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Impact of β-Galactomannan on health status and immune function in rats

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Impact of β-Galactomannan on health status and immune function in rats

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NOMENCLATURE

GIT	Gastrointestinal tract
GALT	Gut-associated lymphoid tissue
IBD	Inflammatory bowel disease
LP	Lamina propria
IEC	Intestinal epithelial cell
MLN	Mesenteric lymph node
DC	Dendritic cell
APC	Antigen presenting cell
PAMP	Pathogen-associated molecular pattern
TLR	Toll-like receptor
PRR	Pattern recognition receptor
Treg	Regulatory T-cell
nTreg	Natural regulatory T-cell
iTreg	Induced regulatory T-cell
IPEX linked	Immunodysregulation polyendocrinopathy enteropathy X-syndrome
IBS	Irritable bowel syndrome
T1DM	Type 1 Diabetes Mellitus
IDO	Indoleamine-pyrrole 2,3-dioxygenase
β-GM	Beta-galactomannan
NSP	Nonstarch polysaccharide
SBM	Soybean meal

CTL	C-type lectin receptor
LPS	Lipopolysaccharide
PHGG	Partially hydrolyzed guar gum
GG	Guar gum
tDC	Tolerogenic dendritic cell
ER	Endoplasmic reticulum
UPR	Unfolded protein response

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ABSTRACT

Intestinal health and the maintaining the integrity of the intestinal barrier are critical to maintaining overall health. Research conducted over the years has consistently shown an unhealthy gut is detrimental to one's overall well-being and is associated with a number of disease states including obesity, autism, Inflammatory bowel disease, and other autoimmune disorders. Chronic intestinal inflammation is an innate immune response that can disrupt the intestinal barrier causing it to become leaky and leaving the host susceptible to a plethora of environmental pathogens. Inflammation is also an energy draining physiological process that also causes health complications.

Consumption of nonstarch polysaccharides, such as the guar gum containing β-galactomannan, have been shown to stimulate the innate immune response characterized by high levels of inflammatory cytokines. In livestock, studies have regularly shown the negative side effects of soybean meal β-galactomannan on health and immune function including impaired nutrient absorption, stunted growth, and inflammation. *In vivo* studies have also elucidated the immunostimulatory effects of mannans. Gums containing β-galactomannan are commonly used as thickeners, stabilizers, and binders in food industry. Therefore, it is essential to elucidate the extent of inflammation β-galactomannan may cause in order to protect the health of consumers.

Our study was conducted to further characterize the impact of guar gum derived β-galactomannan on health and immune status in Sprague-Dawley

rats. As expected, rats fed β-galactomannan gained less weight throughout the course of the study compared to control rats and also consumed less. These effects, however, were not accompanied by an increased inflammatory cytokine profile. B-galactomannan consumption did not affect inflammatory cytokines IL-12a, IL-12b, or IL-6 nor did it affect the anti-inflammatory cytokine IL-10 in the ileum. Even more perplexing was that in the jejunum, β-galactomannan increased IL-10 mRNA transcript abundance and decreased IL-12a mRNA levels. Based on our experiment, β-galactomannan did not stimulate an innate immune response

CHAPTER 1: LITERATURE REVIEW

Introduction to Gut Immunology

The gastrointestinal tract (GIT) functions to digest and absorb nutrients. However, because it is normally the first point of contact with microbes, it is also the largest immune organ in the body and plays a critical role in immune homeostasis. Upon exposure to pathogens, self-antigens, and dietary antigens, the GIT may mount either an immunogenic or tolerogenic response.[1] Typically, interactions between gut-associated lymphoid tissue (GALT) and food promote oral tolerance of these dietary antigens via anergy, deletion of T cells, or the induction of regulatory T cells.[2] In fact, recent studies have investigated the induction of oral tolerance as a potential means of treating food allergies and other autoimmune disorders.[3] Nonetheless, misdirected immune responses and impaired gut integrity may result in devastating gut related disorders, such as inflammatory bowel diseases (IBD) and food allergies which exacerbates disruptions in intestinal epithelial integrity.[3] Gut health has also been linked to other disease pathologies such as chronic heart failure and autism.[4,5] Oral tolerance involves anergy, deletion of T cells, or the induction of regulatory T cells.[2]

Gastrointestinal architecture

The small intestine is lined with villi, microvilli, and invaginations, also known as “crypts”. Villi are fingerlike projections, which increase surface area for

better absorption of nutrients and contain blood vessels to transport food systemically. The Lamina Propia (LP) of the villi is a layer of connective tissue just under the epithelium of the mucous membrane. Microvilli protrude from the enterocytes, or intestinal epithelial cells (IECs), collectively forming the brush border to further enhance nutrient absorption. IECs are held together by tight junctions which function to prevent attachment and subsequent invasion of pathogenic microbes.[6] The intestinal crypts function in cell replication, to replenish the epithelial layer, and in mucous production. Throughout the intestinal mucosa, dome-like structures known as Payer's patches are enriched with lymphoid tissue and are key sites for the development of tolerogenic and immunogenic responses. Microfold cells, or M cells, are specialized epithelial cells that function in antigen uptake by cells within Payer's patches. Lymphatics from both the Payer's patch and LP drain into a mesenteric lymph node (MLN) where the majority of naïve T cells are activated to promote differentiation into effector T cells. Within the Payer's patches, there is an array of immune cells including T cells, B cells, dendritic cells (DC), and Macrophages.

Collectively, the immune system of the digestive tract is most often referred to as gut-associated lymphoid tissue, or GALT. The constant interaction between GALT, dietary antigens, and normal flora of the GIT promotes an environment characterized by sustained low-level inflammation.[7] Environmental and genetic-based challenges against the protective epithelial barrier may increase intestinal permeability and disrupt inflammatory homeostasis in the GIT. Such challenges may result in aberrant immunological responses of pathological

significance. In particular, food allergies are associated with compromised intestinal epithelium function and hypersensitive immune function.[8,9]

Dendritic cells

Dendritic cells are the major antigen presenting cells (APC) of the immune system that bridge innate and adaptive immunity. Most DC subsets express pattern recognition receptors that recognize pathogen-associated molecular patterns (PAMPs). Upon antigen recognition, DCs are activated to produce cytokines, carry out phagocytosis of the pathogen, and migrate to the MLN for maturation. Failure of the DC to mature and migrate results in impaired differentiation of effector T cells.[10] As surveillance cells, DCs can aggregate below M cells of the gut to detect invading pathogens that have crossed the epithelial barrier. However, the majority of immature DCs reside in the lamina propria, and can be classified into two subsets: CD103+ DC and CX_3CR1 DC.[11]

The most discernable difference between these subsets of DCs is in their migratory ability. Upon activation by toll-like receptor (TLR) signaling, CD103+ DCs travel via the hymphatics to the MLN where antigen presentation induces differentiation of naïve T cells into specific effector T cells which is part of the adaptive immune response. In contrast, the CX_3CR1 subset is a residential DC population that is incapable of migration to MLN and has poor antigen presentation abilities. Schulz et al.[11] demonstrated clear migratory differences using fluorescent-labeled Dextran. Mice in the R848 inflammatory group showed mobilized CD103+ DC in lymph vessels and no change in location of the

CX_3CR1 DC. Yrlid et al.[12] also reported R848 signaling via TLRs enhanced migration of CD103+ DC from the lamina propria to MLN. Increased expression of costimulatory molecules CD40 and CD86 upon activation and maturation was seen in CD103+DC but not in the CX_3CR1 cells.[11] Furthermore, CX_3CR1 DCs failed to promote naïve T cell differentiation. These recent findings indicate intestinal CX_3CR1 DCs may only possess innate function and serve as the first line of defense in the gut.

These subsets of DCs also differ in their physical functionality. CX_3CR1 DCs have the ability to extend their dendrites between enterocytes by opening tight junctions and in doing so, are able to directly sample luminal contents of the gut.[13] These dendritic extensions can be detected under normal conditions and will multiply in the presence of bacterial species. [13,14]

Pathogen associated molecular patterns of a pathogen can illicit an innate immune response and inflammation by binding to a pattern recognition receptor (PRR) on a dendritic cell or other innate leukocyte. Under steady state conditions, low-level inflammation is present and controlled without causing a pathological response. When intestinal DCs are stimulated by self-antigens, dietary antigens, or commensal microbes, production and secretion of anti-inflammatory IL-10 to control proinflammatory stimuli and promote tolerance is enhanced. IL-10 produced by the DC itself has an autocrine effect by binding to IL-10 receptors and preventing DC maturation and subsequent release of IL-12.[15] Secretion of IL-10 by other innate leukocytes and Tregs also causes inhibition of DC maturation, and thus promote a tolerogenic environment. Harmless antigens

may also cause unnecessary intestinal inflammation and lead to impaired gut function and nutrient utilization.[16,17] Proinflammatory cytokines such as IL-12, IL-6 and TNF-α are produced and secreted by activated and matured dendritic cells. Therefore, it is imperative that DCs develop appropriate immune responses to stimuli. Both IL-10 and IL-12 have been implicated in signaling adaptive immunity. IL-10 promotes Treg development while IL-12 leads to the development of effector T cells such as Th17, Th1, and Th2.

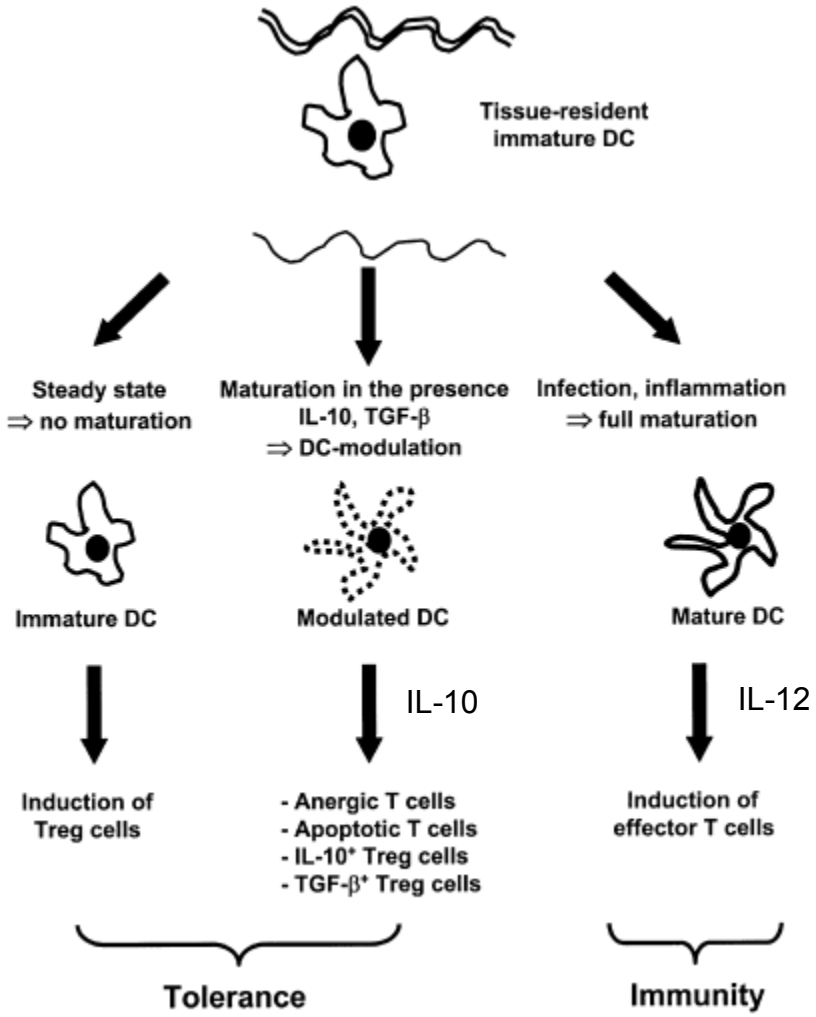

Figure 1: Immature dendritic cells survey intestinal contents during steady state. If no inflammatory signals maturation does not occur and Tregs can be induced. Infection and inflammation causes DCs to mature and induce effector T cells. If a DC matures in the presence of IL-10, TGF-β, and a tolerant microenvironment, maturation if altered the DC becomes tolerogenic, or modulated. tDCs can cause anergy of T cells, apoptosis of T cells, or differentiation into IL-10 or TGF-β producing Tregs. From Mahnke et al.[76]

Regulatory T Cells

Gershon and Kondo were the first to propose the hypothesis that a subset of T cells existed with a primary role in blunting rather than enhancing immune responses.[18] Research surrounding the concept of "Suppressor" T cells, as they were originally named, came to an abrupt halt due to flaws in the initial experimental designs. However, in the early-90's, advanced molecular tools led scientists to again propose the concept of suppressor T-cells which were reintroduced as regulatory T- cells (T_{regs}).[18] The Sakaguchi lab demonstrated the importance of Treg cells using a mouse model based on thymectomy to remove the site of natural Treg cell production.[18] Thereafter, mice were reconstituted with all T cell subsets with or without Treg cells. The mice devoid of Treg cells presented with systemic autoimmune disease whereas their counterparts remained healthy. This early experiment has spawned much research on these immunosuppressive cells, but some mechanisms are still not clearly defined.

Many researchers agree two classes of regulatory T cells exist: natural regulatory T cells (nTreg) and induced regulatory T cells (iTreg). nTreg cells (CD4+,CD25+,Foxp3+) are thought to be antigen specific and develop from naïve nTregs produced by the thymus.[19] The issue of whether nTregs are innate in nature or antigen specific (adaptive) is still controversial. Interactions between MHCII on the DC and the T- cell receptor on the surface of the naïve Treg cell leads to development and activation of nTreg cells. These cells are important in controlling low-level immune responses in the periphery where they are located. nTreg cells express CD25, also known as the α chain of the IL-2 receptor, and

Foxp3.[6] Foxp3 is a transcription factor crucial for the induction, maintenance, and function of Treg cells.[20] Mutations in the Foxp3 gene lead to impaired or incomplete Treg cell production causing Immune dysregulation polyendocrinpathy enteropathy X-linked Syndrome (IPEX), a rare but fatal systemic autoimmune disease.[21] More than 80% of IPEX patients suffer from multiple autoimmune diseases including Irritable Bowel Syndrome (IBS), enteropathy, dermatitis, allergies, and Type 1 Diabetes Mellitus (T1DM) and ultimately die early in life. nTreg cells also express CTLA-4 which is an inhibitory receptor that downregulates effector T-cell response and may also modulate DC function.[22] Studies have shown that CTLA-4 blocking antibodies and CTLA-4 deficient Treg cells attenuate suppression of effector T cells.[23,24] Non-regulatory T-cells (CD4+,CD25-,Foxp3-) have very low levels of CD25 and will usually lack Foxp3, although this remains a topic of discussion among researchers. In the lymph node, upon exposure to an antigen via an APC and in combination with IL-10 and TGF-β, the CD4+ cell differentiate into iTreg cells.[25] iTreg will also be induced by tolerogenic DCs and cytokine IL-10 to express Foxp3 via TGF-β and CD25, making it difficult to determine whether a Treg cell originated from a naïve nTreg cell or was induced by APC and cytokines. Therefore, it is believed the majority of antigen specific Tregs are iTreg cells.[26,27]

The development of immune tolerance via Tregs

Regulatory T cells have the ability to promote immune tolerance via multiple mechanisms, including inhibitory cytokine production, direct disruption of

effector T-cells, modulation of dendritic cells, and cytolysis. These distinct mechanisms, which can become redundant, are all required for maximal Treg cell function.[22]

Production of inhibitory cytokine IL-10

Cytokines are small proteins produced by host cells, particularly leukocytes. These signaling molecules can be characterized as pro-inflammatory or inhibitory (anti-inflammatory). Upon exposure to an antigen, CD4+CD25+Foxp3+ Treg cells will produce and secrete cytokines IL-10 ad TGF-β.[19] IL-10 protects the host by limiting inflammatory responses and inducing tolerogenic DCs by limiting IL-12 production.[28] Treg cell depletion of IL-10 expression increases both lung allergic inflammation and hypersensitivity.[29] IL-10 produced by regulatory T-cells is also essential in preventing irritable bowel disease (IBD).[22] Although IL-10 is not the only mechanism by which Tregs can dampen immune responses, it has been consistently linked to Treg activation. Present literature supports the hypothesis that IL-10 from Treg cells is dependent on both the organism itself and the disease state of the host. IL-35 is another inhibitory cytokine thought to be required for maximal Treg cell function but the implications of IL-35 are still unclear.[30]

Disruption of effector T-cells (CD4+)

Regulatory T cells can directly suppress target effector T cells that elicit an inflammatory immune response in the host. As mentioned previously, Tregs

express CD25, the IL-2 receptor α chain, which can drive “cytokine deprivation mediated apoptosis”. CD25 supports local IL-2 consumption by Tregs which starves developing effector T cells that are dependent on IL-2 for survival.[31,32] However, this mechanism is still vigorously debated and a final consensus opinion will require additional research.

Treg cells release adenosine by converting ATP to AMP via ectonucleotidases CD39 and CD73.[33] Ligation between adenosine and the A_{2a} receptor present on effector T cells results in a signal transduction pathway upregulating second messenger cAMP concentrations.[34,35] Elevated intracellular levels of cAMP suppresses the immunogenic activity of effector T cells. Additionally, adenosine may indirectly suppress effector T cells through modulation of DC maturation and the promotion of a tolerogenic phenotype. Regulatory T cells may also increase intracellular cAMP concentrations by transferring adenosine through gap junctions of effector T cells.[36] As with other mechanisms, additional studies are required to support and extend existing data regarding this particular mechanism before it is viewed as a bona fide action of Tregs.

Not only does the adenosine-A_{2a} receptor complex effect effector T cell functioning, it has also been shown to suppress production of the proinflammatory IL-6.[37] IL-6 is essential for preventing Treg generation and inducing differentiation of CD4+ naïve T cells into proinflammatory Th17 cells.[38] If an imbalance occurs and Th17 outweighs Tregs, the local host environment will favor an immunogenic response rather than promoting tolerance. This

inflammatory response is necessary for riding the host of pathogens but is unnecessary, and detrimental, when dealing with dietary antigens, self-antigens, and commensal microbiota.

Modification of dendritic cells

Although mediating the immune response by direct interaction with effector T cells is beneficial, Tregs often do so through DC mechanisms. Tregs can condition DCs by interactions between CTLA4 and dendritic cell CD80 and/or CD86. Upon contact, expression of indoleamine 2,3-dioxygenase (IDO) is upregulated and acts as a strong regulatory molecule by inducing the catabolism of tryptophan into apoptotic metabolites which can suppress effector T cells.[39,40] Tregs also possess the ability to directly downregulate expression of CD80 and CD86 on DC.[41] Abberant expression of these costimulatory molecules leads to unresponsive, or anergic, effector T cells.[42]

Promotion of cytolysis

The final way Tregs promote tolerance is by deleting antigen specific T cells, thus eliminating an unwarranted immunogenic response against harmless antigens. Perforin and granzyme produced by Tregs are packaged into granules and released to induce apoptosis of effector T cells.[43] Perforin disrupts the cell membrane while granzyme, a protease, affects cellular proteins. Fas ligand, used by iTregs, also promotes apoptosis of effector T cells.[44]

The preceding modes of action of Tregs lead to immune tolerance by promoting: (i) Suppression of immune cells, (ii) deletion of effector T cells and/or (iii) anergy also called immune unresponsiveness.[19] Depending on the situation a specific mechanism will be used to suppress immunogenic responses.[45] It is crucial to retain a balance between Tregs and effector T cells. When the appropriate ratio is disrupted and effector T cells, such as Th17 or Th1, in the gut, become the majority, immune responses will produce inflammation. Conversely, an unbalanced tolerogenic environment diluted by Tregs would leave the host susceptible to harmful pathogens.

β-Galactomannan

β-Galactomannan (β-GM) is a non-starch polysaccharide (NSP) fiber shown to have adverse effects on the digestion and absorption of other nutrients, most likely due to its ability to impair the epithelial barrier function.[46] The anti-nutritive effects associated with β-galactomannan may be secondary to its activation of inflammatory immune responses.[47] Structurally, galactomannans consist of a mannose main chain (β-1,4-mannopyranose) with galactose side chains. These NSP are typically isolated

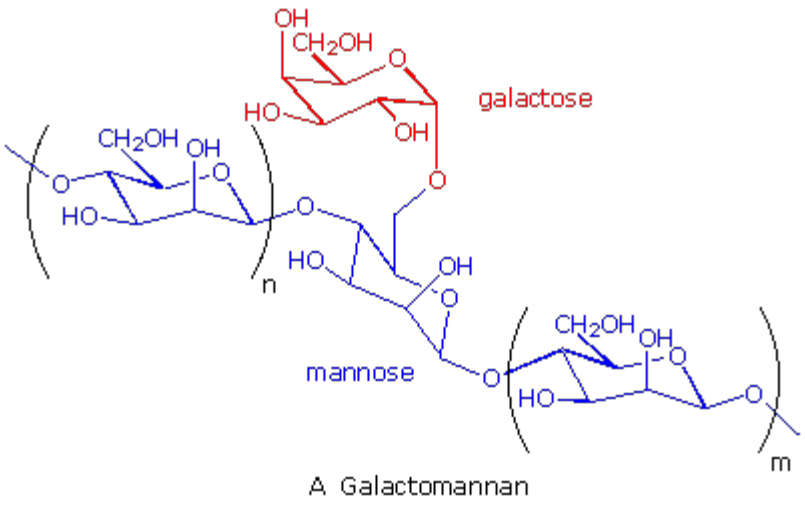

Figure 2: β-galactomannan structure

from the endosperm of leguminous plant seeds or microbial sources.[48] The diversity of galactomannans varies based on the galactose:mannose ratio (gal:man) with galactose molecules specifying the solubility of each source.[49] Table 1 shows the gal:man ratio of various galactomannan gums and sources. Several industries, including the food industry, rely on the important chemical properties of galactomannans to promote thickening, gelling, binding, emulsifying, increased water holding capacity, and suspension.[48] Animal feed ingredients, including soybean meal (SBM), also contain β-GM in sufficient quantities (1.02-1.51%) to be implicated in reduced weight gain, diminished performance, and impaired absorption of glucose and water in poultry.[50] Similar detrimental consequences of dietary β-GM have also been shown in swine.[51,52]

Consumption of β-Galactomannan elicits an immunogenic immune response

NSPs, including galactomannan, are produced by leguminous plants and extracellularly by pathogenic microorganisms to enhance their virulence.[53] Therefore, it is completely reasonable to hypothesize that oral administration of plant derived β-GM will possess immunogenic properties. Indeed the literature to date has indicated strong immunostimuatlory effects of mannan polysaccharides. Of particular medical importance, acemannan reportedly reduces mortality rates of sarcomas in mice and stimulates DC maturation and subsequent T cell activation required to fight infection and disease.[54.55] Galactomannans also induce the production of innate inflammatory cytokines, including IL-12, IL-6, and IFN-γ, and cause DC maturation.[56]

It is of great importance to maintain balance when it comes to the gastrointestinal immune system. The GIT is constantly exposed to both pathogenic and non-pathogenic materials and is responsible for mounting the proper immune response. Eliciting an inflammatory response against harmless antigens, such as plant derived β-GM, is detrimental and causes several destructive side effects when chronically activated. Disruption to the epithelial barrier, impaired nutrient absorption, and reduced growth due to nutrients being partitioned to support the immune response are potential mechanisms.[57,58]

Dendritic cells can sample luminal contents for dietary factors, such as galactomannan, which causes antigenic or immunogenic responses by opening tight junctions between epithelial cells, antigen direct entry into the Peyer's patches through M cells, or transportation of antigen directly into the lamina propria. In fact, Rescigno et al.[59] found that expression of DC transepithelial projections increased when bacteria were present. Galactomannan is recognized by PRRs but the exact PRR galactomannan signals through remains unclear. Some researchers speculate cross-talk between TLRs and C-type lectin receptors (CTLs) are required for the immune repsonse.[60] CTLs are carbohydrate recognition domains able to recognize subtle differences in arrangement and branching of carbohydrates.[60] The main function of CTLs is to internalize pathogens for degradation and enhance antigen processing and presentation.[61] CTLs are thought to promote tolerance by inducing Treg and Th2 production.[62] Geijtenbeek et al.[63] demonstrated that when antibodies were used to block the DC-SIGN, a CTL, tolerance was compromised and proinflammatory

IL-12 concentrations were restored. TLRs are able to recognize foreign carbohydrate structures and initiate an intracellular signaling pathway (i.e., the MyD88 pathways), which can lead to production and secretion of inflammatory cytokines. Communication between TLRs and CTLs may be critical for immune tolerance and activation. When a foreign antigen binds concurrently to CTLs and TLRs, the TLR can override the tolerant effect of the CTL and evoke an immune response, even if the antigen is innocuous galactomannan found in food.[63] After TLR and CTL activation, intracellular signaling leads to the production of proinflammatory cytokines IL-12, IL-6, IL-1, TNF-α and chemokines.[55] This innate response causes acute inflammation in the gut characterized by infiltration of leukocytes and other immune modulators into the tissue.

Dendritic cells also trigger the adaptive immune response. After TLR signaling occurs, DCs increase expression of MHC for antigen presentation, and increase the production of co-stimulatory molecules, IL-12, adhesion molecules, chemokine receptors, and have diminished ability for antigen phagocytosis.[64,65,55] Mature DCs migrate via lymph to the MLN and induce differentiation of naïve CD4+ cells into effector T cells, Th1 and Th17, to assist in clearing the infection.

Consequences of intestinal inflammation

Persistent insults to the gut epithelia, such as continuous intake of galactomannan, may result in a long-term inflammatory state known as chronic inflammation. Numerous studies have reported on the inverse correlation between inflammation and body weight.[17] Several mechanisms have been

implicated as the root cause of the decrease in weight gain during periods of gut inflammation. Kanno et al.[66] showed villous atrophy in histological samples collected from swine undergoing immune challenge. Because the villi are essential for effective nutrient absorption, compromised uptake of nutrients was likely. Indeed, measures of glucose, sodium, and chloride transport indicated all to be significantly reduced. This reduction of nutrient absorption may exaggerate catabolic pathways in the host leading to reductions in weight gain or even weight loss. Furthermore, compromised nutrient absorption may be coupled with repartitioning of nutrients away from growth to support immunological pathways.[57] Finally, another contributor to suppressed weight gain during inflammation is the direct regulation of metabolic pathways by certain proinflammatory cytokines such as TNF-α and IL-6. One study administered inflammatory cytokines to rats at physiological levels which led to altered consumption and consequently weight loss.[67]

A more detrimental effect of intestinal inflammation can lead to various pathologies. Chronic stimulation of TLRs in gut epithelial cells and leukocytes can cause a decrease in epithelial cell migration and proliferation resulting in aberrant intestinal restitution.[68] In TLR4 knockout mice, enterocyte apoptosis rates were ameliorated, which implicates this PRR in intestinal cell apoptosis.[68] When IECs become apoptotic, barrier integrity is compromised leaving hosts susceptible to pathogenic material and opportunistic commensals.[57] Invasion of these microorganisms exacerbates inflammation in order to protect the host but this protective response only further disrupts intestinal integrity and creates a

vicious cycle. It is possible, as has been demonstrated in the literature, that plant-derived galactomannan provokes superfluous innate immune responses and inflammation.[47] Regular consumption may cause chronic inflammation and, as previously noted, lead to epithelial damage and defects.

Therapeutic effects of mannans

Cancer and cancer treatments, such as chemotherapy, can significantly weaken the immune system which is a critical component needed to help fight diseases associated with tumors. It seems logical to hypothesize that the inflammatory effects observed during mannan administration may help boost the immune system in times of need. Immunomodulator acemannan has been researched extensively for its ability to activate the immune system. Bacterial LPS is commonly used to treat tumors but its use also results in unfavorable side effects such as pyrogen-associated toxemia, otherwise known as toxic shock syndrome.[69] Studies reveal LPS and acemannan both stimulate production of inflammatory cytokines IL-1 and TNF-α with acemannan having a more powerful effect.[70] Compared to control mice, acemannan treated mice presented greater tumor regression which could be due to the stimulatory effects of inflammatory cytokines.[70]

The seed Fenugeek contains galactomannan that may possess anti-diabetic properties. Evans et al.[71] found fenugeek consumption inhibited glucose absorption and other studies revealed that fenugeek galactomannan decreased the post-prandial glucose response.[72]

Clinical use of a non-toxic, plant derived therapeutic agent to treat pathologies is a promising alternative to toxic medications currently used to fight different disease states. Although the therapeutic effects of mannans are promising, contradictory evidence is present in the literature. Some studies show no significant therapeutic effect of mannans. The different outcomes of mannan consumption may be due to variances in structure such as the galactose:mannose ratio in various galactomannan sources. Further investigation is required to fully elucidate its therapeutic effects.

Enzymatically modified β-GM may have anti-inflammatory effects and promote tolerance

Humans and other species lack the enzymes necessary to digest the non-starch polysaccharide β-GM but this altered structure can alleviate the immunogenicity of the antigen and is necessary to induce anti-inflammatory effects and promote a tolerogenic environment.[73] The animal industry developed particular interest in β-GM and its adverse effects in livestock such as swine and poultry. Some researchers found β-mannanase, an enzyme capable of digesting β-GM, is capable of increasing lean gain and average daily gain compared to their counterparts who did not consume a feed with the added enzyme.[52] Due to conflicting data, the effects of β-mannanase in livestock remains unclear.

Partially hydrolyzed guar gum (PHGG) supported an anti-inflammatory environment revealed by statistically lower levels of IgG in MLNs and reduced IgA levels in serum, compared to guar gum (GG).[74] Pre-feeding rats PHGG diminished protein and mRNA levels of the inflammatory cytokine TNF-α after

being administered DSS to induce colitis. Weight loss and reduced infiltration of immune cells were also observed which supports the hypothesis that PHGG restrains the mucosal inflammatory response.[75]

It is possible that enzymatically digested β-GM promotes a tolerogenic environment by preventing maturation of immature DCs and differentiation of Tregs from naïve T cells. Immature dendritic cells are highly capable of antigen uptake. During steady state conditions in the gut there is lack of inflammatory signals, such as inflammatory cytokines and chemokines.[76] Recognition of dietary or self antigens by the PRRs (TLRs and CLRs) of the dendritic cell triggers an intracellular signal that leads to the translocation of the transcription factor NF-κB to the nucleus which promotes synthesis of IL-10 mRNA. This anti-inflammatory cytokine is critical for promoting tolerance in the gut and other host tissue. In this scenario, low amounts of IL-12, an inflammatory cytokine, are produced and secreted. Release of IL-10 by immature DCs has an autocrine effect and blocks maturation of the immune cell, which is characterized by reduced expression of MHCII, costimulatory molecules, adhesion molecules, and IL-12 production.[77] It has been demonstrated that DCs, in the presence of IL-10 antibodies, matured and became capable of activating naïve T cells to develop into effector T cells eliciting an immunogenic response.[78] The secreted IL-10 is also an important signaling molecule in adaptive immunity for the differentiation of naïve T cells into IL-10 producing iTregs upon antigen presentation. TGF-β is another immunosuppressive and anti-inflammatory cytokine produced by immature DCs and iTregs.

Modulated dendritic cells travel to the MLNs to promote T cell anergy or apoptosis while also inducing Treg production. The immature dendritic cells present the harmless antigen on MHCII to a naïve CD4+ T cells receptor in the presence of IL-10 and TGF-β. The result is production of IL-10 or TGF-β secreting iTregs. Induced Tregs can suppress immune cells, which supports gut homeostasis and tolerance.[78] IL-10, secreted from any immune cells or IECs, inhibits maturation of DCs leading to the conversion of immunogenic DCs to tolerogenic DCs.[79]

Plenty of research has yet to be completed in order to pinpoint the exact mechanism enzymatically digested β-GM promotes tolerance but the above pathways appear to be a sound hypothesis.

Roles of IL-10 in immune tolerance

Interleukin-10 (IL-10) is a pleiotropic cytokine with both immunosuppressive and anti-inflammatory effects. Several leukocytes and lymphocytes can produce and secrete IL-10 such as monocytes, macrophages, T cells, and dendritic cells. IL-10 secretion can dampen the immune system and prevent excessive collateral damage during times of immunogenic challenge and promote a tolerogenic environment upon host exposure to harmless antigens. Preliminary research has revealed that administration of IL-10 as a therapeutic agent is 100 times more efficient than Cyclosporin A, a common immunosuppresor.[80] Absence of IL-10 causes uncontrolled chronic inflammation and impairs tolerance to dietary and self antigens.[81] IL-10 knockout mice

presented chronic IBD, anemia, and compromised growth rates compared to wild-type mice.[81] These deleterious outcomes, to a lesser extent, were even present in IL-10 knockouts housed in a germ-free environment highlighting the importance of IL-10 in the tolerance of normal enteric antigens. Injections of IL-10 ameliorated inflammation, weight loss, and improved survival rates.

Mechanistically, IL-10 has both direct and indirect effects on many immune cells. In the case of antigen specific T cell differentiation and proliferation, IL-10 can directly inhibit the production of IL-2, a cytokine responsible for the proliferation of activated T cells.[79] IL-10 can also indirectly affect naïve CD4+ T cell activation by downregulating APC expression of MHCII, adhesion molecules, and costimulatory molecules (CD40, CD80, CD86).[64,82] Inflammatory cytokine and chemokine production is repressed by IL-10. Without co-stimulatory molecules, inflammatory cytokines such as IL12, and chemokines migration and activation of T-cells will not occur. Suppression of DC IL-12 production by IL-10 prevents stimulation of NK cells and impairs Th1 development in the periphery.[83] Presence of IL-10 modulates DCs and promotes a tolerogenic function. These tolerogenic DCs (tDCs) produce low levels of inflammatory cytokines and high levels of anti-inflammatory cytokines, particularly IL-10.[84] tDCs interact with naïve T cells in the lymph nodes leading to anergy, apoptosis, or induction of Tregs.

IL-10 also exerts its effects on non-immune cells such as the goblet cells of the intestinal tract, which are responsible for producing mucins, an important component of mucus. During an inflammatory challenge, defective folding of

proteins may occur leading to endoplasmic reticulum (ER) stress and activation of the unfolded protein response (UPR) and exacerbation of intestinal inflammation. Chronic ER stress in goblet cells disrupts mucin production therefore depleting the protective mucus barrier of the epithelium.[85] IL-10 maintains mucin secretion by promoting correct protein folding, which suppresses ER stress and UPR activation.[85] UPR transcription factor ATF6 translocation to the nucleus is blocked by IL-10 signaling.[86]

Prebiotic role of hydrolyzed galactomannan

The use of probiotics and prebiotics to improve gut health has become increasingly popular among Americans. Prebiotics, which are typically non-digestible oligosaccharides, are utilized by microbiota of the GIT to promote growth of selected beneficial bacteria. Appropriate microbial members of the intestine, such as lactobacillus and bifidobacterium, of the intestine displace undesirable microflora, inhibit pathogens, and improve immunological status of the host.[87-89] Clinical studies have revealed administered partially hydrolyzed guar gum, prebiotic galactomman, increased bifidobacterium concentrations in feces in comparison with control feces and significantly increases lactobacillus.[90,91]

Commensal gut microbiota are capable of preventing pathogen attachment to epithelial cell and may do so by acting as receptor decoys on intestinal epithelial cells.[92] Consequently, the host can excrete this pathogen-prebiotic complex. Shoaf et al.[92] tested numerous prebiotic oligosaccharides and

their ability to reduce adherence of *Escherichia coli in vitro*. Galactomannan oligosaccharides prohibited *Escherichia coli* adherence to epithelial cells to the greatest extent in comparison to other prebiotic oligosaccharides.[92] Other pathogens such as, *Salmonella enterica*, also elicit an immune response upon attachment characterized by production of inflammatory cytokines and chemokines. Prebiotic galactomannan treatment reduced IL-6 and CXCL8 production by 40% and 30%, respectively, compared to negative control cultures of medium.[93] Using natural prebiotic galactomannan to improve gastrointestinal health is an exciting and intriguing area of research that will hopefully lead to reduced reliance on clinical medications that can cause adverse side effects and toxicities.

CHAPTER 2: IMPACT OF PLANT DERIVED β-GALACTOMANNAN ON HEALTH AND IMMUNE STATUS OF SPRAGUE-DAWLEY RATS

Abstract

Intestinal inflammation triggered by dietary antigens or immunogens is an energy draining physiological process that compromises gut function and health. Guar gum, a common food additive, contains β-galactomannan, which is also found on the surface of many microbial pathogens. Previous studies, both *in vivo* and *in vitro*, have shown β-galactomannan possesses immunostimulatory capabilities. In livestock, β-galactomannan reduced weight gain, impaired nutrient absorption, and increased inflammatory cytokines. In this study, male Sprague-Dawley rats received either the control diet or the experimental diet containing guar gum β-galactomannan (100g/kg) in place of cellulose. After 3, 7, 14, and 21 days rats were sacrificed and the small intestine was collected for analysis. Rats receiving β-galactomannan gained significantly less weight (9.4%; $p<0.05$) and exhibited reduced food intake compared to control rats (12.3%; $p<0.05$). Based on previous studies it was expected that this result was owing to an inflammatory state; however, mRNA abunance of inflammatory cytokines IL-12a, IL-12b, and IL-6 in the ileum were not affected by β-galactomannan consumption. Moreover, the anti-inflammatory cytokine IL-10, as well as Unfolded Protein Response (UPR) markers ATF-6 and PERK were without effect. Contrary to our hypothesis, β-GM increased IL-10 and decreased IL-12 mRNA abundance in the jejunum. Based on our study, guar gum β-galactomannan reduced overall

weight gain and dietary intake in rats, but did not invoke an innate immune response.

Introduction

Dietary antigens and immunogens can cause gut inflammation, and if chronic or repeated exposures occur, gut function and health become compromised. Locust bean gum, guar gum, tara gum and fenugreek gum are common industrial food additives used primarily as thickeners, binders, and stabilizers.[94] These seed gums are a major source of β-galactomannan (β-GM), a non-starch polysaccharide (NSP) composed of a β-(1,4)-mannan backbone with α-(1,6) side chains. The galactose to mannose ratio is variable between galactomannan sources leading to various functional properties. Animal studies[46,49,51,52] indicate that β-galactomannan may contribute to the anti-nutritional aspects of soybean products which include inflammation and diminished growth and nutrient utilization. Furthermore, there are indications that Ig profiles may be altered in animals consuming guar gum[54]. These studies underscore a key question regarding dietary β-galactomannan, that being whether consumption of this NSP induces an innate immune response and invokes a pro-inflammatory response in the gut. Due to their commercial use and prevalence in microbial sources, it is important to characterize and understand the immunological effects and health consequences of galactomannan consumption. Consequently, the aim of the present study was two-fold. First, we sought to test the hypothesis that consumption of significant

quantities of β-GM (provided as guar gum) invokes an innate immune response and promotes inflammation in the gut, and reduces feed intake and growth in rats. Secondly, we determined whether gastrointestinal disturbances associated with consumption of guar gum were linked with increased expression of markers of the unfolded protein response (UPR).

Materials and Methods

Animals. All protocols were approved by the Institutional Animal Care and Use Committee and performed according to Iowa State University Laboratory Animal Resources Guidelines. Six-week-old male Sprague-Dawley rats (N=96; Harland Teklad, Indianapolis, IN) were obtained at 148-171 grams. Rats were singly housed in plastic cages in a room with a 12 hour light-dark cycle, and given ad libitum access to food and water. The experiment was carried out with two replicate groups of rats started on dietary treatments 3 weeks apart. The rats were maintained on a soy free pellet until started on experimental diets (see table 2.1). Each treatment group within replicate was further divided into four groups that were indicated by days on diets until sacrifice: day 3 (n=7), day 7 (n=7), day 14 (n=7), and day 21 (n=7). The second trial of mice (n=40) were fed a the same generic soy pellet diet but were acclimated for four weeks before being randomly divided into the two dietary treatment groups (control n=20, β-GM n=20) and assigned to a sacrifice time: day 3 (n=5), day 7 (n=5), day 14 (n=5), and day 21 (n=5). On their respective sacrifice days, rats were fasted for six hours before being euthanized by CO_2 asphyxiation for sample collection. Whole blood was

obtained immediately via cardiac puncture and centrifuged for isolation of plasma. Small intestine, spleen, and epididymal fat pad tissue was collected and frozen on dry ice prior to storage at -80°C.

RNA extraction and isolation. RNA was isolated from the ileum and jejunum a using Quick Gene RNA tissue kit SII (Kurabo, Osaka, Japan). The manufacturer's instructions were followed with minor changes. A small amount of tissue was pulverized using liquid nitrogen, a mortar bowl and pestle. A pea-sized amount of the crushed tissue was place in a tube containing 500 μl of LRT and β-mercaptoethanol mixture. Samples where then homogenized using a standard sonicator for 30 seconds and then centrifuged for three minutes at 17,000 x g. The centrifuge step was completed at room temperature. Following centrifugation, 385 μl of supernatant was removed from samples and transferred into a new tube. After the transfer, 175 μl of SRT was added and samples were vortexed for 15 seconds. Next, 140 μl of 99% EtOH was added to the tube and subsequently vortexed again for 1 minute. Samples were pipetted into cartridges and placed into a QuickGene-810 Autogen (FujiFilm, USA) for further processing. Trace DNA was removed from RNA using a TURBO DNA-free™ kit (Invitrogen, Carlsbad, CA). To determine the concentration and purity of RNA samples, the NanoDrop ND-1000 Spectrophotometer was used.

cDNA synthesis and quantitative real-time PCR. First strand cDNA was made in a 20 μl mixture using a BioRad iCycler. First, 1 μl RNA, 1 μl 10 mM dNTP mix

and distilled water to 12 μl. Mixture was heated to 65°C for 5 minutes and chilled on ice. 4 μl 5x First-Strand buffer, 2 μl 0.1 M DTT, and 1 μl RNaseOUT™ Recombinant Ribonuclease Inhibitor was added to the initial mixture and gently mixed. Mixture was incubated at 37°C for 2 minutes. Following incubation, 1 μl of M-MLV Reverse Transcriptase was added and the resulting mixture was incubated at 37°C for 50 minutes followed by an inactivation step for 15 minutes at 70°C.

Quantitative real-time polymerase chain reactions (qPCR) were performed using Roche Light Cycler 96 in triplicates. Each 20 μl PCR mixture contained 10 μl Fast Start Essential DNA Green Master 2x concentration (Roche), 100μM forward primer (Integrated DNA Technologies), 100μM reverse primer (Integrated DNA Technologies), 8 μl RNase free water, and 1 μl of cDNA. The 40 cycle amplification protocol consisted of an initial denaturation at 95°C for 10s, followed by 10s annealing step at 56-60°C, depending on gene, and extension at 72°C for 10s.

Statistics. Statistics were calculated using the SAS 9.4 software (SAS, Cary, NC). Data from both replicates were combined for analysis unless otherwise noted. Means of treatment group were compared to control using the student's t-test. Differences were considered significant at $p<0.05$ and a tendency at $p<0.10$.

Results

β-galactomannan supplementation lowered total weight gain and total feed intake. Rats receiving the β-GM diet gained, on average, 9.4% less weight than control rats ($p<0.05$, Fig. 1A). However, total feed intake of rats consuming β-GM was also significantly reduced ($p<0.05$) by about 12% (Fig. 1B).

β-galactomannan reduced dietary intake throughout the 21 day experiment. Analysis of the interaction between diet and sacrifice day revealed rats fed β-GM regularly ate less than control rats during the duration of the study. Rats fed β-GM consumed less than rats fed the control diet ($p<0.05$, Figure 2). On day fourteen we observed the biggest discrepancy in feed intake between treatments revealing rats consuming β-GM ate 36g less. By day 21 the difference in feed intake between the two groups decreased to 28g, roughly. Diet x sacrifice day interaction $p<0.10$.

Dietary treatment did not change cytokine mRNA abundance in the Ileum. β-GM did not impact anti-inflammatory IL-10 mRNA levels (Fig. 3A). Likewise, transcript abundance of inflammatory cytokines IL-6, IL-12a, and IL-12b were not affected by β-GM treatment (Fig. 3B, 3C, 3D).

Diet did not affect ileal mRNA abundance of transcription factors PERK and ATF-6. Although it appears β-GM decreased mRNA abundance of the

transcription factors PERK and ATF-6 not significant differences were identified. β-GM did not alter PERK or ATF-6 mRNA levels (Fig. 4).

β-GM treatment reduced jejunum IL-12a mRNA transcript abundance. Analysis of mRNA transcript abundance for IL-12a in the jejunum revealed significant alterations in rats fed β-GM relative to control rats ($p<0.05$) (Fig. 5). β-GM supplementation resulted in lower mRNA levels of IL-12a.

Neither dietary treatment significantly impacted IL-12b transcript abundance in the jejunum of rats. Although the data suggests β-GM increased abundance of IL-12b mRNA, analysis revealed no significant differences between dietary groups (Fig. 6).

IL-10 transcript abundance in the jejunum was greater in rats fed β-GM compared to control rats. Data for IL-10 transcript abundance revealed increased IL-10 mRNA levels in the jejunum of rats fed β-GM.

Discussion

β-GM has been associated with unnecessary immune system stimulation and subsequent weight loss; however, the current literature is controversial and scarce. Here, we hypothesized that consumption of β-GM would trigger an unwarranted innate immune response.

Dendritic cells (DCs) are capable of monitoring luminal contents and orchestrating a tolerogenic or immunogenic immune response. Depending on the intestinal micromileu, subtype, and maturation, DCs and their cytokine profile induce differentiation of naïve CD4+ T-cells into immunogenic (Th1, Th2, Th17) or tolerogenic (Treg) T-cells.[95] Research shows NSPs, which are innocuous food antigens, may evoke a superfluous inflammatory immune reaction which has been characterized by enhanced macrophage activation, increased NF-κB expression, raised levels of serum IgA, and high IgG and IgM activity in mesenteric lymph nodes.[47,52,74] Because inflammation is an energy draining process, nutrient partitioning favors immunological processes instead of anabolic pathways which often leads to decreases in body weight due to wasting.[16] Persistent inflammatory insults to gut epithelial have also been shown to cause villi atrophy and consequently impaired nutrient absorption, ER stress, increase autophagy of mucosal cells, reduced brush border enzyme activity, aberrant intestinal restitution, compromised barrier integrity, and susceptibility to pathogenic and opportunistic commensals.[58,66,68,96] In livestock, studies have demonstrated that by enzymatically breaking down β-GM in soybean meal with β-mannase, fewer β-GMs are able to induce an immune response and adverse side effects are alleviated.[52,97,98]

Inflammatory cytokines are a hallmark of the innate immune response and have been shown to be produced after β-GM is recognized by PRRs on DCs and macrophages.[99] The resulting immunogenic DC can induce effector T-cell differentiation. Inflammatory cytokines are necessary T-cell induction and for

recruiting inflammatory cells the site of infection. As immune cells aggregate, even more inflammatory cytokines are generated along with reactive oxygen species (ROS). Both these factors are capable of activating endoplasmic reticulum (ER) stress in mucosal cells and consequently the unfolded protein response (UPR).[100] PERK and ATF-6 are two important genes involved in regulating the UPR.[85,86] Based on this concept, it would be expected that the mRNA abundance of both transcription factors would be elevated in rats fed β-GM. However, our data reveals no significant differences between dietary treatments.

Livestock studies have consistently documented decreased weight gain in β-GM feeding studies. This inverse relationship between β-GM and growth inhibition is rational considering the adverse effects the inflammatory response has on intestinal epithelium. Any damage to barrier function, such as reduced villi height, can lead to malabsorption of key nutrients and most of the nutrients that still get absorbed will be utilized by the body to provide energy for immunological processes. Our study shows consumption of β-GM reduced total weight gain in rats by ~11% which is exactly what we had expected. Further analysis of our data revealed that β-GM also reduced total feed intake by ~12%. It is difficult to determine if the β-GM-mediated weight reduction is actually due to inflammation caused by β-GM or if an unfavorable taste of β-GM resulted in decreased food intake and consequently reduced weight gain. The largest difference in food intake occurred on day 14, where control rats, on average, were consuming ~16% more than those receiving the β-GM diet. This

discrepancy in dietary intake may be the result of innate immune responses in rats fed β-GM.

If inflammation is actually the culprit affecting weight reduction in this study, it is reasonable to assume inflammatory cytokine (IL-6, IL-12a, IL-12b) transcript abundance would be higher in rats fed β-GM and the anti-inflammatory cytokine IL-10 would be lower. Contrary to our hypothesis and existing literature, there was no diet effect on any cytokine (IL-6, IL-12a, IL-12b, IL-10) mRNA abundance in the ileum. Even more perplexing was that we observed significant changes in jejunum cytokine mRNA between diet groups and the results are opposite of what we anticipated. IL-12a transcript abundance decreased in rats fed β-GM whereas IL-10 increased. Previous literature reports the β-GM increases inflammatory cytokines and decreases anti-inflammatory cytokines.[47] This suggests its likely that β-GM is captured by C-type lectin receptors (CTLs) such as Mannose receptor (MR) and DC-SIGN.[101] When MR and DC-SIGN recognize high mannose containing antigens, such as β-GM, the dendritic cell will capture and present them without activating the cell.[102] CTLs may also interfere with TLR signaling and as a result inhibit DC maturation.[102]

Various studies have shown IL-10 is the primary cytokine produced following activation of MR and DC-SIGN on DC.[101-104] The autocrine effect of IL-10 on DCs modulates maturation resulting in tolerogenic DCs. An alternative explanation for increased IL-10 and decreased IL-12a is that the elevated IL-10 could be an adaptive response after prior exposure to β-GM in order to down regulate inflammation in the jejunum.

Future studies should consider using similar fiber types when comparing diet effect. In our study, guar gum is a soluble fiber and cellulose, used in the control diets, is an insoluble fiber. Because guar gum is soluble, it attracts water and will turn into a gel-like consistency during digestive processes. This gel can slow gastric emptying and signal satiety causing the rat to consume less and may be way our guar gum fed rats weighed less than rats fed the control diet.

Overall, our study showed inflammatory cytokine and UPR transcription factor mRNA abundance was not significantly increased in rats consuming β-GM. We can therefore conclude, from these inflammatory markers, there is no inflammatory response associated with β-GM intake. In the jejunum, β-GM surprisingly increased anti-inflammatory cytokine IL-10 transcript abundance. Cytokine IL-10 could possibly promote tolerance in the jejunum. Although, our study contradicts present research, it should be noted many of the experimental designs use mannans or galactomannans of different configurations from sources other than guar gum which could account for the variances in immune response.

Table 1: Galactose to mannose ratio of different galactomannan

Source	Gal:Man Ratio
Guar gum	1:1.7
Soybean meal	1:1.8
Fenugreek gum	1:1.1
Carob bean gum (Locust bean gum)	1:3.5
Tara Gum	1:3.0

Table 2: Ingredient composition of the control and β-galactomannan diets fed to rats

Components[a]	Control diet (g/kg)	β-GM diet (g/kg)
Sucrose	100.0	100.0
Maltodextrin	132.0	132.0
Corn Starch	327.5	327.5
Casein	200.0	200.0
Cellulose	100.0	0.0
Guar Gum	0.0	100.0
Corn Oil	90.0	90.0
Premix[b]	50.5	50.5

[a] All diet ingredients were purchased from Harlan Teklad (Madison, WI), choline bitartrate (Sigma Aldrich).

[b] Premix provided at 50 g/kg diet (g/kg diet): AIN-93 vitamin mix, 10; AIN-93 mineral mix, 35; L-Cystine, 3; Choline Bitartrate, 2.5; TBHQ, 0.014.

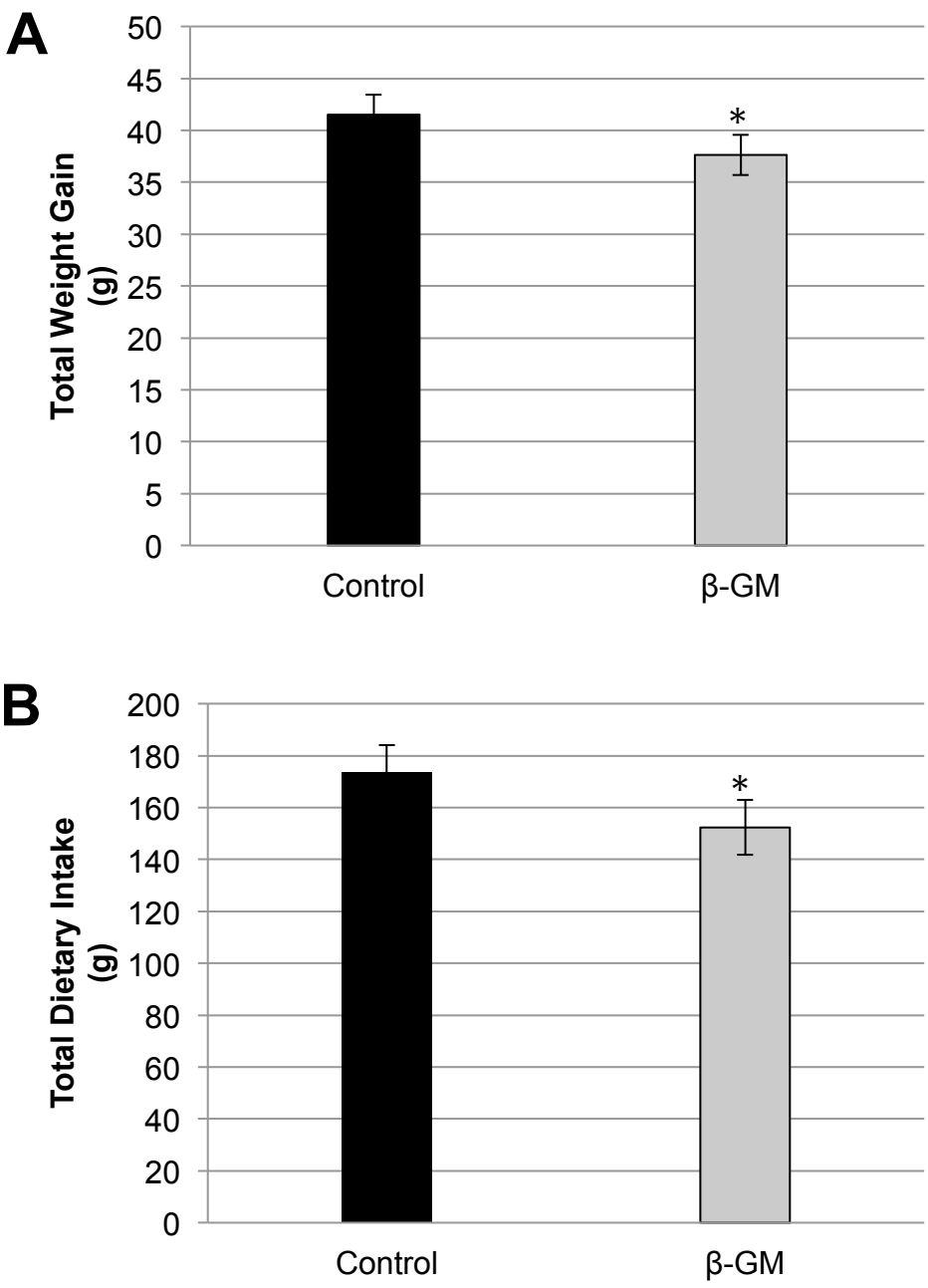

Figure 1: β-galactomannan supplementation causes reduced weight gain and reduced dietary intake. Male Sprague-Dawley rats (N=96) were randomly assigned to two different experimental replicates: Replicate 1 (n=56), Replicate 2 (n=40). Rats in each replicate received a control diet or a diet containing beta-galactomannan (100g/kg). Rats in both dietary groups were randomly assigned one of four sacrifice days (day 3, 7, 14, 21). Bars represent pooled data from rats in replicate 1 and replicate 2. Values shown are group means ±SE. Asterisk indicates significant difference in group mean when compared to the control using a student's t-test ($p<0.05$).

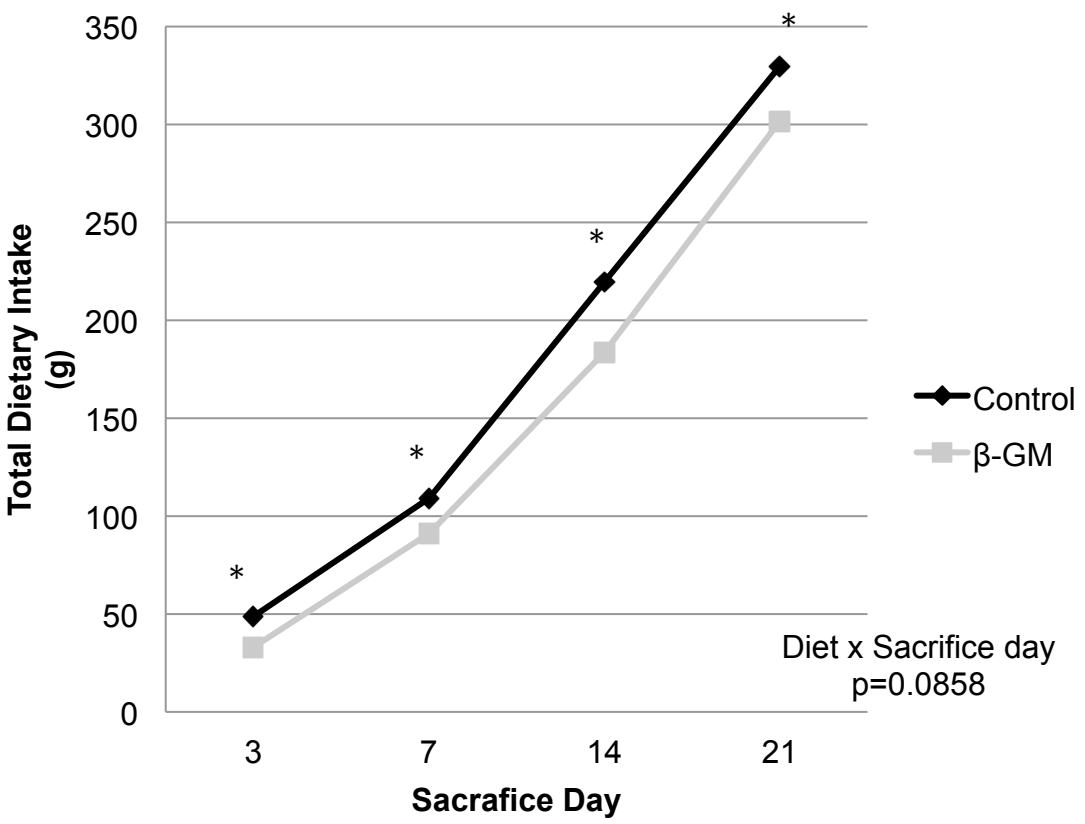

Figure 2: Rats fed β-GM ate less throughout the three weeks study compared to control mice. Male Sprague-Dawley rats (N=96) were randomly assigned to two different experimental trials: Replicate 1 (n=56), Replicate 2 (n=40). Rats in each replicate received a control diet or a diet containing beta-galactomannan (100g/kg). Rats in both dietary groups were randomly assigned one of four sacrifice days (day 3, 7, 14, 21). β-GM reduced dietary intake throughout the course of the 21 day experiment. The largest discrepancy in dietary intake occurs on day 14. Rats consuming β-GM ate ~36g less than control rats on day 14. Values shown are group means ±SE. Asterisk indicates significant difference in group mean when compared to the control using a student's t-test ($p<0.05$). Diet x sacrifice day interaction p=0.0858.

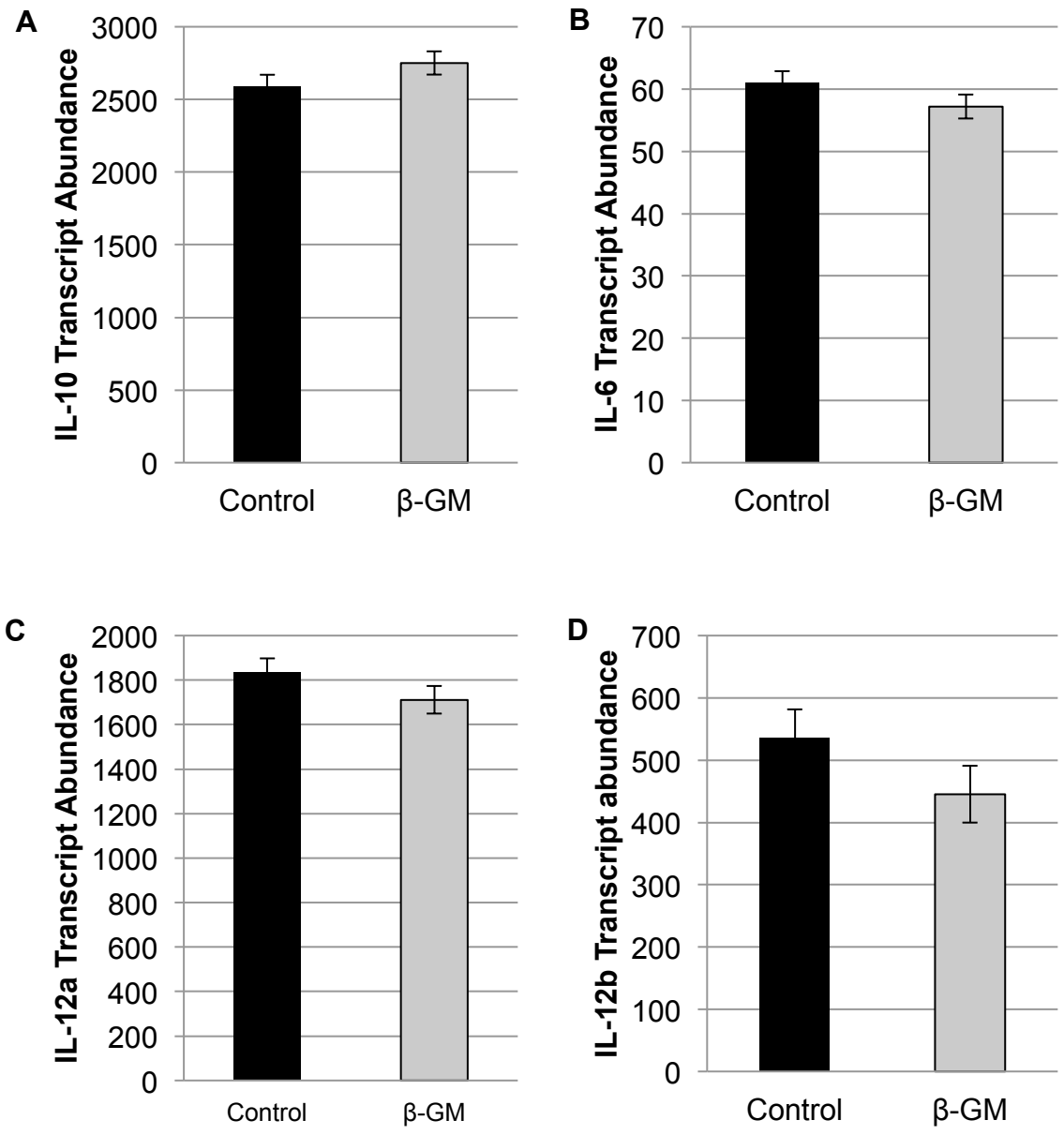

Figure 3: β-GM did not alter ileal IL-10, IL-6, IL-12a, or IL-12b transcript abundance. IL-10 (A), IL-6 (B), IL-12a (C), IL-12b (D). Male Sprague-Dawley rats (N=96) were randomly assigned to two different experimental replicates: Replicate 1 (n=56), Replicate 2 (n=40). Rats in each replicate received a control diet or a diet containing beta-galactomannan (100g/kg). Rats in both dietary groups were randomly assigned one of four sacrifice days (day 3, 7, 14, 21). Bars represent pooled data from rats in replicate 1 and replicate 2. Means between the dietary groups were not statistically significant. Values shown are group means ±SE.

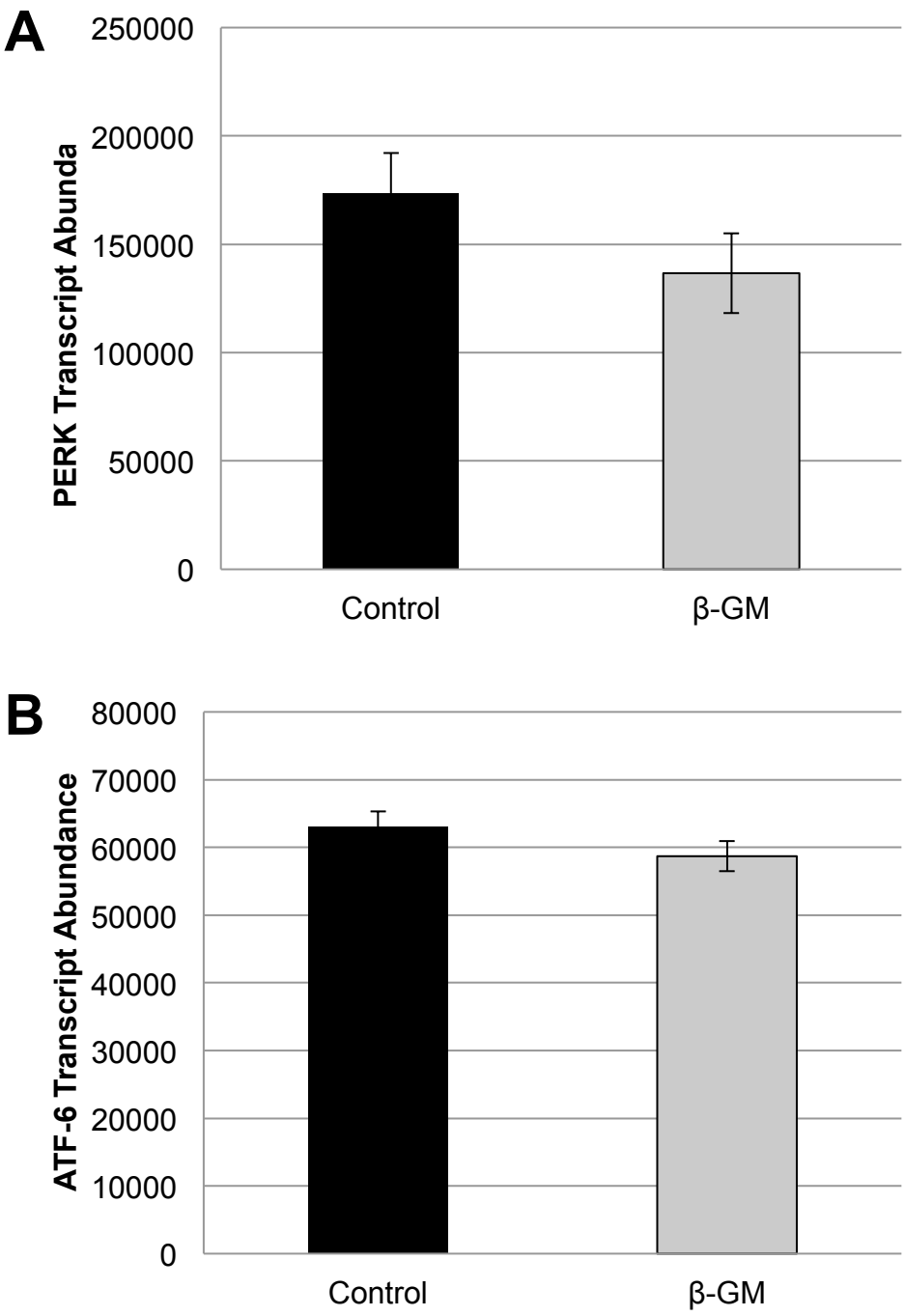

Figure 4: β-GM did not alter ileum mRNA transcript abundance of the PERK or ATF-6. Male Sprague-Dawley rats (N=96) were randomly assigned to two different experimental replicate: Replicate 1 (n=56), Replicate 2 (n=40). Rats in each replicate received a control diet or a diet containing beta-galactomannan(100g/kg). Rats in both dietary groups were randomly assigned one of four sacrifice days (day 3, 7, 14, 21). Bars represent pooled data from rats in replicate 1 and replicate 2. Means between the two dietary groups were not statistically significant. Values shown are group means ±SE.

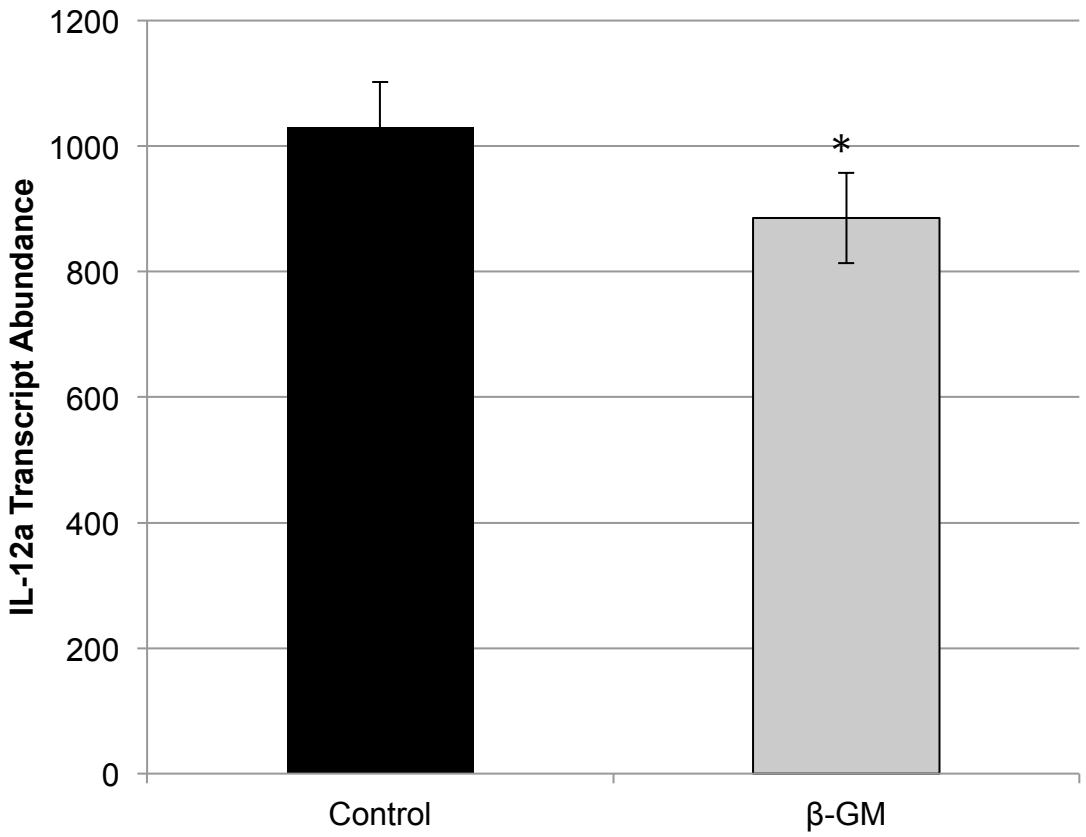

Figure 5: β-GM treatment reduced jejunum IL-12a mRNA transcript abundance. Male Sprague-Dawley rats (N=96) were randomly assigned to two different experimental replicates: Repliate 1 (n=56), Replicate 2 (n=40). Rats in each replicate received a control diet or a diet containing beta-galactomannan (100g/kg). Rats in both dietary groups were randomly assigned one of four sacrifice days (day 3, 7, 14, 21). Bars represent pooled data from rats in replicate 1 and replicate 2. Values shown are group means ±SE. Asterisk indicates significant difference in group mean when compared to the control using a student's t-test ($p<0.05$).

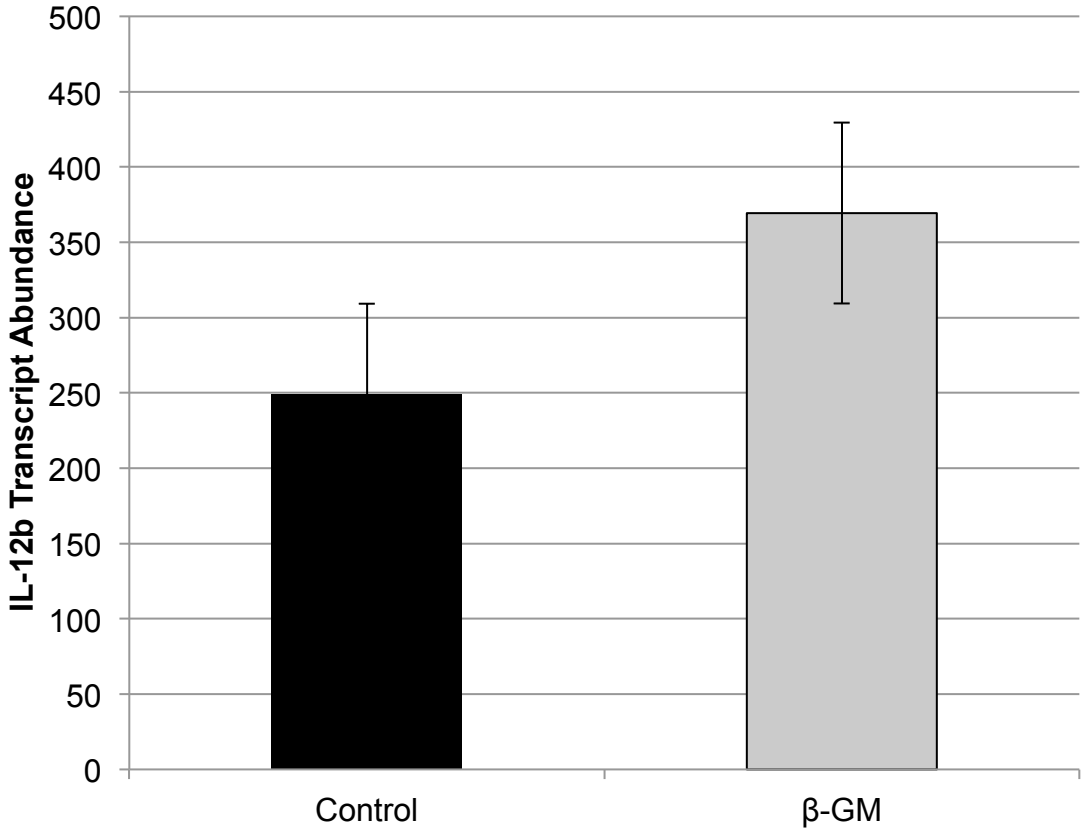

Figure 6: Neither dietary treatment significantly impacted IL-12b transcript abundance in the jejunum of rats. Male Sprague-Dawley rats (N=96) were randomly assigned to two different experimental replicates: Replicate 1 (n=56), Replicate 2 (n=40). Rats in each replicate received a control diet or a diet containing beta-galactomannan (100g/kg). Rats in both dietary groups were randomly assigned one of four sacrifice days (day 3, 7, 14, 21). Bars represent pooled data from rats in replicate 1 and replicate 2. Means between the dietary groups were not statistically significant. Values shown are group means ±SE.

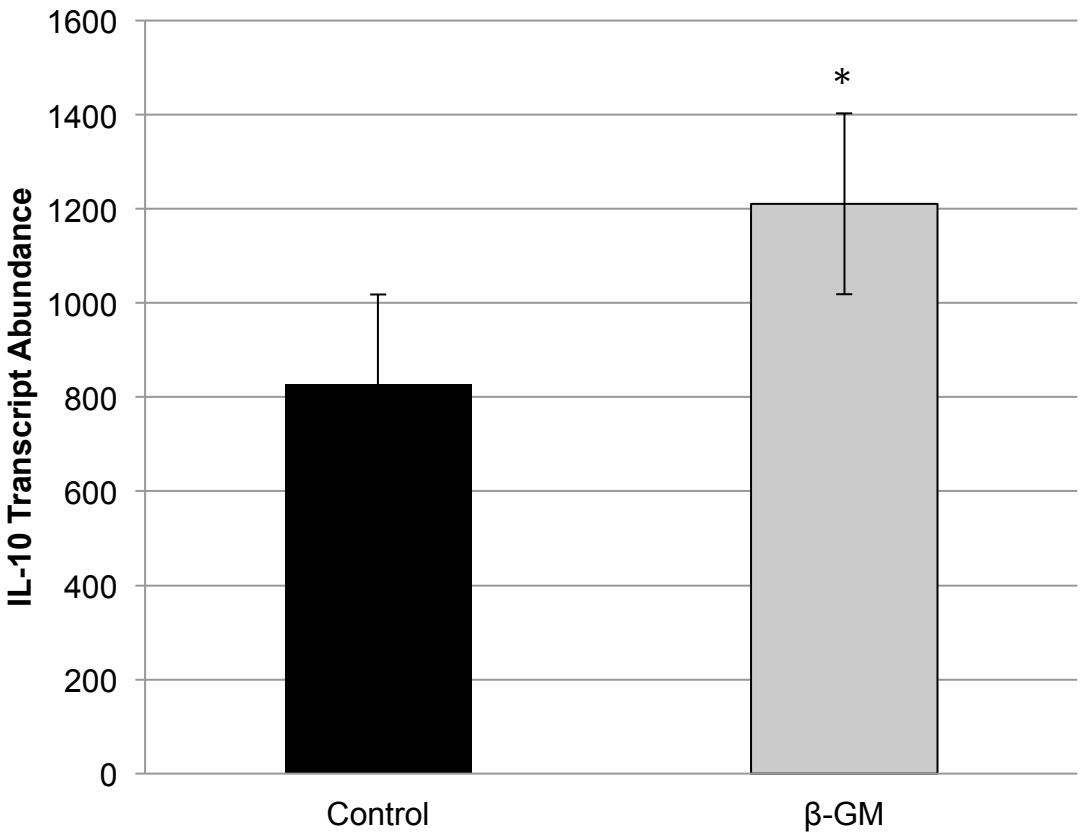

Figure 7: IL-10 transcript abundance in the jejunum was greater in rats fed β-GM compared to control rats. Male Sprague-Dawley rats (N=96) were randomly assigned to two different experimental replicates: Replicate 1 (n=56), Replicate 2 (n=40). Rats in each replicate received a control diet or a diet containing beta-galactomannan (100g/kg). Rats in both dietary groups were randomly assigned one of four sacrifice days (day 3, 7, 14, 21). Bars represent pooled data from rats in replicate 1 and replicate 2. Values shown are group means ±SE. Asterisk indicates significant difference in group mean when compared to the control using a student's t-test ($p<0.05$).

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