolate Characterization and Quantification

The sole glucosinolate, sinigrin, averaged 85.7 and 86.9 µmoles/g of CCWG-15-32 for the two detection methods (Table 7). The median value was higher than the mean due to one lot containing almost half the amount of sinigrin as the other four lots. This is reflected by the low minimum value as compared to the maximum value. The much lower sinigrin level in the one lot may have resulted from the soil conditions at that site.

Table 7. CoverCress CS (CCWG-15-32) glucosinolates (Sinigrin, 100% dry matter basis) – EPL Bio Analytical Services

| | | | Standard | | Range | |
|---|---|---|---|---|---|---|
| **Variable** | **Units** | **Mean** | **Deviation** | **Median** | **Minimum** | **Maximum** |
| Sinigrin – MS | µmoles/g | 85.7 | 16.83 | 90.7 | 56.2 | 98.6 |
| Sinigrin – UV | µmoles/g | 86.9 | 23.97 | 95.7 | 45 | 102 |

Minerals and Vitamins

Table 8 contains the results of the mineral analyses. EPL reported consistently higher mineral levels than Dairyland Laboratories, with the exception of zinc. The discrepancy between labs is unexplained. Generally, the values for the five lots had symmetric distribution. One location had a sulfur level that was 50% of the others. This lower sulfur value corresponds to the lower glucosinolate level of the sample from the same location. Glucosinolates are secondary sulfur compounds commonly found in brassica species (Aghajanzada *et al*., 2014), which suggests there is a plausible relationship between both values being lower at this site. The reason for the lower level at one location as compared to the other four locations is not clear but may have been a result of the soil fertilization. As expected, mean copper values were approximately 800 ppm because of the addition of copper sulfate. The analytical results confirm that the target level of 800 ppm copper was achieved.

Table 8. CCWG-15-32 minerals (100% dry matter basis)

| Analytical | | | | Standard | | Range | |
|---|---|---|---|---|---|---|---|
| **Lab[1]** | **Variable** | **Units** | **Mean** | **Deviation** | **Median** | **Minimum** | **Maximum** |
| EPL | Calcium | % | 0.849 | 0.079 | 0.826 | 0.791 | 0.982 |
| DLL | Calcium | % | 0.72 | 0.108 | 0.69 | 0.60 | 0.88 |
| EPL | Phosphorus | % | 0.991 | 0.138 | 1.060 | 0.840 | 1.135 |
| DLL | Phosphorus | % | 0.70 | 0.123 | 0.78 | 0.56 | 0.81 |
| EPL | Magnesium | % | 0.378 | 0.039 | 0.388 | 0.335 | 0.428 |
| DLL | Magnesium | % | 0.29 | 0.010 | 0.30 | 0.28 | 0.30 |
| EPL | Potassium | % | 0.951 | 0.073 | 0.923 | 0.909 | 1.080 |
| DLL | Potassium | % | 0.74 | 0.114 | 0.71 | 0.63 | 0.93 |
| EPL | Sulfur | % | 1.008 | 0.230 | 1.120 | 0.600 | 1.140 |
| DLL | Sulfur | % | 0.87 | 0.230 | 0.98 | 0.47 | 1.05 |
| EPL | Sodium | % | 0.005 | 0.003 | 0.004 | 0.003 | 0.009 |
| DLL | Sodium | % | 0.00 | 0.003 | 0.00 | 0.00 | 0.01 |
| EPL | Chloride | ppm | 185.10 | 35.379 | 186.50 | 143.00 | 238.50 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| DLL | Chloride | ppm | 400 | 90 | 500 | 300 | 500 |
| EPL | Iron | ppm | 102.18 | 12.350 | 108.50 | 83.850 | 112.00 |
| DLL | Iron | ppm | 40 | 55.1 | 29 | 16 | 98.5 |
| EPL | Copper | ppm | 820.70 | 17.101 | 814.00 | 806.00 | 845.50 |
| DLL | Copper | ppm | 808 | 68.7 | 811 | 704 | 890 |
| EPL | Manganese | ppm | 31.150 | 4.019 | 29.650 | 27.000 | 37.650 |
| DLL | Manganese | ppm | 27 | 2.6 | 26 | 23.5 | 30 |
| EPL | Zinc | ppm | 37.710 | 2.086 | 39.200 | 35.400 | 39.250 |
| DLL | Zinc | ppm | 55 | 11.4 | 55 | 43 | 72 |

[1] Analytical Lab: EPL – EPL Bio Analytical Services; DLL – Dairyland Laboratories

Table 9 contains the vitamins as analyzed by EPL for the five lots. Generally, the values for the five lots had symmetric distribution. Nutritionists and feed formulators, as common practice, do not use the vitamin content of feedstuffs in formulating diets due to the variability, stability, low levels in the feed ingredient and the low cost of supplementing vitamins. The Canola Council of Canada in their Canola Feed Guide (2019) state, "As is recommended with most natural sources of vitamins in animal feeds, users should not place too much reliance on these values and use supplemental vitamin premixes instead."

Table 9. CCWG-15-32 vitamins (100% dry matter basis) – EPL Bio Analytical Services

| | | | Standard | | Range | |
|---|---|---|---|---|---|---|
| Variable | Units | Mean | Deviation | Median | Minimum | Maximum |
| Folic Acid | mg/kg | 3.3 | 1.35 | 3.2 | 1.8 | 5.3 |
| Niacin | mg/kg | 51.2 | 10.52 | 48.6 | 42.1 | 69.3 |
| Vitamin B1 (Thiamine) | mg/kg | 5.4 | 0.25 | 5.3 | 5.2 | 5.8 |
| Vitamin B2 (Riboflavin) | mg/kg | 2.7 | 0.54 | 2.6 | 2.3 | 3.6 |
| Vitamin B6 (Pyridoxine) | mg/kg | 10.6 | 1.69 | 10.3 | 8.8 | 13.4 |
| Alpha-Tocopherol | mg/kg | 118.0 | 10.86 | 121.5 | 99.0 | 125.5 |
| Beta-Tocopherol | mg/kg | 1.8 | 0.07 | 1.8 | 1.7 | 1.9 |
| Delta-Tocopherol | mg/kg | 2.3 | 0.16 | 2.3 | 2.1 | 2.5 |
| Gamma-Tocopherol | mg/kg | 53.4 | 14.65 | 48.6 | 40.3 | 78.5 |

Sinapine and Mycotoxins

Sinapine, like glucosinolates, is an anti-nutrient found in modern oilseed rape and canola meals. Sinapine is metabolized in animals to trimethylamine, which is further metabolized by trimethyl oxidase. Certain brown egg laying strains of hens have been reported to have lower hepatic trimethyl oxidase activity apparently leading to accumulation of trimethylamine in some brown eggs and an associated "fishy taint". As summarized by Rymer & Short (2003), sinapines are present in modern rapeseed at levels of 12-23 g/kg seed (Huisman & Tolman, 2001). They are converted in the large intestine to trimethylamine, which apparently produces an undesirable fishy odor in the eggs of certain brown egg chickens. Some of these types of birds lack the ability to produce trimethylamine oxidase (Jeroch, 2008), and as a result the trimethylamine accumulates in the eggs of these birds. This odor in eggs occurs at levels of 0.8 mg/kg diet (Fenwick, 1982). The concentrations of sinapine in CCWG-15-32 were <0.05% DM basis as reported by EPL in all five lots. This is much lower in comparison to canola meal where the Canola Council of Canada (2015) reports sinapine levels of 1.0% as is basis (with an average 12% moisture content) or 1.14% on a 100% DM basis.

The following mycotoxins were below the limits of detection in all samples from all of the five lots: Aflatoxin B1, B2, G1, G2; T-2 Toxin; Ochratoxin A; Deoxynivalenol (DON), 3-Acetyl-DON; 15-Acetyl-DON; Zearalenone; Fumonisin B1, B2, B3; Ergosine; Ergotamine; Ergocornine; and Ergocryptine. For Ergocristine, which is a natural ergot alkaloid, four lots were below the levels of detection. For one lot, one of the two replicates was below the level of detection and the second had 15.9 ppb on a dry matter basis with level of detection at 1.5 ppb.

**Study B – B3WG-15-32**

Proximate Analyses

Table 10 contains the results of proximate analyses. Just as with the CCWG samples, it was postulated that Dairyland Laboratories's method extracted more fat from the product than EPL's method. In addition, there was greater variation noted with EPL's fat values than with Dairyland Labs. Fat in the sample may affect the fiber analysis with higher fat resulting in inflated crude fiber, ADF and NDF values. Moisture levels are high due to the addition of the copper sulfate solution to the CoverCress grain. Since the mean and median values are similar for all proximates, the values from the five lots are evenly distributed or symmetrical in distribution.

Table 10. CoverCress CS (B3WG-15-32) Proximates (100% dry matter basis)

| Analytical Lab[1] | Variable | Units | Mean | Standard Deviation | Median | Range Minimum | Range Maximum |
|---|---|---|---|---|---|---|---|
| EPL | Moisture | % | 19.6 | 0.24 | 19.6 | 19.3 | 19.9 |
| DLL | Moisture | % | 16.6 | 0.38 | 16.7 | 16.1 | 17.1 |
| EPL | Dry Matter | % | 80.4 | 0.24 | 80.5 | 80.1 | 80.7 |
| DLL | Dry Matter | % | 83.4 | 0.38 | 83.3 | 82.9 | 83.9 |
| EPL | Crude Fiber | % | 20.0 | 2.82 | 21.0 | 15.6 | 22.6 |
| DLL | Crude Fiber | % | 12.1 | 0.71 | 12.5 | 10.9 | 12.5 |
| EPL | Acid Detergent Fiber | % | 25.8 | 2.73 | 25.3 | 22.1 | 28.7 |
| DLL | Acid Detergent Fiber | % | 17.8 | 2.04 | 16.8 | 15.9 | 20.2 |
| EPL | Neutral Detergent Fiber | % | 26.9 | 3.32 | 27.7 | 22.0 | 30.2 |
| DLL | Neutral Detergent Fiber | % | 22.6 | 1.48 | 21.9 | 21.3 | 24.4 |
| EPL | Total Dietary Fiber | % | 28.8 | 1.04 | 28.7 | 27.7 | 30.3 |
| EPL | Crude Protein | % | 25.4 | 1.27 | 25.0 | 24.1 | 27.4 |
| DLL | Crude Protein | % | 23.9 | 0.97 | 24.1 | 22.5 | 25.1 |
| EPL | Crude Fat | % | 32.7 | 1.62 | 33.0 | 30.4 | 34.8 |
| DLL | Crude Fat | % | 37.0 | 1.07 | 37.0 | 35.7 | 38.5 |
| EPL | Carbohydrates | % | 36.3 | 2.12 | 36.8 | 32.7 | 38.1 |
| EPL | Ash | % | 5.6 | 0.45 | 5.4 | 5.1 | 6.2 |
| DLL | Ash | % | 6.0 | 0.86 | 6.1 | 4.6 | 6.7 |

[1] Analytical Lab: EPL – EPL Bio Analytical Services; DLL – Dairyland Laboratories

Amino Acid Analyses

Table 11 contains the amino acid profile of B3WG-15-32 when expressed on a 100% dry matter basis and Table 12 contains the amino acid profile of B3WG-15-32 when expressed on a

351 protein basis. The amino acid values from the five lots showed a symmetrical distribution with
352 the means and median values being similar. Dairyland had a higher percentage of total amino
353 acids when total amino acids were expressed as a percent of crude protein (88.1% versus 83.1%).
354 Table 11. CoverCress CS (B3WG-15-32) Amino Acids (100% dry matter basis)

| Analytical Lab | Variable | Units | Mean | Standard Deviation | Median | Range Minimum | Range Maximum |
|---|---|---|---|---|---|---|---|
| EPL | Alanine | % | 1.13 | 0.023 | 1.14 | 1.11 | 1.15 |
| DLL | Alanine | % | 1.00 | 0.034 | 0.99 | 0.97 | 1.06 |
| EPL | Arginine | % | 1.39 | 0.067 | 1.39 | 1.30 | 1.46 |
| DLL | Arginine | % | 1.57 | 0.052 | 1.55 | 1.51 | 1.63 |
| EPL | Aspartic Acid | % | 1.93 | 0.054 | 1.91 | 1.91 | 2.03 |
| DLL | Aspartic Acid | % | 1.92 | 0.031 | 1.93 | 1.88 | 1.96 |
| EPL | Cystine | % | 0.38 | 0.022 | 0.38 | 0.36 | 0.41 |
| DLL | Cysteine | % | 0.38 | 0.017 | 0.39 | 0.36 | 0.41 |
| EPL | Glutamic Acid | % | 3.83 | 0.110 | 3.81 | 3.68 | 3.95 |
| DLL | Glutamic Acid | % | 3.59 | 0.133 | 3.59 | 3.40 | 3.75 |
| EPL | Glycine | % | 1.38 | 0.064 | 1.38 | 1.30 | 1.46 |
| DLL | Glycine | % | 1.45 | 0.041 | 1.45 | 1.39 | 1.51 |
| EPL | Histidine | % | 0.54 | 0.022 | 0.54 | 0.52 | 0.57 |
| DLL | Histidine | % | 0.47 | 0.015 | 0.47 | 0.45 | 0.49 |
| EPL | Isoleucine | % | 0.93 | 0.019 | 0.93 | 0.90 | 0.94 |
| DLL | Isoleucine | % | 0.73 | 0.047 | 0.74 | 0.68 | 0.80 |
| EPL | Leucine | % | 1.63 | 0.046 | 1.63 | 1.56 | 1.68 |
| DLL | Leucine | % | 1.59 | 0.059 | 1.58 | 1.53 | 1.68 |
| EPL | Lysine | % | 1.28 | 0.035 | 1.28 | 1.24 | 1.34 |
| DLL | Lysine | % | 1.12 | 0.032 | 1.13 | 1.09 | 1.17 |
| EPL | Methionine | % | 0.37 | 0.029 | 0.38 | 0.33 | 0.41 |
| DLL | Methionine | % | 0.33 | 0.019 | 0.33 | 0.31 | 0.36 |
| EPL | Phenylalanine | % | 0.99 | 0.060 | 0.98 | 0.92 | 1.08 |
| DLL | Phenylalanine | % | 1.04 | 0.033 | 1.04 | 0.99 | 1.08 |
| EPL | Proline | % | 1.22 | 0.043 | 1.23 | 1.17 | 1.27 |
| DLL | Proline | % | 1.36 | 0.020 | 1.37 | 1.33 | 1.38 |
| EPL | Serine | % | 0.93 | 0.035 | 0.93 | 0.89 | 0.99 |
| DLL | Serine | % | 0.99 | 0.019 | 0.99 | 0.97 | 1.02 |
| EPL | Threonine | % | 1.03 | 0.030 | 1.04 | 0.99 | 1.07 |
| DLL | Threonine | % | 1.06 | 0.025 | 1.05 | 1.03 | 1.10 |
| EPL | Tryptophan | % | 0.36 | 0.016 | 0.37 | 0.33 | 0.37 |
| DLL | Tryptophan | % | 0.47 | 0.059 | 0.47 | 0.42 | 0.56 |
| EPL | Tyrosine | % | 0.49 | 0.032 | 0.49 | 0.45 | 0.54 |
| DLL | Tyrosine | % | 0.74 | 0.018 | 0.74 | 0.72 | 0.75 |
| EPL | Valine | % | 1.23 | 0.019 | 1.23 | 1.20 | 1.26 |
| DLL | Valine | % | 1.17 | 0.063 | 1.17 | 1.11 | 1.27 |

355 [1] Analytical Lab: EPL – EPL Bio Analytical Services; DLL – Dairyland Laboratories

356

357 Table 12. CoverCress CS (B3WG-15-32) Amino Acids as Percent of Protein

| Analytical Lab | Variable | Units | Mean | Standard Deviation | Median | Range Minimum | Range Maximum |
|---|---|---|---|---|---|---|---|
| EPL | Alanine | % | 4.46 | 0.164 | 4.48 | 4.20 | 4.60 |
| DLL | Alanine | % | 4.20 | 0.118 | 4.19 | 4.05 | 4.38 |
| EPL | Arginine | % | 5.49 | 0.160 | 5.44 | 5.31 | 5.70 |
| DLL | Arginine | % | 6.59 | 0.138 | 6.59 | 6.43 | 6.74 |
| EPL | Aspartic Acid | % | 7.64 | 0.450 | 7.73 | 6.97 | 8.12 |
| DLL | Aspartic Acid | % | 8.05 | 0.220 | 7.99 | 7.81 | 8.33 |
| EPL | Cystine | % | 1.50 | 0.104 | 1.50 | 1.41 | 1.67 |
| DLL | Cysteine | % | 1.62 | 0.076 | 1.62 | 1.49 | 1.69 |
| EPL | Glutamic Acid | % | 15.13 | 0.711 | 15.35 | 13.90 | 15.75 |
| DLL | Glutamic Acid | % | 15.07 | 0.172 | 15.09 | 14.88 | 15.27 |
| EPL | Glycine | % | 5.45 | 0.094 | 5.41 | 5.36 | 5.56 |
| DLL | Glycine | % | 6.09 | 0.087 | 6.06 | 6.01 | 6.20 |
| EPL | Histidine | % | 2.14 | 0.043 | 2.14 | 2.08 | 2.19 |
| DLL | Histidine | % | 1.99 | 0.027 | 1.99 | 1.96 | 2.02 |
| EPL | Isoleucine | % | 3.66 | 0.124 | 3.72 | 3.45 | 3.74 |
| DLL | Isoleucine | % | 3.07 | 0.125 | 3.09 | 2.87 | 3.19 |
| EPL | Leucine | % | 6.42 | 0.162 | 6.50 | 6.13 | 6.51 |
| DLL | Leucine | % | 6.69 | 0.081 | 6.71 | 6.56 | 6.78 |
| EPL | Lysine | % | 5.05 | 0.330 | 5.08 | 4.53 | 5.35 |
| DLL | Lysine | % | 4.71 | 0.121 | 4.69 | 4.55 | 4.85 |
| EPL | Methionine | % | 1.47 | 0.083 | 1.49 | 1.38 | 1.57 |
| DLL | Methionine | % | 1.39 | 0.050 | 1.38 | 1.33 | 1.45 |
| EPL | Phenylalanine | % | 3.89 | 0.072 | 3.91 | 3.80 | 3.96 |
| DLL | Phenylalanine | % | 4.35 | 0.044 | 4.38 | 4.30 | 4.40 |
| EPL | Proline | % | 4.83 | 0.136 | 4.87 | 4.60 | 4.95 |
| DLL | Proline | % | 5.73 | 0.164 | 5.71 | 5.48 | 5.91 |
| EPL | Serine | % | 3.68 | 0.062 | 3.69 | 3.60 | 3.76 |
| DLL | Serine | % | 4.16 | 0.116 | 4.13 | 4.06 | 4.36 |
| EPL | Threonine | % | 4.08 | 0.103 | 4.11 | 3.91 | 4.18 |
| DLL | Threonine | % | 4.44 | 0.098 | 4.38 | 4.36 | 4.58 |
| EPL | Tryptophan | % | 1.41 | 0.058 | 1.44 | 1.34 | 1.48 |
| DLL | Tryptophan | % | 1.98 | 0.174 | 1.93 | 1.80 | 2.21 |
| EPL | Tyrosine | % | 1.93 | 0.051 | 1.97 | 1.87 | 1.97 |
| DLL | Tyrosine | % | 3.09 | 0.073 | 3.08 | 2.99 | 3.18 |
| EPL | Valine | % | 4.85 | 0.170 | 4.92 | 4.56 | 4.98 |
| DLL | Valine | % | 4.89 | 0.180 | 4.98 | 4.59 | 5.04 |

358 [1] Analytical Lab: EPL – EPL Bio Analytical Services; DLL – Dairyland Laboratories

359

360

361 Fatty Acid Characterization

362 Tables 13 and 14 contains the fatty acid profile of B3WG-15-32 when expressed on
363 a 100% dry matter basis and percent of total fatty acids, respectively. In general, there was
364 good agreement between laboratories. The fatty acid values from the five lots showed a
365 symmetrical distribution with the means and median values being similar. The major fatty
366 acids are oleic (~43% of the total fatty acids) followed by linoleic (~32% of the total fatty
367 acids) and linolenic (~18% of the total fatty acids). These three fatty acids comprise over
368 90% of the fatty acids in B3WG-15-32. No high polyunsaturated long chain fatty acids
369 such as EPA and DHA were detected. Results confirm that B3WG-15-32 contains
370 negligible levels of erucic acid. Dairyland Laboratories did not detect it and EPL reported
371 erucic acid to be less than 0.5% of the total fatty acids.

372

373 Table 13. CoverCress CS (B3WG-15-32) Fatty Acids (100% dry matter basis)

| Analytical Lab | Variable | Units | Mean | Standard Deviation | Median | Range Minimum | Range Maximum |
|---|---|---|---|---|---|---|---|
| EPL | Myristic (C1 4:0) | % | 0.02 | 0.002 | 0.02 | 0.01 | 0.02 |
| DLL | Myristic (C14:0) | % | 0.00 | 0.000 | 0.00 | 0.00 | 0.00 |
| EPL | Palmitic (C16:0) | % | 0.62 | 0.039 | 0.62 | 0.56 | 0.67 |
| DLL | Palmitic (C16:0) | % | 1.17 | 0.051 | 1.16 | 1.11 | 1.24 |
| EPL | Palmitoleic (C16:1) | % | 0.02 | 0.002 | 0.02 | 0.02 | 0.03 |
| DLL | Palmitoleic (C16:1) | % | 0.00 | 0.000 | 0.00 | 0.00 | 0.00 |
| EPL | Heptadecanoic (C17:0) | % | 0.01 | 0.001 | 0.01 | 0.01 | 0.01 |
| DLL | Heptadecanoic (C17:0) | % | 0.00 | 0.000 | 0.00 | 0.00 | 0.00 |
| EPL | Heptadecenoic (C17:1) | % | 0.00 | 0.000 | 0.00 | 0.00 | 0.00 |
| DLL | Heptadecenoic (C17:1) | % | NA[2] | NA | NA | NA | NA |
| EPL | Stearic (C18:0) | % | 0.13 | 0.010 | 0.13 | 0.12 | 0.14 |
| DLL | Stearic (C18:0) | % | 0.26 | 0.017 | 0.27 | 0.24 | 0.28 |
| EPL | Oleic (C18:1) | % | 6.75 | 0.430 | 6.74 | 6.17 | 7.38 |
| DLL | Oleic (C18:1) | % | 13.61 | 0.930 | 13.84 | 12.35 | 14.86 |
| EPL | Linoleic (C18:2) | % | 5.16 | 0.411 | 5.14 | 4.77 | 5.84 |
| DLL | Linoleic (C18:2) | % | 9.57 | 0.733 | 9.83 | 8.57 | 10.44 |
| EPL | Linolenic (C18:3) | % | 2.91 | 0.250 | 2.83 | 2.68 | 3.34 |
| DLL | Linolenic (C18:3) | % | 5.16 | 0.423 | 5.28 | 4.72 | 5.70 |
| EPL | Nonadecenoic (C19:1) | % | 0.00 | 0.000 | 0.00 | 0.00 | 0.00 |
| DLL | Nonadecenoic (C19:1) | % | NA | NA | NA | NA | NA |
| EPL | Arachidic (C20:0) | % | 0.02 | 0.001 | 0.02 | 0.02 | 0.02 |
| DLL | Arachidic (C20:0) | % | 0.00 | 0.000 | 0.00 | 0.00 | 0.00 |
| EPL | Eicosenoic (C20:1) | % | 0.14 | 0.009 | 0.14 | 0.12 | 0.14 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| DLL | Eicosenoic (C20:1) | % | 0.23 | 0.014 | 0.23 | 0.21 | 0.25 |
| EPL | Eicosadienoic (C20:2) | % | 0.02 | 0.003 | 0.02 | 0.02 | 0.03 |
| DLL | Eicosadienoic (C20:2), Eicosatrienoic(C20:3) | % | 0.00 | 0.000 | 0.00 | 0.00 | 0.00 |
| EPL | Behenic (C22:0) | % | 0.00 | 0.000 | 0.00 | 0.00 | 0.00 |
| DLL | Behenic (C22:0) | % | NA | NA | NA | NA | NA |
| EPL | Erucic (C22:1) | % | 0.07 | 0.022 | 0.06 | 0.05 | 0.11 |
| DLL | Erucic (C22:1) | % | 0.00 | 0.000 | 0.00 | 0.00 | 0.00 |
| EPL | Lignoceric (C24:0) | % | 0.00 | 0.000 | 0.00 | 0.00 | 0.00 |
| DLL | Lignoceric (C24:0) | % | 0.00 | 0.000 | 0.00 | 0.00 | 0.00 |
| EPL | Nervonic (C24:1) | % | 0.05 | 0.005 | 0.05 | 0.04 | 0.06 |
| DLL | Nervonic (C24:1) | % | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |

[1] Analytical Lab: EPL – EPL Bio Analytical Services; DLL – Dairyland Laboratories
[2] NA: Not Analyzed
The following fatty acids were below the level of detection for Lab EPL: Capric (C10:0), Caproic (C6:0), Caprylic (C8:0), Docosadienoic (C22:2), Docosahexaenoic (C22:6), Eicosatrienoic (C20:3), Eicosapentaenoic (C20:5), Gamma Linolenic (C18:3), Heneicosanoic (C21:0), Heptadecenoic (C17:1), Lauric (C12:0), Myristoleic (C14:1), Nonadecanoic (C19:0), Nonadecenoic (C19:1), Pentadecanoic (C15:0), Pentadecenoic (C15:1), Tricosanoic (C23:0); for Lab DLL: Arachidonic (C20:4), Docosahexanoic acid (C22:6); Eicosapentaenoic acid (C20:5).

Table 14. CoverCress CS (B3WG-15-32) Fatty Acids as a Percent of Total Fatty Acids

| Analytical Lab | Variable | Units | Mean | Standard Deviation | Median | Range Minimum | Range Maximum |
|---|---|---|---|---|---|---|---|
| EPL | Myristic (C14:0) | % | 0.10 | 0.011 | 0.10 | 0.09 | 0.11 |
| DLL | Myristic (C14:0) | % | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| EPL | Palmitic (C16:0) | % | 3.88 | 0.042 | 3.89 | 3.84 | 3.95 |
| DLL | Palmitic (C16:0) | % | 3.89 | 0.079 | 3.91 | 3.77 | 3.96 |
| EPL | Palmitoleic (C16:1) | % | 0.15 | 0.018 | 0.16 | 0.12 | 0.17 |
| DLL | Palmitoleic (C16:1) | % | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| EPL | Heptadecanoic (C17:0) | % | 0.03 | 0.018 | 0.03 | 0.00 | 0.05 |
| DLL | Heptadecanoic (C17:0) | % | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| EPL | Heptadecenoic (C17:1) | % | 0.07 | 0.011 | 0.08 | 0.06 | 0.09 |
| DLL | Heptadecenoic (C17:1) | % | NA | NA | NA | NA | NA |
| EPL | Stearic (C18:0) | % | 0.84 | 0.039 | 0.84 | 0.77 | 0.88 |
| DLL | Stearic (C18:0) | % | 0.88 | 0.040 | 0.87 | 0.83 | 0.93 |
| EPL | Oleic (C18:1) | % | 42.32 | 2.261 | 42.40 | 39.00 | 45.35 |
| DLL | Oleic (C18:1) | % | 45.40 | 2.134 | 45.34 | 42.22 | 48.16 |
| EPL | Linoleic (C18:2) | % | 32.42 | 1.425 | 32.50 | 30.25 | 34.25 |
| DLL | Linoleic (C18:2) | % | 31.89 | 1.431 | 31.86 | 29.84 | 33.87 |
| EPL | Linolenic (C18:3) | % | 18.25 | 0.793 | 18.15 | 17.35 | 19.50 |
| DLL | Linolenic (C18:3) | % | 17.18 | 0.737 | 17.24 | 16.44 | 18.26 |
| EPL | Nonadecenoic (C19:1) | % | 0.03 | 0.018 | 0.03 | 0.00 | 0.05 |
| DLL | Nonadecenoic (C19:1) | % | NA | NA | NA | NA | NA |
| EPL | Arachidic (C20:0) | % | 0.13 | 0.013 | 0.13 | 0.13 | 0.016 |
| DLL | Arachidic (C20:0) | % | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| EPL | Eicosenoic (C20:1) | % | 0.85 | 0.024 | 0.84 | 0.82 | 0.87 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| DLL | Eicosenoic (C20:1) | % | 0.77 | 0.042 | 0.78 | 0.70 | 0.82 |
| EPL | Eicosadienoic (C20:2) | % | 0.03 | 0.002 | 0.03 | 0.03 | 0.03 |
| DLL | Eicosadienoic (C20:2), Eicosatrienoic(C20:3) | % | 0.00 | 0.000 | 0.00 | 0.00 | 0.00 |
| DLL | Arachidonic (20:4) | % | 0.02 | 0.018 | 0.02 | 0.00 | 0.05 |
| EPL | Behenic (C22:0) | % | 0.06 | 0.003 | 0.06 | 0.06 | 0.07 |
| DLL | Behenic (C22:0) | % | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| EPL | Erucic (C22:1) | % | 0.43 | 0.109 | 0.38 | 0.36 | 0.62 |
| DLL | Erucic (C22:1) | % | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| EPL | Lignoceric (C24:0) | % | 0.07 | 0.005 | 0.07 | 0.06 | 0.07 |
| DLL | Lignoceric (C24:0) | % | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| EPL | Nervonic (C24:1) | % | 0.31 | 0.025 | 0.30 | 0.30 | 0.36 |
| DLL | Nervonic (C24:1) | % | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |

[1] Analytical Lab: EPL – EPL Bio Analytical Services; DLL – Dairyland Laboratories
[2] NA: Not Analyzed
The following fatty acids were below the level of detection for Lab A: Arachidonic (C20:4), Capric (C10:0), Caproic (C6:0), Caprylic (C8:0), Docosadienoic (C22:2), Eicosatrienoic (C20:3), Gamma Linolenic (C18:3), Heneicosanoic (C21:0), Lauric (C12:0), Myristoleic (C14:1), Nonadecanoic (C19:0), Pentadecanoic (C15:0), Pentadecenoic (C15:1), Tricosanoic (C23:0); for Lab B: Arachidonic (C20:4), Docosahexanoic acid (C22:6); Eicosapentaenoic acid (C20:5).

## Glucosinolate Characterization and Quantification

Sinigrin averaged 105.4 µmoles/g of B3WG-15-32 on a 100% dry matter basis and was similar among lots (Table 15). Sinigrin was measured using the Ultraviolet method (UV).

Table 15. CoverCress CS (B3WG-15-32) Glucosinolates (Sinigrin, 100% dry matter basis) -EPL Bio Analytical Services

| | | | Standard | | Range | |
|---|---|---|---|---|---|---|
| Variable | Units | Mean | Deviation | Median | Minimum | Maximum |
| Sinigrin - UV | µmoles/g | 105.4 | 4.21 | 104.3 | 101.4 | 110.7 |

## Minerals, Sinapine, and Mycotoxins

Table 16 contains the results to the mineral analyses. EPL and Dairyland Laboratories results varied. The discrepancy between labs is unexplained. Generally, the values for the five lots had symmetric distribution. The high level of copper is due to the addition of copper sulfate. Target addition of copper was 800 ppm. The analytical results confirm that the target levels were achieved.

Table 16. CoverCress CS (B3WG-15-32) Minerals (100% dry matter basis)

| Analytical Lab[1] | Variable | Units | Mean | Standard Deviation | Median | Range Minimum | Range Maximum |
|---|---|---|---|---|---|---|---|
| EPL | Calcium | % | 0.93 | 0.074 | 0.95 | 0.84 | 1.01 |
| DLL | Calcium | % | 1.25 | 0.154 | 1.31 | 0.99 | 1.39 |
| EPL | Phosphorus | % | 0.99 | 0.135 | 0.96 | 0.80 | 1.14 |
| DLL | Phosphorus | % | 0.68 | 0.134 | 0.71 | 0.48 | 0.81 |
| EPL | Magnesium | % | 0.38 | 0.091 | 0.37 | 0.30 | 0.48 |
| DLL | Magnesium | % | 0.35 | 0.087 | 0.30 | 0.27 | 0.44 |
| EPL | Potassium | % | 1.11 | 0.083 | 1.05 | 1.05 | 1.23 |
| DLL | Potassium | % | 1.04 | 0.117 | 1.05 | 0.89 | 1.17 |
| EPL | Sulfur | % | 1.17 | 0.061 | 1.15 | 1.12 | 1.26 |
| DLL | Sulfur | % | 0.94 | 0.064 | 0.96 | 0.86 | 1.01 |
| EPL | Sodium | % | 0.00 | 0.000 | 0.00 | 0.00 | 0.00 |
| DLL | Sodium | % | 0.01 | 0.004 | 0.01 | 0.01 | 0.02 |
| EPL | Chloride | ppm | 399.4 | 85.334 | 432.00 | 275.50 | 473.00 |
| DLL | Chloride | ppm | 800 | 25 | 700 | 600 | 1200 |
| EPL | Iron | ppm | 107.85 | 48.471 | 84.95 | 63.90 | 162.50 |
| DLL | Iron | ppm | 72 | 30.7 | 57 | 40.5 | 113 |
| EPL | Copper | ppm | 959.70 | 29.641 | 955.00 | 930.50 | 1001.5 |
| DLL | Copper | ppm | 1052 | 257.8 | 1087.5 | 633 | 1315 |
| EPL | Manganese | ppm | 36.96 | 3.254 | 38.30 | 31.80 | 39.75 |
| DLL | Manganese | ppm | 46 | 6.5 | 47.5 | 39 | 55 |
| EPL | Zinc | ppm | 38.88 | 4.672 | 40.20 | 33.65 | 45.00 |
| DLL | Zinc | ppm | 46 | 15.6 | 43 | 34 | 73 |

[1] Analytical Lab: EPL – EPL Bio Analytical Services; DLL – Dairyland Laboratories

The concentrations of sinapine in B3WG-15-32 were <0.05% as reported by EPL in all five lots. This is in comparison to canola meal where the Canola Council of Canada (2015) reports sinapine levels of 1.0% as is basis (with an average 12% moisture content) or 1.14% on a 100% DM basis.

No mycotoxins were detected in the five lots. All mycotoxins were below the limits of detection in all samples from all of the five lots: Aflatoxin B1, B2, G1, G2; T-2 Toxin; Ochratoxin A; Deoxynivalenol (DON), 3-Acetyl-DON; 15-Acetyl-DON; Zearalenone; Fumonisin B1, B2, B3; Ergosine; Ergotamine; Ergocristine; and Ergocornine; and Ergocryptine.

422

423 Vitamins

424 Table 17 contains the vitamins as analyzed by EPL for the five lots. Generally, the
425 values for the five lots had symmetric distribution.

426

427 Table 17. CoverCress CS (B3WG-15-32) Vitamins (100% dry matter basis) – EPL Bio Analytical
428 Services

| Variable | Units | Mean | Standard Deviation | Median | Range Minimum | Range Maximum |
|---|---|---|---|---|---|---|
| Folic Acid | mg/kg | 5.6 | 0.67 | 5.2 | 5.0 | 6.6 |
| Niacin | mg/kg | 40.2 | 2.06 | 40.6 | 37.1 | 42.5 |
| Vitamin B1 (Thiamine) | mg/kg | 5.2 | 0.06 | 5.2 | 5.1 | 5.3 |
| Vitamin B2 (Riboflavin) | mg/kg | 4.7 | 0.29 | 4.6 | 4.3 | 5.0 |
| Vitamin B6 (Pyridoxine) | mg/kg | 6.1 | 0.17 | 6.1 | 5.9 | 6.3 |
| Alpha-Tocopherol | mg/kg | 181.8 | 24.28 | 174.5 | 160.5 | 218.0 |
| Beta-Tocopherol | mg/kg | 3.1 | 0.13 | 3.1 | 2.9 | 3.4 |
| Delta-Tocopherol | mg/kg | <1.25 | | <1.25 | <1.25 | <1.25 |
| Gamma-Tocopherol | mg/kg | 13.4 | 1.75 | 12.5 | 11.9 | 15.8 |

429

430 **Conclusions**

431

432 The results of these extensive compositional and nutritional analyses show:

433 ● The composition and nutritional components of CCWG-15-32 and B3WG-15-32
434 lots were generally consistent with very good process control and low inter-lot
435 variability
436 ● As expected by loss of function of the FAE1 gene, the fatty acid profile of the
437 CCWG-15-32 and B3WG-15-32 lots showed negligible erucic acid levels
438 ● As expected by loss of function of the TT8 gene, mean ADF and crude fibers
439 levels were in acceptable ranges for broiler diets
440 ● Sinigrin (2-propenyl glucosinolate) was the only detectable glucosinolate
441 consistent with previous published results in field pennycress seed or meals and
442 total glucosinolate concentration in CCWG-15-32 ranged from 45 to 102 µmoles/g
443 on 100% DM basis. Total glucosinolate concentration in B3WG-15-32 ranged
444 from 101.4 to 110.7 µmoles/g on 100% DM basis
445 ● Total copper levels achieved target levels of 800 ppm

- Other anti-nutrients (sinapine) and mycotoxins were below the imit of detection or quantification

The main purpose of CoverCress whole grain as a feed ingredient in animal diets (e.g. broilers) is as an energy source. Determination of energy value is complex, but is related to the fat, protein, and fiber content. Further, the degree of unsaturation of the fatty acids is a factor in energy. While there are some slight differences in total fat and the fiber profile, the overall nutritional value of CCWG for energy is expected to be similar to that of canola seed (see Table 18 below). Levels of the four key limiting amino acids in broiler diets, methionine, lysine, threonine and valine are similar between CCWG and canola. The low-erucic acid phenotype expected with loss of function of FAE1 was achieved as shown by the low to non-detectable levels in CCWG. The nutritional value in terms of energy (total fat, profile of fatty acids, crude fiber, ADF, NDF) of B3WG is comparable to that of CCWG. Crude fiber levels were variable across lots and between CCWG and B3WG. This may be due to assay or natural variability within the plants. Levels of the four key limiting amino acids in broiler diets, methionine, lysine, threonine and valine were lower for the B3WG as compared to the CCWG. The difference is mostly like due to the lower amino acids as a percent of protein in the B3WG as compared to the CCWG (88.1% versus 97% of the protein for B3WG and CCWG, respectively.

Table 18**.** Key Compositional Parameters for Canola Whole Grain, CCWG and B3WG

| **Component** | **Canola[1]** | **CCWG-15-32 Mean[2] [range]** | **B3WG-15-32 Mean[2] [range]** |
|---|---|---|---|
| Fat % DM | 39.1 | 36.3 [35.5-36.7] | 37.0 [35.7-38.5] |
| Erucic acid % TFA | <0.1 | <0.1 | <0.1 |
| Oleic % of TFA | 61.4 | 40.07 [38.61-41.06] | 45.4 [42.22-48.16] |
| Linoleic % TFA | 20.1 | 34.48 [34.17-34.94] | 31.89 [29.84-33.87] |
| Linolenic % TFA | 9.3 | 16.55 | 17.18 |

| | | | |
|---|---|---|---|
| | | [16.18-17.67] | [16.44-18.26] |
| Total saturated %TFA | 7.0 | 5.0 | |
| Total mono-unsaturated %TFA | 64.4 | 41.6 | |
| Total poly-unsaturated % TFA | 28.6 | 53.4 | |
| | | | |
| Protein %DM | 24.5 | 25.5 [22.7-26.7] | 23.9 [22.5-25.1] |
| Methionine % protein | 1.93 | 1.88 [1.74-2.03] | 1.39 [1.33-1.45] |
| Lysine % protein | 5.66 | 5.51 [5.30-5.68] | 4.71 [4.55-4.85] |
| Threonine % protein | 3.97 | 4.91 [4.62-5.26] | 4.44 [4.36-4.58] |
| | | | |
| Valine %protein | 4.40 | 5.69 [5.48-6.14] | 4.89 [4.59-5.04] |
| Crude Fiber %DM | 13.3 | 23.0 [20.9-24.9] | 12.1 [10.9-12.5] |
| ADF | 18.1 | 13.9 [12.1-15.8] | 17.8 [15.9-20.2] |
| NDF | 25.7 | 20.3 [17.3-24.5] | 22.6 [21.3-24.4] |
| | | | |
| Sulfur % DM | 0.45[3] [0.36-0.55] | 0.87 [0.47-1.05] | 0.94 [0.86-1.01] |
| Total glucosinolates µmoles/g on DM basis UV method | 9.8 | 86.9 [45-102] | 105.4 [101.4-110.7] |

[1]Values from Dairy One feed Composition Laboratory; means of whole canola seed samples from crop years 2004 - 2020. https://www.dairyoneservices.com/feedcomposition/

[2]From the respective 5-lot study, DLL values

[3]From https://www.grainscanada.gc.ca/en/grain-research/export-quality/oilseeds/canola/2020/index.htm

The composition and nutritional components of C3WG-15-32 lots were generally consistent with very good process control and inter-lot variability. These results will enable eventual development of permissible ranges for key nutritional analytes and guaranteed levels for specific components (i.e. commercial specifications) like total crude protein, crude fiber, crude fat, copper and sulfur. A glucosinolate specification will be needed to ensure safe consumption depending on the level of inclusion in the diet and sensitivity of the animal species. In these lots, the mean level of glucosinolates was slightly higher in B3WG than CCWG, but ranges for the two overlapped, indicating the difference is likely due to natural variability.

The current study shows that low-erucic acid, lower-fiber pennycress (CoverCress) produces a consistent compositional phenotype that may enable the seed to be consumed as an energy source for various animal species.

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**Conflict of Interest**

Shawna Lemke and Gary Hartnell were paid by CoverCress Inc for their consulting services. Chris Aubach is an employee of CoverCress Inc.

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