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INVESTIGATION INTO THE ACIDIC PROTEIN FRACTION OF BOVINE WHEY AND ITS EFFECT ON BONE CELLS

A THESIS PRESENTED IN PARTIAL FULFILMENT OF

THE REQUIREMENTS FOR THE DEGREE OF

MASTERS OF SCIENCE IN CHEMISTRY

AT MASSEY UNIVERSITY,

NEW ZEALAND

BERNADETTE JANE MULLAN

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Abstract

Milk is provided to new borns as their first food source and it contains essential nutrients, vitamins and other beneficial components, such as enzymes and antibodies that are required for rapid growth and development of the new born and for sustained growth over time.

Milk contains two main types of proteins; casein proteins and whey proteins. Although casein proteins account for up to 80% of the proteins found in bovine milk, it is the whey protein that has become of high interest because of its bioactive content. Whey, a very watery mixture of lactose, proteins, minerals and trace amounts of fat, is formed from milk when the milk is coagulated and/or the casein proteins are removed from the milk.

Bovine whey protein, including both the acidic and basic fractions (low and high isoelectric point, respectively), has previously been studied *in vitro* (cell based) and *in vivo* (using rats) for its impact on bone to determine if it can help improve bone mineral density and help reduce the risk of developing bone diseases, such as osteoporosis.

Bone is constantly undergoing a remodelling process of being dissolved and reformed and the two main cell types responsible for this bone remodelling process are mature osteoclasts, which dissolve (resorb) bone, and osteoblasts, which reform the bone.

Prior work has shown that acidic protein fractions derived from different sources of whey protein concentrate (WPC) have both *in vivo* and *in vitro* activity on bone, particularly anti-resorptive properties. However, the component(s) which confer activity have not yet been identified. In this thesis, work was undertaken to better understand the analytical composition of three types of WPC (cheese, mineral acid and lactic acid) and their associated acidic protein fractions and relate this to bone activity in the hope of identifying where the activity lies. Bone activity was assessed using *in vitro* screening with osteoblast cells (MC3T3-E1) and osteoclast cells (RAW 264.7).

Comparison of the cell-based bone activity of the parent WPCs and corresponding acidic fractions indicated that the acidic fractions derived from both mineral acid and lactic WPC were superior in their ability to inhibit osteoclast development. Although compositional data was complex and definitive correlations with both bone bioactivities could not be made, it appeared that elements common to both the acidic fractions were a higher proportion of GLYCAM-1 and bone sialoprotein-1 (osteopontin). Further studies to more closely investigate the bone bioactivity of the acidic fractions are warranted.

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Abbreviations

α-Lac	Alpha-lactalbumin
AF	Acid fraction
AOAC	Association of Official Analytical Chemists
ASE	Accelerated solvent extraction
ASG	Analytical Services Group
β-Lac	Beta-lactoglobulin
BMD	Bone mineral density
BME	Beta-mercaptoethanol
BSA	Bovine serum albumin
BT	Breakthrough (fraction)
CPPs	Casein phosphopeptides
CR05	Mineral acid WPC 80 manufactured October 2007
DMSO	Dimethyl sulfoxide
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme-linked immunosorbant assay
EPA	Eicosapentaenoic acid (C20:5N-3)
ESI	Electrospray ionization
FCS	Fetal calf serum
FPLC	Fast protein liquid chromatography system
FRC	Fonterra Research Centre, Palmerston North
FSOT	Fused silica open tubular
GLYCAM 1	Glycosylation-dependent cell adhesion molecule 1
GMP	Glycomacropeptide
GP	Gel permeation
GS19	Cheese WPC 80 manufactured February 2008
HBS-EP	10mM HEPES, 150mM NaCl, 3mM EDTA, 0.005% Surfactant P20
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
HESI	Heated electrospray ionization
HPAEC	High performance anion exchange chromatography

HPLC	High performance liquid chromatography
HRP	Horseradish peroxidase
HS15	Lactic acid WPC 80 manufactured March 2008
ICP-OES	Inductively coupled plasma optical emission spectrophotometry
ID	Internal diameter
IDF	International Dairy Federation
IgA	Immunoglobulin A
IGF-I	Insulin-like growth factor-I
IgG	Immunoglobulin G
IgM	Immunoglobulin M
ISO	International Organization for Standardization
KCl	Potassium chloride
LC	Liquid chromatography
LF	Lactoferrin
LOD	Limit of detection
LOQ	Limit of quantitation
L-PC	Lysophosphatidylcholine
L-PE	Lysophosphatidylethanolamine
LTQ	Linear trap quadrupole
MBP	Milk basic protein
MEMα	Minimal essential medium α
MFGM	Milk fat globule membrane
MS	Mass spectrometry
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NCBI	National Centre for Biotechnology Information
NMKL	Nordic Committee on Food Analysis
NPN	Non-protein nitrogen
NZDRI	New Zealand Dairy Research Institute
NZTM	New Zealand Testing Methods
OPN	Osteopontin
PAGE	Polyacrylamide gel electrophoresis
PC	Phosphatidylcholine
PE	Phosphoethanolamine
P*i*	Isoelectric point

PI	Phosphatidylinositol
PP3	Proteose peptone component 3
PP5	Proteose peptone component 5 (β-casein-5-phosphate 1-105/107)
PP8	Proteose peptone component 8
PS	Phosphatidylserine
PUFA	Polyunsaturated fatty acid
PVDF	hydrophilic polyvinylidene fluoride
RANK-L	Receptor activator for nuclear factor κ B ligand
RAW 264.7	Mouse leukaemic monocyte macrophage cell line
RP-HPLC	Reverse phase high performance liquid chromatography
RP-LC-MS/MS	Reverse phase-liquid chromatography-mass spectrometer
SDS	Sodium dodecylsulfate
SM	Sphingomyelin
SPP 1	Secreted phosphoprotein 1
SPR	Surface plasmon resonance
TEM	Transmission electron microscope
TFA	Trifluoroacetic acid
TGF-β1	Transforming growth factor-β1
TGF-β2	Transforming growth factor-β2
TMB	3,3,5,5-tetramethylbenzidine
TN	Total nitrogen
TRAP	Tartrate-resistant acid phosphatase
Tris-HCl	2-Amino-2-(hydroxymethyl)-1,3-propanediol, hydrochloride (tris(hydroxymethyl)aminomethane hydrochloride)
Trp Hyd	Trypsin hydrolysate
UF	Ultrafiltered
UV-Vis	Ultraviolet - visible
WPC	Whey protein concentrate
WPC 80	Whey protein concentrate 80
WPI	Whey protein isolate