Benefits of the application of a beta glucan formulation APG® 3-6 in animal health care and performance

A bibliographic overview and summary of research notices

2nd, revised edition

Authors:

Prof. Dr. Péter GÁLFI DVM, DSc, head of departmant

Szent István University, Faculty of Veterinary Science,
Department of Pharmacology and Toxicology

Dr. György CSIKÓ, DVM, CSc, deputy head of department

Szent István University, Faculty of Veterinary Science,
Department of Pharmacology and Toxicology

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1. Introduction

Beta glucans are complex carbohydrates of D-glucose monomers linked by β-glycoside bonds. Each type of beta glucan has a unique structure in which glucose molecules are linked together in different ways, giving them physical properties with respect to molecular mass, solubility, viscosity, and three-dimensional configuration. There are several sources of beta glucans, including yeast, bacteria, fungi and cereals, such as oats, barley and rye. The physical structures and biological activity varies with each glucan source.

Beta (β) 1,3-glucan is widely used as a dietary supplement, with well-established stimulating effects on the immune defence system. Since the 1940s, scientists have been evolving the scientific evidence of the remarkable abilities of a simple substance derived from baker's yeast to effectively potentiate and activate the immune response and to work through nutritional potentiating of the immune response in the body's war against cancer, ulcers, radiation exposure, infection, and trauma. Beta glucans are known to possess antitumor and antimicrobial activities by enhancing the host immune function. More than 800 publications have reported that β1,3-glucans, either soluble or particulate, exhibit immunomodulatory properties.

Although, many beta glucans have been observed to demonstrate significant bioactivity *in vivo*, only the 1,3/1,6 linkages of yeast beta glucan are known to spark the greatest degree of biological immuno-activity, making it the most potent immune enhancer. Beta 1,3/1,6 glucan (1,3/1,6 β-glucan) purified from *Saccharomyces cerevisiae* (Baker's yeast) has been widely recognized since the 1960s as an immune enhancer with clinically useful antitumor, haematopoietic and anti-infective properties. Since then numerous studies were conducted to evaluate the effects of *β*-glucan on immune response antibacterial activity and growth performance.

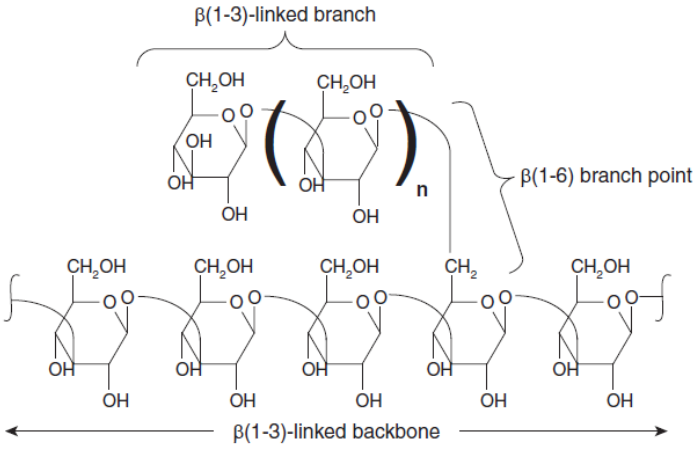
The purpose of this paper is to provide an overview of the role of APG™ 3-6, a purified yeast derived 1,3/1,6 β-glucan in supporting health and performance of animals.

2. Physical and chemical properties

Beta glucans are complex carbohydrates,obtained from different sources, including yeast, bacteria, fungi and cereals, such as oats, barley and rye. Chemical Abstracts Service Registration Number(CASRN) of beta glucan is 9041-22-9 (TOXNET). The physical structures and biological activity varies with each glucan source. The unique structure includes the frequency of side chains, the side chain lengths, and the ratio of different glycoside linkages that contribute to primary, secondary, and tertiary structure.

The basic structure, found in bacterial beta glucans, consists of linear glucose polymers with β(1,3) linkages. Some, such as oat and barley glucans, are primarily linear, with large regions of β(1,4) linkages separating shorter stretches of β (1,3) structures. Others (e.g., mushroom) have short β(1,6)-linked branches coming off of the β (1,3) backbone.

Yeast beta glucans have β(1,6) branches that are further elaborated with additional β(1,3) regions. These seemingly minor structural differences can have large implications for the activity of the beta glucan.



For example, differences in the length of the polysaccharide chain, the extent of branching and the length of those branches can result in the difference between material extractable by hot water (mushroom beta glucan) and an insoluble cell wall component (yeast beta glucan) **(Yan J. et al., 2005)**.

Baker's Yeast beta glucan, (1-3),(1-6)-*/3-d*-glucan, poly-(1-6)-/3-d-glucopyranosyl-(1,3)-*/3-d* glucopyranose, is a light beige to tan fine powder. This ingredient is the result of the fermentation of food-grade baker's yeast *(Saccharomyces cerevisiae)* and later lysis through a thermal process. The cell wall

component is separated from the yeast extract using centrifugation. Then, the cell wall isolate undergoes a caustic treatment to strip the mannosylated cell wall proteins that are linked to the cell wall and to remove the residual cellular lipids. After that, the isolate undergoes an acid treatment, which results in the removal of most of the chitin. Lastly, the yeast wall slurry undergoes flash sterilization, followed by pH adjustment steps, which results in the final dry product. It is comprised mainly of (1,3)/(1,6) branched glucan polymers, and trace amounts of protein and lipid. Small amounts of 3-(1,6)-glucan and chitin are also expected to be present in the final product **(FCC 7, 2010)**.

According to the **Biothera's** declarations **APG® 3-6** water-insoluble (WGP) gluco polysaccharide powder is **beta 1,3/1,6 glucan** isolated from the cell wall of baker's yeast (*Saccharomyces cerevisiae*). The product consists of only purified yeast cell wall isolate and contains no additional ingredients or added excipients, fillers, binding agents or flow agents. AGP 3-6 is a powder typically composed of ≥ 70% yeast derived gluco polysaccharide (beta 1,3/1,6), with ≤ 10% glycogen and ≤ 8% moisture. AGP 3-6 powder does not support microbial growth. The shelf life of AGP 3-6 was established as ≥5 years, stored in closed, sealed packages in a dry controlled environment (21 C° and 50% RH).

3. Mechanism of action

Vetvicka V. et al., (2002) supposed that through specific interactions between the orally applied β1,3-glucan and β1,3-glucan receptors on M-cells within Peyer's patches in the intestinal mucosa that a systemic signal provided by cytokines is elicited by the gut-associated lymphatic system that stimulates the innate immune systemcomponents (macrophages, neutrophils, and NK cells) to a higher functional level, increasing the first line of host defense mechanisms. For these experiments we focused on three important cytokines, IL-2, IFN-γ, and TNF-α. All of these cytokines play an important role not only in physiological processes, but also in bioregulation of host defense reactions. IL-2 is a cytokine produced by activated CD4 and some CD8 T lymphocytes. In addition to being the major T cell growth factor, IL-2 also stimulates: growth and differentiation of cytotoxic T cell precursors, NK cells, differentiation of activated human B-lymphocytes, and activation of monocytes. TNF-α is a pleiotropic cytokine secreted primarily by monocytes/macrophages and T lymphocytes, respectively. TNF-α is the principal mediator of natural immunity against gram-negative bacteria and a key mediator of inflammatory responses and septic shock. IFN-γ, sometimes called immune interferon, is produced mainly by T lymphocytes as a result of antigenic or mitogenic stimulation. The activities of IFN-γ are many, including induction of MHC expression, macrophage activation, and effects on the differentiation of lymphocytes.

Orally consumed whole glucan particles are uptaken through Peyer's Patches in the small intestine and subsequently engulfed by macrophages. The Peyer's Patches are a specialized immune tissue in the intestine that sample ingested material to prepare the body for potential pathogenic challenges. It has been shown that yeast β-glucan that is consumed with the diet is recognized as a carbohydrate that is present on pathogens but not animal cells and consequently "sampled". **Hong et al.(2004)** demonstrated that at the Peyer's patch, M-cells transport yeast β-glucan across the epithelial lining of the small intestine and deliver the material to macrophage cells. The macrophage cells ingest the glucan particle through the process of phagocytosis. Macrophage cells transport the engulfed glucan particle to

the various immune organs of the lymphatic system including, bone marrow, spleen and thymus. The glucan particle is digested by the macrophage over a period of days at these sites. The smaller soluble, macrophagedigested β-glucan (fragment) is the bioactive form. It is released by the macrophage and binds to an abundant species of white blood cells known as neutrophils by attaching to the complement receptor 3 (CR3). Neutrophils are the key immune cell for the clearing of pathogenic cells. These **neutrophils are now "primed" to respond to an immune challenge such as a bacterial infection**. This leads to the next step in understanding the mode of action, the role of the primed neutrophils and antibodies.

Soluble low molecular weight yeast glucans can prime CR3 directly to trigger neutrophil degranulation after receipt of the second signal from iC3b bound to the target cell surface. However, the small molecular weight R-glucans were shown to be rapidly excreted by the kidneys, thus limiting their bioactivity and clinical utility. In contrast, an orally administrated particulate WGP β-glucan (this is the human counterpart of APG 3-6) is expected to have a longer half-life *in vivo* and is highly desirable for its clinical utility. It was hypothesised that particulate β-glucans are processedand digested into small fragments that can prime CR3. Indeed, this hypothesis is supported by recent studies in mice **(Yan J. et al., 2005)**.

The trafficking process of WGP yeast β-glucan can be divided into three steps. **The first step is the phagocytosis phase**. Orally fed WGP is ingested by gastrointestinal macrophages that transport them to lymphoid organs. Within 3 days of daily oral administration of WGPs, macrophages in the spleen and lymph nodes contain WGPs. After 4 days, WGP yeast β-glucans are observed in the bone marrow. The uptake of WGP by macrophages does not require CR3, as characterised by the similar percentage of WGP-containing macrophages in wild-type versus $CR3^{-/-}$mice. The CR3-independent uptake of orally administered WGPsmay illustrate a potential role for other particulate β-glucan receptors, including Dectin-1, in the phagocytosis phase. Dectin-1 is a C-type lectin with an immunoreceptor tyrosine-based activation motif, and some Dectin-1-mediated functions have been observed to be MyD88-

dependent. Dectin-1 is expressed on most myeloid cells and mediates phagocytosis of non-opsonised particles containing limited bioavailable β-glucan (e.g., Zymosan) and some live pathogenic yeast.

The second step is the processing and priming phase. WGP-glucans are digested to release small fragments that are concentrated at the edges of the cytoplasm near the membrane. In vitro experiments conducted with cultures of the macrophage cell line J774 have demonstrated that the macrophages have begun breaking down the particles at day 3. Complete macrophage degradation of all visible cytoplasmic WGPs requires >13 days, *in vitro*. The soluble, biologically active components of WGP are released into the culture medium and can be measured using a (1,3)-glucan-specific bioassay. Moreover, these small fragments of WGP yeast glucans are able to prime CR3 and kill iC3b-opsonised tumour cells. The processing of WGP by macrophages is presumably through an oxidative-dependent pathway; as macrophages do not have glucanase. WGP-glucans can also stimulate macrophages to secrete cytokines such as TNF-α and IL1, -12 and -6. The production of TNF-α and IL-12 is CR3-dependent, whereas the secretion of IL-6 is MyD88 pathway-dependent. IL-1β secretion is partially dependent on the CR3 pathway, but completely dependent on the MyD88 pathway (J. Yan et al., unpublished data). These pro-inflammatory cytokines can potentially enhance the activation of adaptive immunity such as antigen presentation and T cell activation. Thus, the administration of WGP glucan links the activation of both innate and adaptive immunity.

The final step is the effector phase. The β-glucan-primed neutrophils are chemoattracted by leukotriene B_4 (LTB_4) released from tumour endothelial cells (or to c3a and c5a at infection sites), migrate into the tumour milieu and engage iC3b-opsonised tumour cells for cytotoxicity. *In vivo* experimental evidence indicated that C5a derived from complement activation initiates a cascade of chemoattractants and that the C5a-dependent chemotaxis of neutrophils is dependent on signal amplification by LTB_4**(Yan J. et al., 2005)**.

Binding of β-glucan to specific receptors (either CR3 or Dectin-1) activates macrophages. The activation consists of several interconnected processes including increased chemokinesis, chemotaxis, migration of macrophages, degranulation leading to increased expression of adhesive molecules, and adhesion to the endothelium. In addition, β-glucan binding triggers intracellular processes, characterized by the respiratory burst after phagocytosis of invading cells (formation of reactive oxygen species and free radicals), the increase of content and activity of hydrolytic enzymes, and signaling processes leading to activation of other cells and secretion of cytokines **(Vetvicka V. et al., 2008).**

The binding of the WGPfragment (this is the same active material from the same proprietary strain that is used to manufacture APG 3-6) to specific receptors on the surface of innate immune cells is the trigger which primes these cells to quickly respond to foreign challenges in a variety of ways.

Enhanced Chemotaxis

Neutrophils which bound WGP to the CR3 receptor were found to migrate more quickly towards sources of the complement fragment C5a which is commonly produced at a site of infection **(Tsikitis V.L. et. al., 2004)**. This effect was blocked when neutrophils were pretreated with an antibody that specifically blocked the CD11b subunit on CR3 that binds WGP. This clearly demonstrated that the binding of WGP to the CR3 receptor site on neutrophils specifically increased their ability to navigate towards the site of a foreign challenge. **Li B. et. al. (2007)** also reported that in vitro, neutrophils primed by binding WGP had an increased rate of chemotaxis toward opsinized targets.

Enhanced Phagocytosis

Waksull E.D. et. al. (1998) reported increased phagocytosis of *S. aureus* in vitro with neutrophils treated with a soluble version of WGP. In an independent 2003 Phase I human clinical study sponsored by Biothera, human volunteers were dosed for 10

consecutive days with one 250 milligram capsule of Wellmune WGP (Biophage Research 2003, unpublished data). The subjects were monitored for 30 days during which clinical observations were recorded. The Wellmune WGP was safe and well tolerated, as evidenced by the lack of significant changes in key blood and liver enzymes. Wellmune WGP significantly increased phagocytic capacity, the ability of the innate immune cells to eat and destroy foreign intruders. After 10 days of treatment, Wellmune WGP had increased the percentage of immune cells able to phagocytose one particle from 63.8% to 83.2% ($P<0.05$). The number of highly phagocytic cells increased from 37.3% to more than 50% ($P<0.05$). These results show that taking Wellmune WGP enhanced the human immune system to defend the body against a challenge. Wellmune WGP increased the plasma cytokine TNF-α, which plays an important role in regulating the body's immune response. There was no significant increase in cytokine IL-1, which can cause fever, chills and muscle aches associated with other immune- enhancing supplements.

Enhanced Oxidative Burst

Oxidative burst (aka respiratory burst or degranulating cytotoxicity) is the second killing mechanism used by innate immune cells against foreign challenges. In this mechanism macrophages and neutrophils undergo a transient increase in oxygen consumption that causes the production and release of cytotoxic oxygen metabolites (e.g. superoxide ion oxygen and hydrogen peroxide) **(Janeway C.A. et al., 2005)**. Binding of the active WGP fragment to the CD11b subunit on CR3 has been shown to induce enhanced oxidative burst against hyphal forms of yeast (**Lavigne L.M. et. al., 2006**). Treatment with an antibody specific for the CD11b/CD18 receptor blocked this activation. Numerous studies in murine tumor models have shown that priming neutrophils with WGP induces a cytotoxic response toward targets that were opsonized by a monoclonal antibody (**Hong F. etal., 2003; Vetvicka V. et al., 1996; Lavigne L.M. et al., 2006**).

4. Safety aspects

Single dose and repeated dose toxicity

Babicek K. et al. (2007) investigated the toxicity of WGP® 3–6 (former brand name of Wellmune WGP that is compositionally similar to AGP 3-6), a yeast-derived b-glucan ingredient, during single-dose acute and sub-chronic toxicity studies in rats. For the acute study, Fisher-344 rats were administered WGP® 3–6 via gavage at a dose of 2000 mg/kg body weight, and any evidence of toxicity was monitored over a 14-day period. WGP® 3–6 was well tolerated, indicating that the LD50 value is greater than 2000 mg/kg body weight. For the sub-chronic study, Fisher-344 rats (10/sex/group) were randomly allocated to receive daily gavage treatment with WGP® 3–6 at doses of 0, 2, 33.3, or 100 mg/kg body weight. Control and high-dose satellite recovery groups of each sex also were included. Full toxicological monitoring and endpoint investigations were performed throughout and upon completion of the study. No negative effects on animal weights or food consumption attributable to WGP® 3–6 were evident at any dose. In addition, no mortality, clinical pathology, functional/behavioral, microscopic, or gross observations indicating toxicity were observed. Sporadic changes in some biochemical and hematological parameters were observed; however, since the effects were within the physiological ranges in historical controls, were not dose-responsive, or were not observed in both sexes, they were determined to be of no toxicological significance. In conclusion, no adverse or toxic effects were observed after subchronic oral administration of 2, 33.3, or 100 mg/kg body weight/day of WGP® 3–6 in Fisher-344 rats, and therefore, a no observed adverse effect level (NOAEL) of 100 mg/kg body weight/day, the highest dose tested, was determined.

Genotoxicity, mutagenicity

The beta glucans were tested for their potential genotoxicity. The IP applied 100 mg/kg b.w. dose in CD-1 male mouse did not cause *in vivo*chromosomal aberrations neither in bone marrow nor in spermatogonial cells **(TOXNET)**.

Summary of additional safety studies (based on Biothera's non published data)

The results from the Ames test with APG 3-6 showed no abnormal results. The count of colonies in APG 3-6-treatments was less than 2 times that in controls, and no dose response relationship was determined. It was concluded that APG 3-6 is not mutagenic in the tested *Salmonella typhimurium* bacteria.

The results of the micronucleus assay were normal as expected. The ratio of PCE/RBC in the treatment (both APG 3-6 and cyclophosphamide) groups was not less than 20% of negative control, indicating no cytotoxicity occurred under the current experimental conditions. The incidence of micronuclei in bone marrow PCE was significantly higher in both genders following cyclophosphamide treatment than that in the negative control and APG 3-6 treated groups. No significant differences were detected between the negative control and APG 3-6 treated groups. Results indicate that APG 3-6 does not cause chromosomal aberration in mouse bone marrow PCE.

The results of sperm malformation were normal as expected. Cyclophosphamide (positive control) caused significant increase in sperm malformation. The incidence of sperm malformation was comparable between the negative control and APG 3-6 treatment. Results demonstrate that APG 3-6 does not cause sperm malformation under the test conditions.

The potential of APG 3-6 to cause genotoxicity was investigated in the Ames assay, micronucleus test in bone marrow of mice following oral administration by gavage, and sperm malformation in mice following oral administration by gavage. Results demonstrate that treatment with APG 3-6 does not increase in gene mutation (Ames assay), chromosomal aberration (micronucleus assay), or sperm malformation. Therefore, it is concluded that APG 3-6 is not mutagenic, or genotoxic when given orally.

Other possible toxic effects

Beta-1,3-glucan is an immunologically-active glucose polymer that occurs in the cell wall of mould fungi. It is important agent causing the development of pulmonary diseases, both of an inflammatory and an allergic nature. β-1,3-glucan can induce Th1 as well as Th2-driven immune responses. Recently, it was demonstrated that automobiles are potentially a significant source of β-1,3-glucan and endotoxin exposure that could be of importance for asthmatics **(Dutkiewicz J. et al., 2011**).

Purity of the product is vital, since protein contaminants, as seen in the earlier-used source Zymosan, can cause untoward immune reactions **(Blaylock L.R. 2002)**.

Hence APG 3-6 product consists of only purified yeast cell wall isolate, and thus the route of application is oral, this kind of side-effect has minimum relevance.

5. Clinical uses

Antitumor activity

Preclinical studies

Oral administration of Beta Glucan increased the production of three important cytokines (IL-2, IFN-γ, and TNF-α) and inhibited growth of cancer cells *in vivo.* Beta Glucan treatment reduces the threat of cancer, slowing down the progression of tumor growth in a preclinical colon cancer model. This observation extends the large body of preclinical and clinical work done in Japan demonstrating the oral anti-tumor activity of mushroom β1,3-glucans to yeast β1,3-glucan. These published studies have demonstrated that β1,3-glucan immunotherapy leads to the activation of the innate immune cells (macrophages, neutrophils (PMN) and natural killer (NK) cells), the stimulation of tumoricidal activities, production of cytokines, and the generation of enhanced cell-mediated responses. Suzuki and colleagues have reported the stimulation of activated macrophages by the administration of SSG22 and NK-type lymphokine-activated killer cells by the combined administration of lentinan and IL-2. The stimulation of tumoricidal activities in PMN by a linear bacterial β1,3-glucan has also been reported. A number of clinical studies have demonstrated synergy between oral β1,3-glucan immunotherapy, and traditional radiation and chemotherapeutic cancer treatment options (**Vetvicka V. et al., 2002**).

The data presented in the review**of Yan J. et al. (2005)** suggest that the complement system can be manipulated in such a way that it can substantially contribute to maximise the therapeutic efficacyof antitumor mAbs. β-Glucan primed CR3-dependent cytotoxicity represents a novel mechanism by linking innateimmune CRY cells with adaptive immunity to eliminateiC3b-opsonised tumour cells. The experimental animal observations and a few clinical studies have demonstrated a significant therapeutic efficacy in murine breast, liver metastasis,lung, lymphoma tumour models and paediatric neuroblastoma patients. The demonstration of cellular

and molecularmechanisms of action for the combined WGP β-glucan withantitumor mAbs not only improves the prospect of immunotherapeutic treatment, but also increases the number of mAbsthat can be useful in the clinic. All of the antitumor mAbsthat are able to activate complement can be used in combination with beta-glucan for tumour therapy. Another exciting prospect is the combining of β-glucan with existing tumourvaccine strategies, as most tumour vaccines elicit humoral responses in addition to CTL responses, although antibodieshave an unappreciable effect in vaccine-alone models.

Preclinical animal studies havedemonstrated that strategies to improve the chemotaxis ofneutrophils into the tumour microenvironment in the settingof combined WGP and antitumour mAb immunotherapy should improve therapeutic efficacy for this therapy.Traffickingof immune cells, including macrophages, dendritic cells andCTLs, into the tumour microenvironment has proven to bedifficult. However, mechanistic studies demonstratethat C5a is a potent chemoattractant for neutrophil recruitment.It is feasible to combine β-glucan-mediatedtumour therapy with recombinant C5a (rC5a), synthesisedC5a agonist or cytokines to increase neutrophil recruitment,such as granulocyte colony-stimulating factor. Nevertheless,the combined β-glucan withantitumour mAb immunotherapy has demonstrated promising results in the therapeutic setting and might be curative for the cancer patientstreated with this approach.

Antimicrobial activity

Beta Glucan shows promise as a prophylactic treatment to support the immune system and reduce the risk of anthrax infection. Orallyadministered Beta Glucan has strong anthrax-protective effects. Oral Beta Glucan treatment significantly increased the number of surviving animals as well as prolonged survival time of lethally infected animals. Dose ranging studies to date have demonstrated that daily prophylactic doses of 2-20 mg/kg Beta Glucan provides a maximal anthrax-protective effect in mice **(Vetvicka V. et al., 2002)**.

Research conducted on the synergy of β-glucan with antibiotics has demonstrateda strong synergism between antibioticsand Biothera's β-Glucan. In one studyit was demonstrated that glucan and a common antibiotic increased the ability of guinea pigs to resist septicinfection by antibiotic resistant bacteria.An alternative interpretation of the datasuggests that use of APG 3-6 allows forthe use of lower doses of antibiotic tomaintain animal health. In this study PGG-glucan and cefazolin was administered, alone and in combination, to guinea pigs inoculated with isolates of staphylococci. Guinea pigs receiving both PGG-glucan and cefazolin had 50% infective doses that were 8- to 20-fold higher than those obtained with cefazolin alone and 100- to 200-fold higher than those obtained with PGG-glucan alone. PGG-glucan and cefazolin are synergistic in their ability to prevent staphylococcal wound infection(**Kaiser A.B. et al.,1998**).

APG 3-6 wasadministered to mice that weresubsequently infected with high doses ofpathogenic bacteria (*E. coli* and *S. aureus*) (unpublished study of Biothera). Because the β-glucan actssynergistically with antibodies (seemode of action), a septic infection wasprevented in the treatment group vs. thecontrol group, all of which died ofbacterial sepsis.

The initial cold/flu study was completed using normal healthy subjects living in a standard community environment **(Feldman S.H.I. et. al., 2009)**. This was a 12-week, randomized, double blind, placebo-controlled, parallel-group trial comparing Wellmune WGP®(counterpart of APG 3-6) beta-glucanand placebo, in a healthy population during cold/flu season. Study duration was 90 days and included evaluation by medical staff within 24 hours of cold onset. Although there were no differences in the incidence of symptomatic respiratory infections (SRI's), no subjects in the WGP group missed work or school due to colds, while subjects with colds in the placebo group missed an average of 1.38 days and had a significantly lower average fever score. General Health Component Summary score was improved significantly in the Wellmune group after 90 days as compared to baseline

A recently completed clinical study with Wellmune WGP was a 90 day, placebo-controlled, double-blinded design that evaluated the effects on symptoms

associated with upper-respiratory tract infections (URTIs) and psychological well-being **(Talbott S.M. et.al., 2010**). One hundred twenty-two healthy subjects (32 men, 90 women) aging in range from 18-74 (mean age was 38 ± 12 years) self-administered a placebo or 250 mg daily dose of Wellmune WGP during a twelve-week trial period. During the course of the twelve-week reporting period of this study, subjects in the treatment group (250 mg WELLMUNE WGP per day) reported fewer URTI symptoms compared to Placebo (7.3% versus 17.3%, i.e. 42% fewer URTI symptoms), better overall well-being (Global Mood State), and superior mental/physical energy levels (Vigor) based on the POMS survey.

Allergy control

Sato H. et al. (2012)investigated the effects of water-soluble low molecular-weight β -(1,3-1,6) D-glucan isolated from*Aureobasidiune pullulans* LA1 strain black yeast(LMW-β-glucan) on mast cell-mediated anaphylacticreactions. Although it is known that LMW-β-glucan hasanti-tumor, anti-metastatic and anti-stress effects, the roles of LMW-β-glucan in immediate-type allergicreactions have not been fully investigated. Authors examinedwhether LMW-β-glucan could inhibit mast cell degranulation and passive cutaneous anaphylaxis (PCA).LMW-β-glucan dose-dependently inhibited the degranulation of both rat basophilic leukemia (RBL-21I3) andcultured mast cells (CMCs) activated by calciumionophore A23187 or IgE. However, LMW-β-glucanhad no cytotoxicity towards RBL-2113 cells and CMGs.Furthermore, orally administered LMW-β-glucan inhibited the IgE-induced PCA reaction in mice. Theseresults show LMW-β-glucan to be a possible compoundfor the effective therapeutic treatment of allergic diseases.

Wellmune was found to modulate the immune system when less of an immune response was needed (**Talbott S.M. et al., 2012**).
This was concluded based on the results of a randomized, placebo-controlled, double-blind study, which compared the effects of daily supplementation for 4 week with 250 mg Wellmune WGP® β-1,3/1,6- Glucan (WGP) or with placebo 250 mg/day (rice flour) on physical and psychologicalhealth attributes of self-described

"moderate" ragweed allergy sufferers.Compared with placebo, WGP reduced total allergy symptoms(28%), symptom severity (52%), and symptom rating on the VAS (Visual Analog Scale) (37%)(P<0.05), but had no effect on serum IgE levels.As measured by the Profile of Mood States,WGP increased participants' rating of vigor (10%), but reduced tension (34%),depression (45%), anger (41%), fatigue (38%), and confusion (34%)(P<0.05). Study participants given WGP reported increased physical health(11%), energy (19%), and emotional well-being (7%) compared with study participantsgiven the placebo (RAND SF-36 Medical Outcomes Study). The WGPgroup also reported decreased sleep problems (53%), reduced nasal symptoms(59%), eye symptoms (57%), non-nasal symptoms (50%), activities (53%),emotions (57%), and improved quality of life (QOL) (56%), as well asimproved global mood state (13%). Supplementation with WGP for 4 weeksimproved allergy symptoms, overall physical health, and emotional well-beingcompared with placebo in self-described "moderate" ragweed allergy sufferersduring ragweed allergy season.

Other biological activities

Immune system

Sorocinova J. et al., (2011) studied specific immune response to vaccine Enterisol against *Lawsonia intracellularis* in weaned piglets from sows long-term fed an immunomodulating food additive (IMUNOL P) based on **beta-glucane** and compared with positive and negative control animals. Evaluation within the groups was focused on the selected production indices of sows and piglets, selected indices of blood immune profile (CIg, IMA, SI), and blood serum levels of specific antibodies against Lawsonia intracellularis. In the experimental group of sows, the results showed higher numbers of piglets in the litter, comparable numbers of dead-born and died piglets, as well as birth body weight. However, the average daily weight gain

after the weaning was significantly ($p<0.05$) higher in the group of vaccinated piglets, as well as in piglets from sows receiving **beta-glucane**. The same tendency was recorded for the average values of CIg. The immunostimulation effect of the **beta-glucane** based additive combined with vaccination significantly ($p<0.01$) manifested by the changes in the index of leukocyte metabolic activity (IMA) and less markedly in lymphocyte stimulation index (SI), when higher average values were recorded in the groups with combination of **beta-glucane**, as well as in the groups with the vaccination only. The least favourable effects were recorded in the group without both **beta-glucane** and vaccination (A-G; v). The effect of Enterisol oral vaccine was confirmed by estimation of specific antibodies by ELISA test. At both samplings, the groups of non-vaccinated weanlings (A a D) showed minimum levels of antibodies compared with both vaccinated groups (B a C). In the vaccinated groups, highly positive findings were recorded without a significant difference between **beta-glucane** stimulated (C+G; v+) and non-stimulated (B-G; v+) groups.

Depending on various factors, dairy cows, as well as calves are exposed to immunosuppressive conditions. An effort to control consequent health disorders through immunomodulation might use natural polysaccharides including beta glucane. Several authors reported favourable immunomodulation effects of beta glucane in animals, e. g. in laboratory animals, dogs and cats, swine, horses. Based on study of **Reichel P. et al. (2011)** immunomodulating effect of energetic food admixture based on glycerol and containing beta-1,3/1,6-D-glucane (IMUNOL H) was manifested by changes in concentrations of immunoglobulins and their classes in the blood serum of cows and calves. These concentrations in the experimental animals showed significantly ($p< 0.01$) favourable tendency during post-partal period than in the control animals with recorded immunosuppression.

Uchiyama H. et al. (2012) examined the immunomodulatory activity of the β–(1/3),(1/6)-D-glucan extracellularly produced by *Aureobasidium pullulans*, and its use for health supplements. To examine the effects of oral administration of the β–(1/3),(1/6)-D-glucan to domestic animals, a small scale study was conducted using Holstein cows and newborn Japanese Black calves.Holstein cows of which somatic

cell count was less than 3 x 10^5/ml were orally administered with orwithout the β–(1/3),(1/6)-D-glucan-enriched *A. pullulans* cultured fluid (AP-CF) for 3 months, and the propertiesof milk and serum cytokine expression were monitored. Somatic cell counts were not significantly changed by oraladministration of AP-CF, whereas the concentration of solid non fat in the milk tended to increase in the AP-CFadministered cows. The results of cytokine expression analysis in the serum using ELISA indicate that theexpressions of tumor necrosis factor-α (TNF-α) and interleukin (IL)-6 in all cows which were orally administered withAP-CF became slightly lower than that of control cows after the two-month treatment. On the other hand, IL-8expression tended to indicate a moderately higher level in all treated cows after the three-month administration ofAP-CF in comparison with that of the control cows. Peripartum Japanese Black beef cows and their newborn calveswere orally administered with AP-CF, and bacterial flora in the intestines of the calves were analyzed by T-RFLP(terminal restriction fragment length polymorphism). The results suggest that bacterial flora are tendentiouslychanged by oral administration of AP-CF.

Several university and private commercial studied have evaluated the benefits of APG 3-6 on performance of calves receiving milk replacer. A critically important aspect of calf management is the early feeding regimen that typically depends on quality milk replacer to maintain animal health. In several studies completed by the USDA-ARS and at Purdue University in the U.S., APG 3-6 has been demonstrated to increase general immune health as part of a diet that includes milk replacer. In one study researchers reported the following: “The objective of this study was to examine the innate immune response in peripheral blood and tissues, after 21 d of oral feeding of beta-glucan with or without ascorbic acid. Results indicate, that feeding betaglucan results in an up regulation of innate cell surface proteins associated with immune activation, and is further modulated by ascorbic acid**(Cary D.C. et al., 2005).**

A second study**(Eicker S.D. et al., 2005)**by the same research group measured the impact of various immune biomarkers. The general observation was that APG 3-6

improved biomarkers that are related to the immune health of milk-replacer fed calves.

It is important to note that a leading producer of milk-replacer products in the U.S. has recently launched a major product containing Biothera's APG 3-6. The U.S. partner has conducted multiple animal studies at their research facilities and concluded that APG 3-6 provides 5 health and growth performance benefits (results to date are unpublished and confidential)

Wound healing

Vetvicka V. et al. (2011)evaluated the possibility whether individual β(1-3)-D-glucans will have an activity in less studied areas suchas adipogenesis and inflammation. The results showed thatof the tested β(1-3)-D-glucans, yeast-derived insolubleGlucan #300, strongly inhibited adipogenic differentiation,supported wound healing and significantly lowered skinirritation.To allow investigation of the effect of β(1-3)-D-glucanon the wound healing process, simple scratch-wound modelwas used. The effect of exogenously added β(1-3)-Dglucanshas been tested on the regeneration of monolayer.We have seen the remarkable influence of the presence ofthree types of β(1-3)-D-glucan on the regeneration ofmonolayer. The remaining β(1-3)-D-glucans were significantlyless active. Taken together, study showed thatwith respect to naturalβ(1-3)-D-glucans, there is a clearyes-or-no effect suggesting that highly purified and highlyactive β(1-3)-D-glucans will have pleiotropic biologicalimpact, whereas poorly isolated and/or less active β(1-3)-D-glucans will have only mediocre properties.

Neurologic effects

Six randomized, double-blind, placebo-controlled clinical studies were identified in which consumption of a blend of plant-derived polysaccharides showed positive effects on cognitive function and mood in healthy adults. A separate controlled clinical study observed improvements in well-being with ingestion of a yeast beta-glucan. Numerous animal and *in vitro* studies have demonstrated the

ability of individual saccharide compounds and polysaccharide-rich extracts to modify behavior, enhance synaptic plasticity, and provide neuroprotective effects.
Although the mechanisms by which exogenous saccharides can influence brain function are not well understood at this time, the literature suggests that certain naturally occurring compounds and polysaccharide-rich extracts show promise, when taken orally, in supporting neurologic health and function. Additional well-controlled clinical studies on larger populations are necessary, however, before specific recommendations can be made **(Nelson E. D. et al., 2012)**.

Gastro intestinal and metabolic effects

Yeasts and their glycan components can have a beneficial or adverse effect on intestinal inflammation. The non-pathogenic yeastis widely prescribed for thetreatment of antibiotic-induced gastrointestinal disorders and *Clostridium difficile*-associated enteropathies, has been shown to be an alternative approach to counterbalance the equilibrium of the intestinal microflora and modulate the innate immune defence. In the study of **Jawhara S. et al. (2012)** mice received a single oral challenge of *Candida albicans* and were then given 1.5% dextran-sulphatesodium (DSS) for 2 weeks followed by a 3-day restitution period. *S. cerevisiae* strains (Sb, Sc1 to Sc4), as well as mannoprotein (MP) and beta glucan crude fractions prepared from Sc2 and highly purified b-glucans prepared from *C. albicans* were used in this curative model, starting 3 days after *C. albicans* challenge. Mice were assessed for the clinical, histological and inflammatory responses related to DSS administration. Strain Sc1-1 gave the same level of protection against *C. albicans* as Sb when assessed by mortality, clinical scores, colonization levels, reduction of TNFa and increase in IL-10 transcription. When Sc1-1 was compared with the other *S. cerevisiae* strains, the preparation process had a strong influence on biological activity. Interestingly, some *S. cerevisiae* strains dramatically increased mortality and clinical scores. Strain Sc4 and MP fraction favoured *C. albicans* colonization and inflammation, whereas b-glucan fraction was protective against both. Surprisingly, purified beta glucans from *C. albicans* had the same protective effect. Thus, some yeasts appear to be strong modulators of intestinal inflammation. These effects are

dependent on the strain, species, preparation process and cell wall fraction. It was striking that beta glucan fractions or pure beta glucans from *C. albicans* displayed the most potent antiinflammatory effect in the DSS model.

The fermentability of β-glucans and their ability to form highly viscous solutions in the human gut may constitute an additional mechanism of action or part of the basis of their health benefits. Consequently, the applicability of β-glucan as a food ingredient is being widely considered with the dual purposes of increasing the fiber content of food products and enhancing their health properties. It is clear that *β*-glucan is an important food component in the modulation of metabolic dysregulations associated with the metabolic syndrome. However, dose, form, molecular weight, and the carrier food of *β*-glucan shape its effect. The physiological effects of *β*-glucan are mainly attributed to its physicochemical and structural characteristics interacting with the gastrointestinal tract, as reflected by its ability to generate viscous solutions at low concentrations in the upper part of the gastrointestinal tract and to undergo fermentation in the colon **(El Khoury D. et al., 2011).**

*Beta g*lucans extracted from barley, which mainly contains *β*-(1,3-1,4)-d-glucan, are used extensively as supplements and foodadditives due to their wide biologic activities, including a reduction in blood lipid level. In this study, the antioxidant activityof *β*-glucan was examined to assess potential new benefits associated with *β*-glucan, because oxidative stress is considered oneof the primary causal factors for various diseases and aging. *β*-Glucan extracted from barley was found to possess significantantioxidant activity. The amount of antioxidant activity was influenced by different physiologic properties (e.g., structure andmolecular size) of *β*-glucan, which varied depending on the source and extraction method used. The antioxidant activity of *β*-glucan was significantly higher than that of various polymers that are used as food additives. These results indicate that *β*-glucanhas promise as a polymeric excipient for supplement and food additive with antioxidant and other benefits, which may contributeto enhancing health and beauty**(Kofuji K. et al., 2012).**

There is increasing global acceptance that viscous soluble fibers lower serum LDL cholesterol (LDL-C),but most evidence for this comes from studies in Caucasians. To see if oat b-glucan lowers LDL-C in Caucasiansand non-Caucasians we conducted a post-hoc analysis of the results of a randomized, controlled, double-blind,multi-center clinical trial whose primary aim was to determine if molecular-weight (MW) influenced the LDL-Cloweringeffect of oat beta-glucan.

Wolever T.M.S. et al. (2011) studied Caucasian and non-Caucasian subjects with LDL-C-C ≥ 3.0 and ≤ 5.0 mmol/L (n = 786 screened, n = 400ineligible, n = 19 refused, n = 367 randomized, n = 345 completed, n = 1 excluded for missing ethnicity). Patients wererandomly assigned to consume cereal containing wheat-fiber (Control, n = 74:13 Caucasian:non-Caucasian) or 3 ghigh-MW (3H, 2,250,000 g/mol, n = 67:19), 4 g medium-MW (4 M, 850,000 g/mol, n = 50:17), 3 g medium-MW (3M,530,000 g/mol, n = 54:9) or 4 g low-MW (4 L, 210,000 g/mol, n = 51:12) oat beta glucan daily for 4 weeks. LDL-C after4 weeks was influenced by baseline LDL-C ($p<0.001$) and treatment ($p = 0.003$), but not ethnicity ($p = 0.74$). In allsubjects, compared to control, 3 H, 4 M and 3 M reduced LDL-C significantly by 4.8 to 6.5%, but 4 L had no effect.Compared to control, the bioactive oat beta glucan treatments (3H, 4M and 3M) reduced LDL-C by a combined mean(95% CI) of 0.18 (0.06, 0.31) mmol/L (4.8%, n = 171, $p = 0.004$) in Caucasians, a value not significantly different fromthe 0.37 (0.09, 0.65) mmol/L (10.3%, n = 45, $p = 0.008$) reduction in non-Caucasians. Authors conclude that oat beta glucan reduces LDL-C in both Caucasians and non-Caucasians; there wasinsufficient power to determine if the magnitude of LDL-C-lowering differed by ethnicity.

Growth performance

Two experiments were conducted to evaluate the efficacy of *β*-glucan (Glucagen, Enbiotec Company, Seoul, Korea) on growth performance, nutrient digestibility, and immunity in weanling pigs (**Hahn T.W. et al., 2006**). In Exp. 1, 210 weanling pigs (6.38 ± 0.92 kg of BW) were fed dietary *β*-glucan (0, 0.01, 0.02, 0.03, or 0.04%) for 5 wk. In Exp. 2, 168 pigs (6.18 ± 1.31 kg of BW) were fed no *β*-glucan or antibiotics (T1), 0.02% *β*-glucan (T2), only antibiotics (T3), or 0.02% *β*-glucan with antibiotics (T4) for 8 wk. In Exp. 2, the antibiotics fed were apramycin and carbadox in phase I (0 to 2 wk) and carbadox and chlortetracycline in phase II (3 to 8 wk). During Exp. 2, the performance study was conducted for 5 wk, and the immune response was tested until 8 wk. In Exp. 1, there was a trend for a linear increase (P = 0.068) in ADG as the dietary *β*-glucan concentration increased in the diet. The digestibilities of DM, GE, CP, ether extract, Ca, and P increased linearly ($P < 0.05$) in the *β*-glucan-supplemented pigs.

In Exp. 2, the overall ADG was greater ($P < 0.05$) in treatment T4 compared with the control group (T1). Also, except for P, this group showed greater ($P < 0.05$) nutrient digestibilities than the control group. In Exp. 2, at d 15, 24, and 46 antibody titers were measured by ELISA against *Pasteurella multocida* type A and D after vaccination with atrophic rhinitis, and they differed significantly ($P < 0.05$) with no particular trend. Flow cytometry was used to determine porcine lymphocyte subpopulations at 4 and 8 wk of Exp. 2. There was an increase in CD4 cells ($P < 0.05$) and a trend for an increase in CD8 cells ($P < 0.10$) at 8 wk in pigs fed the T2 diet compared with the other groups. Overall, increasing the dietary concentrations of *β*-glucan did not improve ADG without antibiotic, and in weanling pigs antibiotics seem to be more effective in improving nutrient digestibilities and growth performance than *β*-glucan.

In a performancestudy (unpublished dataof Biothera) 1200 pigletswere distributed intotwo treatment groups of 600 animals;one received APG 3-6 and one served asthe control group. Treatment andcontrol animals were housed in separaterooms in the same barn with 40 animalsper group, ear-tagged according

toweight (cutoff weight was 4 kg).Piglets were further separated accordingto normal and light weight piglets.Treatment animals received 0.075% APG3-6 during the first 14 days postweaning.The effect of Biothera's APG3-6 health additive was evaluated on average daily gain, total weight gain andmortality. Pigs fed 0.075% beta-glucan during the first 14 days after weaning showed increasedADG, especially after the 2nd week in the nursery.Reduced mortality and significant increase in final weight of LW pigs fed betaglucanwas evident in first study but not in replicate 2.

In another swine study (Donkers Family Farm Inc.) 2000 piglets were observed (unpublished dataof Biothera, 2007).The evaluation consisted of one control group, which contained one thousand head,and one treatment group, which also contained one thousand head. Housing andenvironmental conditions were identical for both treatment and control groups.Both groups were weaned at approximately twenty-one days of age. Both groupswere sorted and penned by routine farm protocol via size and weight. Producttreatment began on 19 March for the treatment group, via a commercial watermedicator, using the prescribed level of medication. Pigs were allowed ad libfeeding of commercial crumbles nursery diets, formulated according to pig weightand days in the nursery. A clean supply of water was allowed at all times usingnursery pig nipple type waters. Temperature and humidity were monitored andmaintained at comfortable levels through out the duration of the evaluation.Weight gain, mortality rate, and fallout rates were the elements calculated forevaluation.All pigs were removed from the nursery after 42 days, which is the standardnumber of nursery retention days, based on routine farm pig flow, and transferredto a finishing site.Reduced mortality (control 4.3%, treated 3.6%) and increase in body weight of pigs fed betaglucanwas evident.
Results were consistent with the first study, higher weight gain, reduced mortality and decreased fallout rate (fallout is a term meaning that the piglets had to be removed from the nursery for health reasons) for the APG treated group.

In a most recently conducted study **(Sweeney T. et al., 2012)**a total of thirty-two, 49-day-old pigs, with an initial body weight of 15.3 (SD 1.32) kg, wererandomly assigned to one of the fourdietary treatments as follows: (T1) basal diet (control, n =8);(T2)

basal diet supplemented with 250 parts per million(ppm) laminarin from *Laminaria digitata* (n=8); (T3) basal diet supplementedwith 250 ppm laminarin from *L. hyperborea* (n=8);(T4) basal diet supplemented with 250ppm b-glucansfrom *S. cerevisiae* (n=8). Experimental feeding continued for28 days*ad libitum*.It is noteworthy that the level of inclusion of b-glucans usedin the present experiment is very low (250 ppm) comparedwith the levels required for cereal b-glucans (20–40 g/kg)in order to show a biological response.

There were minimal effects on animal performance (foodintake, daily gain or food conversion ratio) or digestibilitycoefficients of DM, organic matter, ash, nitrogen or grossenergy, with b-glucan inclusion from either the seaweed oryeast sources.

There was a significant reduction ($P<0.05$) in Enterobacteriaceaein the ileum and colon with b-glucans derived from*S. cerevisiae*, *L. hyperborea* and *L. digitata*.

The expression of cytokine markers, IL-6 ($P<0.05$) and IL-8($P<0.01$), was lower in the ileum than in the colon of pigs.There was a significant interaction between gastrointestinalregion and b-glucan source in the expression of the cytokinemarkers IL-1 α ($P<0.001$), IL-10 ($P<0.05$), TNF- α ($P<0.05$)and IL-17A ($P<0.001$). b-Glucans did not stimulate anypro- or anti-inflammatory cytokine markers in the ileal epithelial cells. In contrast, the expression of a panel of proandanti-inflammatory cytokines (IL-1α, TNF- α, IL-10 and IL-17A) was down-regulated in the colon following exposureto b-glucans from all the three sources.

Authors concluded that b-Glucans derived from *L. hyperborea*, *L. digitata* and*S. cerevisiae* all reduced the Enterobacteriaceae populationin the ileum and colon without influencing the lactobacilliand bifidobacteria populations. This was associated with areduction in the expression of a number of pro-inflammatorycytokine genes in the colon. However, the data suggest thatthe soluble b-glucans from *L. digitata* may be acting via adifferent mechanism than the insoluble b glucans from*L. hyperborea* and *S. cerevisiae*.

6. Conclusions

Beta 1,3/1,6 glucan, the active substance of APG 3-6 powder has a well-established mechanism of action:

-Beta glucan, more characteristically APG 3-6 makes innate immune cells find infections more quickly, increases the phagocytic activity and oxidative burst activity of innate immune cells and activates neutrophils to more quickly kill a foreign challenge.

- An increased antimicrobial activity can be measured in neutrophils isolated from humans who have taken Wellmune WGP (compositionally similar to APG 3-6)orally. These findings parallel the animal and human in vitro studies and establish that the activation of innate immune cells can be measured in humans who consume Wellmune WGP orally.

Beta 1,3/1,6 glucan possesses a well-recognised chemical structure, including complete physical chemical characterisation.

The product APG 3-6 powder is subject to high safety level, and chemical as well as microbiological stability.

The use of APG 3-6 as an immunomodulator in animal health has a sound scientific basis. Accordingly the following beneficial clinical actions can be expected by administration of APG 3-6 in veterinary medicine:

- **antitumor activity**
- **antimicrobial activity**
- **modification of immune functions, e.g. anti-stress, anti-allergy activities, stimulation of maternal immunity and efficacy of vaccination**
- **mild growth-promotion like effect**

According to the recommendation of Pharmacology and Toxicology Department, APG 3-6 powder may be applied as medicinal substance (feed additive or feed ingredient) alone or adjunct to antitumor, antimicrobial and anti-inflammatory agents in both farm and companion animals.

7. List of references

Babicek K. et al., 2007: Toxicological assessment of a particulate yeast (1,3/1,6)-b-D-glucan in rats *Food and Chemical Toxicology* 45 p. 1719-1730.

Blaylock R. L., 2002: Yeast β-1,3-glucan and Its Use Against Anthrax Infection and in the Treatment of Cancer, Editorial commentary.*JANA* Vol. 5, No. 2.

Cary D.C. et al., 2005: Modulating immune function of neonatal dairycalves fed beta-glucan with and without ascorbic acid. 38th annual meeting ofSociety of Leukocyte Biology, Abstract 161.

Dutkiewicz J. et al., 2011: Biological agents as occupational hazards – selected issues. *Annals of Agricultural and Environmental Medicine* Vol 1, No 1

Eicher S.D et al., 2005: Modulation of Toll-like Receptors 2 and 4 and Interleukin-1 RNAexpression of blood leukocytes by beta-glucan and ascorbic acid in Neonatal Calves., 38th annual meeting of Society of Leukocyte Biology, Abstract 162.

El Khoury D. et al., 2011: Beta Glucan: Health Benefits in Obesity andMetabolic Syndrome Review Article. *Journal of Nutrition and Metabolism,* Article ID 851362, 28 pages doi:10.1155/2012/851362

FCC (Food Chemicals Codex) 7th Edition 2010

Feldman, S.H.I. et al., 2009: Randomized phase II clinical trials of Wellmune WGP® for immune support during cold and flu season. *J. Appl. Res. Clin. Expt. Ther*. 9 p. 30-42.

Hahn T.W. et al., 2006: Effects of supplementation of *β*-glucans on growth performance, nutrient digestibility, and immunity in weanling pigs. *J ANIM SCI* 84. p. 1422-1428.

Hong, F. et al., 2003: ß-Glucan Functions as an Adjuvant for Monoclonal Antibody Immunotherapy by Recruiting Tumoricidal Granulocytes as Killer Cells. *Cancer Res*. 63. p. 9023-9031.

Hong F. et al., 2004: Mechanism by which orally administered b-1,3 glucans enhance the tumoricidal activity of antitumor monoclonal antibodies in murine tumor models. *J. Immunol.* 173. p. 797-806.

Janeway C.A. et al., 2005: In: *Immunobiology*, 6th Edition

Jawhara S. et al., 2012: Modulation of Intestinal Inflammation by Yeasts and Cell Wall Extracts: Strain Dependence and Unexpected Anti-Inflammatory Role of Glucan Fractions. *PLoS ONE* (www.plosone.org) Volume 7. Issue 7. e40648

Kaiser A.B. et al., 1998: Synergism between poly-(1-6)-beta-D-glucopyranosyl-(1-3)-beta-D-glucopyranose glucan and cefazolinin prophylaxis of staphylococcal wound infection in a guinea pig model. *Antimicrob AgentsChemother*. 42. (9). p. 2449-2451

Lavigne L.M. et al., 2006: ß-Glucan is a Fungal Determinant for Adhesion-Dependent Human Neutrophil Functions. *J. Immunol.* 177. p. 8667-8675.

Kofuji K. et al., 2012: Antioxidant Activity of *β*-Glucan. *International Scholarly Research Network, ISRN Pharmaceutics*Volume 2012, Article ID 125864, 5 pagesdoi:10.5402/2012/125864

Li B. et al., 2006: Yeast ß-Glucan Amplifies Phagocyte Killing of iC3b-Opsonized Tumor Cells via Complement Receptor 3-Syk- Phosphatidylinositol 3-Kinase Pathway. *J. Immunol.* 177. p. 1661-1669.

Nelson E.D. et al., 2012: Neurologic effects of exogenous saccharides: A review of controlled human, animal, and in vitro studies, Review. *Nutritional Neuroscience* 15. 4. p. 149-162.

Reichel P. et al., 2011: Feeding of Energetic Food AdmixtureContaining Beta Glucane and its Effects onSelected Blood Serum Indices in Cows andTheir Calves in Peri-Partal Period. *Veterinarska Stanica* 42 - SUPPLEMENT 1, p. 58-64.

Sato H. et al., 2012: Inhibitory Effects of Water-Soluble Low-Molecular-Weight ß-(1,3-1,6) D-GlucanIsolated from *Aureobasidi.um pullulans* 1A1 Strain Black Yeaston Mast Cell Degranulation and Passive Cutaneous Anaphylaxis. *Biosci. Biotechnol. Biochem..* 76. (1). p. 84-88.

Sorocinova J. et al., 2011: Comparison of health and production efficiency of beta-glucanadministration and enterisol vaccination in swine (Summary). *Slovenský Veterinársky Časopis* 36. (5). p. 279-281.

Sweeney T. et al., 2012: Effect of purified b-glucans derived from *Laminaria digitata*, *Laminaria hyperborea* and *Saccharomyces cerevisiae* on piglet performance, selectedbacterial populations, volatile fatty acids and pro-inflammatory cytokinesin the gastrointestinal tract of pigs. *British Journal of Nutrition* 108. p. 1226-1234.

Talbott S.M. et al., 2010: Beta-glucan supplement reduces upper respiratory tract infections and improves mood state in healthy stressed subjects. *FASEB J*. 24. p. 922-931.

Talbott S.M. et al., 2012: β-Glucan supplementation, allergy symptoms, and quality of life in self-described ragweed allergy sufferers.*Food science and nutrition*. Published by Wiley Periodicals, Inc.doi: 10.1002/fsn3.11, 12 pages

TOXNET; Toxicology Data Network http://toxnet.nlm.nih.gov/

Tsikitis V.L. et al., 2004: ß-glucan affects leukocyte navigation in a complex chemotactic gradient.*Surgery* 136. p. 384-389.

Uchiyama H. et al., 2012: A small scale study on the effects of oral administration of the β-glucan produced by*Aureobasidium pullulans* on milk quality andcytokine expressions of Holstein cows, and onbacterial flora in the intestines of Japaneseblack calves. BMC Research Notes 5. p. 189-197.

Vetvicka, V. et al., 1996: Soluble beta-gluco polysaccharide polysaccharide binding to the lectin site of neutrophil or natural killer cell complement receptor type 3 (CD11b/CD18) generates a primed state of the receptor capable of mediating cytotoxicity of iC3b-opsonized target cells. *J. Clin. Invest*. 98. p. 50-61.

Vetvicka V. et al., 2002: Yeast β-1,3-glucan and Its Use Against Anthrax Infection and in the Treatment of Cancer.*JANA* Vol. 5, No.2.

Vetvicka V. et al., 2008:A Comparison of Injected and Orally Administered β-glucans JANA Vol. 11, No. 1, p. 42-49.

Vetvicka V. et al., 2011: β(1-3)-D-glucan affects adipogenesis, wound healing and inflammation. *Orient Pharm Exp Med.* DOI 10.1007/s13596-011-0024-4

Wakshull E.D. et al., 1999: PGG-Glucan, a soluble beta1,3/-glucan, enhances the oxidative burst response, microbicidal activity, and activates an NF-kB-like factor in human PMN: Evidence for a glycosphingolipid beta-1,3/-glucan receptor.*Immunopharmacology* 41. p. 89-107.

Wolever T.M.S. et al., 2011: Bioactive oat b-glucan reduces LDL cholesterol inCaucasians and non-Caucasians. *Nutrition Journal* 10. p. 130-133.

Yan J. et al., 2005: Yeast whole glucan particle (WGP) beta glucan in conjunction with antitumour monoclonal antibodies to treat cancer. *Expert Opin. Biol. Ther.* 5(5) p.691-702.