SCIENCE PERFECTED IN MARKET™

Anagenix.

Livaux® & Immunity

Livaux® is a high-quality powdered ingredient derived entirely from New Zealand gold (Gold3) kiwifruit. The proprietary processing technology gently converts the nutrient-dense gold kiwifruit into a free-flowing powder which is packed full of the key bioactives from the whole fruit.

Research has shown that gold kiwifruit is able to enhance the immune response, control inflammation and help ameliorate the symptoms of colds and flus. These effects are via the direct action of kiwifruit polyphenols and vitamins as well as indirect actions of kiwifruit carbohydrates and polyphenols which are fermented by colonic microbiota, promoting the growth of beneficial microbes and the production of short chain fatty acids (SCFAs).

Livaux® has been shown *in vitro* to affect changes in the number of immune cells and the production of antimicrobial compounds. It has also been shown by way of computer modelling to reduce inflammation. Livaux® contains essential gold kiwifruit carbohydrates, vitamins and polyphenols which are thought to be responsible for the immune responses observed in the *in vitro, in vivo* and human studies described below.

Livaux® also supports a healthy digestive system by promoting bowel regularity and increasing the levels of good bacteria in the gut. A healthy digestive system in turn supports a healthy immune system.

Whole gold kiwifruit research

In 2011, Skinner et al reported that a gold kiwifruit puree enhances both type 1 and 2 T-helper immune responses via enhanced production of IgG antibody and the cytokine, IL-5, in mice. A gold kiwifruit juice concentrate, which contained less dietary fibre and polyphenols, had no effect on the immune response in the same mouse model.

Following this research, Hunter, et al., 2012 and Skinner, 2012 reported the results of two clinical trials which examined the effects of gold kiwifruit consumption on cold and flu symptoms (Tables 1 and 2).

Table 1: Study of older adults (≥ 65 years) given either gold kiwifruit or banana to determine effect on the duration and severity of upper respiratory tract infections (URTI).

PARTICIPANTS	INTERVENTION	PARAMETERS MEASURED	RESULTS
N = 32 **Community-dwelling people** **≥ 65 years old**	Subjects were randomly assigned to receive either: • 2 fresh gold kiwifruit plus 2 freeze-dried gold kiwifruit; or • 2 freeze-dried bananas Daily for 4 weeks 4 week washout before switching to other intervention	Duration and severity of symptoms of upper respiratory tract infections (URTI) as recorded by subjects using a questionnaire.	Duration of sore throat and head congestion were significantly ($P < 0.05$) reduced with gold kiwifruit compared to banana: • Sore throat: 2.01 days vs 5.42 days • Head congestion: 0.88 days vs 4.69 days The severity of head congestion was also significantly reduced with kiwifruit ($P = 0.015$) Tendency for runny nose, blocked nose, hoarseness and chest congestion to also be reduced in terms of duration and severity, but difference did not reach significance.
		Plasma antioxidant status (e.g. vitamin C and carotenoid concentration)	Consumption of gold kiwifruit led to significantly higher plasma concentrations of vitamin C, α-tocopherol, and lutein/zeaxanthin, and higher erythrocyte concentrations of folate.
		Immune function and inflammatory markers	No significant difference.

Table 2: Study of pre-school children (2 – 5 years) given either gold kiwifruit or banana to determine effect on the symptoms of colds and flu-like illnesses.

PARTICIPANTS	INTERVENTION	PARAMETERS MEASURED	RESULTS
N = 66 Children aged 2 – 5 years attending day care	Subjects were randomly assigned to receive either: • 2 servings of fresh gold kiwifruit (150 g total); or • 2 servings of banana (110 g total) 5 days a week for 4 weeks, then switched to other intervention	Symptoms of colds and flu-like illnesses as recorded by parents using a questionnaire (Canadian Acute Respiratory Illness and Flu Scale).	Overall incidence of a cold or flu-like illness was considerably reduced by nearly 50% when the children ate kiwifruit compared to banana. Significant improvements in a number of symptoms with kiwifruit consumption. Children: • Had a better appetite (P = 0.01) • Did not feel so unwell (P = 0.04) • Had more energy (P = 0.01) • Cried less (P = 0.04) • Had less severe headaches (P = 0.02) and sore throats (P = 0.04)

A summary of *in vitro, in vivo* and human studies on kiwifruit with respect to immunity was published in 2013 (Skinner, et al., 2013). In addition to the work above, the publication discussed the following:

- *In vitro* studies
  - Direct antimicrobial effect of extracts from gold kiwifruit has been shown.
  - Green kiwifruit was shown to increase the production of gastrointestinal defensins (human beta defensin-1 and -2).
    - This increase is thought to be mediated through SCFA production.
    - Gold kiwifruit was not evaluated in this study for its effect on defensins, however gold kiwifruit is known to stimulate the production of SCFAs.
  - Green and gold kiwifruit significantly reduced the intensity of barrier damage *in vitro.*
  - Aqueous extracts of green and gold kiwifruit reduced the levels of proinflammatory cytokines (TNF-α and IL-1β) in LPS-stimulated human monocytes and whole blood.
- *In vivo* studies
  - Rats fed 100 g of kiwifruit had a higher percentage of primed γδ T cells compared to control rats.
  - Kiwifruit extracts significantly enhanced intestinal mucosal and serum antibody responses to vaccines and promoted IFN-γ and natural killer cell activity.
- Human studies
  - Healthy, but fatigued-feeling adults were given either 3 gold kiwifruit, 3 bananas, a vitamin C supplement or no treatment for four weeks.
    - Gold kiwifruit consumption led to an increase in the % of CD8+ T cells and an increase in the numbers of naïve T cells and CD8+ CD28+ T cells.
    - There was also a significant reduction in the age of T cells in the gold kiwifruit group compared to baseline, whereas there was an increase with the banana group. No significant effect was seen with vitamin C or no treatment.
    - Subjects reported feeling significantly less fatigue, less skin dryness, better skin condition and less eye fatigue following kiwifruit consumption.

Livaux® specific research

Livaux® Gastrointestinal Effects - Clinical Study

The gastrointestinal and immune systems are invariably linked. The gastrointestinal system is responsible not only for digestion, which produces the essential nutrients the body needs to function, and eliminating waste, but also for protecting us from infection. If the digestive system is not working optimally then this can impact the performance of the immune system.

Livaux has been shown in a clinical study to support a healthy digestive system by:

- Acting as a precision prebiotic, increasing the abundance of the beneficial gut bacteria, *Faecalibacterium prausnitzii* (*F. prau*), to restore balance to the gut microbiome (Blatchford, et al., 2017); and,
- Increasing stool frequency to help maintain bowel regularity (Ansell, et al., 2015).

Health benefits of F. prau

Of the thousand or so bacterial species that reside in the human gut (primarily the large intestine), only a few are very special. *F. prau* is one of those very special bacterial species (Lopez-Siles, Duncan, Garcia-Gil, & Martinez-Medina, 2017) (Velasquez-Manoff, 2015). *F. prau* accounts for ~5% of the total faecal microbiota in healthy individuals but can increase to ~15% in some, making it one of the most abundant bacteria in the healthy human intestinal microbiota (Miquel, et al., 2013) (Martin, Bermudez-Humaran, & Langella, 2018).

Two of the key properties of *F. prau* is that it is a major butyrate-producer and has anti-inflammatory effects (directly and indirectly via butyrate and other metabolites).

The major end products of fermentation by *F. prau* are formate, lactate and significant amounts of butyrate (> 10mM *in vitro*). As butyrate plays a major role in gut physiology (it serves as the major energy source for colonocytes), intestinal cell lifecycle (stimulates growth and apoptosis) and immunity (anti-inflammatory, induces apoptosis in cancer cells), it follows that *F. prau* may impact on these functions (Miquel, et al., 2013).

Other metabolites produced either by or in the presence of *F. prau* include salicylic acid, shikimic acid and raffinose. Salicylic acid and shikimic acid are anti-inflammatory molecules. Shikimate is a precursor for folate and aromatic amino acids (tyrosine etc). Raffinose plays a role in maintaining gut permeability (Martin, Bermudez-Humaran, & Langella, 2018) (Ferreira-Halder, de Sousa Faria, & Andrade, 2017).

In *in vitro* and *in vivo* studies, *F. prau* and the molecules it secretes have been found to have potent anti-inflammatory action, affecting cytokine levels and intestinal permeability (Miquel, et al., 2013)(Qiu, Zhang, Yang, Hong, & Yu, 2013) (Martin, et al., 2015).

Over the last decade, an increasing number of studies have reported on *F. prau* depletion in various diseases/health concerns, including inflammatory bowel disease (IBD, i.e. Crohn's disease and ulcerative colitis), irritable bowel syndrome (IBS), colorectal cancer, diabetes, psoriasis, atopy, multiple sclerosis, Parkinson's disease and depression. The findings of these studies indicate that *F. prau* has a crucial role to play in maintaining gut physiology and overall host wellbeing.

Livaux® direct immune system effects

Background

γδ T cells are a unique population of immune cells (lymphocytes) which defend mucosal integrity and coordinate appropriate immune responses to infection. They are distributed throughout the body but are at their highest levels in the mucosal tissues, such as in the intestines and lungs. Priming (activation) of these cells is linked to subsequent increases in their numbers and may enable them to respond more quickly to pathogens.

As part of the ageing process, the number of γδ T cells decline in healthy individuals and possibly contribute to the higher susceptibility to infection and chronic/inappropriate inflammation.

Phenolic extracts from kiwifruit have been shown to prime γδ T cells in rats *ex vivo*. Unpublished *in vitro* data from Plant and Food showed that digested Gold3 kiwifruit led to increased γδ T cell priming.

Undigested polyphenols and carbohydrates can be fermented by the gut bacteria to produce metabolites (i.e. SCFAs) which can enhance antimicrobial β-defensin production in colonic cells. β-defensins are secreted by colonic cells and their antimicrobial activity keeps pathogenic bacteria in check and prevents bacterial infection.

Plant & Food Research experiment

Livaux® was subjected to simulated gastrointestinal digestion and fermentation and changes to γδT cell numbers and defensin production from human cells was evaluated *in vitro*.

The addition of Livaux® to the cells led to significant increases in the number of γδ T cells and the secretion of β-defensin 2.

CytoSolve in silico study

The CytoSolve *in silico* study investigated how Livaux® affects gut motility by way of computer modelling. As part of this study, it was found that the bioactive components within Livaux® support healthy gut motility and the immune system by affecting three major physiological processes:

- Mucus production
- Control of inflammation
- Faecal bulking

Mucus production

Mucus serves as a protective barrier for the gut lining and as a lubricant to facilitate the passage of stool. Maintaining a thick layer of mucus is therefore important for allow stools to pass freely and protect against infection.

Livaux® contains the amino acid, leucine, which directly targets the mucus production pathway, stimulating the production of mucin 2, thereby helping to maintain the mucus layer.

Control of inflammation

Two key pathways involved in the inflammatory response are the oxidative stress pathway and TNF-α induced nitric oxide (NO) synthesis from inducible nitric oxide synthase.

Livaux® contains key bioactives which influence these inflammatory pathways, namely vitamins E, A and C and the polyphenol, epicatechin.

Using the CytoSolve computer model, a daily dose of Livaux® led to a reduction in both reactive oxygen species (by-product of oxidative stress) and inducible NO, indicating that Livaux® is able to help control inflammation.

Faecal bulking

Livaux® contains non-digestible, fermentable components in the form of dietary fibre and polyphenols. These components are fermented by the good bacteria in the gut to produce short chain fatty acids (SCFAs), such as propionate, which stimulate the production of PYY and GLP-1. These compounds increase gut transit time, facilitating the optimal absorption of water and other nutrients to promote healthy stool formation and good faecal bulk.

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