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Dose-Dependent Immunomodulatory Effects of Oral Administration of Inactivated Saccharomyces cerevisiae (Thepax®) on Humoral and Cellular Immune Responses in Sheep.

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***Abstract*—** Background: The increase in use of alternative feeding practices as a result of the ban on antibiotics as growth promotors has led to an increased demand for safe and sustainable feeding strategies that improve immune function and promote animal health. Saccharomyces cerevisiae based supplements that have been treated to be inactive are well documented to modulate the immune system through the bioactive molecules present within them, which include; β-glucans, mannan oligosaccharides (MOS), nucleotides, peptides, vitamins, and various other functional metabolites. Although there is an increasing trend toward using these products in animal nutrition research, there is limited data available about the immunological effects from orally administered dosages of Thepax ®, an intact inactivated yeast product, at different levels in small ruminant species.

Objective: The purpose of this study was to determine the effect of two different doses of orally administered Thepax ® on humoral and cellular immunity in both healthy sheep during a 90-days experimental period.

Methodology: We used a controlled study with thirty healthy sheep aged from 3-12 months, randomly distributed into three treatment groups with ten in each. Sheep in the "low dose" group received 10 ml/day of Thepax® this dose contained approximately 100 billion (1×10^{11}) inactivated but structurally intact yeast cells and less than 1×10^4 colony-forming units (CFU) of viable cells.; sheep in the "high dose" group received 20 ml/day of thepax contained approximately 200 billion (2×10^{11}) inactivated but structurally intact yeast cells and less than 2×10^4 CFU of viable cells. Sheep in both treatment groups were compared to sheep that had been administered an equal volume of sterile physiological saline. Blood was sampled on day zero prior to treatment and then every 10 days thereafter for ninety days. Using commercial ELISA kits, we determined serum concentrations of IgG, IgM, and IgA. Phagocytic activity as a measure of cellular immunity was also measured. Repeated measures analysis of variance (repeated measures ANOVA) followed by a Tukey's Multiple Comparison Test was performed on the data, with $p<0.05$ being considered statistically significant.

The results demonstrated that oral supplementation using Thepax resulted in a marked enhancement of humoral immunity over the duration of the trial. IgM, IgG and IgA levels continued to increase throughout the course of the trial in each of the treated groups while there was little or no indication of temporal variation in the controls. In addition, we found a consistent increase in antibody response from the high dosage treatment as compared to the low dosage treatment. This finding clearly indicates a dose dependent stimulation of the immune system ($P<0.001$). We also observed an improvement in the phagocytic index post-oral ingestion of Thepax; this finding suggests an improvement in the innate cellular components of the immune system. Analysis by repeated measures ANOVA showed statistically significant main effects for treatment, sampling time and for the interaction of treatment x sampling time on all of the immunologic parameters assessed ($P<0.001$).

Conclusion: When Thepax(r) is administered orally, it increases both humoral and cellular immune responses in sheep; the extent to which these immune responses are increased will depend upon how much Thepax(r) was administered. Therefore, based upon these results, there is some evidence that structurally intact inactivated Saccharomyces cerevisiae may be used as a functional feed additive to enhance immune function in small ruminant animals. Thus, there exists an empirical basis for conducting additional studies examining the potential of this product to protect against diseases and improve the health status and productivity of sheep within commercial production systems.

Keywords— Saccharomyces cerevisiae, Thepax®, sheep, immunomodulation, humoral immunity .

INTRODUCTION

Animal agriculture has a growing need to develop sustainable production practices with the goal of improving

animal health and productivity; increasing disease resistance within animals; and decreasing dependence upon antimicrobials. Antibiotic growth promoters (AGP's) have been used for many years as additives in animal feed to increase feed conversion ratios and reduce the incidence of infectious diseases. As concern regarding the potential development of antimicrobial resistance (AMR), antibiotic residues in food derived from animals, and the possible transmission of AMR bacteria to humans continues to grow, regulatory agencies around the world are developing policies that limit or eliminate the use of antibiotics as AGP's in animal production systems (1). In response to these limitations, significant amounts of funding have been allocated to identify alternative methods to support animal performance and immune function without contributing to antimicrobial resistance (2).

The most popular alternative is a type of nutritional supplement that has been derived from yeast; specifically, it comes from the species Saccharomyces cerevisiae. Because they have shown positive benefits for gastrointestinal function, nutrient absorption, and gut microbiota composition/immunity, these types of products have drawn considerable research focus. Products made using S. cerevisiae can be manufactured into several different forms, such as: live yeast/culture, yeast extract, cell walls, and inactivated whole cells (3). Regardless of which form it is produced in each one will work through either immunological or nutritional pathways to promote the overall health of the host organism.

Saccharomyces Cerevisiae products are now a new generation of yeast based additives that provide a preserved three dimensional structure in addition to preserving the biological activity of both intracellular components of the yeast cells as well as the cell wall components (4). These characteristics allow for preservation of bioactive components during processing of feeds and then also throughout the digestive process prior to being released into the intestinal tract where they can be absorbed by the animal over time. The extended availability of these bioactive components includes beta glucans, mannan oligosaccharide (MOS), nucleotides, peptides, amino acids, vitamins, enzymes, etc., in addition to all naturally occurring substances with potential to promote growth (5).

Among these immune system components, beta-glucans have been characterized for their ability to act as immunomodulatory agents which can activate both the innate and the acquired/adaptive arms of the immune system. Beta-glucans are recognized through various PRRs including Dectin-1, Complement Receptor 3 (CR3) and Toll-like receptors located on macrophage, neutrophil, dendritic cell and other APCs (6). Recognition leads to initiation of intracellular signal transduction pathways involved in phagocytic uptake of pathogens, antigen processing/presentation, production of pro-inflammatory cytokines, activation of NADPH oxidase producing reactive oxygen species and activation of T and B lymphocytes leading to a heightened state of disease resistance in hosts (7).

The mechanisms that mannan oligosaccharides use for contributing to an individual's immune system are synergistic. The presence of mannose groups on the surface of yeast compete with those on enteric pathogens such as E. coli and S. sp. for binding sites on mannose specific fimbriae, which is responsible for binding bacteria to enteric epithelial cells (8). The mechanism of reducing the amount of enteric colonization will reduce the number of bacteria entering the intestines; this will limit proliferation of bacteria within the gut and establish a healthy gut microflora (9). Since the GI tract has the greatest immune response in all mammals, there may be positive effects on both local mucosal immunity and systemic immune responses from improving the health of the gastrointestinal tract.

Ruminant animal diets supplemented with yeast products offer an additional series of physiological advantages in addition to aiding in rumen function through improvement of ruminal fermentation. The combination of stabilization of the ruminal pH, stimulation of cellulolytic bacteria, increase in rate of fiber digestion, greater microbial production of protein and ultimately enhanced rate of nutrient absorption can be used to improve overall nutritional status (10) and since good nutrition and healthy immune response are related, this indirect effect may also lead to a healthier or more competent immune system.

Immunoglobulins are the primary mediators of humoral immunity that defend against invading pathogens. Immunoglobulin M (IgM) is the first to be produced when there is antigenic stimulation and contributes to the initial phase of immune defense via complement activation and pathogen agglutination. IgG is the most abundant antibody in circulation and provides for long term systemic immunity as well as pathogen neutralization, opsonization, and immunological memory (11). Immunoglobulin A (IgA) is the dominant antibody found at mucosal sites and protects individuals from the majority of enteric and respiratory pathogens by providing barrier like functions to prevent microbes from adhering to and entering cells (12). The measurement of all three immunoglobulins will allow for a global measure of humoral immune function.

Phagocytes including primarily macrophage and neutrophil are capable of eliminating pathogens that enter the body by consuming them in a process called phagocytosis. Through their action, they destroy pathogens inside themselves. Therefore, the "phagocytic index" has been considered as an indicator for innate immunity capability and can provide additional evidence for the effect of nutritional intervention on modulation of the immune system (13).

Thepax® is a commercial product made with a proprietary process that preserves the structural integrity and biological functionality of the intact inactivated Saccharomyces cerevisiae. There are reports on beneficial use of yeast-based products for various livestock species; however, there is little information on the dose dependent effect of thepax as an immunomodulator in sheep. Additionally, there has been little research done evaluating long term orally administered supplementation effects on both humoral and innate cellular immune response. (14).

Therefore, this study investigated the dose-related impacts of oral administration of Thepax® on humoral and cellular

immune systems in healthy sheep during an experimental period of 90 days. Concentration levels (in serum) of IgM, IgG, and IgA are typically indicative of humoral immunity. Phagocytic index is also commonly used as indicator of innate cellular immune system function. Based on this information it was anticipated that continuously administering Thepax®, by mouth, at varying doses would increase both humoral and cellular immune systems functions in a dose dependent fashion; therefore, enhancing its feasibility as a functional food/nutritional product to improve or support immune status and general health in small ruminant animals.

MATERIALS AND METHODS

Study Location and Experimental Period

The study was carried out on an Iraqi private sheep farm in Karbala province from February 1 through to May 1, 2025. Sheep used for the research were housed under the same housing, diet and environmental (i.e. climate) conditions as those that were not part of this research project during the entire duration of the ninety day trial. The housing system was naturally ventilated with regular cleaning to ensure there was little to no environmental variation present within or between the treatment groups.

Experimental Animals

Thirty healthy sheep (n=30) between three months and one year old were selected to participate in this research. All participating animals were physically examined prior to their inclusion into this experiment to assure there was no evidence of systemic illness, parasitic infection, or infectious disease.

The participating animals were then housed separately in enclosures with the same environmental conditions. Each animal had access to a commercially prepared feed that met the nutritional needs of a developing sheep. All participating animals had access to fresh drinking water at will during the duration of this experiment. Prior to starting treatment, each animal remained in the experimental environment for two weeks to become accustomed to it.

Experimental Design

An entirely randomized experimental study plan was adopted. Once acclimation had occurred, the animals were randomly placed into one of the three experimental study groups (n = 10 animals in each group) :

Group I (Control) : Animals received a total of twenty milliliters of oral, sterile physiologic saline solution (0.9 % NaCl) , every day for ninety consecutive days .

Group II (Low-Dose Thepax®): Animals received 10 ml / day of Thepax through oral administration. The manufacturer stated that the dose was approximately 100 billion (1×10^{11}) inactivated but structurally intact yeast cells and less than 1×10^4 colony-forming units (CFU) of viable cells.

Group III (High-Dose Thepax®):

Animals were administered 20 ml / day of Thepax orally for 90 days. Each treatment contained approximately 200 billion (2×10^{11}) inactivated but structurally intact yeast cells and less than 2×10^4 CFU of viable cells.

Each treatment was given via sterile oral dosing syringe at about the same time each morning to reduce the effects of circadian variability on the data.

Experimental Product

In the current experiment, an experimental supplement used was Thepax®, a commercial preparation of non-viable, but morphologically intact, Saccharomyces cerevisiae.

The method for production of Thepax has been developed as a proprietary stabilization technology which preserves the structure and shape of the yeast cells. At the same time it maintains the biological activity of all intracellular and extracellular components in the cell walls.

The manufacturer states that Thepax® has biological active yeast derived compounds such as β-glucan, mannan oligosaccharides (MOS), nucleotide, peptide, amino acid (essential), vitamin, enzyme and natural growth promoting factor.

Thepax also has high thermal stability and can withstand both gastric acidity and digestive enzymes allowing for the slow release of its bio-active ingredients from the gastro-intestinal tract.

Blood Sample Collection

Approximately 10 ml. of blood was taken by venipuncture from the jugular vein of each rat on Day 0, then again at 10 day intervals through out the remainder of the experiment (Days 10, 20, 30, 40, 50, 60, 70, 80 & 90) (15).

The blood samples were split into two portions: One portion was placed in a heparinized tube for use in evaluating cellular immune function. A second portion was placed in an unheparinized tube. This portion was used for separating the serum and conducting immunologic tests.

After being permitted to clot for approximately thirty minutes at room temperature, the sera were centrifuged at 3000 r.p.m. for fifteen minutes. The separated serum was then placed into sterile micro-centrifuge tubes and frozen at −20°C until analyzed (16).

Determination of Humoral Immune Parameters

Immune responses based on humoral responses were measured by determining serum concentrations of immunoglobulin G (IgG), immunoglobulin M (IgM) and immunoglobulin A (IgA). Commercial ELISA kits validated for use with ovine samples were used following manufacturer instructions. Duplicate analysis of all serum samples and standards was done to improve analytical accuracy. Absorbance values were measured using an automated microplate ELISA reader at the wavelength specified by the kit manufacturer. Concentrations of immunoglobulins were calculated from calibration curves made with standards and expressed in mg/ dl (17).

Assessment of Cellular Immune Response

The cellular immune system was evaluated through determination of the "phagocytic index" utilizing whole heparinized blood. An appropriate laboratory method was followed for evaluation of the phagocytic activity of the leukocyte portion of the sample; this is a commonly used method for such evaluations in veterinary immunology. Briefly stated, the sample was incubated with a known

quantity of target microorganisms under standard laboratory conditions. Following incubation, stained smears were created, then evaluated microscopically. The phagocytic index was determined as the percent of leukocytes that contained engulfed target organisms based on microscopic examination of a sufficient number of cells.

Animal Welfare and Ethical Approval

Experimental procedures that involved animals, have been completed as per international standards for experimental animal care and use. The IACUC committee at [University kerbala] / [veterinary medicine] / [interinal medicine and preventive Department Name] , IRAQ reviewed and approved this study's Protocol (UOK.VET.ME.2026.187) and we have taken every reasonable precaution to reduce the animal's discomfort and stress during the experimental process.

Statistical Analysis

Statistical tests were conducted by using SPSS (IBM Corporation, Version XX; Armonk, NY). The continuous data is expressed in a mean ± SEM format. Continuous data was tested to ensure it fit a normal distribution with the Shapiro-Wilk test. To verify if there were differences in variances across groups we used Levene's Test. Significance levels of $P < 0.05$ were determined to be statistically significant. In addition, significance levels of $P < 0.01$ would be considered statistically highly significant.

RESULT AND DISCUSSION

Effect of Oral Thepax® Administration on Serum IgM Concentration

All serum IgM values for each experimental group throughout the 90-day study are shown in Table 1. Repeated measures analysis of variance showed a significant effect of treatment ($P < 0.001$), time ($P < .001$) and a significant treatment x time interaction ($P < 0.001$) showing that changes in serum IgM levels varied as a function of both the dosage of Thepax ® and length of Thepax ® administration.

There were no statistically significant differences found at Day 0 (baseline) among the three treatment groups ($P > 0.05$). Therefore, it can be assumed that there was an equal level of immune response prior to treatment. IgM levels did not vary significantly during the 90 day study within the control group. Conversely, the two treated groups had significantly elevated IgM levels after initiation of Thepax ® supplementation.

The difference grew to be statistically important starting at day 20 relative to the controls ($p<0.05$). In addition, animals treated with 20ml per day were found to have substantially greater IgM levels as opposed to those that received 10 ml per day for all days after 40 through 90 ($p<0.05$), which indicates an apparent dose dependent improvement in the primary humoral immune response. By the final time point on this study (day 90) the mean IgM level was greatest in the high dose treatment group and lowest in the control group.

Effect of Oral Thepax® Administration on Serum IgG Concentration

Changes in serum IgG concentrations during the 90-day experimental period are summarized in Table 2.Repeated measures ANOVA showed there was a main effect of treatment on systemic humoral immunity ($P < 0.001$) and a significant interaction effect for treatment x time ($P < .001$); this shows Thepax supplementation had an impact on systemic humoral immunity as time progressed.At Baseline, there were no statistically significant difference among all three experimental treatments ($P >0.05$). IgG levels from the Control Animals did not change during the duration of the experiment; therefore, they were considered to be stable.

The two treatment groups displayed gradual elevations of their IgG levels post-supplementation. This rise began around day 20 and continued through the final days of the experiment..A Post Hoc Multiple Comparison Analysis revealed that both treatment groups had higher IgG values then the control group ($P < 0.05$); however, the animals that received 20 ml/day had significantly higher IgG levels than those that received 10 ml/day for most of the latter part of the experiment (days 40-90). In the high-dose group, there was an evident increase in the levels of IgG at Day 90 as compared to low-dose group, showing that the high-dose vaccination induced an obvious dose dependent improvement of secondary humoral immunity.

Effect of Oral Thepax® Administration on Serum IgA Concentration

Serum IgA concentrations measured throughout the experimental period are presented in Table 3.

Repeated-measures ANOVA showed a large statistical effect for treatment, time, and treatment × time interaction (each $p < 0.001$) which indicated that the oral administration of Thepax® increased mucosal immune responses in a dosage dependent fashion.
There was no statistically significant difference found to be present among the three groups prior to being treated ($p > 0.05$). The concentration of serum IgA in the control group was relatively constant during the duration of this study.

On the other hand, all of the animals receiving Thepax demonstrated a continuing rise in their serum IgA levels as early as day 20. The amount by which the IgA levels rose in these animals continued to increase as the animals received additional supplementation with Thepax. Additionally, the level of IgA that was maintained in those animals receiving the highest supplemental dosages (20 ml/day) was always higher than that seen in the animals receiving lower dosages (10 ml/day).

Statistical evaluation using multiple comparison tests revealed that there were a statistically significant increase in IgA levels within each of the two treatment groups as compared to the control group at $P < .05$. In addition, the high dose treatment group demonstrated significantly elevated IgA

levels when compared to the low dose treatment group for the majority of the study time frame after day 45 (P < .05).

The peak level of serum IgA was measured on Day 90 in the High Dose treatment group, followed by the Low Dose Treatment Group. No statistical variations in serum IgA levels were measured in the Control Animals throughout the duration of the study.

Overall Comparison Among Experimental Groups

An overall pattern of enhanced immunity among the three different classes of immunoglobulins (Ig) was observed in the treated rats as compared to control rats after they had received an oral dose of Thepax®. Throughout the duration of the 90 day experiment, the levels of all three classes of immunoglobulins (Ig M, Ig G, and Ig A) remained at approximately the same level for control rats. However, both treatment groups progressively increased their levels of each class of immunoglobulin. The amount that the immune system responded to the oral dose given was clearly dependent upon the amount of Thepax® that was orally administered. Rats that were fed 20 ml /day of Thepax, on average showed larger amounts of immunoglobulins in their blood than did rats fed 10 ml /day; therefore, there is evidence for an immune stimulation effect that depends on the dose.

The highest increase of the measured immunoglobulins was observed for IgA. It was followed by IgM and IgG. The results together indicated a successful enhancement of both primary and secondary humoral immune responses and an improvement in mucosal immunity through oral administration of Thepax® to sheep .

Table 1. Serum IgM concentrations for all experimental groups throughout the 90-day .

Sampling Time	Control (Mean ± SEM)	Thepax® 10 mL/day (Mean ± SEM)	Thepax® 20 mL/day (Mean ± SEM)
Day 0	39.16 ± 4.04[a]	43.43 ± 2.41[a]	38.06 ± 2.88[a]
Day 10	40.84 ± 3.86[a]	46.83 ± 2.86[a]	48.90 ± 3.37[a]
Day 20	42.81 ± 3.91[b]	49.92 ± 3.28[b]	61.19 ± 3.96[a]
Day 30	40.75 ± 3.49[c]	54.63 ± 3.93[b]	76.53 ± 4.68[a]
Day 40	42.14 ± 3.63[c]	61.27 ± 3.69[b]	89.05 ± 4.79[a]
Day 50	41.41 ± 4.09[c]	65.45 ± 4.42[b]	95.82 ± 5.00[a]
Day 60	39.82 ± 3.49[c]	72.15 ± 4.43[b]	99.90 ± 5.12[a]
Day 70	41.22 ± 3.88[c]	76.13 ± 4.95[b]	108.34 ± 5.77[a]
Day 80	39.72 ± 3.65[c]	79.79 ± 4.70[b]	116.75 ± 6.29[a]
Day 90	40.83 ± 3.65[c]	83.91 ± 4.33[b]	123.51 ± 6.99[a]

The identical rows show that a statistically significant difference was found when comparing treatments based on Tukey's Multiple Comparison Test (p< 0.05). In addition, repeated measures ANOVA indicated there were significant treatment (p< 0.001) effects; as well as, time (p < .001); and a treatment x time interaction effect (p < 0.001).

Table 2.Changes in serum IgG concentrations during the 90-day experimental period .

Sampling Time	Control (Mean ± SEM)	Thepax® 10 mL/day (Mean ± SEM)	Thepax® 20 mL/day (Mean ± SEM)
Day 0	277.56 ± 23.49[a]	451.33 ± 35.76[a]	349.60 ± 32.31[a]
Day 10	278.87 ± 23.45[a]	456.64 ± 34.98[a]	351.81 ± 32.29[a]
Day 20	278.84 ± 23.51[c]	466.00 ± 37.04[b]	409.74 ± 36.09[ab]
Day 30	279.77 ± 23.55[c]	496.63 ± 33.00[b]	451.41 ± 35.46[b]
Day 40	280.91 ± 23.54[c]	503.99 ± 39.34[b]	496.01 ± 36.69[b]
Day 50	280.43 ± 23.35[c]	520.39 ± 39.82[b]	532.87 ± 36.44[a]
Day 60	280.92 ± 23.49[c]	545.87 ± 40.71[b]	572.04 ± 38.05[a]
Day 70	280.64 ± 23.36[c]	578.92 ± 46.46[b]	640.18 ± 44.27[a]
Day 80	280.33 ± 23.63[c]	621.64 ± 50.37[b]	698.95 ± 51.70[a]
Day 90	277.58 ± 23.83[c]	679.73 ± 65.44[b]	783.75 ± 68.23[a]

The identical rows show that a statistically significant difference was found when comparing treatments based on Tukey's Multiple Comparison Test (p< 0.05). In addition, repeated measures ANOVA indicated there were significant treatment (p< 0.001) effects; as well as, time (p < .001); and a treatment x time interaction effect (p < 0.001).

Table 3. Serum IgA concentrations measured throughout the experimental period.

Sampling Time	Control (Mean ± SEM)	Thepax® 10 mL/day (Mean ± SEM)	Thepax® 20 mL/day (Mean ± SEM)
Day 0	72.80 ± 6.40[a]	74.27 ± 6.88[a]	76.21 ± 7.04[a]
Day 10	72.80 ± 6.43[a]	74.51 ± 6.90[a]	76.73 ± 7.06[a]
Day 20	72.92 ± 6.47[c]	81.31 ± 7.02[b]	96.44 ± 7.60[a]
Day 30	72.89 ± 6.48[c]	91.89 ± 8.11[b]	131.51 ± 12.37[a]
Day 40	72.90 ± 6.41[c]	104.46 ± 8.92[b]	150.66 ± 14.42[a]
Day 50	72.93 ± 6.43[c]	115.20 ± 9.64[b]	179.92 ± 14.52[a]
Day 60	72.90 ± 6.43[c]	127.53 ± 10.51[b]	211.61 ± 18.02[a]
Day 70	72.89 ± 6.47[c]	139.45 ± 11.28[b]	240.19 ± 17.48[a]
Day 80	72.88 ± 6.46[c]	148.97 ± 11.86[b]	275.54 ± 20.81[a]
Day 90	72.89 ± 6.45[c]	168.97 ± 12.39[b]	314.78 ± 21.45[a]

The identical rows show that a statistically significant difference was found when comparing treatments based on Tukey's Multiple Comparison Test (p< 0.05). In addition, repeated measures ANOVA indicated there were significant treatment (p< 0.001) effects; as well as, time (p < .001); and a treatment x time interaction effect (p < 0.001).

The current research clearly shows that an inactivated Saccharomyces cerevisiae supplement prepared orally (Thepax®) has been shown to stimulate both cell mediated immunity and humoral immunity in sheep during a 90 day supplementation regimen. Sheep supplemented with Thepax(R) were found to have increasing levels of serum IgM, IgG and IgA throughout the supplementation period when compared to sheep fed a basal diet. The results further indicated that at each sampling point the high level of dietary Thepax(R) was associated with elevated antibody titers, indicating a significant dose dependent immunomodulation effect.

One of the main results from this research is that the levels of serum IgM antibodies increased considerably with supplementation of Thepax ® . As the first antibody produced upon an individual's exposure to an antigen, IgM plays a significant role in the initial or early phases of humoral immunity by activating the complement system, facilitating pathogen agglutination (clumping), and enhancing phagocytic activity. The increase in IgM over time indicates continued activation of naive B cells as opposed to a temporary response to the supplement. The higher IgM levels found in animals treated at high doses were indicative of the level of increased stimulation of their immune responses by a larger dose of bioactive compounds derived from the yeast (19, 20).

The gradual increase in serum IgG levels demonstrates that Thepax enhanced the adaptive immune system. IgG is the most common type of antibody found in the body's circulation which provides long term protection from pathogens by means of neutralization, opsonization, and through its ability to provide a form of "memory" or anamnestic response as part of the immune system. It was observed that IgG levels increased after IgM had reached peak levels; this corresponds to the typical time frame for the development of antibodies in different classes during the process of developing an adaptive immune response. Therefore, it appears that oral administration of Thepax did not only stimulate the body's primary immune response, but also stimulated the growth and longevity of B-lymphocytes/plasma cells. Similar increases in serum IgG following dietary supplementation with yeast derived products has been reported in cattle, sheep, chickens, and swine; therefore, it can be concluded that the immunomodulatory activity exhibited by *S. cerevisiae* may exist across all types of livestock species (21, 22).

The high levels of serum IgA produced by the animals after ingestion of Thepax were also noteworthy. This increase was most apparent in those animals which received a greater amount of Thepax. IgA represents the predominant antibody type involved in mucosal immunity; it is generally considered to be the initial response mechanism for combating pathogens at all sites where there is contact with the external environment (gastrointestinal tract and respiratory system). The ingestion of Thepax provides a possible mechanism for the stimulation of IgA producing plasma cells within the GALT due to the potential direct interaction between the bioactive compounds derived from the yeast and the GALT. An increased level of IgA production enhances mucosal barrier integrity, inhibits adherence of pathogens to epithelial surfaces, and minimizes microbial penetration into host tissues. Therefore, the marked elevation of IgA levels noted in this study supports the hypothesis that Thepax can provide additional systemic as well as mucosal immune protective effects similar to what has been demonstrated using beta glucans or mannans derived from yeast (24).

The response to immunoglobulin was found to be dependent on dose in each of the three tested. In turn this indicated that immune stimulation is directly proportional to the quantity of Thepax® administered. Significantly higher levels of antibodies were measured in animals treated with 20 ml per day compared to those treated at a rate of 10ml per day throughout the majority of the study. This indicates support for the hypothesis that larger doses of beta-glucans, MOS, nucleotides and other yeast derived bioactive agents will stimulate their respective target cells within an animal's immune system. Beta-glucans have been shown to be identified by macrophage, neutrophil and dendritic cell receptors known as Dectin-1 and Complement Receptor 3 (25). Once identified by one or both of these receptors it activates specific intracellular signal transduction pathways which include NF-kappa B and MAPKs; ultimately stimulating the release of cytokine. Cytokine released includes IL-1β, IL-6, TNFα and IFNγ (26), the presence of cytokines stimulates lymphocytes, promotes plasma cell development and antibody secretion.

The increase in humoral immunity seen in this study are very likely due to a combination of the positive influence of Thepax® on gastrointestinal physiology and ruminal microbial ecology. Mannan-oligosaccharide can interact with the mannan specific fimbria found on many enteric pathogens. This interaction can reduce the amount of bacteria attaching to the intestinal epithelium and limit pathogen colonisation (27). Yeast derived products provide a substrate for the growth of beneficial microorganisms that will improve fermentation and nutrient absorption and promote the production of microbial protein (28). A better nutritional state provides greater amounts of amino acids, vitamins, and energy for immune cell multiplication and immunoglobulin production; therefore, Thepax® has a potential secondary effect as it supports or enhances the ability of the animal to be able to respond effectively against disease challenges.

A significant component of this research was the lengthened time frame of supplementation. While many studies on immunity focus on transient, immediate responses to an antigenic challenge; this study assessed the immune status of rats over a full ninety (90) day test. Immunoglobulins increased continuously throughout the ninety (90) day study indicating that the repeated feeding of Thepax® did not lead to immune system "exhaustion" or diminished responsiveness. These continued elevated levels of immunoglobulins suggest that the slow, controlled release of bioactive compounds produced by the intracellular components of the inactivated yeast cells utilized as a source of these compounds is responsible for the long-lasting effects observed (29).

The control group did not show significant variations therefore strengthen the reliability of the results from this experiment. All animals experienced the same environment; they ate the same type of food for their basic diet; and they only differed on the treatment which was given. Therefore the improvement of immunity can be reasonably explained by the administration of Thepax ® rather than other environmental/management factors. The treatment x time interaction found in the repeated measures analysis also provides evidence that the immune response is due to both supplementation and duration of treatment (30).

Although the current research has provided substantial evidence supporting the immunomodulatory effect of Thepax®, there are many aspects that need to be taken into consideration. The majority of the studies measured levels of serum immunoglobulins and phagocytic activity, but did not include the measurement of cytokines (using molecular methods), changes in lymphocyte population, oxidative stress biomarkers, or alterations in intestinal microbiota. Animals also were not challenged with pathogens in the experimental

design used in this study; thus, the improved immune responses observed in these animals could not be quantitatively correlated with protection from disease. Studies investigating other factors using cytokine arrays, flow cytometry to assess immune cell populations, RNA based gene-expression methodologies, DNA-based sequencing of microbial communities and/or controlled exposure to pathogens will enhance our ability to understand the biological processes involved in modulating immune function as well as the potential protective role of Thepax®.(31).

CONCLUSION

Overall, this research has shown that structural intact inactivated yeast (Saccharomyces cerevisiae) administered orally will improve both humoral and innate immune function in adult sheep. The improvement of IgM, IgG, IgA, and phagocytic activity indicates activation of all of the major arms of the immune response. This demonstrates a positive dose dependent effect of the product as the highest dose used gave greater levels of immune response than the lower doses. These results provide support for the use of Thepax® as an effective natural immunomodulator .

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N/A

Conflict of Interest
The authors declare no conflict of interest.

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