

Selenium-enriched yeast supplementation significantly increased lymphocytes response to mitogens, which reached the upper limit of the usual range for adults after 6 months. This study demonstrates the immunostimulant properties of selenium-enriched yeast in elderly subjects (Figure 1)

Of the various chemical forms of selenium available, selenium-rich yeast, such as Lallemand's LALMIN®Se, is recognized for its superior bioavailability and is better suited than mineral forms of selenium for dietary supplementation. In 2008, EFSA's Scientific Panel recognised that yeast with high levels of organically bound selenium (selenium incorporated within aminoacids, the same form found in our organism) was up to twice as bioavailable than inorganic forms (6). For this reason, LALMIN Immune has been formulated using LALMIN®Se, an inactivated whole cell yeast rich in bioavailable selenium produced to high quality standards. 350mg of LALMIN® IMMUNE contains 60µg of organically-bound selenium.

The immune power of Vitamin D

Vitamin D also plays important roles in immune response. Vitamin D deficiency is correlated with a higher susceptibility to infections due to impaired localized innate immunity and defects in antigen-specific cellular immune response.

Vitamin D plays a regulatory role in the immune response and is involved in immunomodulation during excessive inflammatory response. Vitamin D receptors are found in peripheral mononuclear cells and in both T-helper 1 and T-helper 2 cells. Calcitriol (1,25(OH)2D), the hormonally active form of vitamin D, reduces the inflammatory response of Th1 cells, suppresses antigen presentation by dendritic cells, suppresses proliferation and immunoglobulin production and delays the differentiation of B cell precursors into plasma cells, exerting an inhibitory action on the adaptive immune system. 1,25(OH)2D increases expression of cathelicidin, an antimicrobial peptide thought to be important for the innate immune system (7).

Poor vitamin D status has been linked to the development of a number of different type-one mediated (Th1) autoimmune diseases (8).

Certain studies have linked high occurrence of flu during the winter to vitamin D deficiency, and several European surveys have shown that up to 75% of the European population's vitamin D intake could be below the recommended daily allowances (RDA).

The role of vitamin D in the immune system and inflammatory response has been recognised by EFSA scientific panel in 2010, who concluded that: "a cause and effect relationship has been established between the dietary intake of vitamin D and contribution to the normal function of the immune system and healthy inflammatory response".

LALMIN® Immune contains LALMIN® VitaD Yeast, an inactivated dried whole cell yeast containing elevated and standardised levels of non-synthetic vitamin D2, produced thanks to a unique process involving the conversion by yeast cells of natural endogenous ergosterol. 350mg of LALMIN® IMMUNE provides 10µg vitamin D.

Antioxidants for a well-functioning immune system

The presence of free radicals leading to oxidative reactions in the organism is physiological. It is a natural mechanism of defence against bacteria, also involved in normal cell functions. But these reactions must be kept under control, which is done thanks to two types of anti-oxidative mechanisms, either by directly neutralizing free radicals (anti-oxidants) or by reducing oxidised molecules (enzymatic systems).

Immune reactions can produce excessive free radicals. In particular, phagocytosis of a microbe by macrophages produces excessive superoxide anions (O₂⁻) responsible for killing the bacteria (9).

Unfortunately, the formation of these oxidants may also damage local tissue. In the body, oxidative stress and inflammation state are in a delicate equilibrium. In case of high

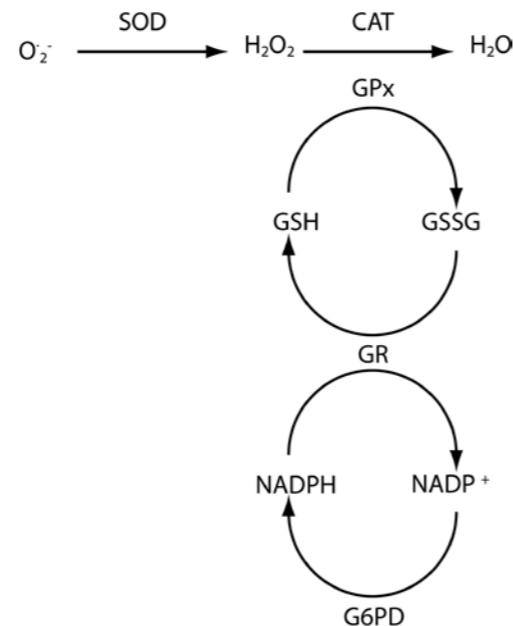
inflammatory status, oxidative stress due to generation of free radicals will cause damage at cell level, but also at tissue level (Figure 2). Hence, there is a need for antioxidant nutrients in order to prevent local oxidative injury.

Figure 2: Oxidative stress and inflammation



The role of selenium as an indirect component of the antioxidant network is well established. As mentioned earlier, the antioxidant system comprises of low molecular weight antioxidants and enzymatic systems such as enzymes from the glutathione peroxidase family, which catalyses the reduction of hydrogen peroxide or organic hydroperoxides using reduced glutathione as a co-substrate (Figure 3). These and other selenoenzymes, such as the thioredoxin reductases, also involved in antioxidant defence are selenium-dependent and can respond to selenium supplementation.

Figure 3. Glutathione redox cycle. GPx = Glutathione peroxidase, GSH = Glutathione, GSSG Oxidized glutathione,



GR = Glutathione reductase, NADP = Nicotinamide adenine dinucleotide phosphate, NADPH = Reduced nicotinamide adenine dinucleotide phosphate, G6PD = Glucose 6-phosphate dehydrogenase

Supplementation with selenium-enriched yeast has been shown to have a beneficial effect on oxidative stress. For example, a nine months double-blind randomised, placebo-controlled trial carried out in healthy men with Selenium enriched yeast showed the effect of supplementation on biomarkers of oxidative damage (10). In the supplemented group, investigators observed a significant 32% increase in blood glutathione levels (P<0.05) and a significant decrease in prostate specific antigen (PSA) (P<0.001), suggesting a protective effect of Se-enriched yeast supplementation against prostate cancer.

To complement Selenium enriched yeast's anti-oxidative

potential, LALMIN Immune is also a source of yeast beta-1,3/1,6-glucans, whose powerful anti-oxidant activity has been shown to protect cellular integrity from oxidative injury following an excessive activation of the immune response (11).

All beta-glucans are not the same: their biological activity is linked to their chemical structure, and related to their origins. Hence, it is important to differentiate yeast beta-glucans from beta-glucans from oat or barley. Yeast cell wall beta-glucans are generally recognised for their immunomodulating effects and beneficial health effects. They also have a strong anti-oxidant power. The yeast cell wall beta-glucans used for the production of LALMIN Immune, called Glucans 30, contain standardised levels of activated (exposed) beta-1,3/1,6-glucans. Glucans 30 is issued from a unique production process developed by Lallemand to extract and expose yeast cell walls beta-glucans in order to ensure optimal biological activity on human cells. The beta-glucans in Glucans 30 are extracted and then activated using a biological process (enzymatic process, no use of solvents, no alkaline extraction). 350mg of LALMIN® IMMUNE provides 270mg of activated yeast beta-1,3/1,6-glucans.

Possible EFSA Health Claims

The components in LALMIN® IMMUNE are documented and recognised for their beneficial health effects. They have been granted positive immune health claims by EFSA (4,7). Hence,

- (1) Eva S. Wintergersta, Silvia Maggini, Dietrich H. Hornig, 2007. Contribution of Selected Vitamins and Trace Elements to Immune Function. *Ann Nutr Metab* 51:301-323
- (2) D.E. Godar, S.J. Pope, W. Burgess Grant, M.F. Holick. 2011. Solar UV Doses of Young Americans and Vitamin D3 Production *Environmental Health Perspectives* <http://dx.doi.org/10.1289/ehp.1003195>
- (3) Rayman MP. The importance of selenium to human health. *Lancet*. 2000 Jul 15;356(9225):233-41.
- (4) EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA); Scientific Opinion on the substantiation of health claims related to selenium and ... protection of DNA, proteins and lipids from oxidative damage (ID 1292), and maintenance of the normal function of the immune system (ID 1750) pursuant to Article 13(1) of Regulation (EC) No 1924/2006. *The EFSA Journal* 2010;8(10):1727.
- (5) Peretz A. et al., 1991. Lymphocyte response is enhanced by supplementation of elderly subjects with selenium-enriched yeast. *Am J Clin Nutr* 53:1323-8
- (6) Scientific Opinion of the Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food (AFC) on a request from the Commission on Selenium-enriched yeast as source for selenium. *The EFSA Journal* (2008) 766, 1-43.
- (7) EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA); Scientific Opinion on the substantiation of health claims related to vitamin D and normal function of the immune system and inflammatory response (ID 154, 159)... pursuant to Article 13(1) of Regulation (EC) No 1924/2006. *EFSA Journal* 2010; 8(2):1468
- (8) Cantorna et al., 2004. Mounting evidence for vitamin D as an environmental factor affecting autoimmune disease prevalence. *Exp Biol Med* (Maywood). 229(11):1136-42.
- (9) Khanna et al., 2005. Imaging of Chronic Granulomatous Disease in Children. *RadioGraphics*. 25:1183-1195.
- (10) El-Bayoumy K, Richie JP Jr, Boyiri T, Komninou D, Prokopczyk B, Trushin N, Kleinman W, Cox J, Pittman B & Colosimo S (2002) Influence of selenium-enriched yeast supplementation on biomarkers of oxidative damage and hormone status in healthy adult males: a clinical pilot study. *Cancer Epidemiol Biomarkers Prev* 11, 1459-1465.
- (11) Sener G. et al., 2005. Protective effect of beta-glucans against oxidative organ injury in a rat model of sepsis. *Int. Immunopharmacol.* 5(9): 1387-96.

Learning from the mistakes of others

The Top 5 Good Manufacturing Practices Nonconformances

Despite a struggling economy, the US nutritional products industry was estimated to have reached 117 billion in 2010, a 6% growth over the previous year. With 65% of US adults, roughly 150 million people, labeling themselves as supplement users, the opportunity for nutritional product manufacturers has not gone unnoticed. In fact, the number of nutritional supplements on US store shelves has surged from 4,000 to an estimated 75,000 since the Dietary Supplement Health and Education Act, or DSHEA, was passed in 1994.

This fantastic growth and proliferation of new products has created new challenges for the industry in terms of standardizing safety and quality, not to mention the regulatory hurdles that

products containing LALMIN® IMMUNE could bear health claims based on:

"Rich in Selenium, which contributes to the protection of cell constituents from oxidative damage", or:

"A source of Selenium, which contributes to the normal function of the immune system", or:

"High in Vitamin D, which contributes to the normal function of the immune system and healthy inflammatory response."

Conclusion

LALMIN® IMMUNE is formulated using premium quality yeast-based ingredients, recognised for their immunomodulatory and immune support functions (Selenium, Vitamin D), and activated yeast beta-1,3/1,6-glucans, shown to protect the cells from the oxidative stress which can occur during excessive immune response. Altogether these effects have the potential to protect the body. Moreover, as a 100% yeast based ingredient, LALMIN® Immune also represents a source of yeast natural's essential nutrients. Indeed, its processing conditions allow preserving the essential nutrients naturally present in yeast (B Vitamins, minerals, amino acids...), bringing additional nutritive value to this unique formulation which can be used alone or in combination with other health ingredients to formulate natural, EFSA claims bearing dietary supplements or food products destined to the immune health market.

come with a globalized supply chain. To establish quality-control standards for US nutritional products, the US Food and Drug Administration introduced Good Manufacturing Practices (GMP) regulations in 2007. GMPs require that the processes by which nutritional supplement ingredients are produced are safer and free of possible contamination. This includes all aspects of the manufacturing process, from raw material control to finished product release, as well as training of personnel, qualification and validation of testing equipment and methods, product traceability and facility maintenance.

Why does this matter to a Europe-based nutritional product manufacturer? As of last year, every organization that wishes to participate in the booming US nutritional products industry must demonstrate compliance to these GMP regulations. Although European nutritional product manufacturers are no strangers to meeting rigorous regulations, they have the unique challenge of demonstrating compliance to both EU and US regulations, which have few similarities.

The cost of noncompliance is steep and many manufacturers are still struggling to comply as evident by a 42% increase in warning letters issued by the US FDA in 2010. Noncompliance with US GMPs can trigger a number of undesirable outcomes: public warning letters, products labeled as adulterated, seizure by authorities, injunction from manufacturing, and damage to brand and company reputation.

With Warning Letters and other regulatory action hitting an all time high, it's take time to reflect and ask "What can we learn from the mistakes of others?" NSF International, a global provider of

nutritional supplement auditing, training and certification, examined GMP nonconformances over the last two years to help better serve their customers and help them avoid costly mistakes. The following are the top five GMP nonconformances found by FDA inspectors including practical solutions for avoiding the same fate.

#1 GMP Nonconformance: Failed to perform identity testing of incoming ingredients

Nutritional product manufacturers are required to test incoming ingredients and products to confirm their identity. Manufacturers can avoid this nonconformance by performing this type of testing in house or outsourcing to an accredited lab. Relying on a supplier's certificate of analysis (CofA) is not sufficient in meeting this requirement unless you qualify that supplier. We'll get into more detail about supplier qualification in #5.

NSF International provides test-only identity testing services through their accredited laboratories in the US, China and Europe. If a method for a unique ingredient has not been developed, NSF can also provide method development and validation services to ensure a method is followed that is "fit for purpose."



Purchase your copy of NSF's GMP Resource Book <http://bit.ly/p77Ln0>

#2 GMP Nonconformance: Failed to set appropriate Specifications

Manufacturers must establish a specification for any point, step, or stage in the manufacturing process where control is necessary to ensure the quality of the dietary supplement. This includes establishing a specification for identity; purity, strength and composition; limits of contaminants that could lead to adulteration of finished product; labels and packaging. Manufacturers can avoid this nonconformance by working closely with their quality assurance team to set specifications in each of these areas.

NSF-DBA, an NSF International company with over 25 years pharmaceutical and dietary supplements expertise, provides consulting to help companies develop these documents in compliance to GMP regulations.



Sign up for NSF's GMP training classes <http://bit.ly/paDsh6>

#3 GMP Nonconformance: Failed to adopt written procedures

GMPs require manufacturers to demonstrate that proper measures are in place to ensure nutritional supplements are processed and manufactured in a consistent manner and meet quality standards. This includes areas such as personnel, physical plant and grounds, equipment and utensils, production and process controls, holding and distributing, return of dietary supplements, production complaints, records and records keeping.



Find an NSF GMP certified supplier by downloading this free NSF Sourcing Guide. <http://bit.ly/pOvgoc>

Developing and maintaining this level of documentation can be a daunting task. This is why NSF-DBA developed the SOP (Standard Operating Procedure) Template Resource Book. This book provides GMP-compliant SOP and Form templates that can be customized to a specific operation to cover every aspect of production.

#4 GMP Nonconformance: Failed to prepare & follow Master Manufacturing Records (MMR)

Manufacturers can avoid this mistake by preparing and following a written Master Manufacturing Records (MMR) for each unique formulation and batch size of nutritional supplement that they manufacture. MMRs serve as the foundation for which all other documentation is based and helps ensure the uniformity of each batch of nutritional products or ingredients that are produced. MMRs should include information such as the strength, concentration and weight of each nutritional ingredient for each batch size; complete list of components used; the identity and weight of each nutritional ingredient declared on the Supplement Facts label; description of packaging and labeling; description of each stage of production; procedures for sampling; and corrective action plans, among others.

MMRs are the backbone of a good record keeping system. NSF-DBA developed training classes that cover all aspects of GMP compliance, including developing a comprehensive MMR. Training personnel in developing a top-notch MMR is the best way to achieve compliance to this requirement.

#5 GMP Nonconformance: Failed to qualify supplier

The US FDA requires that manufacturers, packagers, labelers, and warehouse and distributors of nutritional supplements to "qualify" each of their vendors but do not provide guidance on how a company is to perform this qualification. Supplier qualification principles also apply to subcontracted services affecting cGMP (manufacturing steps, packaging and labeling, testing and/or calibration services, storage and distribution, etc.). Any supplier qualification program must include two critical components: Clearly defined specifications / requirements for all goods or services being purchased, and; objective evidence to show that your requirements are being consistently fulfilled.

There are a number of ways to qualify a supplier, but a good place to start is with solid training. NSF-DBA offers a course on vendor qualification & auditor training that provides useful tools for setting up a comprehensive and GMP-compliant vendor qualification program. Manufacturers can also rely on third-party GMP certification from a credible organization like NSF International. NSF GMP certification demonstrates that a supplier has been audited and found compliant to US FDA GMPs. To ensure continued compliance, NSF GMP certified facilities are audited twice annually. Sourcing from GMP certified suppliers saves time and costs associated with testing incoming ingredients, and is one of the best ways to satisfy GMP requirements for supplier qualification.

For more information on NSF's GMP resources, visit nsf.org/business/gmp/index.asp or contact David Trosin at dtrosin@nsf.org or +1 734-827-6856.

NSF International's Health Sciences Division offers certification, training, consulting, GMP and GLP testing, R&D and auditing for the pharmaceutical, dietary supplement and medical device industries. The Health Sciences Division includes: NSF Pharmalytica, a GLP & GMP contract laboratory; NSF-DBA, which has more than 25 years of pharmaceutical, dietary supplement, and medical devices training expertise; NSF Reference Standards and NSF Dietary Supplements. NSF International developed the American National Standard for Dietary Supplements (NSF/ANSI 173) and tests and certifies supplements against this standard via the NSF Dietary Supplements Certification Program. NSF's Health Science Division operates globally throughout North America, Europe, Middle East, Africa, Asia and Latin America.



100% YEAST BASED FORMULATION

Complex of 3 natural active ingredients:

- High Bioavailable Selenium
- Natural Vitamin D
- Activated Yeast β-glucans



EFSA 13.1 Health Claims:

"Selenium contributes to the protection of cell constituents from oxidative damage"

"Vitamin D contributes to the normal function of the immune system and healthy inflammatory response"



Health Ingredients

European Sales Office | +45 45 95 08 50
North American Sales Office | +1 514 381 5631

www.lallemmandhi.com