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**Effect of synthetic and bovine milk  
conjugated linoleic acid (CLA) on  
immune function**

**A thesis presented in partial fulfilment of the requirements for  
the degree of Master of Science in Nutrition Science  
at Massey University, New Zealand**

**HUI ZHAO**

**1999**

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## ABSTRACT

CLA is a collective name for a mixture of positional and geometrical isomers of linoleic acid (*c*-9, *c*-12-octadecadioenic acid) which possess conjugated double bonds. CLA occurs in a variety of foods, but is present at higher concentrations in products from ruminants. Milk fat is the richest natural source of CLA. The objective of this research was to examine the immunomodulatory properties of CLA (both synthetic and natural CLA derived from bovine milk fat). Two experiments were conducted at the Milk and Health Research Centre, Massey University, Palmerston North, New Zealand.

The aim of the first experiment was to investigate the dose effect of different concentrations of synthetic CLA (Tonalin) on immune function. Mice were fed either skim milk powder based diet or the same diet supplemented with 0.1, 0.25, 0.5, 1.0 or 2.0% synthetic CLA (Tonalin) by weight. Animals were immunised orally with a mixture of polio vaccine in sodium bicarbonate (25 µl) and subcutaneously with Fluvax and Tetanus toxoid vaccine on days 7 and 21. After 4 weeks feeding, mice were euthanased by isoflurane overdose.

Various immune parameters were measured and the results showed that synthetic CLA (Tonalin) enhanced a range of immune functions. Synthetic CLA stimulated PHA induced T lymphocyte proliferation at 0.25, 0.5 and 1.0% as compared with the control group ( $p < 0.05$ ). Synthetic CLA enhanced macrophage phagocytosis in a dose dependent manner. Synthetic CLA enhanced antibody responses (mucosal and systemic) to vaccines (polio vaccine, Fluvax and Tetanus toxoid). Natural killer cell activity was significantly enhanced in mice fed 0.25 and 0.5% CLA. In general, 0.25% CLA was regarded as the best CLA level which achieved optimal immunoregulating effects.

The aim of the second experiment was to examine the effect of natural CLA derived from milk fat on immune responses in mice. Mice were fed a skim milk powder (SMP) based diet. The control diet was skim milk powder only, without any CLA or milk fat supplementation. The dietary treatments were: ordinary milk fat, fractionated milk fat (1<sup>st</sup> stage), 0.2% synthetic CLA (Tonalin) and CLA enriched milk fat. Animals were fed these

diets for 28 days. Mice were immunised orally with a mixture of polio vaccine/ovalbumin/cholera toxin in sodium bicarbonate on days 7, 14 and 21 and subcutaneously with Fluvax and ADT (Diphtheria and Tetanus toxoid vaccine) on days 7 and 21. Natural CLA was found to stimulate PHA and Con A induced T lymphocyte blastogenesis. Supplementation with natural CLA also led to increased antibody responses to vaccines and increased CD25<sup>+</sup> populations in peripheral blood in mice. Natural CLA also enhanced macrophage phagocytosis. Synthetic CLA enhanced a range of immune functions which is consistent with the results in the first experiment.

It is noted that although the CLA content is low in milk fat, the natural CLA derived from milk fat expressed potent effects in enhancing the growth of immune cells and promoting a range of immune functions in mice.

**Key words:** conjugated linoleic acid (CLA), lymphocyte, macrophage, immunity, milk fat

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## LIST OF ABBREVIATIONS

AA	Arachidonic acid
CD	Cluster differentiation
CLA	Conjugated linoleic acid
Con A	Concanavalin A
CT	Cholera toxin
Ig A	Immunoglobulin A
Ig G	Immunoglobulin G
IFN- $\gamma$	Interferon- $\gamma$
IL-4	Interleukin-4
LPS	Lipopolysaccharide
NK cell	Natural killer cell
OV	Ovalbumin
Polio	Poliomyelitis
PHA	Phytohemagglutinin
PUFA	Polyunsaturated fatty acid
Tet Tox	Tetanus toxoid
Th cell	T helper cell
Ts cell	T suppresser cell
TNF	Tumour necrosis factor