



**‘BIOCLA: Biotechnological Approaches for the  
Development of Functional Foods Enriched in Bioactive  
Lipids’**

by

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## DECLARATION

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## ABSTRACT

The aims of this study were to investigate the potential of microbial cultures of dairy significance to produce conjugated fatty acids exhibiting beneficial anti-microbial and anti-carcinogenic activities. Supplementation of reconstituted skimmed milk with food grade yeast extract, sodium acetate and inulin enhanced production of the health promoting conjugated linoleic acid (CLA) by bifidobacteria. Three novel microbially-produced conjugated fatty acids were isolated and identified using RP-HPLC and GLC-MS. These conjugated fatty acids exhibited potent anti-carcinogenic activity associated with increased cellular lipid oxidation and altered expression of Bcl-2 in the SW480 human colon cancer cell line. These novel conjugated fatty acids also displayed potent inhibitory activity against methicillin resistant *Staphylococcus aureus* (MRSA). It was observed that the pattern of inhibition displayed by the conjugated fatty acids differed substantially from that of their parent unsaturated fatty acids. Furthermore, it was observed that the conjugated fatty acids remained active in the presence of known inactivators of the inhibitory activity of unsaturated fatty acid such as blood serum and  $\alpha$ -tocopherol. Bovine nutrition intervention trials showed that dietary supplementation with  $\omega$ -3 PUFA elevated concentrations of  $\omega$ -3 PUFA and the concentration of CLA and its precursor vaccenic acid. Furthermore, such a diet could be used to enhance the  $\omega$ -3/ $\omega$ -6 PUFA and PUFA/saturated fatty acid profiles of plasma, meat and liver from beef heifers. In conclusion, the results demonstrate that 1) members of the enteric microbiota possess the ability to biosynthesise novel conjugated fatty acids with positive benefits for human health and 2) dietary  $\omega$ -3 PUFA supplementation in cows leads to changes in fatty acid profiles of tissues which positively impact on the nutritive quality of bovine meat.

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## ABBREVIATIONS

ADG	Average daily gain
AIC	Akaike criterion
A.m.u.	Atomic mass units
BCS	Body condition score
BEND	Bovine endometrial cells
BHA	Butylated hydroxyanisole
BHBA	$\beta$ -hydroxybutyrate
BHT	Butylated hydroxytoluene
CWG	Choice white grease
<i>c</i>	<i>cis</i>
CALA	Conjugated $\alpha$ -linolenic acid
CALA1	9, 11, 15-C18:3 or <i>c</i> 9, <i>t</i> 11, <i>c</i> 15-C18:3
CALA2	<i>t</i> 9, <i>t</i> 11, <i>c</i> 15-C18:3
cfu	Colony forming units
CGLA	Conjugated $\gamma$ -linolenic acid
CGLA1	6, 9, 11-C18:3 or <i>c</i> 6, <i>c</i> 9, <i>t</i> 11-C18:3
CGLA2	<i>c</i> 6, <i>t</i> 9, <i>t</i> 11-C18:3
CLA	Conjugated linoleic acid
COX	Cyclooxygenase
COX-2	Cyclooxygenase-2
CSA	Conjugated stearidonic acid
CSA1	6, 9, 11, 15-C18:4 or <i>c</i> 6, <i>c</i> 9, <i>t</i> 11, <i>c</i> 15-C18:4
CSA2	<i>c</i> 6, <i>t</i> 9, <i>t</i> 11, <i>c</i> 15-C18:4
DAD	Diode array detector
dDM	Dietary dry matter
DHA	Docosahexaenoic acid
DM	Dry matter
DMEM	Dulbecco's minimum essential medium
DMI	Dry matter intake
DMOX	4, 4-dimethyloxazoline
DPA	Docosapentaenoic acid
ELISA	Enzyme-linked immunosorbent assay

EPA	Eicosapentaenoic acid
FAD2	$\Delta^{12}$ -oleate desaturase
FAME	Fatty acid methyl ester
FBS	Fetal bovine serum
FHC	Normal human fetal epithelial cell line
FID	Flame ionization detector
GLC	Gas liquid chromatography
GLC-MS	Gas liquid chromatography mass spectrometry
HDL	High density lipoprotein
IDL	Intermediate density lipoprotein
IVF	<i>in vitro</i> fertilisation
IVM	<i>in vitro</i> maturation
LA	Linoleic acid
LAB	Lactic acid bacteria
LDL	Low density lipoprotein
LPS	Lipopolysaccharide
mBHI	Modified brain heart infusion broth
MRD	Maximum recovery diluent
MRSA	Methicillin resistant <i>S. aureus</i>
MRS	De Man, Rogosa and Sharpe
mRSM	Modified reconstituted skimmed milk
MTAD	4-methyl-1, 2, 4- triazoline-3, 5-dione
MTS	(3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium)
NEFA	Non-esterified fatty acid
OLETF	Otsuka Long Evans Tokushima Fatty
PBS	Phosphate buffer saline
PGF <sub>2<math>\alpha</math></sub>	Prostaglandin F <sub>2<math>\alpha</math></sub>
PHVO	Partially hydrogenated vegetable oil
PPAR $\alpha$	Peroxisome proliferator-activated receptor $\alpha$
PUFA	Polyunsaturated fatty acids
RP-HPLC	Reversed phase high performance liquid chromatography
RSM	Reconstituted skimmed milk
SEM	Standard error of mean



SCFA	Short chain fatty acid
SREBPs	Sterol regulatory element binding proteins
SFA	Saturated fatty acids
SGJ	Simulated gastric juice
SPE	Solid phase extraction
<i>t</i>	<i>trans</i>
TSA	Tryptic soy agar
TSB	Tryptic soy broth
UHT	Ultra-high temperature
VLDL	Very-low density lipoprotein
WSB	Whole soybeans

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