Col-ME™

Bioxyne

GTNZ GLOBAL TREASURE NEW ZEALAND

Col-ME, is a unique colostrum compound (colostrum powder and colostrum extract (Immunel from Sterling Technology) developed by Global Treasure New Zealand Limited, a subsidiary of Bioxyne Limited.

Low molecular weight bioactive peptide and growth factors extracted from bovine colostrum whey

Colostrum

Colostrum is the biological fluid produced from the mammary glands of female mammals shortly after giving birth. The fluid has a distinct composition different from the milk produced afterwards. The composition of colostrum provides immediate immune protection to the newborn. In humans, some degree of protective passive immunity is transferred to the fetus during gestation to a higher degree than in a number of other mammalian species, and the human colostrum then further supports the continued development of the newborn's immunity. However, in many other mammals, the newborn baby arrives with no immune protection.

Research provides evidence for transfer of cytokines, immunoglobulin, growth factors, antimicrobial compounds, and maternal immune cells to the newborn via the feeding of colostrum [1-3]. The supportive properties of bovine colostrum when consumed by other mammalian species, including pigs and humans, are well documented in the literature [4-11].

Colostrum chemical composition

Colostral secretions are designed to provide protection critical to neonatal survival during the early days of life as well as sustained growth and development of babies later in their life. Bovine colostrum is especially rich in immune factors, amino acids, nucleotides and growth factors. Some elements are present in higher quantities in bovine colostrum than in human colostrum.

Colostrum versus Immunel

Colostrum whey is the liquid remaining after the removal of casein and fat. Whey protein is a well-known nutritional supplement in the sports arena, including body building. Colostrum whey contains compounds with a direct bactericidal effect. In addition, it also contains compounds that trigger immune defense mechanisms to further help eliminate bacteria and viruses. Immunel is a fat-free, lactose-reduced extract from Colostrum whey, where the immune protective compounds are enriched.

Immunel chemical composition

Immunel was developed to provide a more concentrated delivery of key bioactive compounds. Immunel is a high-potency blend of compounds, allowing multi-facetted mechanisms of actions to support multiple body functions simultaneously. These compounds and their known biological properties are listed in Table 1.

Table 1: Composition of Immunel

Actives	Health/Nutrition Outcomes
Proline-Rich Peptide (PRP)	Immune modulation, cognitive enhancement, thymus regulation
Insulin-like Growth Factor-1 (IgF-1)	Sports nutrition, lean body, cell and tissue repair and rejuvenation
Transforming Growth Factor (TGF)-ƒÀ2	Cell protection, immune enhancement
Sialic Acid	Immune modulation, brain health, prebiotic
Nucleotides	Immune modulation, anti-aging, stamina

Immunel supports innate immune defense mechanisms

The immune modulating compounds in colostrum from farm animals have been receiving attention as substitutes for pharmaceutical drugs in a number of clinical applications [12]. Colostrum provides protection from NSAID-induced intestinal damage [13-16] as well as protection from upper respiratory illness [17-19]. A human study found that nutritional supplementation with colostrum was equally efficient as a vaccine at preventing flu episodes [20].

Immunel consumption reduced the symptoms and severity of bacterial and viral airway infections in rodents

Two animal studies were conducted on Immunel, to test whether Immunel consumption helped reduce the severity of cold and flu symptoms.

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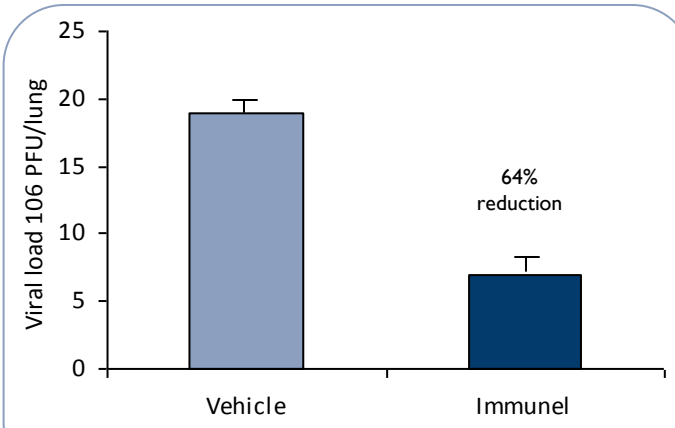

Figure 1: Influenza viral load after oral treatment

In one study, mice were infected with mouse-adapted influenza virus. Animals treated orally with a single dose of Immunel within 24 hours prior to infection showed reduced viral titer in the lungs, compared to control animals (Figure1).

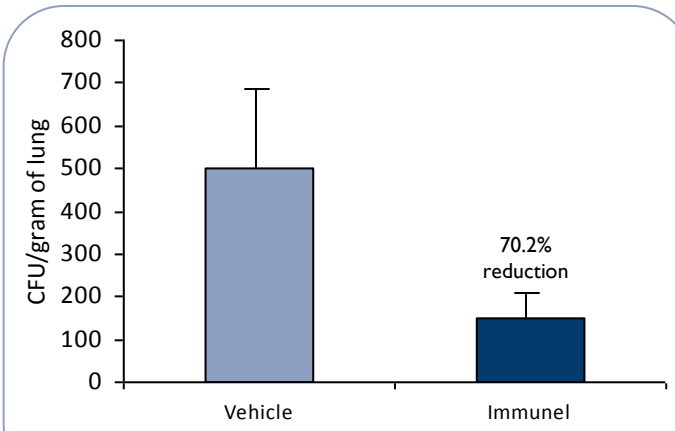

Figure 2: S. pneumonia CFU/lung

In another study, mice were infected with Streptococcus pneumonia, which is a human pathogen and causes infections in the upper respiratory tract, sinuses, and eyes (Figure 2).

Animals treated orally with two doses of Immunel 30 minutes before and 4 hours after infection showed reduced bacterial load at 20 hours after infection, when compared to control animals.

Thus, treatment with Immunel showed enhanced bacterial clearance as a result of antimicrobial activity in the animals. Immunel. supports distinct mechanisms of innate immune defense reactions.

The innate part of our immune defense refers to the cellular defenses that act the quickest and most immediate. Many types of cellular reactions contribute to the immediate efforts to stop microbial invaders from taking hold in our body and causing disease. These mechanisms include

- Phagocytosis, i.e. some of our cells are able to eat bacteria
- Recruiting immune cells into the area of infection
- Killing of our own cells if they have become transformed, such as being infected with a virus.

When Immunel was added to human immune cells in laboratory bioassays, Immunel supported all three mechanisms.

Immunel supports phagocytosis

In order to test this, human polymorphonuclear (PMN) cells were used. The pre-treatment with Immunel acted almost immediately, and within a few minutes after treating phagocytic cells with Immunel, more cells were phagocytic, and each phagocytic cell consumed more particles (Figure 3).

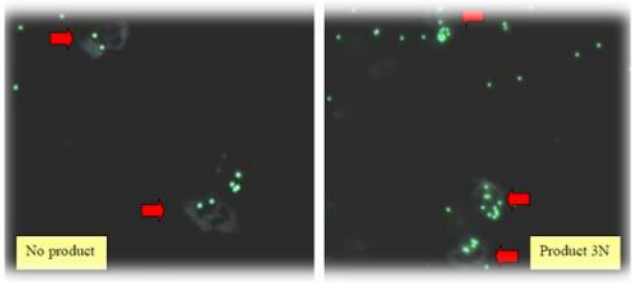

Figure 3: Micro-photographs documenting qualitative number of beads within phagocytes after different treatments. Product 3N refers to Immunel

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Immunel activates Natural Killer (NK) cells

Another type of immune cells that are able to respond immediately to invading pathogens is called Natural Killer (NK) cells. These cells are able to attach to those of our cells that have become invaded by viruses, or transformed into cancer cells. The killing of the transformed cell can happen via cell-cell contact or by secretion of chemicals such as Perforin which helps destroy the malfunctioning target cell.

Treatment of NK cells with Immunel. resulted in an activation of the NK cell. The treated NK cells expressed much higher amounts of an activation marker called CD69, which indicates that the NK cells were activated to be more efficient at attacking target cells (Figure 4).

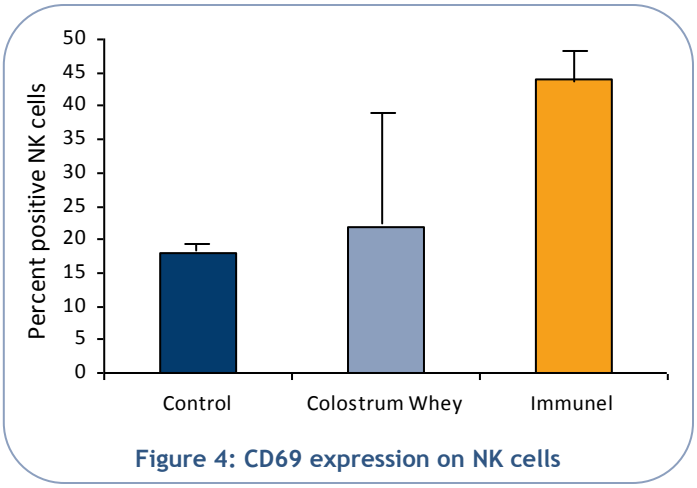

Figure 4: CD69 expression on NK cells

Furthermore, when another well-known stimulus of NK cell activation, namely Interleukin-2 (IL-2) was added to the tests, Immunel and IL-2 acted in synergy and produced higher levels of NK cell activation. This may indicate that when an ongoing immune reaction is happening, and IL-2 is produced, Immunel further supports the immune reaction involving NK cells. Immunel also increased the production of Interferongamma, which is a cytokine that induces further activation of NK cells and other cell types involved in the innate immune defense.

Immunel supports infiltration of phagocytic immune cells in response to microbial challenge

When an invading microbe attacks our cells, and our immune cells respond, chemical signals are sent out to recruit more immune cells to the area. The effective recruitment and increased infiltration of immune cells to an area in need is important for prompt elimination of microbial invaders. When Immunel is consumed it is presented to the immune system from the gut lumen, in conjunction with potentially pathogenic microbes. This may have a protective effect. This was tested in a cell-based bioassay using a dual chamber system. Cells were placed in one chamber adjacent to another chamber where pieces of bacterial proteins were placed. Components from the bacterial fragments could seep into the next chamber and result in an increased movement (recruitment) of immune cells into the chamber containing the microbial signal.

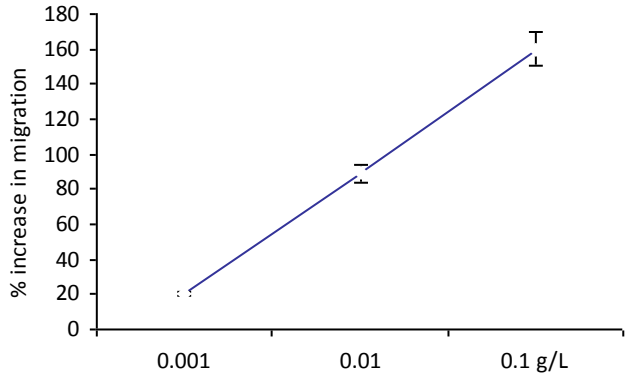

Figure 5: Recruitment of cells by bacterial peptides and Immunel

When Immunel was placed together with the bacterial fragments (Figure 5), an increase in recruitment of immune cells into the chamber containing the bacterial fragments was measured. This may signify potential benefit from consuming Immunel, such as strengthening of the gut immune protection, where one of our largest volumes of potential invaders exists.

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When Immunel was placed in the chamber adjacent to the immune cells (Figure 6), in the absence of bacterial fragments, we also saw an increased recruitment into the chamber containing Immunel. This suggests that when Immunel is consumed, more cells are recruited into the gut wall, in preparation to respond to microbial invasion.

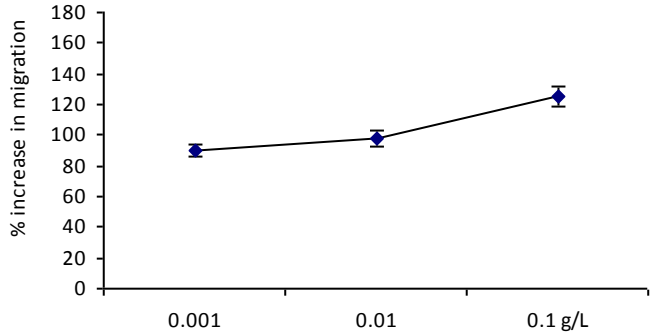


Figure 6: Recruitment of cells by Immunel alone

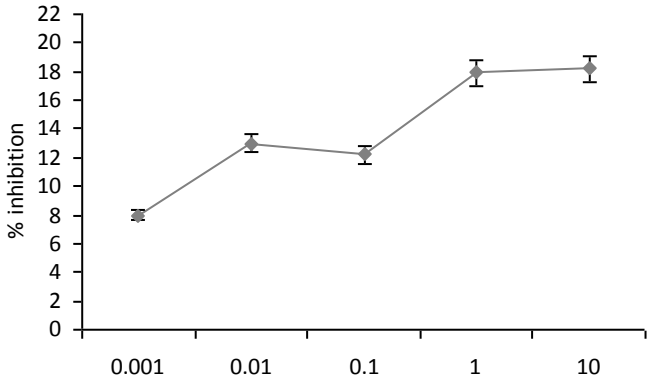


Figure 7: Inhibition of lipoxygenase enzyme activity

Immunel has anti-inflammatory properties by inhibiting Lipoxygenase enzyme activity

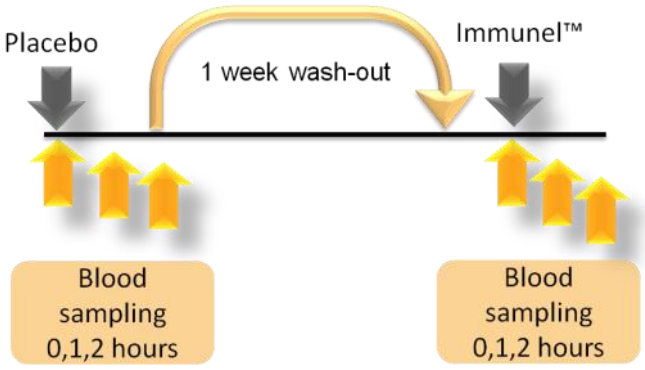
Selective inhibition of Lipoxygenase enzymatic activity points to an anti-inflammatory role (Figure 7). If Immunel only had the property of supporting the function of the immediate immune protection, Immunel would be a less interesting product. However, since Immunel also possesses some natural anti-inflammatory properties from Colostrum, the potentially abrasive result of immune defense reactions are buffered by inhibition of the enzymatic action of Lipoxygenase.

This inhibition may further reduce free radical damage naturally associated with immune defenses, in part since the activity of the Lipoxygenase enzyme further produces additional free radicals.

Human clinical data

Data from a recent human clinical trial further supports that consumption of Immunel. induces potent and rapid changes in markers associated with the innate immune defense.

Study design: A randomized double-blinded placebo controlled cross-over study design was used. Twelve healthy human subjects were tested on two different days at least one week apart. On each test day, subjects were fed either Immunel. or placebo. At baseline and at 1 and 2 hours after consumption, blood samples were drawn.
The shown sequence is an example only; the sequence in which each person consumed Immunel or Placebo for short-term testing was randomized.



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Consumption of Immunel results in rapid increase in phagocytic activity in humans

Consumption of a single dose of Immunel. resulted in a rapid increase in the phagocytic activity of human cells tested ex vivo at different time points after consumption. This was in contrast to the reduced phagocytic activity seen on the day when Placebo was consumed by the same people.

The difference in phagocytic activity between Immunel and Placebo was statically significant at 2 hours after consuming a single dose (P<0.02).

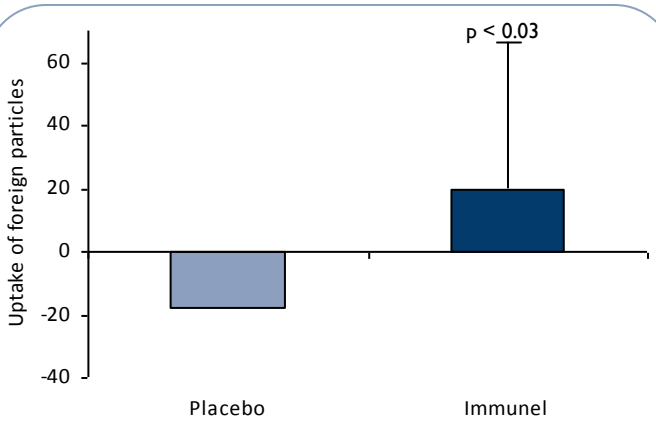

Figure 8: Phagocytic activity

Consumption of Immunel provides a rapid, transient support for immune surveillance of human Natural Killer cells

Natural Killer (NK) cells are an important part of our anti-viral defenses, and these cells work primarily by surveying through tissue looking for target cells, which they then kill, either by contact or by secreting certain chemicals. The NK cells have very little activity in the blood circulation. The study was conducted during the early/mid-morning hours on both study days. The increase in the numbers of circulating Natural Killer (NK) cells, seen with placebo, reflects part of natural circadian (day/night) fluctuations (Figure 9). The delay in this increase, seen after consumption of Immunel., is suggestive of increased NK cell trafficking/homing, as a reflection of increased immune surveillance. This reflects that NK cells are retained in tissue more, scavenging for target cells.

Conclusion

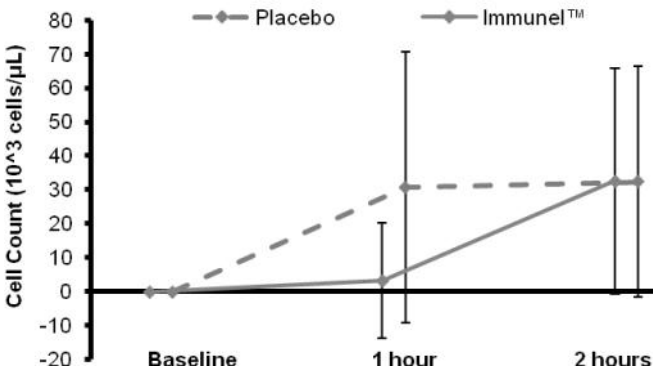

Figure 9: NK Cells in the blood circulation

Mechanistic data, combined with animal studies and human clinical data suggests that Immunel. Induces rapid changes in immune support, including specific mechanisms involved in anti-bacterial and anti-viral defenses

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Research Portfolio

- NIS Laboratory (2006, June) Colostrum and fractions thereof: Exploring the pro- and anti-inflammatory and immunomodultory effect on human leukocyte subsets in vitro. Report #:059 76 051506 Non-published. NIS Labs, Klamath Falls, OR.
- NIS Labs (2006, August) REPORT: PILOT STUDY 2. Colostrum fractions 3L, 3N, and 14: Comparison of immuno-modulatory effect on human leu-kocyte subsets in vitro. Report #: 059 76 051506 Non-published. NIS Labs, Klamath Falls, OR.
- Burleson, Gary, Ph.D. (2007, January 26). Evaluation of three colostrum fractions in the mouse streptococcal model. Study No. BRT 20061127. Burleson Research Technologies, 120 First Flight Lane, Morrisville, NC. Non-published
- Burleson, Gary, Ph.D. (2007, July 27). Evaluation of C-18 in the mouse influenza host resistance model. Study No. BRT 20070526. Burleson Research Technologies, 120 First Flight Lane, Morrisville, NC. Non-published
- Honzel, D. et.al (2008) Comparison of Chemical and Cell-Based Anti-oxidant Methods for Evaluation of Foods and Natural Products: Generating a Multifaceted Data by Parallel Testing Using Erthrocytes and Polymorphonuclear Cells. J. Agric. Food Chem. 56. 8316-8325.
- NIS Laboratory (2009, May 18). Immunel/Immunel Plus: comparative testing of chemoattractant and anti-inflammatory effects in vitro. Report 6: NIS Labs, Klamath Falls, OR.
- NIS Laboratory (2009, August 14). Comparison of 5 colostrum based products in a phagocytosis assay. Report 8. Non-published. NIS Labs, Klamath Falls, OR.
- Christiansen, S. et al (2010, September) Chemical composition and nutrient profile of low molecular weight fraction of bovine colostrum. International Dairy Journal Volume 20, Issue 9, September 2010, Pages 630-636 6th NIZO Dairy Conference - Dairy Ingredients: Innovations in Functionality
- NIS Labs (2011, August) Acute effects of RPEP in vivo. Submitted for publication. Non-published. NIS Labs, Klamath Falls, OR.

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