PIGS FED CAMELINA MEAL INCREASES LIVER EXPRESSION OF DRUG METABOLISM ENZYMES CYP8B1, ALDH2 AND TST.

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Abstract − Camelina is an oil seed crop which can be grown on marginal lands. Camelina seed oil is rich in omega-3 fatty acids (>35%) and g-tocopherol but also contains erucic acid and glucosinolates. Camelina meal is the by-product after the oil has been extracted. Camelina meal was fed to 16d old weaned pigs at 3.7% and 7.4% for 24 days. The trial indicated that camelina meal improved feed efficiency but the liver weights were also significantly increased. Gene expression analysis of the livers, using microarrays, indicated increased expression of phase 1 and phase 2, drug metabolism enzymes. The porcine genes, cytochrome 8b1 (CYP8B1), aldehyde dehydrogenous 2 (Aldh2), and thiosulfate sulfurtransferase (TST) were the most significantly stimulated. Together these indicate the camelina glucosinolate metabolite, methyl-sulfinyldecyl , as being the main xeniobiotic causing increased hepatic metabolism.

Key Words − Camelina, CYP8b1, Glucosinolates, RNA

- INTRODUCTION

Camelina, a member of the family *Brassicaceae* is related to rapeseed. It has commercial value as an oil seed crop for biofuels and bio-lubricants which can be grown on marginal lands. Camelina seed has an oil content of > 40% (dry weight) and this oil is high in omega-3 fatty acids, erucic acid and gamma tocopherol [1]. Camelina meal is the by-product after the oil has been extracted. Camelina meal is currently under consideration 2013, for recognition by the Canadian food inspection agency (CFIA) as feed ingredient in diets for Canadian livestock.

Glucosinolates are considered bitter to humans. The glucosinolates are metabolized into biologically active compounds such as, isothiocyanates, indoles and nitriles, by endogenous plant enzymes called myrosinase or β-thioglucosidases from gut bacteria [2]. Higher doses of glucosinolate metabolite, thiocyanate, can affect the transport of iodine to thyroid. Glucosinoate metabolic products are mainly associated with the induction of Phase I and Phase 2 biotransformation enzymes. Phase 1 enzymes catalyse a variety of hydrolytic, oxidative and reductive reactions, including the cytochrome P450 xenobiotic and toxin metabolizing enzymes. Phase 2 enzymes such as glutathione S-transferase and UDP-glucuronyl transferase form conjugation products with xenobiotics which are readily excreted. When feeding xenobiotics, usually, liver is the most responsive tissue for phase 1 and 2 expression.

Glucosinoates metabolites from camelina, rapeseed and canola differ slightly in the type and quantity. Glucosinolates metabolites from rapeseed and Canadian canola (Brassica napsis) are predominately progoitrin (2-hydroxy-3-butenyl) and gluconapin (3-butenyl) structure [3]. Glucosinolates from camelina are predominantly glucocamelina, which is metabolized into 10-methylsulphinyldecyl isothiocyanate. The structure of 10-methylsulfinyldecyl is closely analogous to sulforaphane which is common to cruciferous vegetables such as broccoli, mustard and cabbage and may be protective against cancer and cardiovascular disease.

Typical seed crushers will extract the oil content down to 4% (dry weight). The Camelian sativa oil is composed of omega-6 linoleic acid, omega-3 linolenic acid but also contains ~5% of the monounsaturated omega-9 fatty acid, erucic acid (C22:1 ω-9). Erucic acid content is a large component of mustard oil and its consumption by human is still controversial, being part of Lorenzo's oil [4] but also implicated in causing thrombocytopenia. Camelina oil is also rich in the antioxidant, gamma tocopherol at over 1000 ug/g, which can improve the palatability. The present study was undertaken as a preliminary investigation of pigs receiving camelina meal as an animal model to assess the benefit of human-health products derived from camelina.

- MATERIALS AND METHODS

The feeding trial was performed in Lacombe Research center piggery in accordance with guidelines of the Canadian Council on Animal Care. The feeding trial was composed of three groups fed either, the Control diet, the 3.7% camelina meal supplemented diet (LOW) diet, or the 7.4% camelina meal supplemented diet (HIGH) for 20 days. The HPLC analysis of camelina for glucocamelinin, 10-methyl-sulfinyldecyl, and 11-methyl-sulfinyldecyl content was made using method AOCS AK 1-92 by Bioprofile Testing Labs (St. Paul, MN, USA). The 27 healthy barrows were started on test feed at age of 16 days. Their weights and feed were monitored daily, until they were reached an age of 40 days. Pigs were euthanized in accordance to CCAC guidelines. Organ tissues were removed post mortem, weighed and stored at -20^{o}C.

Total pig liver RNA was examined for gene expression changes by microarray analysis using, the Rat Drug metabolism: phase 1 array (PARN-068) and the Human Drug Metabolism: phase 2 array (PAHS-069) (SABioscience /Qiagen, Mississauga, ON, Canada). Total RNA was extracted from the livers (100mg) by homogenizing in 5M guanidium isothiocyanate and then binding on silica columns for DNase treatment and washing before collecting the RNA in water according to the manufacturer's methods of the Aurum Total RNA fatty and fibrous tissue kit (BioRad, Mississauga, Ontario).

The genes identified by the arrays were used in the search for their closest porcine equivalent in the National Center for Biotechnology Information (NCBI) U.S. National Library of Medicine, GenBank using the Basic Local Alignment Search Tool (Blast) version 2.2.27 program. Primers were generated using Primer3 program v.0.4.0 to amplify the porcine version of the gene transcripts. Porcine transcripts were confirmed by sequencing the PCR products on a CEQ8000 machine (Beckman Coulter, Mississauga, ON, Canada). The cDNA [100ng] was used in a RT² SYBR Green master mix with 10 uM of each primer run on Mx300P QPCR machine for 40 cycles of 95^{o}C/30sec, 56^{o}C/30sec and 72^{o}C/60sec. Relative gene analysis was based on comparative $2^{-\Delta\Delta Ct}$ method. The reference genes were averaged between internal housekeeping genes, GADPH and β–actin.

Animal responses to the diets were analyzed using ANOVA followed by Duncan's test for differences in the group means. The gene analysis data was calculated using the comparative $2^{-\Delta\Delta Ct}$ method and significance between treatment groups was determined using the t-test. Statistical significance was accepted at $p < 0.05$ and trends were indicated at $p < 0.1$. All data were run on SAS version 8.

- RESULTS AND DISCUSSION

The amount of crude protein in the meal was estimated to be ~363 g/kg. The amount of crude fat in the diet was 143 g/kg, which is still high, considering the oil was extracted. The maximum recommended dose of glucosinolates for monogastric animals such as swine is

approximately 2 umoles per gram of feed. The concentration of glucosinolates was measured to be ~23.70 umol/g in the camelina meal. The molecular weight of glucocamelinin $C_{18}H_{35}O_{10}S_3N$ is estimated to be 521.65 g/mole; therefore at the total content is 12.36 g/ kg of camelina meal. The estimated final concentration of glucocamelinin in the LOW and HIGH, supplemented diets was 0.82 umol and 1.63 umol per gram of feed. Camelina also contains the monounsaturated omega-9 fatty acid, erucic acid. Erucic acid (C20:1 w-9) is a large component of mustard oil and can account to approximately 5% of camelina seed. The meal of Camelina after crushing and processing for oil, is expected to contain less than 4% oil, and therefore erucic acid level of the meal was expected to be less than 0.5%. The allowable amount of erucic acid in canola seed in Canada is set at 2% [5].

Mean initial weights of the pigs was 12.7 ±1.73 kg for the start of trial and at the end of the 24 day trial, the pigs weighed an average of 17.1 ±2.12 kg at age of 40d. The pigs appeared to have no problem consuming the feed. The pigs on HIGH diet was indicating an aversion to the meal with average daily feed intakes of 518.1 ±70.4 as compared to the CON diet 605.9 ± 111.3 but this was only shown as a trend at t-test; $P < 0.1$. There was a significant increase in the livers from the pigs fed the High diet. The liver weights at the end of the trial, was 346.4g/pig for the CON fed pigs, to 418.4g/pig for MED and 427.7g/pig for HIGH fed camelina meal. This indicates extra hepatic activity caused by the camelina meal. However, the same trend, was opposite, with thyroid weight between less in the pigs fed the camelina meal [6]. This may indicate a disruption of iodine absorption and thyroid activity caused by glucoinosinolate in the camelina meal but the iodine level of the pigs was not measured in this trial.

The total RNA from the pigs livers fed the High level of camelina meal was compared with animal fed the CON diets were tested on microarrays representing 168 genes involved in drug metabolism. The rat Drug Metabolism Phase 1 microarray identified the cytochrome P450 - 8b1 (Cyp8b1) and the aldehyde dehydrogenase 2 (Aldh2) gene transcription as being stimulated in the pig livers by camelina meal feeding. The human drug metabolism phase 2 array identified glutathione S-transferase mu 5 (Gstm5) and thiosulfate sulfotransferase (TST), as being significantly up-regulated > 4-fold relative to the control livers, as determined by the RT² profiler PCR data analysis program 3.5.

The genes were investigated using porcine specific primers on the livers of the fed pigs LOW and HIGH amounts of camelina meal. Cyp8b1 mRNA was significantly up-regulated approximately 80-fold in the liver tissue of pigs supplemented with High camelina meal. The transcripts for the TST and Aldh2 were increased approximately 1.8 and 3.2 fold but these were only weakly significant ($P < 0.1$). The Gstm5 transcript was not significantly stimulated by adding camelina to their diet.

Cytochrome P450 8B1 (Cyp8b1) is primary a microsomal sterol hydroxylase involved in bile acid formation. The basic structure of cholic acid is cholesterol which quite different than the metabolites of camelina meal glucosinolates, namely glucocamelina, which are methyl-sulfinyldecyl isothiocyanates. The transcriptional activation Cyp8b1 is probably the same mechanism as caused by broccoli [7]. Metabolites of broccoli glucosinolate contain sulforaphane, the methyl-sulfinylbutane isothiocyanate activates phase 2 drug enzymes including Cyp1A1, through the nuclear factor E2 p-45-related factor 2 (Nrf2) transcription factor. The Nrf2 factor binds the antioxidant response element (ARE) to activate transcription of the respective genes [8]. The same Nrf2 transcription factors have been shown to activate the other phase 2 enzymes, Aldh2, TST and GstM1 in mice [9].

- CONCLUSION

The widely successful development of rapeseed to low erucic, low glucosinolate, food grade canola in 1979 has brought camelina back into commercial interest as an alternative plant that can be grown on marginal land with good oil but some characteristics that might be detrimental if used as animal feed. Camelina is close undeveloped relative of rapeseed with has high erucic acid content and high glucosinolate content and was initially used as a source of oil for soap and lamps. The erucic acid content is difficult to digest and was thought to be cardiotoxic but studies later showed that animal will adjust to the fatty acid and breakdown the lipid in peroxisomes with the ABC transporter. Camelina glucoinsolates can be both anti-nutritional and chemo-protective as well. This trial with camelina meal feeding demonstrated that the expression of phase 1 and 2 xenobiotic detoxifying enzymes are up regulated, as indicated by pig hepatic expression analysis. The glucosinolate content of camelina meal is probably activating the detox enzymes and this may provide anti-carcinogenic benefits.

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