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# Selected physiological activities and health promoting properties of cereal beta-glucans. A review

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**ABSTRACT.** Cereal beta-glucans are linear homoglucose polymers found in endosperm and sub-aleurone layer of grain, consisting mostly of water-soluble fraction and exerting different specific biological activities. In this review beneficial effects of oat and barley purified beta-glucans on immunity, gastrointestinal tract health and inflammatory diseases treatment in animals are presented. Oat and barley beta-glucans interact with innate immune system cells and affect pro- and anti-inflammatory cytokines production leading to alleviation of inflammation. Few recent *in vitro* and *in vivo* studies showed also a prebiotic effect of cereal beta-glucans due to fermentation of these polysaccharides by beneficial gut microbiota. Barley and oat beta-glucans stimulate growth of *Lactobacilli* and positively affect large intestine content of short-chain fatty acids, faecal water content, pH value and decrease ammonia level in animals. Studies on farm animals, especially pigs, show that cereal beta-glucans can beneficially affect gastrointestinal tract functions and immunity of animals mainly due to their prebiotic activity and interactions with immune cell system. Beta-glucans tend to reduce body weight gain and may help to prevent obesity. The health-promoting properties of barley and oat beta-glucans should be taken into account when formulating diets for farm and companion animals.

## Introduction

Beta-glucans are polysaccharide compounds originating from various sources as bacterial exopolysaccharides, component of *Basidiomycota* fungi and yeasts (*Saccharomyces cerevisiae*) cell walls and cereal grains, mainly oat and barley. Physicochemical properties of cereal beta-glucans like molecular mass, degree of branching, water solubility and viscosity depend on their source and extraction method (Lazaridou et al., 2007). The yeast and other fungal beta-glucans differ from cereal beta-glucans. They contain about 53–83% of insoluble fraction, are built from glucose monomers linked with beta-(1,3) and beta-(1,6)-glycosidic bonds and due to such structure presumed as insoluble whereas the cereal beta-glucans are built of linear glucose molecules linked by beta-(1,3) and beta-(1,4)-glycosidic bonds and are water-soluble. Moreover, oat beta-glucan is in 82% built of soluble fraction and exerts very specific biological activity (El Khoury et al., 2012). Cereal beta-glucans as non-starchy polysaccharides of grain are considered as the component of dietary fibre. It is presumed that due to high absorption of water in the gastrointestinal tract they can produce viscous gels not digested in the small intestine. This property of barley and oat beta-glucans exerts documented effects on lipid and glucose metabolism and is beneficial in prevention of coronary heart disease and diabetes. These health-promoting effects of cereal beta-glucans were the basis of the authorized health claims in EU by European Food Safety Authority (Harland, 2014) and in USA by US Food and Drug Administration (FDA, 2016). Although, the very recent research reports put new lights on lipid and glucose metabolism activity of beta-glucans revealing that such activity is more pronounced for grain milling fraction containing beta-glucan as one of components than of purified beta-glucan (Grundy et al., 2016; He et al., 2016).

Studies on bioactivity of beta-glucans revealed their antioxidative, antiviral, immunomodulating and anticancerogenic effects found mostly in fungal beta-glucans. The beneficial effects of cereal beta-glucans on immune system and their role in preventing infections, chronic inflammation or cancer are also well documented, but not sufficiently appreciated.

The aim of this review is to outline the health-promoting effects of purified barley and oat beta-(1,3)(1,4)-glucans with particular emphasis on immunity and prevention of inflammatory diseases, and on mechanisms underlying these activities. The role of cereal beta-glucans as a prebiotic affecting gut health is also discussed. The review deals with the purified compounds (not grain milling fraction) and points that they should be taken into account as a supplement or component of specific diets for farm and companion animals.

## Beta-glucan sources and physicochemical properties

Cereal beta-glucans occur mainly in cell walls in grain endosperm and sub-aleurone layer. Cereal beta-glucans are linear polysaccharides consisting of glucose molecules linked by consecutive beta-(1,4) and single beta-(1,3)-glycosidic bonds. Beta-glucan contents in oat and barley vary depending on cultivar and plant growth conditions. The content of beta-glucans in the whole oat grains amounts 4–7% of dry matter (DM) and in bran 6–9% DM (Beer et al., 1997), whereas in the whole barley grains is 3–11% DM (Skendi et al., 2003).

Among cereal beta-glucans, oat glucans have the highest molecular mass ($65–3100 \times 10^3$ g · mol$^{-1}$) and molecular mass of barley beta-glucan is $31–2700 \times 10^3$ g · mol$^{-1}$ (Lazaridou and Biliaderis, 2007). Chemical structure of beta-glucan is determined using lichenase (beta-(1→3),(1→4)-D-glucan 4-glucanohydrolase), enzyme which catalyses hydrolysis of beta-glucan molecule to oligosaccharides. The molar ratio of tri- to tetrasaccharide (DP3/DP4) is 3.0–4.5 in wheat, 1.8–3.5 in barley and 1.5–2.3 in oat (Lazaridou and Biliaderis, 2007).

The activity of cereal beta-glucan was mainly connected with its ability to form viscous solution and gels (Siurek et al., 2012). Hu et al. (2015) extensively reviewed the characteristics of cereal beta-glucans comprising their distribution, structure, extraction methods, emulsifying, gelling and rheological properties (viscosity, foaming capacity, foaming stability). As the concentration of beta-glucan or its molecular weight rises the viscosity of solution increases. The viscoelastic behaviour is impacted also by the fine structure of polymer chains as it is indicated by the higher viscosity of purified oat beta-glucans than barley beta-glucans of equal size (Johansson et al., 2008). Physical structure of cereal beta-glucans affects not only their viscosity but also metabolic activity which is mediated by receptors corresponding with this structure.

## Prebiotic properties of cereal beta-glucans

Composition of the hind gut microbiota depends on the quantity and characteristics of dietary fibre which is presumed to be the main source of energy for microbiota. Dietary fibre can affect long- and short-chain fatty acids concentrations and profiles as well as induce microbial shifts. It is possible therefore to change the composition of microbial population using nutritional strategies. One of such strategies is to use prebiotic supplements which are not enzymatically digested in the upper part of digestive tract and so stimulate beneficial microbiota in the large intestine leading to favourable health outcomes for the host organism. According to some authors, polysaccharides like beta-glucans, can act as prebiotics and stimulate the growth of *Bifidobacteria* in the colon (Hamaker and Tuncil, 2014). Beeren et al. (2015) designed a method allowing to follow *in vitro* fermentation of the fluorescently labelled beta-glucans in the model simulating human colon digestion. In this model the enzymatic breakdown of beta-glucans begins with quick reaction catalysed by bacterial *endo*-1,3(4)-beta-glucanase breaking down the polysaccharide into trimers and tetramers and which is followed by a slower process catalysed by *exo*-1,3(4)-beta-glucanase leading to generation of 3-*O*-beta-glucosyl-D-glucose (Beeren et al., 2015).

Cereal beta-glucans can be fermented by bacteria in the large intestine and the results of several *in vitro* and *in vivo* animal and human studies suggest that they have also prebiotic activity.

In the study on the effects of addition of cereal beta-glucans to yogurt, beta-glucans improved viability and stability of *Bifidobacterium animalis* ssp. *lactis* during 4-week storage (Vasiljevic et al., 2007). Barley beta-glucan stimulated growth of *Lactobacillus plantarum*, *L. acidophilus* and *L. fermentum* in unstressed conditions and in *in vitro* conditions simulating digestion in the gut. Moreover, food matrices containing barley beta-glucans had positive effect on probiotic-enterocyte interaction (Arena et al., 2014). However, in another *in vitro* study, the authors did not observe support of growth of probiotic strains by oat and barley beta-glucans used as the sole carbon sources in fermentation media. The investigated beta-glucans had also no enhancing effect on the bacteria survival in the *in vitro* simulation of gastrointestinal tract environment and on the bacteria adherence to the intestinal cells (Arena et al., 2016).

Prebiotic properties of cereal beta-glucans were confirmed in the *in vivo* studies. Feeding highly viscous barley beta-glucan to rats caused an increase of caecal content of *L. acidophilus* derived rRNAs comparing with feeding cellulose-based diet (Snart et al., 2006). Also, rats fed with oat-based diet had increased caecal content of acetic, propionic and butyric acids (Drzikova et al., 2005). Dongowski et al. (2002) reported that feeding fibre-rich barley-containing diet to rats caused a decrease of number of coliforms and *Bacterioides*, increase of *Lactobacillus* number and increase of short-chain fatty acids (SCFA) concentration in the colon, caecum and faeces. Positive alterations in faecal water content, pH value, ammonia levels, β-glucuronidase and azoreductase activities, and SCFA concentration in the colon were found in rats treated for 6 weeks with oat and barley glucans by intragastric gavage, with greater effect observed for oat beta-glucan (Shen et al., 2012). In our study carried out on rats with lipopolysaccharide (LPS)-induced enteritis, positive changes in faecal SCFA contents and increased number of lactic acid bacteria were found in rats fed oat beta-glucan supplemented diet indicating prebiotic effect of this compound (Wilczak et al., 2015).

The evidence of prebiotic effect of beta-glucans in humans is scarce but the barley beta-glucan prebiotic potential was confirmed in a randomized, double-blinded, placebo-controlled clinical trial of Mitsou et al. (2010). It was found that daily intake of a cake containing barley beta-glucan was well-tolerated by patients (≥50 years old) and had a bifidogenic effect increasing the *Bifidobacteria* number to the traceable level as compared with a non-detectable level in the control group.

Studies indicating prebiotic properties of cereal beta-glucans stimulate development of novel functional food products. Supplementation of 1.4% of oat beta-glucan to milk fermented with yogurt cultures resulted in an increase of viable cells count during the storage. This type of fermented dairy products combine the probiotic benefits of *Lactobacillus* with hypocholesterolaemic effects of beta-glucans (Lazaridou et al., 2014). Other type of probiotic products containing cereal beta-glucans are multifunctional oat-based fermented foods. Such products are rich in soluble and insoluble dietary fibre, plant fats and probiotic organisms. In a study of Russo et al. (2016), inoculation of a novel oat-based product with the riboflavin-overproducer *L. plantarum* strain led to *in situ* biofortification with riboflavin. In the same study, fermentation of the product with exopolisaccharides producing *L. plantarum* strain led to beneficial changes of the rheological features of the product, although the improvements were lost during storage (Russo et al., 2016).

## Cell receptors of beta-glucans

Beta-glucan molecules have an ability to bind to pattern recognition receptors on immune cells (such as granulocytes, monocytes, macrophages and Natural Killer (NK)-cells) and influence their immunological response, production of multiple cytokines, as well as induce oxidative burst (Hong et al., 2004). Main and the most extensively studied beta-glucan receptors are dectin-1, Complement Receptor 3 (CR-3) and Toll-like receptors.

The role of specific receptors in the recognition of cereal beta-glucan by innate immune cells has not been definitely established. Inconsistency of the results may be due to different purity of beta-glucan isolates studied, differences in their structure, differences between cell types and the fact that the studies were conducted on human, porcine or mice receptors. Most of recent studies lean towards a theory that dectin-1 receptor is able to bind with glucans with long chains of beta-(1,3) bound glucose molecules, thus the cereal beta-glucans as linear beta-(1,4)-glucan with different number of beta-(1,3)-linkages are unable to act *via* dectin-1 pathway. The results of the *in vitro* studies are also inconclusive, as discussed below. Dectin-1 is composed of one C-type Carbohydrate Recognition Domain (CRD) and a cytoplasmic domain with an Immunoreceptor Tyrosine-based Activation Motif (ITAM) (Mikkelsen et al. 2014). Dectin-1 is known to be the main innate immune receptor for beta-(1,3)-glucans, expressed mostly in monocytic cells populations, as evidenced for fungal beta-glucans (Noss et al., 2013). This receptor is also known to collaborate with Toll-like receptor 2 (TRL2) in recognition of beta-glucans and mediation of tumor necrosis factor-α (TNF-α) production (Zheng et al., 2016). Another receptor that is likely to bind with mixed linked cereal beta-glucans and play important role in their immune response regulation is CR3, which is a trans-membrane glycoprotein built of αM (CD11b) and β2 (CD18) integrins. Two subunits are bound with non-covalent linkage and exposed on the surface of the effector cells (Hong et al., 2004).

Recent study conducted by Bose et al. (2014) on yeast derived beta-glucans, showed that in human peripheral blood mononuclear cells (PBMC) dectin-1 was the receptor for whole glucan particle but CR3 was the one for immobilized soluble beta-glucan. Another *in vitro* study on porcine innate immune cells showed that CR3 served as main receptor for 6 different beta-glucans in porcine neutrophils. Results of this study suggest also that multiple receptors were engaged in beta-glucan recognition by porcine macrophages. These results are in accordance with findings of previous *in vitro* study on human cell lines (Baert et al., 2015) and recent study conducted by Zheng et al. (2016) on murine macrophage/monocyte-like RAW264.7 cells.

There is still lack of evidence whether similar pathway is involved in the immunomodulatory activity of cereal beta-glucans. The results of an early *in vitro* study conducted by Tada et al. (2009) suggest a dominant role of dectin-1 as barley beta-glucan receptor in macrophages and neutrophils. On the other hand, in a more recent study on the recognition of glucans by human dectin-1 (using the first sequence-defined glycome-scale microarray), no binding of barley beta-glucan and other mixed-linked glucans by dectin-1 was detected. This phenomenon was explained by the fact that dectin-1 binds solely to glucans with beta-(1,3)-linked linear gluco-oligosaccharides with degree of polymerization (DP)-10 or higher (Palma et al., 2015). In another *in vitro* study (Noss et al., 2013) the beta-glucans of different origin, molecular mass and structure induced strong cytokine response in human whole blood cultures. In this study barley beta-glucan induced strong and oat beta-glucan-moderate interleukin (IL)-6 and IL-8 production. As it is unlikely that this inflammatory response was dectin-1 mediated due to its affinity for structures containing a beta-(1,3)-linked backbone, the authors suggest that observed immunological response may have been CR3-mediated (Noss et al., 2013).

## Immunomodulatory effects and anti-inflammatory activity of cereal beta glucans

Beta-glucans have strong immunomodulatory properties and act as biological response modifier (BRM). In the study of Bermudez-Brito et al. (2015), barley beta-glucan induced an immunological response in human dendritic cells by decreasing the production of IL-8 and elevating expression of CD83 (the activation marker occurring on the surface of dendritic cells). The study was designed to address the cross-talk between specific cells in the mucosa in the presence of different dietary fibres. The immunological response of dendritic cells to barley beta-glucan (following direct contact with intestinal epithelial cells (IECs)) comprised significant decrease of pro-inflammatory cytokines IL-12, IL-6 and IL-8 production. In another *in vitro* study of Mikkelsen et al. (2014) cereal beta-glucan down-regulated LPS-induced IL-12 production and up-regulated LPS-induced IL-10 production in murine dendritic cells. According to this study, impact of cereal beta-glucan on immune response depends on sample preparation, solubility and polysaccharide aggregation and solution. Also Arena et al. (2016) observed that the incubation of human LPS-stimulated THP-1 macrophages with oat and barley beta-glucans decreased the expression levels of some pro-inflammatory cytokines (IL-8, IL-1β, IL-6). These results confirm immunomodulatory properties of cereal beta-glucans and their ability to reduce the pro-inflammatory effect of LPS in the *in vitro* studies.

The results of our *in vivo* study confirm those findings. In the study carried out on rats with LPS-induced enteritis and fed with diets supplemented with two oat beta-glucan fractions varying in molecular mass (MM) we measured selected markers of immune response in the rat colon, blood, spleen, liver and stomach (Błaszczyk et al., 2015; Wilczak et al., 2015; Suchecka et al., 2016). Beta-glucan administration caused a significant decrease of IL-12 production in colon elevated by LPS treatment. Both low and high MM beta-glucan fractions caused a significant decrease in the production of this cytokine, although, the decrease observed after administration of low MM beta-glucan was greater, and the level of cytokine was similar as that found in healthy individuals. The LPS-induced enteritis caused also an increase of IL-10 production in the colon tissue, alleviated by both low and high MM oat beta-glucans administration. These results suggest strong anti-inflammatory activity of oat beta-glucans, which could be recommended for individuals suffering from intestinal inflammatory diseases (Wilczak et al., 2015).

## Cereal beta-glucans in animal nutrition

Cereals and cereal products, such as bran and flour, are widely used as feed for farm animals. Nevertheless, literature available on the nutritional properties of one of the cereal polysaccharide compounds – beta-glucans – is still limited. Taking into account well documented stimulation of the both cellular and humoral immune response and prebiotic properties of cereal beta-glucans it can be presumed that they can be purposely used in both farm and companion animals diet. It seems that physiological effects of cereal beta-glucans are not only linked to their viscosity and receptor mediated cell recognition but also to fermentation by gastrointestinal microbiota. As was described by Metzler-Zebeli et al. (2011), oat beta-glucan is completely fermented in pig's digestive tract and due to this upregulates cytokine synthesis and their activity in the large intestine. However, fermentation rate of these polysaccharides in digestive tract depends on their composition, form, source, cultivar and growing conditions and physical properties (Le Goff et al., 2003; Holtekjølen et al., 2014)

Most of the published studies on the effects of those cereal polysaccharides have been conducted on monogastric mammals, mainly on pigs. New improved methods of isolation of cereal beta-glucans led to redefinition of their role as a feed supplement for monogastric mammals. The beneficial effects of these carbohydrate compounds on digestive tract physiology and animal health are partly related to the increased digesta viscosity and decreased gastric emptying. Moreover, fermented cereal beta-glucan promote growth and metabolic activity of microbiota, mainly by the increase of SCFA and lactic acid synthesis as well as decrease of ammonia production in pig's digestive tract (Jha et al., 2010). All these physiological effects stimulate growth and metabolic activity of health-promoting intestinal microbiota.

Results of other studies show also that beta-glucans of different origin have beneficial effects on body growth and immunity of farm pigs (Dritz et al., 1995; Fortin et al., 2003). In the review article of Vetvicka et al. (2014) it has been stated that barley and oats beta-glucans added to pig feed stimulate animals' growth and enhance their immunity to diseases caused by bacterial, viral and fungal pathogens. The immunostimulatory effects of cereal beta-glucans were based on the regulation of pro- and anti-inflammatory cytokines secretion, phagocytosis, stimulation of immunoglobulin A (IgA) synthesis and a positive impact on intestine microbiota and on gut associated lymphoid tissue (GALT). It was also found that beta-glucans stimulate secretory functions of intestinal mucosa by decreasing its permeability for different types of proteins.

The described effects on digestive system of pigs were most efficiently produced by the insoluble fractions of beta-glucans, which are more abundant in barley than in oat (O'Shea et al., 2010). It was, however, found that oat beta-glucans (as a feed additive) were more effective in increasing *Lactobacillus* spp. and *Bifidobacterium* spp. populations, decreasing pH in different intestine section and reducing unpleasant odours and ammonia in faeces (O'Shea et al., 2010). Those findings have been confirmed by the study of Murphy et al. (2012), who found that an increase of oat beta-glucans content in pig feed causes an increase of *Bifidobacterium* (in ileum, caecum and colon) and *Lactobacillus* (in caecum and colon) populations. Some of the published studies did not find, however any impact of beta-glucans on pigs' immunity (Hiss and Sauerwein, 2003).

In the meta-analysis of 26 published studies, Metzler-Zebeli and Zebeli (2013) concluded that cereal beta-glucans in pig's feed can decrease nutrients digestibility. It is especially important in growing animals and has an impact on body weight gain, and thus on production efficiency. It should be added that oat beta-glucan raised gastric bacterial number and colonic *Lactobacillus* spp. and *Bifidobacterium* spp. in weaned pigs (Pieper et al., 2008) and increased butyrate level in the digestive tract which is favourable for intestinal development. Metzler-Zebeli et al. (2011) found also that oat beta-glucan promotes overall microbiota number and metabolic activity in the stomach and gut of weaned pigs and protects *Lactobacilli* from acid gastric environment. The effects of barley-derived beta-glucans on immune system and intestinal function in weanling pigs were also studied by Ewaschuk et al. (2012). These authors found that barley beta-glucans alter immune and intestinal function, mainly by increase of CD4+ cells and rise of intestinal barrier permeability with no morphological gut changes and no adverse effect on animal growth and clinical health.

The authors admit that the increase of beta-glucans content in feed for adult pigs causes a decrease of ammonia emission and unpleasant odours of faeces, and an increase of butyric acid level in the digesta. Similar conclusion was formulated by Lærke et al. (2014) – dietary beta-glucans, regardless both their source (barley or yeast) and interventions in digestive processes, have a beneficial impact on intestine functions. On the other hand, due to an increased viscosity of digesta they may reduce intestinal absorption of bile acids and lipids, and lower plasma and lymph levels of fatty acids. Moreover, they increase butyrate concentration in the colon and decrease emission of ammonia from manure. The unfavourable effect of feeding beta-glucans to young pigs is the reduction of body weight gain, however this effect is not significant. The impact of beta-glucans on the digestion and absorption of nutrients is limited and requires further studies.

In the small intestine of young pigs the soluble fraction of barley beta-glucans undergoes depolymerization, leading to reduction of their molecular weight by 50%. In the caecum and colon, beta-glucans are present as the low MW compounds, approximately 100 kDa (Holtekjølen et al., 2014).

As already mentioned, beta-glucans excert an impact on immune system cells by interacting with their membrane receptors. The most important of those receptors is dectin-1 present in immune system cells of laboratory rodents and in pig blood neutrophils and macrophages (Shinkai et al., 2016). The Shinkai et al. (2016) study was performed on beta-glucans from *Saccharomyces cerevisiae,* but it can be presumed that the same receptors are involved in interactions with cereal beta-glucans, especially that the presence of those receptors has been confirmed in many organs of pig (Sonck et al., 2009). Shinkai et al. (2016) have also found that there is a significant polymorphism in receptor proteins among different pig populations, and that the interaction between receptors and beta-glucans results in a higher anti-fungal immunity and maintenance of immunological homeostasis in the intestine. The presence of specific beta-glucan receptors in pig's immune cells has been confirmed also by the *in vitro* studies (Baert et al., 2015).

It can be therefore assumed that cereal beta-glucans have some yet undiscovered but significant impact on general health condition in pigs and that the supplementation of feed with purified cereal beta-glucans can stimulate animal immunity and help in disease prevention.

There are only few studies on the effects of dietary beta-glucans derived from cereals, yeast or other fungi in domestic ruminants and their results are not conclusive. Eicher et al. (2011) found that supplementation of yeast beta-glucan plus vitamin C to neonatal calves artificially infected by *Salmonella enteritica* has immunomodulatory effects on the immune cells in lungs, without altering the inflammatory response in the peripheral blood, lymph nodes and spleen. Oral administration of fungal-derived (*Aureobasidium pullulans*) beta-glucans had strong immunomodulatory effect in Holstein cows and Japanese black calves (Uchiyama et al., 2015). Results of study performed on cross-bred steer calves indicated that dietary oat beta-glucans did not influence specific and non-specific immune parameters in healthy animals, but in calves treated with immunosuppressive factor they partially restored some of the immune indices. It may be considered as very important in providing resistance to infectious diseases (Estrada et al., 1999). The results obtained by Grove et al. (2006) indicate that barley beta-glucans stimulate also the immune system of mature cattle.

The depressing effect of beta-glucans on nutrients digestibility and body weight gain may be beneficial for prevention obesity in companion animals (dogs and cats). The immunostimulatory effect is also expected in these animals fed diets supplemented with beta-glucans, mainly of yeast origin. These compounds are used as dietary supplement supporting effectiveness of vaccinations in puppies, especially against canine parvovirus and rabies infection (Haladová et al., 2011). Stuyven et al. (2010) found that oral administration of beta-glucan from *Saccharomyces cerevisiae* changes the profile of secreted immunoglobulins in beagle dogs' serum and thus affects the humoral immunity. Although the related studies concerned activity of fungal beta-glucans, it can be assumed that these polysaccharides derived from cereals can act in similar way. Such hypothesis seems to be confirmed by identification of different isoforms of dectin-1 beta-glucan receptors in a number of mammalian species including such domestic animals as pigs (Baert et al., 2015), sheep (Zhou et al., 2010) and cattle (Willcocks et al., 2006), and in different laboratory rodents.

## Conclusions

The literature regarding application of cereal grains and milling fraction containing beta-glucans in animal nutrition is extensive although studies on the bioactivity of these purified compounds are just starting. In the experiments, mainly the purified yeast or fungal beta-glucans are used, which are structurally different from cereal beta-glucans and reveal several distinct activities. The further research in this field is needed especially regarding immunosupportive activity of beta-glucans, which can help in maintaining good health in farm and companion animals. The another direction, which could be further explored is the body weight lowering effect, which may be beneficial not only for companion animals suffering from human civilization diseases but also for adult farm animals.

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