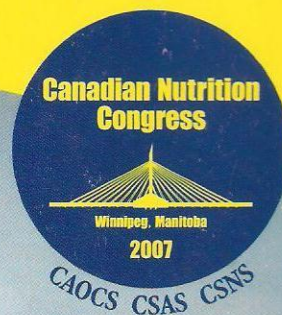


DSN-75

# Canadian Nutrition Congress

Building Bridges in the Nutrition Research Community



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**June 18–21, 2007**

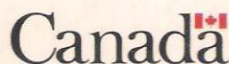
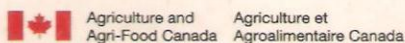
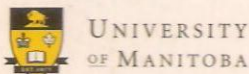
## Program & Proceedings

*A Joint Meeting of*

Canadian Section of the American Oil Chemists' Society

Canadian Society for Nutritional Sciences

Canadian Society of Animal Science



# Contents

Welcome to the Canadian Nutrition Congress .....	1	Intakes of ALA, EPA, DPA $\alpha$ -3 and DHA. ....	23
CNC Organizational Committee .....	1	Monitoring omega-3 fatty acids in clinical populations. ....	23
Scientific Advisory Committee .....	1	Polyunsaturated fatty acid synthases as novel sources of LC-PUFA for human consumption.....	24
Welcome from the Minister of Science, Technology, Energy and Mines.....	2	Soy protein and isoflavones: scientific evidence for possible health claims. ....	24
Participating Societies.....	3	Probiotics – Is there evidence to support health claims? .....	24
Exhibitors.....	4	Health Canada’s position on the use of antimicrobial growth promotants in Canada.....	25
General Meeting Information.....	4	Study of molecular-structural changes of flaxseeds as affected by several moist heat treatment conditions in relation to rumen degradation kinetics in dairy cattle.....	25
Special Events.....	5	Effects of grain-induced subacute ruminal acidosis (SARA) on feeding behaviour of lactating dairy cows. ....	25
Week at a Glance .....	6	Effect of canola seed fibre protein and sugar fractions used as additives in dehydrated alfalfa pellets on palatability and lactation performance of dairy cows.....	26
Scientific Programs.....	8	Hull, hydroxycinnamic acids contents and particle size distribution of various barley varieties in relation to nutrient availability in ruminant. ....	26
2007 Danone Institute’s Distinguished Nutrition Leadership Award .....	8	A further evaluation of the NRC (1996) model energy requirement and DMI equations for western Canadian wintering beef cows. ....	26
Canadian Nutrition Congress Banquet.....	9	Relationships between progeny residual feed intake and dam productivity traits.....	27
<b>Plenary Symposium Speakers</b>		Fat composition of beef as affected by the inclusion of potato processing by-products in corn or barley based finishing diets.....	27
Dale E. Bauman, M.S., Ph.D.....	15	<i>Escherichia coli</i> O157:H7 secreted toxins enhance intestinal colonization in cattle.....	28
Tom Clandinin, Ph.D. ....	15	Genotypic characterization of distinct lineage types of <i>E. coli</i> O157:H7 from feedlots in Alberta. ....	28
Brent D. Flickinger, Ph.D.....	16	<b>Graduate Student Competition Abstracts</b>	
Ronald P. Mensink, Ph.D.....	16	A comparison of high and low insoluble fiber breakfast cereals on blood glucose and food intake in healthy individuals. ....	31
<b>Invited Speakers</b>		Impact of an integrated community-based micronutrient and health programme on anaemia in Malawian preschool children.....	31
Principles of rumen microbiology to optimize carbohydrate and nitrogen fermentation.....	19	Neurotoxicity of methylmercury and the protective effects of antioxidants using rat embryo culture .....	31
Advances in the development of rumen models.....	19	The Effects of the Combination of Dietary Flaxseed Oil and Cyclosporine in a Rat Cardiac Allograft Model.....	32
Practical use of ration models in ruminant nutrition.....	19		
Maternal nutrition and fetal development. ....	20		
Nutrition and reproduction: the importance of omega-3 fatty acids. ....	20		
Methods and limitations in determining trans fat.....	20		
Sustainable feed management strategies to reduce ruminant pathogen shedding in the environment.....	21		
Ecosystems goods and services – a new paradigm for policy makers and researchers to consider.....	21		
How much ALA do humans convert to EPA and DHA and what are the implications?.....	21		
ALA conversion to DHA in the brain. ....	22		
Omega-3 fatty acids and neurological health. ....	22		
Infectious disease models to study gut health in pigs. ....	22		
Pig gut microbiology and contribution to intestinal development. ....	23		

High protein consumption reduces body fat but does not alter mechanical abilities of bone in female rats. .... 32

**Major component responsible for the tumor regressing effect of flaxseed, alone or combined with tamoxifen, and mechanism of action. .... 32**

Majority of women at childbearing age may not be meeting their folate requirements from diet alone based on mandated levels of folic acid fortification. .... 33

Anti-steatotic effects of trans-10,cis-12 conjugated linoleic acid are associated with reduced hepatic levels of adipophilin, but not perilipin, in the *falfa* zucker rat. .... 33

Microbial programming: The effect of early microbial colonization on post-weaning performance in the pig ..... 34

Effect of phytase supplementation on performance, bone strength, and nutrient digestibility in newly-weaned pigs that are liquid fed high-moisture corn based diets..... 34

Changes in indices of sulphur amino acid (SAA) metabolism in response to graded levels of vitamin B<sub>6</sub> repletion in vitamin B<sub>6</sub> depleted piglets. .... 34

Fecal vs. rectoanal mucosal swab sampling for detection of experimentally inoculated *Escherichia coli* O157:H7 in feedlot cattle orally treated with bacteriophage..... 35

Effect of crab and lobster meal supplementation in diets for laying hens on productive performance and fatty acid composition of egg yolks. .... 35

Urea-nitrogen recycling and microbial protein production in beef heifers fed diets varying in levels of crude protein and ruminally-degradable protein..... 35

Relationships between residual feed intake and infrared thermography and glucocorticoid levels in feedlot steers from three different sire breeds. .... 36

Induction of subacute ruminal acidosis (SARA) in Holstein dairy cows by feeding a high grain diet or by feeding alfalfa pellets results in dissimilar immune responses. .... 36

Association of a marker in the vitamin D receptor gene with Marek's disease resistance in chickens. .... 37

## CNC Poster Session Abstracts – Series 100

Economic values of beef production traits in Manitoba. .... 41

Economics and net merit optimization in the development of a composite beef cattle line..... 41

Prion protein gene polymorphisms in purebred sheep breeds of British Columbia. .... 41

Variability of the Aleutian mink disease virus in Nova Scotia..... 42

Reproductive management strategies to improve pregnancy rate following Ovsynch/TAI protocol in dairy cows and heifers..... 42

Effects of modulating the NO/cGMP pathway on cGMP levels in bovine cumulus-oocyte complexes. .... 42

Effects of hot boning and moisture enhancement on tenderness of cull cow beef. .... 43

## CNC Poster Session Abstracts – Series 200

Laying Hens in Conventional and Enriched Cages. .... 47

The behaviour of early-weaned piglets following transport: Effect of season and weaning weight. .... 47

Early metritis reduces subsequent milk production in Holstein dairy cows..... 47

Non-invasive detection of infectious laryngotracheitis (ILT) in poultry using infrared thermography. .... 48

Early disease detection: Implications for industry efficiency, food quality and safety..... 48

## CNC Poster Session Abstracts – Series 300

Community analysis of antibiotic resistant microbes in hog manure and groundwater..... 51

Increased turnover of brush border enzymes is induced by microbial colonization..... 51

Reduction of pathogens in ruminant manure using sainfoin, a phytochemical rich forage. .... 51

Effects of a mixed culture of lactic acid-producing bacteria on fermentation and aerobic stability of barley silage. .... 52

*In vitro* metabolism of flaxseed lignans by bovine fecal microflora. .... 52

*In vitro* metabolism of flaxseed lignans by bovine ruminal microflora. .... 52

Effects of Tween 80 and fibrolytic enzymes on *in vitro* rumen fermentation characteristics of *Leymus chinensis*. .... 53

Effects of supplementation of feedlot ration with rare earth elements on rumen fermentation and digestion in a continuous culture..... 53

Development of a composting system for disposal of cattle carcasses and manure in the event of an infectious disease outbreak..... 53

Effects of *Ascophyllum nodosum* phlorotannins and terrestrial tannins on *Escherichia coli* and *E. coli* O157:H7. .... 54

Dietary soybean trypsin inhibitor-related changes in intestinal bacteria. .... 54

## CNC Poster Session Abstracts – Series 400

*Phlorotannins* from *ascophyllum nodosum* (brown seaweed) inhibit *in vitro* ruminal digestion of forage..... 57

Effects of *ascophyllum nodosum* phlorotannins on *in vitro* ruminal digestion of barley grain..... 57

Evaluating mitigation practices in livestock systems: an illustration of a whole-farm approach..... 57

Effect of propylene glycol administration on protein catabolism in transition dairy cows. ....	58	Enhanced hepatic ABCG5/G8 expression contributes to increased hepatobiliary and ileal cholesterol excretion in response to guar gum consumption in pigs fed an atherogenic diet. ....	67
Life cycle assessment of dairy systems in Nova Scotia. ....	58	Consuming an arachidonic acid and docosahexaenoic acid beverage for 7 months has a positive effect on blood parameters and visual perception among children 5–7 years of age. ....	68
Effect of dry matter intake on visceral organ mass and the protein expression of ATP Synthase and Na <sup>+</sup> /K <sup>+</sup> -ATPase in steers. ....	58	Fat-1 mouse retinas are rich in docosahexaenoic acid and n-3 very long chain fatty acids (VLCFA, C24-C26) and have increased rod and cone mediated retina function. ....	68
Effect of barley variety on hull content and comparison among various barley varieties. ....	59	Serum trans fatty acids are elevated in prostate cancer patients. ....	68
Effect of barley variety on ferulic acid and para-coumaric acid concentrations and comparison among various barley varieties. ....	59	Efficacy of EPA/DHA supplementation to more favorably modify postprandial triglyceride surges in hypercholesterolaemic patients on statin therapy. ....	69
Effects of lasalocid or monensin on in situ ruminal biohydrogenation of unsaturated fatty acids. ....	59	Eicosapentaenoic acid and docosahexaenoic acid enriched fish oil modifies inflammatory cytokine production and T-lymphocyte phenotypes in the JCR:LA-cp rat, a model of obesity and insulin resistance. ....	69
Intraindividual variation of in vitro metabolism of lignans in ruminal fluid and feces. ....	60	Garlic and juniper berry oils improve fatty acids accumulation in differentiating 3T3-L1 adipocytes while trans 10, cis 12 conjugated linoleic acid depresses it. ....	69
Effects of winter feeding systems on soil compaction, soil nutrients, and cost of production. ....	60	Effect of bio-formed conjugated linoleic acids (CLA) as high CLA beef on growth and adipose tissue development of rats. ....	70
Metabolic acidosis, D-lactic acidosis, and sodium bicarbonate therapy: effects on CSF pH, D-lactate clearance and neurological status. ....	60	Acute supplementation of trans vaccenic acid improves plasma lipid profiles in the obese and insulin resistant JCR:LA-cp rat. ....	70
Productivity and environmental sustainability of grassland receiving liquid hog manure. ....	61	Conjugated linoleic acids: What can explain the discrepancy between animal and human studies? ....	71
Effects of prepartum feed restriction and fat supplementation on periparturient dairy cow performance. ....	61	Study of the digestion of conjugated linoleic acids from different sources in a dynamic in vitro gastrointestinal model. ....	71
Twenty-four hour plasma glucose and insulin profiles and glucose tolerance in dairy cows fed at 0900h or 2100h. ....	61	Peyer's patch cells proliferate less in response to ovalbumin and soy in vitro when arachidonic acid and docosahexaenoic acid are included in the weaning diet of piglets. ....	71
Subacute ruminal acidosis (SARA) induced by feeding a pelleted diet affects feeding behaviour of lactating dairy cows. ....	62	Potential of eggs in enhancing the <i>cardioprotective/health</i> effects of carbohydrate restricted diets (CRD) in weight loss interventions. ....	72
Effects of barley grain processing and source of oilseed on milk fatty acid composition in dairy cows. ....	62	Chronic effects of CLA:c-9,t-11-enriched butter oil and n-3 polyunsaturated fatty acids (PUFA) supplementation in a rodent of obesity and insulin resistance: Effects on dyslipidemia and renal pathophysiology. ....	72
Chemical characteristics and in-situ rumen degradation of canola fractions in comparison with commercial canola meal and soy meal. ....	62	Stearidonic acid increases red blood cell and cardiac levels of epa in dogs. ....	73
Predicted energy values and nutrient supply to dairy cattle from canola fractions: Comparison with commercial canola meal and soy meal. ....	63	Low dietary n-6/n-3 fatty acid ratio reduce cardiovascular risk in mice regardless of the origin of n-3 fatty acids. ....	73
Pregnancy establishment and loss in dairy cows fed a flaxseed-based ration: observations from two field trials. ...	63	Short-term effects of dietary t-10, c-12 conjugated linoleic acid on energy intake, and visfatin expression in mice fed low and high fat diets. ....	73
Effects of feeding at 2100 vs. 0900 H on post-feeding intake patterns, nutrient digestibility and nitrogen balance in lactating cows. ....	64		
<b>CNC Poster Session Abstracts – Series 500</b>			
Use of canola, sunflower or flax affects meat lipid peroxidation and fatty acid composition in beef cattle. ....	67		
Do antibiotics alter the biohydrogenation of unsaturated fatty acids in barley-finished beef? ....	67		

Fatty acid profile and sensory characteristics of table eggs from laying hens fed diets containing microencapsulated fish oil (MFO).....	74
Feeding dried distillers grain (DDG) induced attenuation of ischemic cell death in adult rat ventricular myocytes. ....	74
Rats treated chronically with NMDA may be a model of upregulated brain arachidonic acid metabolism in bipolar disorder.....	74
Total alkyl and alkenyl ether composition of meat from beef fed pasture or concentrate or retail meats.....	75
Supplementation of an extract from <i>Ascophyllum nodosum</i> seaweed to media improves cellular monounsaturated fatty acids accumulation in differentiating 3T3-L1 adipocytes. ....	75
Fatty acids profile in breast milk of lactating mother consuming a galactogogue, coleus amboinicus lour.....	75

### CNC Poster Session Abstracts – Series 600

Circulating fatty acid profile and bone resorption in gilts fed dietary flax prepubertally.....	79
Hypor pigs need more lysine than recommended to express growth potential.....	79
Protein and gender interaction on net energy in pigs. ....	79
Decreased growth and muscle protein synthesis in macronutrient-restricted piglets are mediated by decrease in translation initiation signals rather than altered proteolysis. ....	80
Extracts derived from degradation of wheat and flaxseed non-starch polysaccharides by carbohydrase enzymes enhance fluid absorption in enterotoxigenic <i>Escherichia coli</i> infected piglet jejunal segments. ....	80
Nutrient digestibility in finishing pigs fed phytase-supplemented barley-based diets containing soybean meal or canola meal as a protein source. ....	80
Supplementation of phosphorus deficient corn/barley/soybean meal-based diets with phytase: Effect on nutrient utilization and manure P content.....	81
The effects of restrictive feeding and gender on pig growth performance, carcass and meat quality.....	81
Dietary phosphorus levels for liquid feeding high-moisture corn based diets to growing-finishing pigs. ....	81
Value of liquid feeding corn based diets to growing-finishing pigs.....	82
Fermentation as means to enhance the nutritional of high-moisture corn for liquid-fed starter pigs. ....	82
Effect of the raw potato starch on growth performance and gut microbial population of piglets fed diets containing pea protein isolate.....	82
Effect of dietary protein level on performance, plasma urea N and fecal consistency score in piglets challenged with <i>Escherichia coli</i> K88.....	83

Effect of dietary protein level on intestinal microbial population and metabolites in piglets challenged with <i>Escherichia coli</i> K88.....	83
The small intestinal alkaline phosphatase digestive capacity is reduced despite of an increase in its mrna abundance in the piglet with bowel inflammations. ....	83
True crude protein digestibility (CP) and the fecal endogenous CP outputs in diets for weaned pigs determined by the substitution method.....	84
Impact of probiotics on homeostasis of vitamins B <sub>9</sub> and B <sub>12</sub> and modulation of immunity and resistance to <i>E. coli</i> infection in weaned pigs. ....	84
Pyridoxine (vitamin B <sub>6</sub> ) and tryptophan modulate immune response of weanling pigs? .....	85
Feeding full-fat oilseeds as partial replacement for fish meal and fish oil in practical diets for rainbow trout fingerlings. .	85
Evaluation of shellfish by-products as alternative dietary sources of large particle calcium for laying hens. ....	85
Evaluating the efficacy of lysozyme as a feed supplement for heavy hen turkeys.....	86
Evaluating the <i>in vitro</i> solubility of by-products of the Atlantic shellfish industry as potential calcium sources for laying hens. ....	86
Feeding management practices for liquid-fed newly-weaned pigs using multiple trough sizes and feeding times.....	86

### CNC Poster Session Abstracts – Series 700

Effects of single nuclear polymorphisms of ATP-binding cassette G5/G8 and Niemann-Pick C1 Like 1 on the responsiveness of hypercholesterolemic individuals to plant sterol intervention. ....	89
A novel <i>in vitro</i> model for determining antioxidant properties of human milk.....	89
Dietary conjugated linoleic acid (CLA) isomers influence blood pressure in fa/fa Zucker rats by modulating the transition from large to small adipocytes. ....	89
Proximate analysis and protein digestibility-corrected amino acid score (PDCAAS) of hemp seed and hemp seed products.....	90
Homocysteine induces oxidative stress leading to endothelial dysfunction.....	90
Factors affecting glycemic response, satiety and food intake after pulse consumption in young men. ....	91
Antioxidants properties of human milk fats.....	91
Trends in beverages intake of Canadians within last three decades; are they similar to the trend in the United States? 91	91
A telenutrition monitoring system for cardiovascular information monitoring. ....	92
Bitter melon ( <i>Momordica charantia</i> ) extract induces apoptosis in 3T3-L1 preadipocytes.....	92

Mycosporine-like amino acid composition, antioxidant and antiproliferative activities of the edible red alga, <i>Palmaria palmata</i> (Dulse).....	92
Quantitative detection of napin in yellow mustard ( <i>Sinapis alba</i> L.) Seeds by an enzyme-linked immunosorbent assay. ...	92
A high protein diet at the upper end of the acceptable macronutrient distribution range (AMDR) leads to kidney glomerular damage in normal female sprague-dawley rats.	93
Relative bioactivity of a new coenzyme Q10 formulation. ..	93
The role of nutrition in space flights.....	93
Systemic production of t cell cytokines in acute weanling malnutrition: <i>in vivo</i> reflection of type 2 polarization. ....	94
Relationship between protein intake and lean body mass in young adults.....	94
Expression of Jejunal Excitatory Amino Acid Carrier 1 (EAAC1) in piglets with bowel inflammation. ....	94
Systemic production of interleukin-10 <i>in vivo</i> during weanling acute protein and energy deficit. ....	95
Neurotoxicity of polychlorinated biphenyls in combination with methylmercury and the protective effects of antioxidants using rat embryo culture.....	95
Trends in the Healthy Eating Index of Singaporeans: 1998 vs. 2004. ....	96
Effect of the transition from high school to university on anthropometric and lifestyle variables in males. ....	96
Impact of oral supplements on the self-selected energy intake of seniors in long-term care at risk of unintentional weight loss: the good, bad, and ugly. ....	96
A qualitative exploration of how children perceive dieting and weight. ....	97
Food patterns of Canadian healthy full term infants. ....	97
High parenteral arginine maintained blood flow but not small intestinal growth compared to high enteral arginine in TPN-fed miniature piglets. ....	98
Small birth weight miniature piglets are more susceptible to the development of obesity, fat intolerance, and hypertension in adulthood. ....	98
The next generation project: early growth assessment of offspring of aboriginal mothers with pre-pregnancy type 2 diabetes. ....	98
Molybdenum content of infant formula. ....	99

## Canadian Workshop on Conjugated Linoleic Acid (CLA)

Welcome from organizing committee .....	103
Canadian Workshop on Conjugated Linoleic Acid: Keynote Speaker, Margot Ip .....	104

Conjugated linoleic acid prevents cardiac hypertrophy in spontaneously hypertensive heart failure rats and in neonatal cardiac myocytes. ....	105
Cherry pit oil as a source of 9,11,13-cis, trans, trans-octadecadienoic acid. ....	105
Dietary conjugated linoleic acid (CLA) isomers influence blood pressure in <i>fal/fa</i> Zucker rats by modulating the transition from large to small adipocytes. ....	105
Summary of uses of CLA and production using base catalysts. ....	106
Dietary trans vaccenic acid may favourably alter immune function in JCR:LA-CP rats. ....	106
Total alkyl and alkenyl ether composition of meat from beef fed pasture or concentrate or retail meats.....	106
Chronic effects of CLA:c-9,t-11-enriched butter oil and n-3 polyunsaturated fatty acids (PUFA) supplementation in a rodent model of obesity and insulin resistance: Effects on dyslipidemia and renal pathophysiology. ....	107
Acute effects of CLA on adiponectin mass and oligomer oomposition in 3T3-L1 adipocytes. ....	107
Conjugated linoleic acids: What can explain the discrepancy between animal and human studies? .....	108
The effects of feeding c9t11 and t10c12 conjugated linoleic acid isomers on the abnormal immune response in the Zucker <i>fal/fa</i> rat.....	108
Stereoselective preparation of <i>cis</i> and <i>trans</i> -vaccenic acid. ....	108
Anti-steatotic effects of <i>trans</i> -10, <i>cis</i> -12 conjugated linoleic acid are associated with reduced hepatic levels of adipophilin, but not perilipin, in the <i>fal/fa</i> Zucker rat.....	109
Protective lipid-lowering effects of acutely supplemented <i>trans</i> -vaccenic acid in the obese and insulin resistant JCR: LA-cp rat. ....	109
Milk enriched with conjugated linoleic acid fails to alter blood lipids, body weight and composition in moderately overweight, hyperlipidemic individuals. ....	109
Canadian Workshop on Conjugated Linoleic Acid Attendees .....	111
Index of Authors .....	115
Canadian Nutrition Congress Attendees.....	121
Winnipeg Convention Centre floor plan .....	128

to findings where chronic administration of antimanic drugs to rats decreases turnover of arachidonic acid in brain phospholipids, suggest that excessive NMDA signaling via arachidonic acid may be a contributing factor to the manic phase of bipolar disorder. The results suggest that chronic NMDA-treated animals may be a model of upregulated arachidonic acid turnover in bipolar disorder.

#### CNC\_527

##### Total alkyl and alkenyl ether composition of meat from beef fed pasture or concentrate or retail meats.

Mayer, F.<sup>1</sup>, Kramer, J.K.G.<sup>2\*</sup>, Nürnberg, K.<sup>3</sup> and Steinhart, H.<sup>1</sup>

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Beef meat was obtained from animals raised on pasture, fed a total mixed ration (barn), or obtained from retail stores April 2006 in Hamburg, Germany. The lipid composition of beef meat contains a mixture of *O*-acyl and *O*-alkenyl ether moieties linked to glycerol or *N*-acyl moieties attached to sphingosine. For the complete analysis of beef lipids a combination of derivatization techniques were used taking into consideration conjugated double bond moieties that isomerize under acid condition, *O*-alkenyl ethers that are stable under alkali conditions, *N*-acyl bonds that require extensive acid-catalyze treatment, and *O*-acyl lipids that are easily converted under either acid or base condition. In addition, the lipid moieties in ruminants are a complex mixture of isomers differing in chain length (C10 to C26), normal and branch chains, number of double bonds (0 to 6), and geometric and positional isomers. Prior and complimentary separation techniques were used to separate geometric isomers and fatty acid methyl esters from dimethylacetals (DMA) derived from the *O*-alkenyl ether moieties. Two GC temperature programs were employed using the highly polar 100 m capillary columns to adequately resolve most of the positional and geometric isomers. This is the first report that quantitates and characterizes total lipids of beef meat. The meat from pasture-fed beef shows high levels of 11*t*-18:1, 9*c*11*t*-CLA and 11*t*13*c*-CLA, while beef raised under intensive conditions show increased amounts of 10*t*-18:1, 7*t*9*c*-CLA, 9*t*11*c*-CLA and 10*t*12*c*-CLA. The retail beef appeared to be an equal mixture of the two. The total DMA content was higher in pasture (1.6%) than barn fed beef (2.5%), and showed a similar pattern in the *trans*-18:1 isomer composition.

#### CNC\_528

##### Supplementation of an extract from *Ascophyllum nodosum* seaweed to media improves cellular monounsaturated fatty acids accumulation in differentiating 3T3-L1 adipocytes.

He, M.L., Wang, Y., You, J.S., Mir, P.S., McAllister, T.A.

Agriculture and Agri-Food Canada, Lethbridge, Alberta, Canada T1J 4B1

This study was designed to determine effect of a mixture that was extracted with 80% ethanol from *Ascophyllum nodosum* seaweed on cell differentiation and fatty acid accumulation in 3T3-L1 adipocytes. The 3T3-L1 preadipocytes in the density of  $2 \times 10^4$  per ml were seeded to 48 wells, distributed in two 24-well plates and allowed to proliferate to confluence. Cells were treated with media containing 0, 12.5, 25, 50, 75, 100 micro g per ml media of the seaweed extract during both early (day 1-2) and intermediate & late differentiation periods (day 3-8). Dexamethasone, methyl-isobutylxanthine and insulin (DMI) containing media were applied during the early period to induce cell differentiation. Cells were visualized in two sub sets of the wells with Oil Red-O stain and those in the remaining wells were harvested on day 8 for determination of cellular fatty acid. Supplementation with the seaweed extract increased ( $P < 0.01$ ,  $n = 6$ ), concentration of cellular monounsaturated fatty acids including myristoleic acid (C14:1), palmitoleic acid (C16:1) and oleic acid (C18:1) without affecting ( $P > 0.05$ ,  $n = 6$ ) the cell number and total cellular fatty acid concentration. The ratios of C14:0/C14:1 fatty acid and C16:0/C16:1 fatty acid declined ( $P < 0.05$ ,  $n = 6$ ) in dose-dependent manner. The results suggest that the extract from *Ascophyllum nodosum* seaweed may contain certain bioactive factors which could increase desaturation of those *de novo* synthesized fatty acids.

Key words: adipocytes, fatty acids, seaweed

#### CNC\_552

##### Fatty acids profile in breast milk of lactating mother consuming a galactagogue, coleus amboinicus lour.

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Traditional knowledge of medicinal plants is an integral part of a culture's understanding of health and disease. Every culture on earth has herbalist lore, a storehouse of knowledge handed down over the generations. An example of this is the traditional practice among the Batakese lactating women in North Sumatra, Indonesia, of consuming the leaves of Torbangun (*Coleus amboinicus* Lour) during the first 30 days after giving birth. They believe that this plant stimulates breast milk production. The intervention study conducted in Simalungun, North Sumatra, Indonesia, showed that the mother subjects in the Torbangun Group produced more milk than did subjects in the Reference Group. Breast milk contains a full complement of all polyunsaturated fatty acids (PUFA), including the two essential PUFA, linoleic acid (C18:2- $\omega$ 6) and  $\alpha$ -linolenic acid, and also a range of long chain (LC) PUFA that have been shown to have benefits for both preterm and term infants.

The present study reports fatty acid composition in breast milk of lactating mother who received one of three different supplements, namely Torbangun leaves, Moloco+B12™ Tablets and Fenugreek Capsules for 30 days. The subjects were to be followed up for another 30 days after the supplementation was completed.

Results collected from this study showed that the linoleic acid (C18:2- $\omega$ 6) content in transitory milk (Day 8) of the Torbangun Group was significantly lower than for the Reference Groups. Further, it was observed that the linoleic acid content of the Torbangun Group remained increase until Day 60 and the increase was significantly higher than for the Reference Group. The residual effect of Torbangun supplementation during the first month of lactation can be seen even after the supplementation was ended for one month. The observations in the present study of the increase in milk production in the Torbangun Group has confirmed the beliefs amongst Bataknese people that Torbangun can be used as galactogogue in humans.

Key words: Torbangun, Bataknese, Coleus amboinicus Lour, Indonesia



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***FATTY ACIDS PROFILE IN BREAST MILK OF  
LACTATING MOTHER CONSUMING A  
GALACTOGOGUE, COLEUS AMBOINICUS LOUR***

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**Drh. Rizal Damanik, M.Rep.Sc., Ph.D**

The paper is presented at the Canadian Nutrition Congress on  
June 18-21, 2007 in Winnipeg Canada.

## ABSTRACTS

### **FATTY ACIDS PROFILE IN BREAST MILK OF LACTATING MOTHER CONSUMING A GALACTOGOGUE, *COLEUS AMBOINICUS* LOUR.**

**DAMANIK, RIZAL\***

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Traditional knowledge of medicinal plants is an integral part of a culture's understanding of health and disease. Every culture on earth has herbalist lore, a storehouse of knowledge handed down over the generations. An example of this is the traditional practice among the Batakese lactating women in North Sumatra, Indonesia, of consuming the leaves of Torbangun (*Coleus amboinicus* Lour) during the first 30 days after giving birth. They believe that this plant stimulates breast milk production. The intervention study conducted in Simalungun, North Sumatra, Indonesia, showed that the mother subjects in the Torbangun Group produced more milk than did subjects in the Reference Group. Breast milk contains a full complement of all polyunsaturated fatty acids (PUFA), including the two essential PUFA, linoleic acid (C18:2- $\omega$ 6) and  $\alpha$ -linolenic acid, and also a range of long chain (LC) PUFA that have been shown to have benefits for both preterm and term infants.

The present study reports fatty acid composition in breast milk of lactating mother who received one of three different supplements, namely Torbangun leaves, Moloco+B12™ Tablets and Fenugreek Capsules for 30 days. The subjects were to be followed up for another 30 days after the supplementation was completed.

Results collected from this study showed that the linoleic acid (C18:2- $\omega$ 6) content in transitory milk (Day 8) of the Torbangun Group was significantly lower than for the Reference Groups. Further, it was observed that the linoleic acid content of the Torbangun Group remained increase until Day 60 and the increase was significantly higher than for the Reference Group. The residual effect of Torbangun supplementation during the first month of lactation can be seen even after the supplementation was ended for one month. The observations in the present study of the increase in milk production in the Torbangun Group has confirmed the beliefs amongst Batakese people that Torbangun can be used as galactagogue in humans.

**Key Words:** Torbangun, Batakese, *Coleus amboinicus* Lour, Indonesia

## INTRODUCTION

Breast milk is complex and presents continuous changes. The composition and volume of breast milk varies with each individual mother, the demands made by the infants, the time of the day, the nutritional status of the mother, and whether the milk is suckled or expressed (Emmet & Rogers, 1997). The content of breast milk is changed as lactation proceeds and presumably as the requirement of the infant changes, so the mother's diet is important (Harding, 2001; Mora & Nestel, 2000).

An intervention study was conducted to examine the lactagogue properties of CA and Fenugreek in lactating women in Simalungun, North Sumatra Indonesia. This paper reports the effects of CA and Fenugreek on the fatty acid composition of breast milk, in comparison with those of Moloco+B12™.

## MATERIALS AND METHODS

Of 75 mother subjects recruited, 67 participated for the whole two-month study period. Subjects were divided into three groups: Moloco+B12™ (Reference Group, n = 22), CA Group (n = 23) and Fenugreek Group (n = 22).

Breast milk samples were collected from the subjects for nutrient analysis (macronutrients, minerals and fatty acids) over three occasions. To let infants consume colostrum, the first milk collection was conducted on Day 8 post partum and was used as Baseline for comparison with the information subsequently collected on second and third collections. The second collection was conducted one day after the completion of a 30-day supplementation period (Day 33). And the third collection was conducted at the end of the study period (Day 60). Over the two-month study period, the volumes of the 24-h breast milk intake were recorded on Days 14, 28, 42 and 56 post partum. The terminology "transitory milk" for Day 8 and "mature milk" for Day 33 and 60 sometimes are used in paragraphs in interpreting the data.

The effects of CA and Fenugreek were examined by comparing the percentage changes in the volume and nutritional quality of breast milk (using the values of the first measurements as the baseline, i.e. Day 14 for milk volume and Day 8 for milk nutrient contents) with those of the Reference Group.

## RESULTS

### Breast Milk Quantity

The 24-h breast milk intakes were recorded every 2 weeks over the two-month period, and the results are shown in Table 1.

**Table 1** The breast milk intake during the two-month study period (mL)

Parameter	Reference Group		CA Group		Fenugreek Group	
	n	Mean $\pm$ SD	n	Mean $\pm$ SD	n	Mean $\pm$ SD
Day 14 (baseline)	22	453.8 $\pm$ 192.6	23	361.1 $\pm$ 201.1	22	466.9 $\pm$ 253.0
Day 28	22	385.1 $\pm$ 201.9	23	478.7 $\pm$ 157.0 *	22	400.3 $\pm$ 215.1
Day 42	22	387.4 $\pm$ 188.3	23	439.8 $\pm$ 196.7	22	456.6 $\pm$ 247.1
Day 56	22	385.5 $\pm$ 170.5	23	478.3 $\pm$ 265.0	22	358.5 $\pm$ 135.2

No significant differences from the Reference Group (Moloco+B12™) were observed at Day 14 (ANOVA).

Significant differences from Day 14 within the same group (paired t-test): \*,  $P < 0.05$ .

The information on 24-h breast milk intake was collected for the first time on Day 14 post partum, and was used as the Baseline for comparison with the information subsequently collected on Day 28, 42 and 56. Table 1 shows that although the mean milk volume at Day 14 of the CA Group was 361 mL, and about 100 mL less than that of the Reference and Fenugreek Groups (454 and 467 mL, respectively), statistical analysis showed that there were no significant differences amongst these three groups.

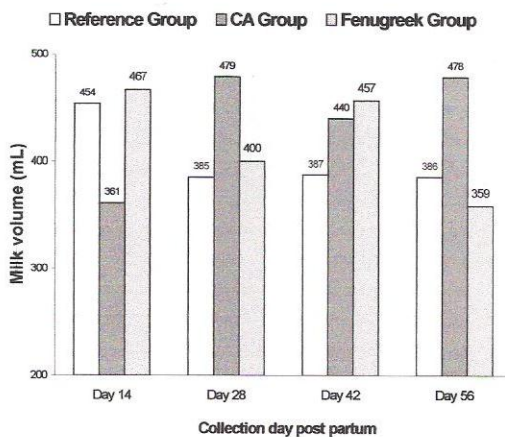


Figure 1  
24-h breast milk intake during the two-month study period

It was observed that during the last two weeks of CA supplementation (from Day 14 to Day 28), the 24-h breast milk intake significantly increased from 361 to 479 mL ( $P < 0.05$ ) or the average increase was 65% (Table 2). This increase was higher than with the groups receiving Moloco+B12™ Tablets or Fenugreek Capsules which were only 10% and 20% respectively. Additionally, it was found that, even after the completion of supplementation, the increase in breast milk intake of the CA Group still remained higher than the other two groups (Table 2).

**Table 2** % Change in breast milk intake during the two-month study period

Parameter	Reference Group		CA Group		Fenugreek Group	
	N	Mean ± SD	n	Mean ± SD	n	Mean ± SD
Changes from Day 14 to						
Day 28	22	9.7 ± 97.7	23	65.2 ± 83.8	22	20.3 ± 105.7
Day 42	22	-1.2 ± 56.1	23	53.5 ± 95.2 *	22	19.6 ± 80.1
Day 56	22	-4.2 ± 44.1	23	68.4 ± 139.1 *	22	5.0 ± 67.2

Significant difference from the Reference Group (Moloco+B12™) (ANOVA): \*,  $P < 0.05$ .

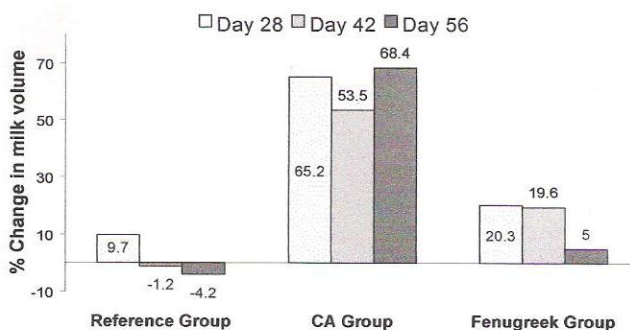


Figure 2  
% Change in 24-h milk volume

### Fatty acid contents

As shown in Table 3, saturated fatty acids constituted 38% of the total fatty acid in the transitory milk (Day 8) of the CA Group. This value was significantly higher ( $P < 0.001$ ) than for the Reference Group (28%). The saturate of lauric acid (C12:0), myristic acid (C14:0) and palmitic acid (C16:0) contents of the transitory milk in the CA Group contributed to a higher content of saturated fatty acids of the CA Group than the Reference Group. The content of these fatty acids in the CA Group was significantly higher than the Reference Group. The saturate of decanoic (C10:0) and stearic acid (C18:0) in the CA Group were comparable to the Reference Group (0.8% vs 0.9% and 4% vs 3.6% respectively).

**Table 3** Saturated fatty acid contents during the two-month study period (% by weight)

Parameter	Reference Group		CA Group	
	n	Mean $\pm$ SD	n	Mean $\pm$ SD
<b>Day 8</b>				
Decanoic (C10:0)	22	0.9 $\pm$ 0.4	22	0.8 $\pm$ 0.4
Lauric acid (C12:0)	22	6.4 $\pm$ 2.7	23	9.7 $\pm$ 6.0 <sup>a</sup>
Myristic acid (C14:0)	22	2.6 $\pm$ 1.0	23	4.5 $\pm$ 2.9 <sup>b</sup>
Palmitic acid (C16:0)	22	15.1 $\pm$ 1.1	23	19.3 $\pm$ 3.1 <sup>d</sup>
Stearic acid (C18:0)	22	3.6 $\pm$ 0.7	23	4.0 $\pm$ 1.1
Total	22	28.6 $\pm$ 5.9	23	38.3 $\pm$ 13.5 <sup>d</sup>
<b>Day 33</b>				
Decanoic (C10:0)	22	1.2 $\pm$ 0.4 §	23	1.5 $\pm$ 0.5 ¶
Lauric acid (C12:0)	22	7.2 $\pm$ 2.3	23	10.9 $\pm$ 5.6
Myristic acid (C14:0)	22	2.4 $\pm$ 0.8	23	3.2 $\pm$ 1.4 ¶
Palmitic acid (C16:0)	22	15.0 $\pm$ 1.4	23	16.6 $\pm$ 2.0 ¶
Stearic acid (C18:0)	22	3.8 $\pm$ 0.5	23	4.0 $\pm$ 0.7
Total	22	29.6 $\pm$ 5.4	23	36.2 $\pm$ 10.2
<b>Day 60</b>				
Decanoic (C10:0)	22	1.2 $\pm$ 0.4 ¶	23	1.0 $\pm$ 0.2
Lauric acid (C12:0)	22	7.7 $\pm$ 2.9	23	6.3 $\pm$ 2.3 <sup>*</sup>
Myristic acid (C14:0)	22	2.5 $\pm$ 0.9	23	2.5 $\pm$ 0.9 ¶
Palmitic acid (C16:0)	22	15.0 $\pm$ 0.9	23	15.9 $\pm$ 1.3 ¶
Stearic acid (C18:0)	22	3.9 $\pm$ 0.6	23	4.0 $\pm$ 0.5
Total	22	30.3 $\pm$ 5.7	23	29.7 $\pm$ 5.2

Significant difference from the Reference Group (Moloco+B12™) at Day 8 (ANOVA): <sup>a</sup>,  $P < 0.05$ ; <sup>b</sup>,  $P < 0.01$ ; <sup>c</sup>,  $P < 0.001$ ; <sup>d</sup>,  $P < 0.0001$ .

Significant difference from Day 8 within the same group (paired *t*-test): <sup>\*</sup>,  $P < 0.05$ ; <sup>¶</sup>,  $P < 0.01$ ; <sup>§</sup>,  $P < 0.001$ ; <sup>¶¶</sup>,  $P < 0.0001$ .

After the completion of supplementation (Day 33), the decanoic acid (C10:0) content in mature milk of the CA Group was significantly higher than Day 8 (0.8 vs 1.5,  $P < 0.0001$ ). The C10:0 content of this group showed a greater rise from Day 8 to Day 33. Statistical analysis showed that the rise in the CA Group was significantly higher than in the Reference Group (Table 7.8). The rise of C10:0 content in the CA Group continued until day 60. However, statistical analysis showed no significant difference in the rise of C10:0 content in breast milk between the CA and the Reference Groups.

Although the lauric acid (C12:0) content in the mature milk of the CA Group on Day 33 was comparable to the transitory milk (Day 8), it was observed that the C12:0 decreased significantly on Day 60 (9.7 vs 6.3,  $P < 0.05$ ). Statistical analysis shows that the decrease of C12:0 in the CA Group was significantly higher than in the Reference Group (Table 4). Correspondingly, the myristic (C14:0) and palmitic (C16:0) contents of breast milk in the CA Group also showed a decline from Day 33 to Day 60. Table 4 showed that the decline of these fatty acids was significantly higher than in the Reference Group.

**Table 4** % Change in saturated fatty acid content during the two-month study period

Parameter	Reference Group		CA Group	
	n	Mean $\pm$ SD	n	Mean $\pm$ SD
<b>% Change from Day 8 to Day 33</b>				
Decanoic (C10:0)	22	50.1 $\pm$ 55.5	23	172.9 $\pm$ 267.3 *
Lauric acid (C12:0)	22	27.4 $\pm$ 54.4	23	48.1 $\pm$ 120.6
Myristic acid (C14:0)	22	4.0 $\pm$ 41.6	23	-18.8 $\pm$ 31.7 *
Palmitic acid (C16:0)	22	-1.3 $\pm$ 8.0	23	-13.0 $\pm$ 11.8 §
Stearic acid (C18:0)	22	4.3 $\pm$ 14.1	23	6.3 $\pm$ 26.4
<b>% Change from Day 8 to Day 60</b>				
Decanoic (C10:0)	22	64.0 $\pm$ 62.2	23	76.6 $\pm$ 148.2
Lauric acid (C12:0)	22	44.2 $\pm$ 76.6	23	-10.4 $\pm$ 65.5 *
Myristic acid (C14:0)	22	13.0 $\pm$ 58.4	23	-30.7 $\pm$ 36.2 †
Palmitic acid (C16:0)	22	-1.7 $\pm$ 9.2	23	-16.0 $\pm$ 13.6 §
Stearic acid (C18:0)	22	8.0 $\pm$ 19.8	23	5.1 $\pm$ 24.6

Significant difference from the Reference Group (Moloco+B12™) (ANOVA): \*,  $P < 0.05$ ; †,  $P < 0.01$ ; §,  $P < 0.001$ .

The unsaturated fatty acid contents of the two comparison groups is shown in Table 5. The unsaturated fatty acids accounted for 29% of the total fatty acids

of transitory milk (Day 8) and 33% of the total fatty acids of mature milk (Day 60) for the CA Group. Most of the unsaturates were monounsaturated fatty acids, which contributed 23% and 26% to the total fatty acid content of transitory and mature milk respectively. In the Reference Group, unsaturated fatty acids accounted for 33% of the total fatty acids of transitory milk and remained at a constant level for mature milk. As with the CA Group, the monounsaturated fatty acids were major fatty acids in the Reference Group. Monounsaturated of palmitoleic (C16:1) and polyunsaturated of linoleic acid (C18:2n-6) and linolenic acid (C18:3n-3) contents in the transitory milk (Day 8) of the CA Group were found to be considerably lower than in the Reference Group.

**Table 5** Unsaturated fatty acid contents during the two-month study period (% by weight)

Parameter	Reference Group		CA Group	
	n	Mean ± SD	n	Mean ± SD
<b>Day 8</b>				
Monounsaturated fatty acids	22	25.7 ± 3.1	23	23.6 ± 5.3
Palmitoleic acid (C16:1)	22	2.0 ± 0.5	23	1.5 ± 0.9 <sup>a</sup>
Oleic acid (C18:1)	22	23.7 ± 2.6	23	22.1 ± 4.4
Polyunsaturated fatty acids	22	7.6 ± 1.3	23	5.6 ± 1.6
Linoleic acid (C18:2n-6)	22	7.1 ± 1.1	23	5.4 ± 1.3 <sup>d</sup>
Linolenic acid (C18:3n-3)	22	0.5 ± 0.2	23	0.2 ± 0.3 <sup>c</sup>
<b>Day 33</b>				
Monounsaturated fatty acids	22	26.6 ± 2.8	23	25.5 ± 3.5
Palmitoleic acid (C16:1)	22	1.9 ± 0.5	23	2.2 ± 0.9 <sup>‡</sup>
Oleic acid (C18:1)	22	24.1 ± 2.3	23	23.3 ± 2.6
Polyunsaturated fatty acids	22	8.2 ± 2.0	23	6.9 ± 1.3
Linoleic acid (C18:2n-6)	22	7.7 ± 1.9	23	6.5 ± 1.2 <sup>‡</sup>
Linolenic acid (C18:3n-3)	22	0.5 ± 0.1	23	0.4 ± 0.1
<b>Day 60</b>				
Monounsaturated fatty acids	22	25.5 ± 2.6	23	26.1 ± 2.8
Palmitoleic acid (C16:1)	22	1.8 ± 0.4 <sup>*</sup>	23	2.1 ± 0.6 <sup>*</sup>
Oleic acid (C18:1)	22	23.7 ± 2.2	23	24.0 ± 2.2
Polyunsaturated fatty acids	22	8.2 ± 1.7	23	7.3 ± 1.4
Linoleic acid (C18:2n-6)	22	7.7 ± 1.6	23	6.9 ± 1.3 <sup>¶</sup>
Linolenic acid (C18:3n-3)	22	0.5 ± 0.1	23	0.4 ± 0.1

Significant difference from the Reference Group (Moloco+B12™) at Day 8 (ANOVA): <sup>a</sup>,  $P < 0.05$ ; <sup>b</sup>,  $P < 0.01$ ; <sup>c</sup>,  $P < 0.001$ ; <sup>d</sup>,  $P < 0.0001$ .

Significant difference from Day 8 within the same group (paired *t*-test): <sup>\*</sup>,  $P < 0.05$ ; <sup>‡</sup>,  $P < 0.01$ ; <sup>§</sup>,  $P < 0.001$ ; <sup>¶</sup>,  $P < 0.0001$ .



The polyunsaturates of the linoleic acid (C18:2n-6) totaled 5.4% of the transitory (Day 8) and 6.9% of the mature milk (Day 60) fatty acids for the CA Group. In the Reference Group, the values were 7.1% and 7.7% respectively.

The linoleic acid (C18:2n-6) content of the CA Group showed a consistent rise until Day 60. This fatty acid rose from 5.4% on Day 8 to 6.9% on Day 60 ( $P < 0.0001$ ). Statistical analysis showed that the rise of the C18:2n-6 content in the CA Group was significantly higher ( $P < 0.0001$ ) than in the Reference Group (Table 6). In addition, the palmitoleic acid (C16:1) of the CA Group also elevated continually until Day 60 (1.5% to 2.1%,  $P < 0.05$ ), and as shown in Table 6, this change was significantly different from that of the Reference Group (83.9% vs -9.5%,  $P < 0.001$ ).

**Table 6** % Change in unsaturated fatty acid contents during the two-month study period

Parameter	Reference Group			CA Group		
	n	Mean	± SD	n	Mean	± SD
% Change from Day 8 to Day 33						
Palmitoleic (C16:1)	22	-3.0	± 24.0	23	94.4	± 140.6 ‡
Oleic (C18:1)	22	1.8	± 10.1	23	9.7	± 27.7
Linoleic (C18:2)	22	10.7	± 30.7	23	26.9	± 34.5
% Change from Day 8 to Day 60						
Palmitoleic (C16:1)	22	-9.5	± 20.2	23	83.9	± 111.8 §
Oleic (C18:1)	22	1.0	± 14.5	23	13.7	± 32.2
Linoleic (C18:2)	22	10.2	± 28.9	23	36.1	± 43.6 *

Significant difference from the Reference Group (Moloco+B12™) (ANOVA): \*,  $P < 0.05$ ; ‡,  $P < 0.01$ ; §,  $P < 0.001$ .

Note: Most of the Linolenic (C18:3) values are zero, and % change values are not calculated.

## DISCUSSION

### Quantity of Breast Milk

A key element defining lactation performance is the total amount of milk produced. The most widely accepted method for measuring milk intake is test weighing, a procedure in which the infant is weighed before and after each

feeding, preferably using a balance scale accurate to  $\pm 5$  g. In this method, milk intake is usually underestimated by approximately 1-5% (Brown et al., 1982) because of evaporative water loss from the infant between weighing.

The findings that the 24-h milk intake of the CA Group increased by 65% from Day 14 to Day 28, while that of Reference and Fenugreek Groups increased only 10% and 20%, respectively, suggest that the breast milk production was enhanced by the CA soup. Furthermore, it was also observed that, even though the supplementation period was already over, the milk volume in the CA Group on Day 56 still remained increased while there was a decline in the milk volume in the other two intervention groups, especially in the last two weeks of the follow-up period. During 2 months lactation, the milk volume in the Fenugreek Group was found to be lowest amongst the Reference and CA Groups (358mL vs 385mL vs 478mL, respectively). However, the nutritional quality of milk samples of the CA Group needs to be further examined.

The effect of CA on the proliferation of mammary secretory cells, which is used an indicator for the activity of the secretory cells in secreting milk (Knight et al., 1984), has already been shown in an animal study (Silitonga, 1993). In that study, lactating mice were supplemented with various doses of a CA extract from Day 2 post partum for 28 days. The 75% and 75.3% increases in DNA and RNA levels in the mice receiving the CA extract 60 g/kg body weight, compared to the 22.5% decrease in DNA and 26% increase in the RNA observed in the mice receiving Moloco+B12™ supplement ( $P < 0.05$ ), indicating that CA could increase the proliferation of mammary secretory cells. It was also found that the effect of CA on the proliferation of secretory cells in the mammary gland was dose-dependent (Silitonga, 1993).

### **Fatty Acid Contents**

It has been shown in several studies that the composition of the fat consumed by the mother will influence the fatty acid composition of milk (Chen et al., 1997; Chulei et al., 1995; Francois et al., 1998; Kneebone et al., 1985).

In the present study, it was anticipated that there would be changes, to some extent, in the fatty acid contents in milk samples of the CA Group. This was because CA was supplemented as a soup that had coconut milk and chicken, apart from CA leaves, as the main ingredients. Fatty acid analysis showed that the CA soup was a good source of saturated fatty acids, especially lauric acid (C12:0), and linoleic acid (C18:2). And indeed, the lauric and linoleic acid contents were increased by 48% and 27%, respectively, at the end of the one-

month CA supplementation. However, the more striking increases were found in decanoic (C10:0, 173%) and palmitoleic (C16:1, 95%) acid contents.

The high increase in decanoic acid (C10:0) and lauric acid (C12:0) suggested that high consumption of saturated acids through coconut milk of CA supplement was associated with the biosynthesis of high levels of intermediate and medium (C6:0 to C12:0) saturated fatty acids. This finding is in agreement with previous reports (Kneebone et al., 1985).

It has been established that fatty acid composition of human milk varies as lactation progresses (Luukkainen et al., 1994; Makrides et al., 1995). From colostrum to mature milk, the contents of essential fatty acids C18:2n-6 and C18:3n-3 in human milk increase, whereas the percentage of long-chain polyunsaturated fatty acid of both the n-6 and n-3 series decrease (Luukkainen et al., 1994). The present study has demonstrated that PUFA concentrations in breast milk of two groups studied changed during lactation. The significance difference was observed in the CA Group where the PUFA concentrations of linoleic acid (LA; C18:2n-6) showed a change from the transitory milk on Day 8 (5.4%) to the mature milk on Day 33 (6.5%) and Day 60 (6.9%).

Compared with other fatty acids, unsaturated fatty acids, especially LC-PUFAs, in milk exhibited a delay in peak values (at 24 hours of ingestion) and showed prolonged elevation in breast milk, possibly because the body pool size of these fatty acids is small (Francois et al., 1998).

Although the essential fatty acid content of the breast milk in the CA Group was lower than the Reference Group, however, it is observed that the C18:2n-6 to C18:3n-3 ratio in this group was higher than in the Reference Group (27% vs 14.2%, respectively). Because linoleic acid (C18:2n-6) and  $\alpha$ -linolenic acid (C18:3n-3) both are known to compete for the same enzyme for further desaturation and chain elongation polyunsaturated fatty acids (LC-PUFAs), the dietary ratio between these fatty acids is more important than the absolute intake of each of these essential fatty acids (Koletzko et al., 1992). The availability of LC-PUFAs (C>20-24), such as arachidonic acid (20:4n-6) and docosahexanoic acid (C22:6n-3), is important for early human growth and development. During late fetal and early postnatal growth, considerable amount of fatty acids (both n-6 and n-3) is incorporated in neural and other tissues. Brain lipids of newborn infants contain about twice as much (n-6) LC-PUFAs as (n-3) LC-PUFAs (Koletzko & Muller, 1990).

The present study has shown that linoleic acid (C18:2n-6) content of breast milk in the CA Group exhibited a significant rise from Day 8 to Day 60 due to consumption of CA supplement. These findings lend support to the presumption that this fatty acid present in the CA supplement might stimulate the secretion of prostaglandin. This fatty acid can be desaturated and elongated to 20-carbon LC-PUFAs (eicosanoids) (Koletzko et al., 1992).

The secretory cell numbers in the mammary gland are positively correlated to milk yield. Therefore, the more secretory cells present, the more prolactin hormone produced in circulation. In the present study the increase of breast milk intake in the CA Group was higher than the other two groups. Thus, the high level of prolactin hormone circulating in the CA subjects would be important to support this presumption. Because oxytocin is essential for milk ejection in humans, the existence of this hormone in the circulation should be known to support this presumption.

## **CONCLUSION**

The observations in the present study of the increase in milk production without compromising the nutritional quality in the CA Group has confirmed the belief amongst Batakese people that CA can be used as a lactagogue in humans. The residual effect of CA supplementation during the first month of lactation can be seen even after the supplementation was ended for one month.

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