These/Thesis

Master of Science

Effect of supplementation with β-glucan of Saccharomyces cerevisiae and chito-oligosaccharides on digestion and growth performance in weanling rabbits

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ABSTRACT

The aim of this work was to study the effect of yeast β-glucans and chito-oligosaccharides supplementation on digestion and growth performance of growing rabbits. Five experimental diets were prepared. A control diet was formulated with 18.1% protein, 33.1% neutral detergent fibre and 13% soluble fibre (on DM basis). Another four diets were obtained by supplementing control diet with β-glucans (200 or 400 ppm. BG2 and BG4) and chito-oligosaccharides (200 or 400 ppm. OT2 and OT4). No antibiotic was used. In the first experiment, 56 rabbits / treatment weighing 510 ± 73.0g weaned at 28 d of age were used to record mortality, and in 42 rabbits / treatment growth traits were recorded. Rabbits fed control, BG4 and OT4 diets (42/treatment), were used to determine the body composition by bioelectrical impedance technique measured at 28, 38 and 63 d of age. Experimental diets were offered from weaning up to 38 d of age. At this moment all rabbits were fed the control diet up to 63 d of age. Another group of 24 rabbits / treatment received from 28 to 38 d of age the five diets containing mordanced ytterbium. They were slaughtered at 38 d of age and ileal digesta collected to quantify ileal digestibility and caecal pH recorded. Faecal digestibility was measured from 36 to 38 d of age (9rabbits/diet). In the second experiment, three hundred thirty-four rabbits were separated from their mothers at 19 d of age (329 ± 65g) and assigned to the control, BG4 and OT4 diets. Rabbits suckled every morning for 10 minutes until 28 d of age. After weaning at 28 d of age, two hundred thirty-one rabbits received the same experimental diets than in the first period (77 rabbits per diet) and were caged individually to determine growth performance. At 19 d of age, 10 rabbits from 10 different litters were slaughtered to determine the digestive parameters (relative weight of total digestive tract, stomach and caecum).At 27 and 38 d of age 30 rabbits(3 rabbits/treatment and litter) from each of the same 10 litters which have been chosen before (at 19 d of age) were slaughtered to determine the same measurements. Diet supplementation with β-glucans and chito-oligosaccharides did not affect ileal and faecal DM digestibility and faecal digestibility of fat, protein and fibre. Similarly, no treatment effect was found on growth traits along fattening period in the two experiments. In the first experiment, the mortality increased in rabbits supplemented with 200 ppm β-glucans compared to 400 ppm β-glucans ($P = 0.048$).In the second experiment, the mortality rates were lower than in the first one, and there were no other effect of treatments. The type of diet did not modify the retention and

efficiency of both nitrogen and energy in the whole animal or in the carcass. However, rabbits fed supplemented diets with chito-oligosacchharides showed a higher faecal excretion of nitrogen (P=0.034) and energy (P=0.076) between 28 and 38d of age. Treatments had no effect on the relative weight of digestive organs. In conclusion, in our condition the supplementation of β-glucans and chito-oligosaccharides does not provide any advantage to growing rabbits.

Keywords: β-glucans, chito-oligosaccharides, growth performance, rabbit.

RESUME

L'objectif de ce travail était d'étudier l'effet de la supplémentation avec β-glucanes de levures et Oligo-quitosans sur la digestion et les performances de croissance des lapins. Cinque aliments expérimentaux ont été préparés. Un aliment contrôle a été formulé avec 18,1% protéines, 33,1% de fibres neutre détergent et 13% de fibres solubles (sur MS). Autres quatre aliments ont été obtenus en complétant l'aliment contrôle avec β-glucanes (200 ou 400 ppm. BG2 et BG4) et Oligo-quitosans (200 ou 400 ppm. OT2 et Ot4). Aucun antibiotique n'a été utilisé. Dans la première expérience, 56 lapereaux / traitement pesant 510 ± 73.0g sevrés à 28 jours d'âge ont été utilisées pour l'estimation de la mortalité, et dans 42 lapereaux / traitement les performances de croissance ont été enregistrés. Les lapins recevant les aliments contrôle, BG4 et OT4 (42 / traitement), ont été utilisés pour déterminer la composition corporelle par la technique d'impédance bioélectrique mesurée à 28, 38 et 63 jours d'âge. Les aliments expérimentaux ont été offerts à partir du sevrage jusqu'à 38 jours d'âge. En ce moment, tous les lapins reçoivent l'aliment contrôle jusqu'à 63 jours d'âge. Un autre groupe de 24 lapereaux / traitement de 28 à 38 jours d'âge ont été alimentés avec cinq aliments marqués avec ytterbium, ils étaient abattus à 38 jours d'âge et le contenue d'iléon a été recueillis pour évaluer la digestibilité iléal ainsi que le pH de caecum a été enregistré. La digestibilité fécale a été mesuré de 36 à 38 jours d'âge (9 lapins / traitement). Dans la deuxième expérience 334 lapereaux ont été séparés de leurs mères à 19 jours d'âge (329 ± 65 g) et attribuée aux aliments contrôle, BG4 et OT4. Les lapereaux allaitent chaque matin pendant 10 minutes jusqu'à 28 jours d'âge. Après le sevrage à 28 jours d'âge, 231 lapins reçoivent les mêmes aliments expérimentaux que dans la première période (77 lapins par traitement) et logés individuellement pour déterminer les performances de croissance. Au 19 jours d'âge, 10 lapereaux provenant de 10 portées différentes ont été abattus afin de déterminer les paramètres digestifs (poids relatif du tube digestif, l'estomac et le caecum). 27 et 38 jours d'âge 30 lapereaux (3 lapereaux / traitement et par portée) de chacun des mêmes 10 portées qui ont été choisis avant (au 19 jours d'âge) ont été abattus afin de déterminer les mêmes mesures. La supplémentation de l'aliment avec des β-glucanes et Oligo-quitosans n'a pas affecté la digestibilité iléale et fécale de la MS ainsi que la digestibilité fécale des lipides, protéines et fibres. De même, aucun effet du traitement n'a été trouvé sur les paramètres de croissance le long de la période d'engraissement dans les deux expériences. Dans la première expérience, la mortalité a augmenté chez les lapins additionné de 200 ppm de β-glucanes par rapport à 400 ppm de

β-glucanes (P = 0,048). Dans la deuxième expérience, les taux de mortalité ont été plus faibles que dans la première, et il n'y avait pas d'effets de traitements. Le type d'aliment n'a pas modifié la rétention et l'efficacité à la fois de l'azote et de l'énergie dans l'animal entier ou de la carcasse. Cependant, les lapins nourris l'aliment complété avec Oligo-quitosans ont montré une excrétion fécale d'azote plus élevé (P = 0,034) et de l'énergie (P = 0,076) dans la période entre 28 et 38 jours d'âge. Les Traitements eu aucun effet sur le poids relatif des organes digestifs. En conclusion, dans nos conditions la supplémentation avec β-glucanes et oligo-quitosans ne fournit aucun avantage pour les lapins en croissance.

Mots clés: β-glucanes, oligoquitosans, croissance, lapin**.**

RESUMEN

El objetivo de este trabajo fue estudiar el efecto de la suplementación con β-glucanos de levaduras y oligoquitosanos sobre los rendimientos productivos de los gazapos. Se formuló un pienso control con un 18,1% proteína, 33,1% fibra neutro detergente y 13% fibra soluble (sobre MS). Se obtuvieron otros cuatro piensos suplementando el pienso control con β-glucanos de levaduras (200 ó 400 ppm.BG2 y BG4) o con oligoquitosanos (200 ó 400 ppm.OT2 y OT4).No se utilizaron antibióticos. En el primer experimento, se utilizaron 42 animales por tratamiento destetados a los 28 d de edad (510 ±73.0 g) para estudiar los parámetros de crecimiento y 56 gazapos / tratamiento para evaluar la mortalidad. Los animales que reciben control, BG4 y OT4 dietas (42 / tratamiento) se utilizaron para determinar la composición corporal mediante la técnica de impedancia bioeléctrica mide a 28, 38 y 63 días de edad. Los 5 piensos experimentales se suministraron de el destete hasta los 38 d de edad, a partir de esta edad se les suministró a todos los animales el pienso control hasta el final del cebo (63 d de edad).Otro grupo de 24 gazapos / tratamiento de 28 a 38 días de edad se alimentaron cinco alimentos marcados con iterbio, que fueron sacrificados a los 38 días de edad y se recogió el contenido ileal para evaluar la digestibilidad ileal y se registró el pH de ciego. La digestibilidad fecal se midió de 36 a 38 días de edad (9rabbits / tratamiento). En el segundo experimento 334 gazapos fueron separados de sus madres a los 19 días de edad (329 ± 65 g) y los atribuyen al control, BG4 y OT4 dietas. Gazapos amamantaron cada mañana durante 10 minutos hasta 28 días de edad. Después del destete a los 28 días de edad, 231 gazapos recibieron las mismas dietas experimentales en el primer período (77 conejos por tratamiento) y se alojaron individualmente para determinar los parámetros de crecimiento. A los 19 días de edad, 10 gazapos de 10 camadas diferentes fueron sacrificados para determinar los parámetros digestivos (peso relativo del tracto digestivo, el estómago y el ciego). A los 27 y 38 días de edad 30 gazapos (3 gazapos / tratamiento y por camada) de cada una de las mismas 10 camadas que fueron elegidos antes de (19 días de edad) fueron sacrificados para determinar las mismas medidas. La suplementación del pienso con β-glucanos y oligoquitosans no afectó ileal y fecal digestibilidad de la MS y la digestibilidad fecal de grasa, proteína y fibra. De mismo, ningún efecto del tratamiento se encuentra en los parámetros de crecimiento a lo largo del período de engorde en ambos experimentos. En el primer experimento, la mortalidad aumentó en conejos suplementado con 200 ppm de β-glucanos en comparación con 400 ppm de β-glucanos (P = 0,048). En el segundo

experimento, la mortalidad fue menor que en el primero, y no hubo efectos de tratamiento. El tipo de pienso no afecta a la retención y la eficacia de ambos nitrógeno y energía en animal o en canal. Sin embargo, gazapos alimentados con dietas suplementadas con oligoquitosanos mostró una mayor excreción fecal de nitrógeno (P= 0,034) y la energía (P = 0,076) en el periodo entre 28 y 38 dias. Los tratamientos no tuvieron efecto sobre el peso relativo de los órganos digestivos. En conclusión, en nuestra condición la suplementación con β-glucanos y oligoquitosanos no proporciona ninguna ventaja a los conejos en crecimiento.

Palabras clave: β-glucanos, oligoquitosanos, crecimiento, conejo.

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ABREVIATIONS KEYS

ERE: Epizootic Rabbit Enteropathy

IgM: Immunoglobulin M

IgG: Immunoglobulin G

GALT: Gut Associated Lymphoid Tissue

CFU: Colony Forming Unit

SR: Similarity Rate

VFA: Volatile Fatty Acid

NH3: Ammonia

CH4: Methane

MALT: Mucosa Associated Lymphoid Tissue

APC: Antigen Presenting Cell

MW: Molecular Weight

NK: Natural Killer

CR: Complement Receptor

ADG: Average Daily Gain

COS: Chito-oligosaccharides

DP: Degree of Polymerization

ADFI: Average Daily Feed Intake

HDL: High Density Lipoproteins

LDL: Low Density Lipoproteins

FCR: Feed Conversion Ration

IL: Interleukin

% : percentage

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Introduction

In rabbit production, the period around weaning (28-35 d of age) is very critical. It is associated with a higher risk of digestive disorders (Lebas et al., 1998; Gidenne and Garcia, 2006).The most common disorder in rabbit production is the Epizootic Rabbit Enteropathy (ERE) which is the first cause of mortality in the European rabbit industry (Dewree et al., 2003). The mortality rates at the onset of the epizooty were high (30 to 80%) (Licois et al., 1998; Marlier and Vindevogel, 1998). The frequency of this problem is high during this period and the use of antibiotics is usual as standard treatment in many commercial farms. However, the wide use of antibiotics has led to the occurrence of antibiotic resistant bacteria (Falcao-Cunha et al., 2007). Consequently, in January 2006 the European Community banned the use of antibiotics as growth promoters.

In this context the research of alternatives to antibiotics is indispensable. Many strategies exist to limit this problem such as the use of high-health animals, feeding strategies and nutrition. Thus, there has been increasing interest in the use of probiotics and prebiotics. Prebiotics are indigestible food ingredients that selectively stimulate the growth of saprophyte bacteria (Van Immerseel et al., 2006).

The supplementation of the diets of young rabbits after weaning with yeast β-glucans enhanced the functionality of the intestinal mucosa and modified the intestinal microbiota of rabbits from a farm with very low mortality (El Abed et al., 2015). These authors showed that β-glucan have a positive effect on the immune system by a higher IL6 expression in the appendix. In rabbit does β-glucans improved maternal humoral immunity during late lactation by increasing serum IgM and IgG without any effect on growth performances of rabbits does and their litters (Wu et al, .2011).

There are no results reported about the use of chito-oligosaccharides in rabbits. Its supplementation in piglets improved growth performance and integrity of the intestinal mucosa, and promoted favorable changes in intestinal microbiota (Yang and al., 2012), limiting the effects of diarrhea caused by E. coli after weaning (Liu et al., 2010).

The aim of this work was to evaluate the influence of yeast β-glucan and chito-oligosaccharides supplementation on digestion, rabbit performance and immune response after weaning in a context of epizootic rabbit enteropathy.

CHAPTER I: LITERATURE REVEW

1. Evolution, composition and characteristics of the gut microbiota of the rabbit after weaning

After birth the rabbit's gut is characterized by a microbial colonization, followed by gradual development of the intestinal microbiota (Fortun-Lamothe and Boullier, 2007). After weaning the colonization of the small intestine is more rapid than that of the stomach and the microflora was more abundant. Rabbit microflora is characterized by the dominance of strictly anaerobic species, the number of facultative anaerobic bacteria low down (10^2 -10^4) and are frequently absent, while strict anaerobic flora remains stable to 10^9 -10^{11} bact /g (Gouet and Fonty., 1979). The cellulolytic flora increases when the animal ingests solid food (around 18 d of age, figure 5) to reach 10^7 bact / g , while the xylanolytic and pectinolytic flora is implanted at a higher level (Boulahrouf et al., 1991). The most cultivable species identified were Eubacterium cellulosolvens for cellulolytic bacteria and Prevotella ruminicola for pectinolytic and xylanolytic bacteria (Boulahrouf et al., 1991).

In healthy rabbits the digestive microbiota was characterized by the absence or low density of Lactobacillus, Streptococcus and Escherichia coli (Ducluzeau, 1969; Gouet and Fonty, 1973; Fonty et al., 1979; Padilha et al., 1996). In the last years, molecular micro-biology techniques have shown a progress in the knowledge of the microbial diversity of digestive ecosystems. In addition, sometimes it varies greatly from one animal to another (Combes et al 2011). The most abundant bacterial community is present throughout the caecum–colon and in hard and soft faeces (10^{10} to 10^{12} bacteria/g), (Gouet and Fonty, 1973; Forsytheand Parker, 1985).Also the ceacal digestive ecosystem is characterized by the presence of anaerobic fungi (Bennegadi et al., 2003) and protozoa (Forsythe and Parker, 1985; Bennegadi et al., 2003).

This caecal diversity is variable according to the age of rabbits, a study of the evolution of caecal microbiota in rabbits shows that the similarity rate (SR) of caecal microbiota at 45 and 52 d of age was 92.5% between them compared to animals at 26, 31 and 38 d of age, showing a SR between 82.3 and 86.7 among them (Delgado et al., 2010). The mainly intestinal flora in the caecum and the ileum consisted in the *phylum Firmicutes, Bacteroidetes* and *Verrumicrobia,* while in ileum there is a high proportion of *Proteobacteria* (Delgado et al., 2012). This micro flora evaluates by the age of rabbits, At

26 d *Firmicutes* and *Bacteroidetes* represented a high proportion of caecal microbiota (47 and 53% respectively). As age increased *Bacteriodetes* was replaced by *Firmicutes* then at 52 d was 85% of the flora majority. On the other hand, in ileum it was shown that the most important family was *Porphyromonadaceae* which appeared in a low proportion in early ages (5%), but increased from 45 to 52 d to represent 37% of the total flora (Delgado et al., 2012).

This particular digestive flora is an important constituent of the defenses of the intestine. It creates a "barrier effect" making it more difficult colonization of the gut by exogenous bacteria by inducing competition for substrates and synthesizing antimicrobial substances (Combes et al., 2013). Furthermore colonization of the gut flora is considered as a strong antigenic stimulus for the maturation of the GALT (Guarner and Malagelada, 2003). Accordingly, to limit the digestive problems in rabbits around weaning it is indispensible to know and control the microbiota because of its barrier effect and its role as immune stimulator.

2. Roles of the digestive microbiota

2.1. Digestion and feed efficiency

In rabbits and monogastric herbivores, the small intestine is the mainly place of the digestion of nutrients through the endogenous digestive enzymes, but for components of plant cell walls (pectins, hemicelluloses, cellulose, etc.) which are hydrolyzed by bacterial enzymes (Fonty and Gouet, 1989). As it is demonstrated in the (Figure 1) the metabolic activities of microbiota are organized in a trophic chain. The first step of the trophic chain corresponds to the hydrolysis of complex polymers by a variety of hydrolases (polysaccharidases, glycosidases, proteases, peptidases) provided by hydrolytic species in smaller compounds (monosaccharides, amino acids, etc.).These later are used by hydrolytic and fermentative species as energy sources (Combes et al., 2013). By fermentation, microbial flora products volatile fatty acid (VFA: acetic acid, propionic acid and butyricacid), ammonia (NH_3) derived from proteolysis, intermediary metabolites (lactic acid, succinic acid, formic acid) and gas (CO_2,CH_4,H_2). The major enzymes of the microbial ecosystem in rabbits are pectinase, xylanase, cellulase and urease (Marounek and Vovk, 1995).Microbial activity may cover 30% to 50% of maintenance energy requirements of adult rabbits by VFA production (Gidenne, 1994).Moreover, in rabbits,

30% to 50% of the digestible organic matter is digested in the caeco-colic segment (Gidenne, 1992; Gidenne et al., 2000).

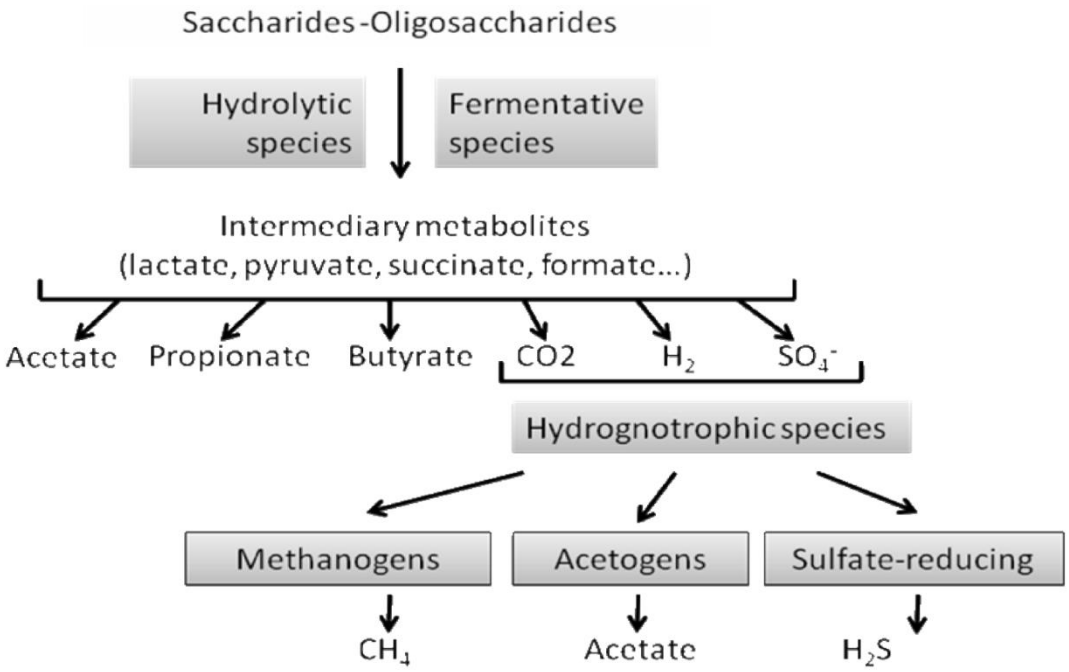

Figure 1: Trophic chain of food carbohydrates according to Bernalier-Donadille (2004)

2.2. Role in defence against infectious agents and in the intestinal immune system

Microbiota has an important role in the barrier function of rabbits; it is involved in immune organs and cell development, diversification of antibodies and mechanisms of oral tolerance (Combes et al., 2013).The concept of barrier function is based on the fact that the microbiota permanently present in the digestive tract and inhibit the colonization of exogenous pathogenic bacteria (Berg, 1996).There are different mechanisms to explain the role of microbiota in gut barrier (Guarner and Malagelada, 2003) :1) the prevention of attachment and entry of pathogenic bacteria to the mucusa by commensal bacteria adherence. Therefore the filamentous bacteria that colonize the ileum reduce the attachment of enteropathogenic E. coli (Heczko et al., 2000). 2) Competition of micro-organisms for nutrients to maintain their ecological niche and habitat by consuming all resources. 3), Inhibition of the growth of competing bacteria by producing antimicrobial substances.

In rabbits, the diversification of the primary repertoire of antibodies continues after birth until 10 – 12 weeks of age (Figure 2), and the microbiota is essential in this diversification (Lanning et al., 2000). Morever, inoculation of several intestinal bacteria in sterile rabbit vermiform appendix showed that *Bacillussubtilis* and *Bacteroides fragilis* together stimulates B-cell proliferation and diversification of genes encoding the immunoglobulin (Rhee et al., 2004). Indeed, these bacteria can improve the immune system of mammalian species by its wall polysaccharides (Mazmanian et al., 2005).

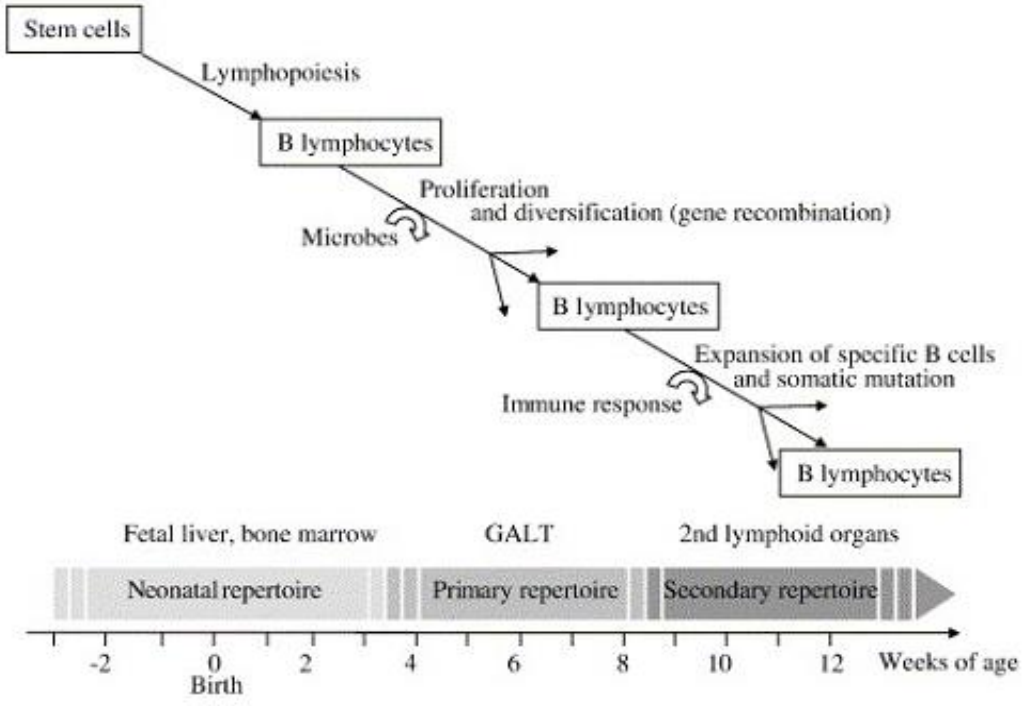

Figure 2: Schematic representation of antibody repertoire development in rabbit from Fortun-Lamothe and Boullier (2007).

3. Gut morphology of rabbit after weaning

The small intestine represents the most important site of exchanges between the external environment and internal environment in the gastrointestinal tract of rabbits. On the interne surface of the intestinal wall we find the mucosa which represents a major site of digestion and absorption of nutrients, as well as an important area of defense against antigenic aggressions.

In rabbits, the post-weaning maturation of the ileal histology is generally related to an increase of digestive enzyme activities (Dojana et al., 1998). Some studies report an

increased villous length and width with age (Yu and Chiou., 1997). In the jejunum the villi length and crypt depth reduced between 26 and 31 d of age, however from 31 to 38 d increased (Gallois et al., 2005). From 38 to 52 d of age, villi length did not change, showing similar values that those observed at 26 d, however the crypt depth increased (Delgado et al., 2012).

4. Immune system in rabbits

4.1. Organization of the rabbit immune system

At birth rabbits are physiologically immature and their lymphoid organs are not fully developed. First lymphatic follicles start to form at the age of 2 weeks. There are two types of lymphoid organs. Primary organs are the bone marrow and thymus which are responsible of the production and maturation of lymphocytes. Secondary organs include the spleen, peyer's patches and the appendix which are responsible of initiation of an immune response (Weih and Caamano, 2003).As it is mentioned in the (Figure 3), at the end of the ceacum we find the vermiform appendix responsible of the production and maturation of B-cells during the first weeks of life (Reynaud and Weill, 1996).

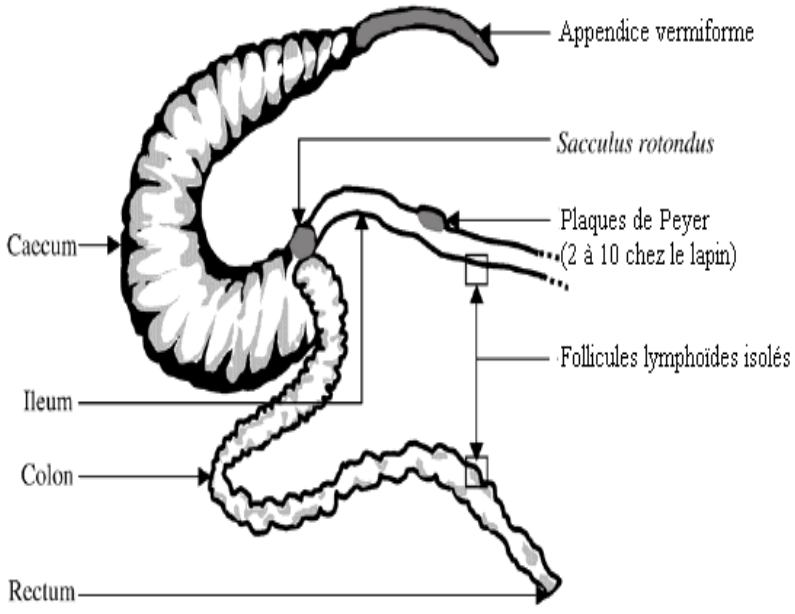

Figure 3: Organization of the gastrointestinal lymphoid tissue of the rabbit (from Fortun-Lamothe and Boullier, 2007)

4.2. Mucosa-associated lymphoid tissues (MALT)

The MALT is lymphoid tissue situated on the surface of all mucosal tissue (Elmore, 2006).The real function of the MALT is to protect mucosal barrier, thus they are strategically placed to act as sentinels but can also become a portal of entry of bacteria (Fry and Donald McGavin, 2012). Indeed it plays a critical role as inductive sites for the initiation of antigen-specific protective immunity against the pathogens penetrating the mucosal membrane (Okada et al., 2011)

5. Immune response

Any immune system firstly involved the recognition of the pathogen of foreign material, secondary the development of reaction against it to eliminate it. We can distinguish two categories of immune response: innate (or non adaptive) immune responses and adaptive immune responses (Roitt et al., 1996). The interaction between these two systems is summarized in the Figure 4.

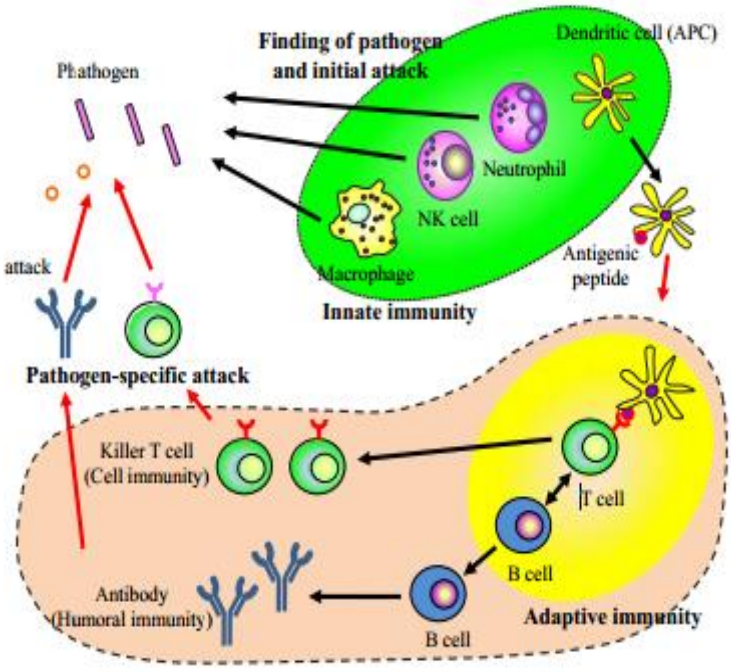

Figure 4: Interactions of innate and adaptive immunity. APC: Antigen presenting cell (Akira, 2012)

6. Epizootic Rabbit Enteropathy (ERE) and digestive disorders

After weaning, the digestive system of young rabbits can be affected by digestive disorders and pathologies as coccidiosis, enteritis and specially Epizootic Rabbit Enteropathy (ERE).

Epizootic rabbit enteropathy appeared in the rabbit farms in western France in 1996 and quickly spread to the rest of the country then to the rest of Europe in the following years. Cases have also been reported in recent years in Mexico (Rodriguez Lara et *al*., 2008).

The ERE is described as a primarily disease affecting young rabbits between 5 and 14 weeks, but can also affect unweaned animals and reproductive does. The first symptoms are appeared by a reduction of feed and water intake, leading to fast dehydration, then the caecum became liquid or compacted and its pH is very acid (Pérez de Rozas et al., 2005).In presence of ERE, histological observations also show major villus destruction and loss of epithelial cells both at ileum and jejunum (Licois et al., 2005; Dewrée et al., 2007; Chamorro et al., 2010).The agent responsible for ERE is not yet identified and many factors are considered as causes for its occurrence such as infectious causes, unbalanced diets, improper antibiotics treatments and environmental conditions (Licois et al., 2005).

The main solution to ERE is the use of antibiotics, but its use has been led by the European rules as preventive treatments. Moreover, nutrition may be an effective solution because of it plays an important role in immunity and the activity of microbial flora, offering a different substrate to bacteria in the terminal ileum and caecum (Gidenne et al., 2003). It therefore seems possible that by changing the quantity or quality of nutrition can affect the strength of rabbits to digestive problems.

Strategies based on the use of prebiotics or probiotics could help strengthen the digestive immune system of rabbits. Prebiotics can influence favorably digestive flora by selective stimulation of certain bacteria. Dietary supplementation with some oligosaccharides as β-glucan could be of interest in rabbits.

7. β-glucans

7.1. Sources, structure and proprieties of β-glucans

B-glucans are naturally occurring polysaccharides with glucose as structural component, linked by β-glycosidic bonds. In nature, β-glucan are produced by a variety of plants, such as oat, barley, and seaweed, baker's and brewer's yeast (Saccharomyces genus), and Echinaceae members (Tokunaka et al., 2000). Each type of β-glucan, generally derived from different sources, has a unique structure in which glucose units are linked together in different ways (Stone and Clarke, 1992). The most important are β-1, 3 and 1, 6 glucan which are derived from the cell wall of yeast *Saccharomyces cerevisiae* (Figure 5).

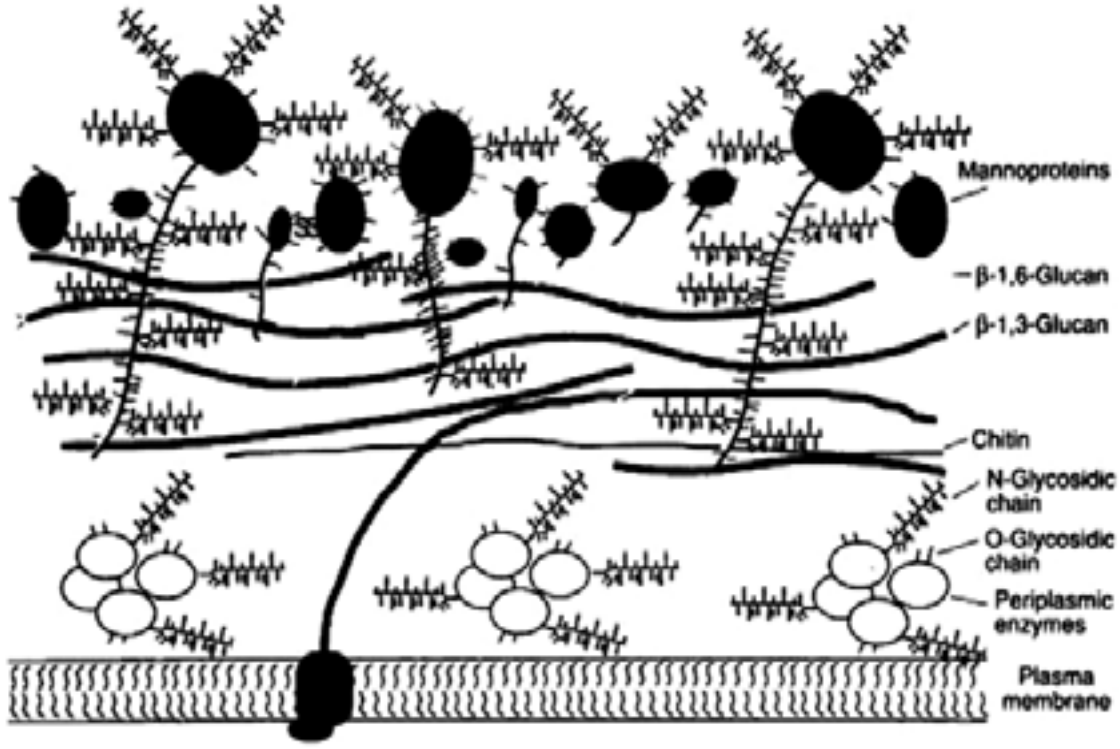

Figure 5: Structure of the cell wall of yeast *Saccharomyces cerevisiae* (Kath and Kulicke, 1999).

Yeast is a well known microorganism that is used in biotechnology since ancient times (Waszkiewicz-Robak and Bartnikowska 2009), and it is a good source of β-glucan. β-glucan in yeast cell walls are a group of molecules containing linear (1,3)-β-glucosyl chains that are joined through (1,6)-linkages (Osumi, 1998; Kath and Kulicke, 1999) (Figure 6).

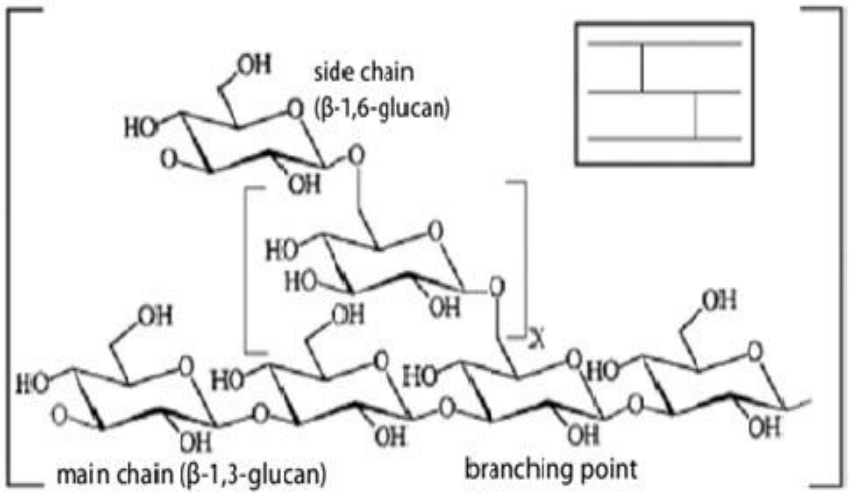

Figure 6: Chemical structure of β-glucan in yeast *Saccharomyces cerevisiae* (Osumi, 1998).

Depending on the source, there are clear differences in macro-molecular structure between β-glucans. Furthermore, besides differences in type of linkage and branching, β-glucan can vary in solubility, molecular mass, tertiary structure, degree of branching, polymer charge and solution conformation (triple or single helix or random coil). However insoluble (1, 3/1, 6) β-glucan have greater biological activity than that of its soluble (1, 3/1, 4) counterparts (Ooi and Liu 2000). For example, studies about mammalian animals have recently suggested that high molecular weight (MW) of β-glucan from fungi directly activate leukocytes, while low MW β-glucan only modulate the response of cells when they are stimulated with cytokines (Brown and Gordon, 2003).

7.2. Mechanism of act of β-glucan in immune system

β-glucans can enhance the functional activity of macrophages and activate antimicrobial activity of mononuclear cells and neutrophils in vitro (Williams, 1997; Zekovic et al., 2005). However, the mechanisms by which they exert their effects are still poorly understood. In Figure 7 is shown an illustration of dietary β-glucan possible pathways from the gut to immune-modulation.

β-glucans may be fermented by the indigenous microbiota, which can lead to changes in bacterial composition and released metabolic compounds (Figure 7, part A).In other side may have a direct interaction with immune competent cells (Figure 7, part B). In the part C of Figure 7, is illustrated the possible uptake mechanisms for β-glucans from the gut lumen.

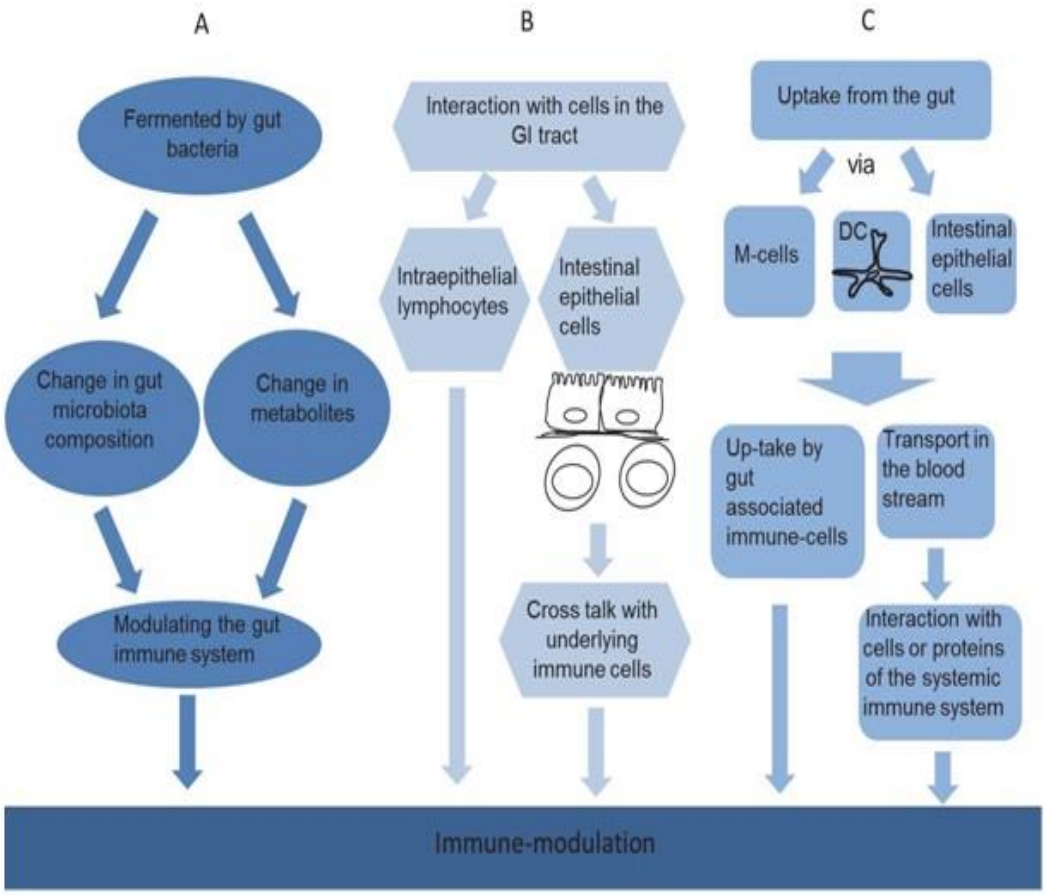

Figure 7: β-glucan possible steps from the intestine to immune-modulation (Anne Rieder, 2013)

7.3. β-glucan receptors

The induction of β-glucan is likely to involve their specific interaction with one or more cell surface receptors. Glucans are thought to mediate their effects via interaction with membrane receptors on macrophages, neutrophils, and NK cells. Till now, four β-glucan receptors have been identified as candidates mediating these activities. It is namely

complement receptor 3 (CR3; CD11b/CD18), lactosylceramide, selected scavenger receptors, and dectin-1 (Czop and Austen, 1985).

Soluble β-glucan can be internalized by intestinal epithelial cells and gut-associated lymphoid tissue (GALT) cells (Rice et al., 2005). However uptake by the latter requires binding to receptors such as Dectin-1 and TLR-2. Once internalized the β-glucan were engulfed by macrophages via specific receptors, such as the Dectin-1 receptor, then transported through the body to the spleen, lymph nodes and bone marrow. Within the macrophages, the β-glucans were broken down into smaller fractions then released from the macrophages nearby of their site of action (Hong et al., 2004). Consequently they act by priming a normal immune response designed to detect and combat invading yeast and certain pathogenic bacteria (Vetvicka et al., 1996; Chan et al., 2009). This defense mechanism is usually triggered through complement receptor CR-3 expressed on phagocytic cells and NK cells, a receptor that recognizes and mediates phagocytosis and destruction of microorganisms and yeasts (Le Cabec et al., 2002).

7.4. Effects of β-glucan on growth performances and immune response

The immune response can be modulated by nutrients like β-glucans. It has been shown to increase the ability to resist bacteria (Chen et al., 2008), viruses (Xiao et *al*., 2004) and protozoa (Yun et al., 2003). The improvements of immunity through β-1, 3 – 1, 6-glucan supplementation were reported in chicken (Chen et al., 2008), swine (Hahn et al., 2006), horse (Krakowski et al., 1999) and rabbit (Chen et al., 2006). The dietary supplementation with 0.128% β-glucan limited the adverse effects caused by coccidial infection in rabbits, improving daily gain and feed conversion rate (Chen et al., 2006).Also, supplementation with 0.064% β-1,3–1,6-glucan reduced serum IgG concentration of does at the last gestation stage but increased serum IgM and IgG concentrations at the last lactation stage and therefore may provide more IgM and IgG through their milk to the young rabbits (Wu et al., 2011). Moreover, supplementation with 0.312g/kg of BW in piglets after birth in milk and then in feed until 4 weeks of age showed that β-glucan increased the body weight and ADG (Eicher et al., 2006). By contrast, there was no effect on growth performances in chickens fed a diet containing 0.1% β-glucan (Chen et al., 2008).In broiler chickens, dietary levels of β-glucan above 0.02% improved growth performance, nutrient retention and immunity (Chae et al., 2006).

8. Chitins, chitosans and chito-oligosaharides

8.1. Sources, structure and proprieties

Lot of oligosaccharides (Fructooligosaccharides, mannanoligosaccharide and Chito-oligosaccharide) have been used as prebiotics to improve animal performance, to enhance immune ability, and to affect gut microbial flora concentrations (White et al., 2002; Lemieux et al., 2003; Smiricky- Tjardes et al., 2003; Flemming et al., 2004). Chito-oligosaccharides are the second most abundant carbohydrates polymers found in nature (Knaul et al., 1999). Chitine is mainly found in the cell walls of fungi and yeasts and the exoskeleton of arthropods and insects (Aam et al., 2010; Minke et al., 1978). The main commercial sources of chitin are the shell waste of shrimps, lobsters, krills and crabs.

Chitin (Figure 8) is a mucopolysaccharide and the supporting material of crustaceans and insects, it is a linear polysaccharide composed of (1 → 4) linked 2-acetamido-2-deoxy-β-d-glucopyranosyl units and occurs naturally in three polymorphic forms with different orientations of the micro fibrils, known as α-, β-, and γ-chitin (Tharanathan, 2003 and Rudall, 1963).

Chitosan is a natural nontoxic biopolymer produced by the deacetylation of chitin. Chitosan (Figure 9) has three types of reactive functional groups, an amino group primary and secondary hydroxyl groups at the C-2, C-3, and C-6 positions, respectively. Chitosan is insoluble in water, organic solvents, and aqueous bases and it is soluble after stirring in acids such as acetic, nitric, hydrochloric, perchloric, and phosphoric (Anthonsen and Smidsrod, 1995; Sankararamakrishnan and Sanghi, 2006).

Chito-oligosaccharides (COS) (Figure 9) are oligomers that can be obtained from chitosan either chemically or enzymatically via synthesis with glycosyl hydrolases (Aam et al. 2010). Chitosans with degrees of polymerization (DPs) <20 and an average molecular weight less than 3900Da are called chitosan oligomers, chito-oligomers, or chito-oligosaccharides (Mourya et al., 2011). COS is soluble in acidic solutions (Shahidi et al., 1999), and it is partially digested in the gastrointestinal tract of monogastric animals (Hirano et al., 1990; Okamoto et al., 2001).

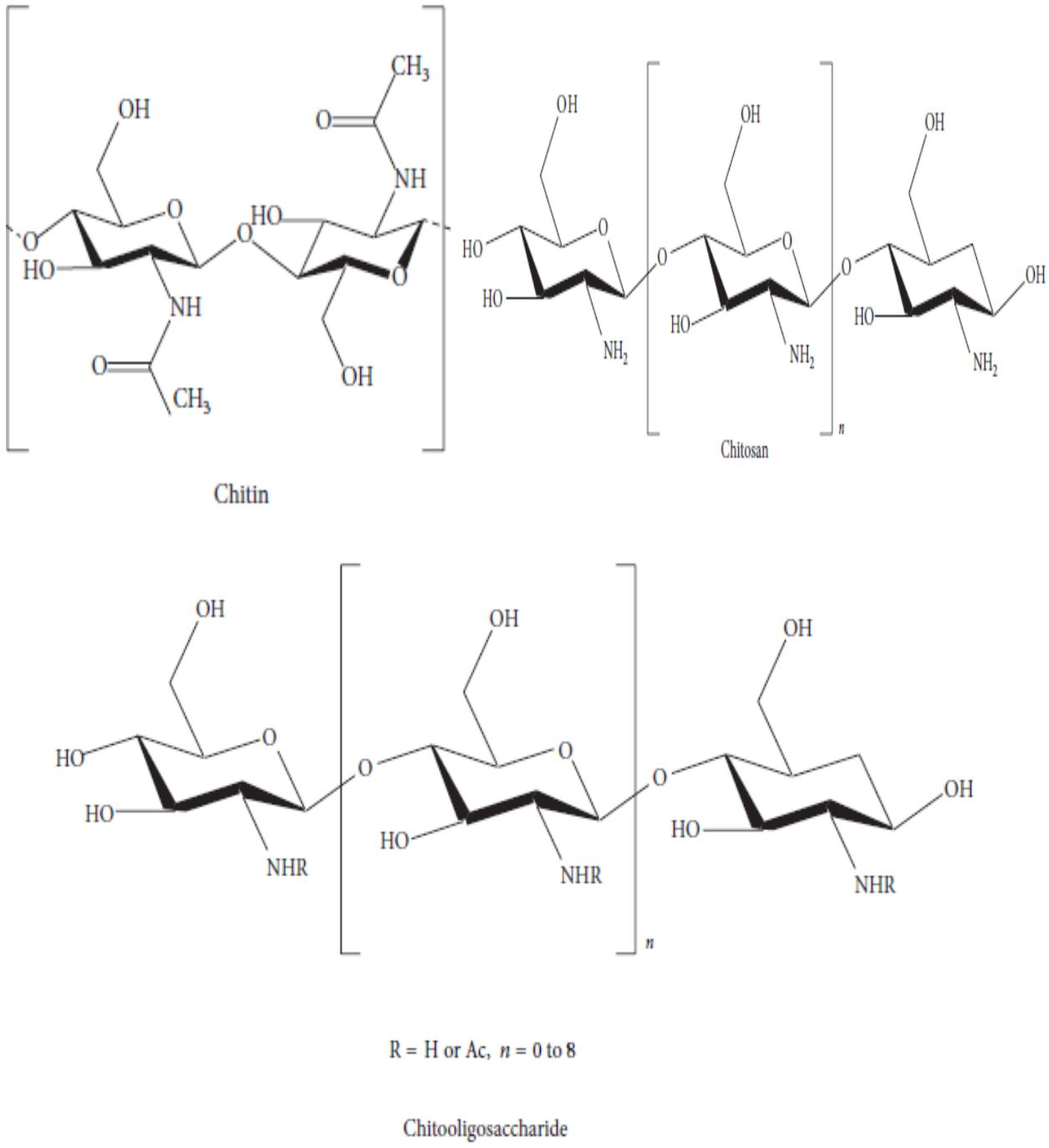

Figure 8. Chemical structure of chitine, chitosan and chito-oligosaccharides (Aam et al,. 2010)

8.2. Effects of chito-oligosaccharides on growth performances and immune response

Chito-oligosaccharides may improve growth performances and immunity in different animals as chickens, pigs and rabbits. Supplementation of diets with 400 and 600 mg/kg of chito-oligosaccharides increase ADG and ADFI, improves gut barrier function, increases the population of *Bifidobacteria* and *Lactobacilli*, and decreases *S.aureus* in the ceacum of weanling pigs (Yang et al,. 2012). In other side COS supplementation at 30 mg/kg had no effects on promoting growth performance and tended to reduce villus height in the duodenum or jejunum. COS at this level may cause an immune and oxidative stress response in small intestine and have compromised the intestinal barrier integrity in weaned piglets (Xiong et al., 2015). Huang et al. (2005) reported that broilers given 50 and 150 mg/kg diet COS have no difference in growth performances comparing to control diets. Contrarily, in a study conducted by Li et al. (2007) a diet supplemented with 50 and 100 mg/kg COS in broiler diet reported higher ADG, ADFI and FCR than controls. However, the use of 0.025% of chito-oligosaccharides in broilers diets decreased LDL-cholesterol levels without any alteration in HDL-cholesterol levels (Onur et al,.2012).Furthermore, dietary supplementation with 160 mg of chito-oligosaccharide/kg reduced incidence of diarrhea and alleviated many of the signs associated with infection in weaned pigs challenged with *E. coli* (Liu et al., 2010).

CHAPTER II: MATERIALS AND METHODS

1. Experimental Diets

A control diet was formulated (Table 1) to meet the nutritive requirements for fattening rabbit according to De Blas and Mateos (2010). Two more diets BG2 and BG4 were obtained by supplementing the control diet with 200 and 400 mg/kg of β-glucans. Another tow diets OT2 and OT4 were obtained by supplementing the control diet with 200 and 400 mg/kg of chito-oligosacharides.

Yeast cell wall β-glucans were supplemented by the inclusion of a mixture made of 40% pure β-glucan (β-glucan-chitin-chitosan complex extracted from *S.cerevisiae* by the Instituto de Estudios Biofuncionales, Madrid, Spain) and 60% calcium carbonate. The β-glucan complex (>95%β-glucan) was isolated from yeast cell walls following the protocol suggested by Pochanavanich and Suntornsuk (2002) with some modifications. Yeast cells were harvested by centrifugation at 8000 g for 30 min, washed twice with distilled water and dried at 65 °C to a constant weight. Dry yeast cells were finely ground, suspended with a NaOH solution (1:30 w/v) and autoclaved at 121°C for 15 min. Alkali-insoluble fractions were collected after centrifugation at 12000 g for 15 min, washed with distilled water and centrifuged again at a neutral pH (pH 7). The chito-oligosaccharides COS was obtained by depolymerization of the chitosan (chitopharm S) from the company Chitinor, the molecular weight of chitopahrm S= 101971 g/mol and for COS = 36432 g/mol.

To evaluate ileal digestibility the five experimental diets (C, BG2, BG4, OT2 and OT4) were supplemented with 0.5% marked alfalfa with ytterbium. The diets (and drinking water) were free from other additives or antibiotics, but contained a coccidiostat (Diclazuril). Chemical composition of experimental diets is shown in Table 2.

Table1: Ingredient composition (g/kg, as fed basis) of control diet

Alfalfa	100
Wheat	260
Soymeal 44	80
Grape seed meal	100
Sunflower meal 28	100
Wheat bran	130
Sugar beet pulp	180
Soybean oil	20
Calcium carbonate	5,5
Dicalcium phosphate	8,5
NaCl	5
DL-Methionine	0,8
L-Lysine	2,7
L-Threonine	2,5
Corrector L-511[1]	5

[1]Provided by Trouw Nutrition-Tecna (Madrid, España), (mg/kg): Mn: 4000; Zn: 11840; Cu: 2000; I: 250; CO: 99; Fe: 15200; Niacin: 4000; Betaine: 10830; Choline: 27500; Vitamin K: 200; Vitamin B1: 200; Vitamin B2: 400; Vitamin B6: 200; Vitamin A: 1675000 UI/Kg; Vitamin D3: 150000UI/Kg; Vitamin E(α-tocopherol acetate): 4000 UI/kg. 200 ppm de Clinacox 0.5% (Diclazuril).

Table2: Chemical composition (g/kg DM) of the experimental diets

Diets	**C**	**BG2**	**BG4**	**OT2**	**OT4**
β-glucan (ppm)	0	200	400	0	0
Chito-oligosaccharides (ppm)	0	0	0	200	400
Dry matter	903	905	903	904	901
Starch	187	188	196	191	187
Crude protein	181	181	182	181	181
CP-TDF	118	127	119	121	121
CP-NDF	92	95	93	91	89
Ether extract	39	40	39	39	40
Total dietary fibre (TDF)	430	435	433	430	431
NDFom	336	334	324	337	323
ADFom	189	188	184	194	183
ADLom	73.7	76.6	70.2	77.3	71.7
Soluble fibre(TDF-aNDFom-cp)	126	128	131	129	135
Gross energy, MJ/kg	18.2	18.3	18.4	18.3	18.3

2. Housing

Rabbits used in the two experiments were cross-bred (New Zealand White × Californian) mixed-sex. Animals were handled according to the principles for experimental animal care published by Spanish Royal Decree 1201/2005 (B.O.E., 2005), and experiments were approved by the Ethics Committee from the Department of Animal Production of the Technical University of Madrid.

During the whole experimental period (Experiments 1 and 2), both feed and water were administrated adlibitum and animals were kept in controlled environmental conditions as follows: temperature maintained at 21 ± 2ºC through heating and cooling systems combined with continue forced ventilation and a cycle of 12 h light: dark was established.

3. Experiment 1

3.1. Growth performances, nitrogen and energy balances and fecal digestibility

Two hundred eighty rabbits 28d-old with a body weight of 510 ± 73.0 g were blocked by litter and randomly assigned to the 5 experimental diets (56 rabbits per diet) and housed individually in flat-deck cages of 600 × 250 × 330 mm. From these rabbits it was recorded growth traits in 42 rabbits / treatment. At 38 d of age the diets were removed and all animals received the same diet (control diet). Individual live weight and feed intake were recorded at 28, 38 and 63 d of age and the health status of rabbits was monitored daily from 28 to 63 d of age.

One hundred twenty six rabbits (506 ± 72.0 g), those used to record growth traits from control, BG4 and OT4 diets (42 / treatment) were used to determine the body composition by bioelectrical impedance technique measured at 28, 38 and 63 d of age. Morbid rabbits were excluded from the experiment. The rabbits were weighted and the dorsal impedance and reactance were recorded on each one using a Quantum II (Model BIA-101®, RJL Systems, Detroit, MI USA). Four electrodes were placed along the spine of the rabbit, a pair to 4 cm from the base of the ears and the other pair to 4 cm from the

base of the tail. Also the distance between the internal electrodes, the length of the animal, the resistance and reactance were measured to determine the impedance value. Using the equations developed by Saiz et al. (2011 a,b and 2013 a,b), the body composition and impedance were calculated. The balances of both nitrogen and energy were estimated following the methodology used by Crespo et al. (2014), from feed digestibility of the three diets (control, BG4 and OT4) and chemical composition (body and carcass) of animals.

The faecal digestibility of the experimental diets was measured on 45 rabbits among those on trial, with an initial body weight of 508 ± 74.1 g (9 rabbits per diet) and housed in metabolism cages measuring 400 × 510 × 330 mm from 28 to 38 d of age according to the European standardized method (Perez et al., 1995). After 7 d of adaptation, faeces were collected daily for three consecutive days (36 to 38 d of age) from each cage and the feed intake was recorded. Faecal samples were stored at −20 °C for analysis. Samples of the feeds offered to the animals were collected at the end of the faecal collection period. At 56 d of age 15 rabbits with a body weight of 1751± 492 g (3 rabbits/diet) from those used in growth traits were chosen to estimate the faecal digestibility of DM, gross energy and crude proteins on the control diet between (56 to 59d of age).

3.2. Ileal digestibility, gut barrier, intestinal microbiota, and immune response

One hundred-twenty one rabbits 28 d of age and a BW of 510 ± 73g (mean ± standard deviation) received the five marked diets (24 per diet) and housed collectively (5 rabbits / cage) until 38d of age.

During two consecutive days (at 37 and 38 d of age), all rabbits were slaughtered by CO_2 inhalation between 19:00 and 21:00 h to minimize the influence of caecotrophy. The body weight at slaughtering and the caecal pH were measured (19 rabbits per treatment). Immediately, 10 cm from middle jejunum were collected (10 per treatment). The first 5 cm were washed with saline solution (0.9 % NaCl), frozen in dry ice and stored at -80ºC until determination of sucrase activity. The other 5 cm were stored in 10% buffered formalin solution (pH: 7.2-7.4) for analysis of mucosa morphology and goblet cell

count. One gram of caecal and ileal content was collected and placed in sterile tubes containing 3 ml of ethanol solution (98%) and stored for microbial analysis (10 per treatment). The last 20 cm of the ileum in each case was excised, emptied and the digesta was frozen in dry ice. Samples were freeze-dried and grounded. Due to the small amounts of samples, samples from rabbits received the same treatments were pooled (8 pools/ treatment) to determinate ytterbium. For the immunological analyses, the appendix was excised and washed with saline solution (0.9% NaCl), longitudinally opened and the mucosa was scraped using a blunt spatula then placed in vials containing 1 ml of RNA-later (Ambion) and stored at -80ºC (10 per treatment).The symptoms of morbidity taken in consideration once animals were slaughtered were the presence of gas in intestinal segments or liquid and compacted caecal content.

4. Experiment 2

4.1. Growth performances

At 19 d of age three hundred thirty-four rabbits with body weight of 329 ± 65g were separated of their mothers. Each litter was divided in to three groups of two or three rabbits according to the litter size. Each group was assigned to one of the three experimental diets (control diet, BG4 and OT4). Rabbits were caged in groups of six or nine rabbits coming from three different litters. They were marked using colored sprays. Every morning rabbits of the same litter were taken and left with their mothers for suckling about 10 minutes and returned back to their cages.

After the first period, at 28 d old two hundred thirty-three rabbits received the same experimental diets in the first period (77 rabbits per diet) were transferred and housed individually in flat-deck cages of 600 × 250 × 330 mm. At 39 d of age all rabbits were fed with control diet until 59 d of age. The health status of rabbits was monitored daily in the whole period of the experiment.

4.2. Gut barrier, intestinal microbiota, and immune response

At 19 d of age 10 rabbits from 10 different litters with a body weight of 328 ± 35g (mean ± standard deviation) were slaughtered by cervical dislocation at 10:00 h. The body weight and the weight of: total digestive tract, stomach and cæcum were measured. Immediately, the first peyer’s patch from the ileum, 5 cm of from middle jejunum and a

part of middle appendix were collected and stored in 10% buffered formalin solution (pH: 7.2-7.4) to determine mucosal and payer patch morphology. Other 5 cm of middle jejunum were washed with saline solution (0.9 % NaCl), frozen in ice and stored at -80ºC until determination of sucrase activity. One gram of caecal content was collected and placed in sterile tubes containing 3 ml of ethanol solution (98%) and stored for microbial analysis. For the ileal content the most animals don't have enough content because they still suckling milk. For the immunological analyses, both of the last part of the appendix and the first part of ileum were excised and washed with saline solution (0.9% NaCl), longitudinally opened and the mucosa was scraped using a blunt spatula then placed in vials containing 1 ml of RNA-later (Ambion) and stored at -80ºC.

At 27 and 38 d of age 30 rabbits with a BW of 500 ± 82g and 1004 ± 85g (mean ± standard deviation) respectively,(3 rabbits/treatments and litter) from each of the same 10 litters which have been chosen in the first slaughter (at 19 d of age) were slaughtered by cervical dislocation at 10:00 h. The samples taken and variables measured were the same that at 19 d of age.

5. Analytical methods

Methods of the AOAC (2000) were used to analyze DM (934.01), ash (967.05), CP (2001.11), starch (996.11) of the feed and faecal contents. Ether extract was determined after acid-hydrolysis treatment (EC, 1998). The total dietary fibre (TDF) was determined with a gravimetric enzymatic procedure with α-amylase, protease, and amylo-glucosidase treatments (Method AOAC 991.43) (Megazyme Int. Ireland Ltd., Wicklow, Ireland). Dietary aNDFom (without sodium sulphite), and ADFom were sequentially determined using the filter bag system (Ankom Technology, New York) according to Mertens et al., (2002) and AOAC procedure (2000, 973.187). Soluble fiber was calculated as TDF-aNDFom-cp (both corrected for ash and protein). The gross energy was measured with an adiabatic bomb calorimeter. Ytterbium concentration of diets and ileal digesta were analyzed by atomic absorption spectrometry (Smith Hieftje 22, Thermo Jarrel Ash) (García et al., 1999).

6. Statistical analysis

The results obtained were analyzed as a completely randomized design in which the main sources of variation were the type and the levels of additives supplementation, using the General Linear Model (GLM) procedure of SAS (Statistical System Institute Inc., Cary, NC). The rabbit was used as the experimental unit in the analyses of growth performances with the weaning weight used as a covariate and litter as block. Mortality rate was analyzed using logistic regression (GENMOD) procedure of SAS.Non-orthogonal contrasts were used to test the effect of the type and level of the additives.

Chapter III: RESULTS

1. Experiment 1

1.1. Growth performances and mortality

Dietary supplementations with β-glucans tended to reduce growth rate compared to control diet from 28 to 38 d of age (P = 0.063.Table3) with no effect on feed intake and feed efficiency. In the second period all rabbits received control diet and those previously supplemented with β-glucan tended to have a higher feed efficiency (P = 0.090).When the whole fattening period was considered no effect of β-glucan supplementation was observed. Dietary supplementation with chito-oligosaccharides at different levels did not modify growth performances of rabbits along the fattening period (46.8 g/d, 113 g/d and 0.417 on average for growth rate, feed intake and feed efficiency) showing no difference with control or β-glucan supplemented groups.

Health condition of rabbits was relatively good during the first ten days of the experiment showing a low mortality (3.9% on average. Table 3), and there was not any effect of treatments. Mortality rate increased from 38 to 63 d of age (20% on average in this period) with no effect of treatments. However, when the whole fattening period was considered, rabbits supplemented with the lower dose of β-glucan (200 ppm) had a higher mortality than those supplemented with 400ppm of β-glucan (42.9 vs. 25.0% respectively. P=0.048).

1.2. Ileal and faecal digestibility

The supplementation of diets with different levels of β-glucans and chito-oligosaccharides did not affect the ileal and faecal apparent digestibility of DM (39.7 and 69.5%.Table 4), and faecal digestibility of gross energy, crude protein, ether extract and total dietary fibre (being on average 67.9, 78.6, 75.4 and 47.2%, respectively). Fecal digestibility of rabbits fed control diet, from 56 to 59 d of age, of gross energy and crude protein were on average 67.1 ± 4.4 and 72.9 ± 4.8%, respectively. Caecal pH tended to be lower for rabbits fed β-glucans compared to the chito-oligosaccharides group (P = 0.074).

1.3. Nitrogen and energy balances

Rabbits selected to record energy and nitrogen balances showed similar growth traits than the corresponding groups. In the first period (28 to 38d), it was observed that the faecal excretion of nitrogen in faeces increased by 16% (P = 0.034) in rabbits fed the diet supplemented with 400 ppm chito-oligosaccharides comparing to those fed the control diet, showing rabbits fed the β-glucan diet an intermediate value (Table 5). The supplementation with chito-oligosaccharides also tended to increase the excretion of energy in faeces in comparison with the control diet (P=0.076). Between 38 and 63d of age it was observed that animals supplemented with 400 ppm β-glucan tended to increase the excreted nitrogen in skin and entrails in comparison with the control diet (P=0.086.Table 6). In the whole period between 28-63d of age there were no effects of β-glucan and chito-oligosaccharide supplementation on ingested and ingested digestible nitrogen. Neither, there was any effect of these additives on ingested and ingested digestible energy. Morever, the retention of both of nitrogen and energy in the animal or in carcass were not affected by the type of treatment (1,16 g/kgPV^{0,75}d and 312 kJ/kgPV$^{0.75}$d, 0,679 g/kgPV^{0,75}d and 186 kJ/kgPV$^{0.75}$d in animal and carcass respectively. Table7).

2. Experiment 2

2.1. Growth performances and mortality

From 19 to 27 d of age suckling rabbits from 400 ppm β-glucan and chito-oligosaccharide groups increased their feed intake respect to control group by 14% (P = 0.015) with no effect on weight gain or feed efficiency (the latter calculated considering only solid feed intake. Table8). There were no effects of β-glucans or chito-oligosaccharides supplementation on growth rate, feed intake and feed efficiency (50.6, 157 g/d and 0.322 g/g respectively) along the fattening period. The mortality rates in this experiment were lower than the first experiment, but there was no effect of treatments on mortality.

2.2. Digestive parameters

The supplementation with β-glucans or chito-oligosaccharides did not affect the development of digestive organs (stomach and caecum. Table10). The relative weight of the total digestive tract and ceacum increased with age ($P < 0.001$. Table10). The relative weight of stomach increased from 19 and 27d of age (7.6 to 10.7 % BW respectively), and then decreased to 6.9 % BW at 38d of age.

The results dealing with mucosa morphology and functionality are not reported due to laboratorial problems.

Chapter III: DISCUSSION

Certain types of oligosaccharides might be a potential alternative of antibiotics in enhancing animal growth and improving the intestinal microbiota (Patterson and Burkholder, 2003). The supplementation with oligosaccharides, such as yeast manano-oligosaccharides and yeast β-glucans have been used to improve growth performance and immunity in young rabbits (El-Abed et al., 2015), or the use of chito-oligosaccharides in piglets (Liu et al., 2008; Xiong et al., 2015).However there is still little knowledge of the possible benefits of β-glucans and chito-oligosaccharides in rabbits. A recent work carried out with very low mortality showed a positive effect of yeast β-glucans supplementation (100 and 200 ppm) on the specific sucrase activity and changed the bacterial community structure at both ileum and caecum, although it seemed more effective in the ileum where the microbiota diversity was reduce (El Abed et al., 2015).

This study was conducted in low sanitary conditions due to the incidence of ERE. The total mortality rate was at 27.8% in the total fattening period. In these conditions, the supplementation yeast β-glucans and chito-oligosaccharides had no effect on growth performances. A similar response was found by Decuypere et al. (1998) that did not observe a positive response of β-glucans in improving health and growth in a low sanitary environment. In the present work, there was no effect of supplementation with β-glucans on growth performances in agreement with El Abed et al. (2015), and with other works in poultry in which reported that the use of 0,02 and 0.04% β-glucan in broilers did not cause any difference between groups for ADG, ADFI and FCR (Chae et al., 2006).Wang et al. (2008) demonstrate that dietary β-1,3/1,6-glucan supplemented at 0, 25, 50, 100 and 200 ppm in pigs for 4 weeks of age did not modify body weight and feed intake. In our experiment, there was a trend to decrease growth rate in rabbits supplemented with yeast β-glucans compared to the control diet. On the opposite, the supplementation with 100 and 150 ppm of yeast β-glucans (fibosel) improved daily weight gain and feed intake in rabbits (García-Ruiz et al., 2008), or similar effects were observed in pigs when supplemented with 0.025% β-glucan (Dritz et al., 1995).

Also in the current study, addition of chito-oligosaccharides had no effect on growth performances. There is no previous result in rabbits with this additive but the results in other non-ruminants are variable. No effect of chito-oligosaccharides was found in broilers (50 and 150 ppm, Huang et al., 2005), or pigs (30 ppm, Xiong et al., 2015, or 0.2 and 0.1%, Kim et al., 2008). However, a level of 0.3% reduced feed intake and improved feed gain in growing piglets (Han et al. 2007b), and the supplementation of broilers diet with 50 and 100 ppm improved growth performance (Li et al., 2007). These discrepancies in response to chito-oligosaccharides may result in part from the different molecular weights and different doses of the COS used in the experiments. The COS serves as an adhesive and carrying agent when its molecular weight is greater than 10^5Da, while at a molecular weight range of 10^3 to10^4Da it reduces the establishment of pathogens in the intestine (Huang et al. 2005).

Accordingly, the reason for differences between these studies and our study could be related to the source for β-glucans, the molecular weight of both β-glucans and chito-oligosaccharides or the type of control diet used. In this work, these additives were studied using a control diet with a high level of soluble fibre that in ERE conditions limit the mortality rate of growing rabbits (Trocino et al., 2013; Martínez-Vallespín et al., 2011). High levels of soluble fiber obtained using sugar beet pulp promotes relevant changes in intestinal microbiota and might decrease the microbial ileal diversity as reported by El Abed et al. (2013). In this context, the addition of potential prebiotics to high soluble fibre diets might provoke no effect or even negative interactions. In this sense, the addition of cellobiose was positive for rabbits fed a low soluble fiber diet, but negative for those fed a high soluble fiber diet (Ocasio-Vega et al., 2015).

The results of the nitrogen and energy balances were similar than those reported by Delgado et al. (2015) with no effect of treatments on the efficiency of retention of nitrogen or energy. However, the nitrogen and energy excreted in faeces increased in rabbits supplemented with chito-oligosaccharides from 28 to 38 d of age. These effects might be related to the reduction of the faecal digestibility of ether extract by β-glucans supplementation (El Abed et al., 2015). In fact, these authors suggest that this effect might be induced by the chitosans associated to β-glucans in the yeast cell wall. According to Xia et al. (2011), chitosan can absorb fatty acids by electrostatic interaction. In the small

intestine, chitosan might precipitate together with the entrapped fat at neutral pH preventing the digestion of fat, as observed in vitro (Zhou et al., 2006). However, in our study no effect on faecal digestibility was found and the main effect was on nitrogen excretion.

The addition of chito-oligosaccharides had no effect on faecal digestibility in piglets (Kim et al, 2008), but improved DM, energy and calcium digestibility in broilers (Li et al., 2007). Similarly, Wang et al. (2005) also reported that dietary supplementation of 125 mg/kg of COS increased growth rate by 5.9% and improved nutrient digestibility by improving gut health. Both of β-glucans and chito-oligosaccharides did not affect ileal digestibility of DM in rabbits at 38d of age in agreement with results of El-Abed et al, (2015).While diets formulated by adding 50, 100, and 150 ppm of chito-oligosaccharides improved the ileal digestibility of different nutrients in broilers (Huang et al., 2005).

There were no effect of β-glucans or chito-oligosaccharideson digestive organ weight and evolution. However, it was found an increase in the relative weight of total digestive tract, and the ceacum between 19 and 27d of age and still increasing until 38 d of age, while the relative weight of the stomach decreased between 27 and 38d of age. The increase of the weight of different parts of the digestive system between 19 and 27d of age are due to the beginning of solid feed intake. These results show that especially between 27 and 38d of age the ceacum continues increasing faster than the rest of digestive tract due to a greater accumulation of digesta in ceacum in this period. These results are common and were previously described by Padilha et al. (1995), Gallois et al. (2005) and Delgado et al, (2010).

Conclusions

The supplementation with yeast β-glucans and chito-oligosaccharides, independently of the dose, do not improve the growth performance or the digestibility of the diets with respect to a control diet with a high content in soluble fiber.

Table3: Effect of β-glucan and chito-oligosaccharides supplementation on growth performance and mortality of rabbits from 28 to 63d of age (Experiment 1).

	Experimental diets[1]						*P value* of contrasts					
	C	BG2	BG4	OT2	OT4	Cov[3]	C vs. BG	C vs. OT	BG2 vs. BG4	OT2 vs. OT4	BG2+BG4 vs. OT2+OT4	SEM
N[2]	28	20	29	27	30							
28-38d												
Live weight, 28 g/d	497	533	517	513	499							
Feed intake, g/d	74	78.2	73.4	79	76.6	<0.001	0.65	0.33	0.37	0.59	0.55	16.7
Weight gain, g/d	52	48.5	47.8	50.3	47.9	<0.001	0.063	0.19	0.91	0.35	0.46	9.62
Feed efficiency, g/g	0.703	0.626	0.682	0.650	0.666	0.42	0.31	0.34	0.34	0.77	0.92	0.2
Mortality[2], %	0.0	10.7	5.36	3.61	0.0	-	0.99	0.99	0.31	0.99	0.99	-
38-63d												
Live weight 38, g/d	1031	986	989	1014	990	<0.001	0.063	0.19	0.91	0.35	0.45	96.2
Feed intake, g/d	128	124	129	131	127	0.009	0.78	0.9	0.52	0.55	0.63	25.7
Weight gain, g/d	43.5	45.6	46.9	46.8	45.8	0.25	0.26	0.23	0.65	0.73	0.98	10
Feed efficiency, g/g	0.345	0.363	0.366	0.358	0.364	<0.001	0.09	0.16	0.83	0.68	0.67	0.049
Mortality[2], %	23.2	32.1	19.6	23.2	21.4	-	0.76	0.89	0.13	0.82	0.59	-
28-63d												
Live weight 63d, g/d	2119	2126	2163	2184	2136	<0.001	0.68	0.49	0.63	0.49	0.76	254
Feed intake, g/d	113	111	113	116	111	<0.001	0.88	0.73	0.73	0.5	0.56	20
Weight gain, g/d	46.2	46.1	47.2	47.8	46.4	0.37	0.68	0.49	0.63	0.49	0.76	7.27
Feed efficiency, g/g	0.413	0.418	0.421	0.414	0.418	<0.001	0.49	0.77	0.81	0.70	0.72	0.04
Mortality[2], %	23.2	42.7	25.0	26.8	21.4	-	0.18	0.91	0.048	0.51	0.13	-

[1]C: control diet. BG2: diet 200 ppmβ-glucans. BG4: diet 400 ppmβ-glucans. OT2: diet 200 ppm chito-oligosaccharides. OT4: diet 400 ppm chito-oligosaccharides. [2]N= number of animals that finished the growth traits from the 42 that started per treatment.N = 56 for mortality.[3]Live weight at weaning (LW28d).

Table4: Effect of β-glucan and chito-oligosaccharides supplementation on the ileal and faecal apparent digestibility of 38 d old rabbits (Experiment 1)

	Experimental diets[1]					P *value* of contrasts					
N[2]	C	BG2	BG4	OT2	OT4	C vs. OT	C vs. BG	OT2 vs. OT4	BG2 vs. BG4	OT2+OT4 vs. BG2+BG4	SEM[2]
Aparent ileal digestibility(38d)											
Dry matter	41.2	37.2	39.7	39.9	40.4	0.15	0.60	0.19	0.83	0.26	3.14
Aparent faecal digestibility(35-38 d)											
Feed intake, g/d	82.1	76.9	84.5	75.7	83.2	0.72	0.84	0.38	0.34	0.83	5.72
Dry matter	69.9	70.3	68.7	70.5	68.3	0.87	0.80	0.25	0.40	0.96	1.37
Gross energy	68.7	68.1	67.3	68.8	66.7	0.56	0.73	0.32	0.43	0.79	1.48
Crude protein	79.3	79.0	77.9	79.2	77.4	0.51	0.57	0.34	0.56	0.91	1.35
Total dietary fibre	47.2	48.6	46.6	48.9	46.4	0.89	0.89	0.44	0.52	0.99	2.18
Ether extract	74.8	76.9	73.6	76.1	75.7	0.56	0.81	0.86	0.14	0.67	1.53
Caecal pH	5.41	5.41	5.34	5.53	5.60	0.20	0.82	0.67	0.65	0.074	0.10

[1]C: control diet. BG2: diet 200 ppmβ-glucans. BG4: diet 400 ppmβ-glucans. OT2: diet 200 ppm chito-oligosaccharides. OT4: diet 400 ppm chito-oligosaccharides. [2]N= 5 pools/treatment for ileal digestibility, N=9 rabbits/diet for faecal digestibility and N= 18/treatment for caecal pH.

Table5: Effect of β-glucan and chito-oligosaccharides supplementation onthe nitrogen and energy balances in rabbits between 28 and 38 d of age (Experiment 1).

	Experimental diets[1]				P-value	
	C	BG4	OT4	SEM	Cov[2]	Treatment
N	26	27	29			
Live weight, 28 g/d	499	516	491			
Feed intake, g/d	73,01	71,5	77,8	3,18	<0.001	0,34
Weight gain, g/d	51,4	46,7	48,6	1,94	<0.001	0,23
Feed efficiency, g/g	0,704	0,688	0,668	0,046	0,43	0,85
Nitrogen balance 28-38 d						
Ingested nitrogen, g/kgPV^{0,75} d	2,36	2,343	2,501	0,095	0,49	0,42
Ingesteddigestible nitrogen,g/kgPV^{0,75} d	1,873	1,825	1,935	0,07	0,49	0,55
Retained nitrogen (animal), g/kgPV^{0,75} d	1,643	1,588	1,576	0,038	<0.001	0,77
Retained nitrogen (carcass), g/kgPV^{0,75} d	0,871	0,845	0,844	0,029	<0.001	0,77
Retention efficiency of nitrogen (animal)	0,698	0,724	0,673	0,044	0,005	0,71
Retention efficiency of digestible nitrogen (carcass)	0,466	0,501	0,457	0,032	0,003	0,58
Excreted nitrogen, g/kgPV^{0,75} d						
Skin and entrails	0,772	0,743	0,731	0,027	0,008	0,54
Faeces	0,486^{b}	0,517^{ab}	0,565^{a}	0,021	0,49	0,034
Urines + heat increment	0,313	0,398	0,424	0,066	0,007	0,38
Energy balance28-38 d						
Ingested energy, kJ/ kgPV$^{0.75}$ d	1482	1480	1580	60,45	0,48	0,39
Ingesteddigestible energy, kJ/ kgPV$^{0.75}$ d	1018	996	1054	40,27	0,48	0,58
Retained energy (animal), kJ/ kgPV$^{0.75}$ d	397	373	373	17,78	<0.001	0,56
Retained energy (carcass), kJ/ kgPV$^{0.75}$ d	183	185	177	10,53	<0.001	0,84
Retention efficiency of energy(animal)	0,267	0,273	0,248	0,018	0,005	0,58
Retention efficiency of digestible energy(carcass)	0,180	0,203	0,169	0,015	0.0002	0,24
Excreted energy, kJ/ kgPV$^{0.75}$ d						
Skin and entrails	215	195	196	11,98	0,27	0,41
Faeces	464^{b}	484^{ab}	526^{a}	19,82	0,49	0,076
Urine + heat increment	620	646	681	39,41	0,29	0,53

[1]C: control diet. BG2: diet 200 ppm β-glucans. BG4: diet 400 ppm β-glucans. OT2: diet 200 ppm chito-oligosaccharides. OT4: diet 400 ppm chito-oligosaccharides.[2]Live weight at weaning (LW28d) for growth parameters and Weight gain between 28 and 38 for both of nitrogen and energy balances.

Table6: Effect of β-glucan and chito-oligosaccharides supplementation onnitrogen and energy balances in rabbits between 38 and 63 d of age (Experiment 1).

	Experimental diets[1]				P-value	
	C	BG4	OT4	SEM	Cov[2]	Treatment
N	26	27	29			
Live weight, 38 g/d	1008	993	963			
Feed intake, g/d	126	128	127	5,16	0,019	0,94
Weight gain, g/d	43,1	47,53	46,17	1,96	0,39	0,27
Feed efficiency, g/g	0,347	0,372	0,366	0,01	<0.001	0,21
Nitrogen balance 38-63 d						
Ingested nitrogen, g/kgPV^{0,75} d	2,356	2,464	2,44	0,07	<0.001	0,53
Digestible nitrogen,g/kgPV^{0,75} d	0,719	0,798	0,78	0,05	<0.001	0,53
Retained nitrogen (animal), g/kgPV^{0,75} d	0,935	1,023	1,026	0,04	<0.001	0,26
Retained nitrogen (carcass), g/kgPV^{0,75} d	0,593	0,611	0,626	0,02	<0.001	0,63
Retention efficiency of nitrogen (animal)	0,395	0,415	0,42	0,015	<0.001	0,50
Retention efficiency of digestible nitrogen (carcass)	0,344	0,334	0,352	0,011	<0.001	0,77
Excreted nitrogen, g/kgPV^{0,75} d						
Skin and entrails	0,342^{ab}	0,411^{a}	0,400^{a}	0,02	0,009	0,086
Faeces	0,637	0,666	0,659	0,018	<0.001	0,53
Urines + heat increment	0,783	0,775	0,754	0,04	0,1	0,89
Energy balance38-63 d						
Ingested energy, kJ/ kgPV$^{0.75}$ d	1468	1536	1521	43,8	<0.001	0,53
Digestible energy, kJ/ kgPV$^{0.75}$ d	986	1031	1020	29,4	<0.001	0,53
Retained energy (animal), kJ/ kgPV$^{0.75}$ d	274	285	296	14,1	<0.001	0,51
Retained energy (carcass), kJ/ kgPV$^{0.75}$ d	183	185	193	8,9	0,0002	0,67
Retention efficiency of energy(animal)	0,184	0,185	0,193	0,007	0,006	0,61
Retention efficiency of digestible energy(carcass)	0,186	0,175	0,190	0,006	<0.001	0,23
Excreted energy, kJ/ kgPV$^{0.75}$ d						
Skin and entrails	90	98	103	5,87	0,002	0,27
Faeces	483	505	499	14,4	0,01	0,53
Urine + heat increment	712	745	724	23,8	0,002	0,67

[1]C: control diet. BG2: diet 200 ppm β-glucans. BG4: diet 400 ppm β-glucans. OT2: diet 200 ppm chito-oligosaccharides. OT4: diet 400 ppm chito-oligosaccharides.[2]Live weight at weaning (LW28d) for growth parameters and Weight gain between 28 and 38 for both of nitrogen and energy balances.

Table7: Effect of β-glucan and chito-oligosaccharides supplementation nitrogen and energy balances in rabbits between 28 and 63 d of age (Experiment 1).

	Experimental diets[1]				P-value	
	C	BG4	OT4	SEM	Cov[2]	Treatment
N	26	27	29			
Live weight, 63 g/d	2176	2091	2117			
Feed intake, g/d	111	112	112	3,39	0,001	0,94
Weight gain, g/d	45,48	47,3	46,86	1,46	0,38	0,66
Feed efficiency, g/g	0,415	0,425	0,42	0,008	0,002	0,72
Nitrogen balance 28-63 d						
Ingested nitrogen, g/kgPV^{0,75} d	2,361	2,427	2,456	0,06	0,0002	0,5
Digestible nitrogen,g/kgPV^{0,75} d	1,766	1,803	1,824	0,04	0,003	0,63
Retained nitrogen (animal), g/kgPV^{0,75} d	1,149	1,177	1,18	0,03	0,001	0,74
Retained nitrogen (carcass), g/kgPV^{0,75} d	0,681	0,672	0,686	0,02	<0.001	0,84
Retention efficiency of nitrogen (animal)	0,487	0,500	0,491	0,01	0,128	0,86
Retention efficiency of digestible nitrogen (carcass)	0,384	0,383	0,38	0,01	0,1	0,98
Excreted nitrogen, g/kgPV^{0,75} d						
Skin and entrails	0,468	0,504	0,494	0,01	0,0028	0,29
Faeces	0,594	0,623	0,632	0,01	0,0002	0,19
Urines + heat increment	0,616	0,626	0,644	0,04	0,3	0,88
Energy balance28-63 d						
Ingested energy, kJ/ kgPV$^{0.75}$ d	1475	1518	1537	36,4	0,002	0,47
Digestible energy, kJ/ kgPV$^{0.75}$ d	997	1019	1030	24,4	0,0002	0,62
Retained energy (animal), kJ/ kgPV$^{0.75}$ d	312	309	317	10,7	<0.001	0,82
Retained energy (carcass), kJ/ kgPV$^{0.75}$ d	186	184	188	6,9	<0.001	0,89
Retention efficiency of energy(animal)	0,209	0,208	0,208	0,007	0,016	0,99
Retention efficiency of digestible energy(carcass)	0,199	0,207	0,183	0,015	0,0002	0,54
Excreted energy, kJ/ kgPV$^{0.75}$ d						
Skin and entrails	126	125	129	4,85	0,004	0,81
Faeces	478	498	507	4,85	0,002	0,22
Urine + heat increment	685	721	711	21,3	0,005	0,47

[1]C: control diet. BG2: diet 200 ppmβ-glucans. BG4: diet 400 ppmβ-glucans. OT2: diet 200 ppm chito-oligosaccharides. OT4: diet 400 ppm chito-oligosaccharides.[2]Live weight at weaning (LW28d) for growth parameters and Weight gain between 28 and 38 for both of nitrogen and energy balances.

Table8: Effect of β-glucan and chito-oligosaccharides supplementation on growth performance of rabbits from 19 to 27 d of age (Experiment 2).

	Experimental diets				*P-value*	
	C	BG4	OT4	SEM	Cov[1]	Treatment
N cages[2]						
Live weight, g/rabbit						
19 d	313	335	313	12		
27 d	502	517	493	13	<0.001	0.29
Period (19 - 27 d)						
Feed intake, g/d	9.33[b]	10.8[a]	10.5[a]	0.36	<0.001	0.015
Weight gain, g/d	20.2	21.9	19.2	1.18	0.032	0.30
Feed efficiency, g/g	0.488	0.518	0.580	0.045	0.83	0.20

[1]Live weight at 19d of age.[2]Six rabbits per cage and 19cages/treatment.

Table 9: Effect of β-glucan and chito-oligosaccharides supplementation on growth performance of rabbits from 29 to 59d of age (Experiment 2).

	Experimental diets[1]				P-value	
	C	BG4	OT4		Cov	Treatment
N[2]	51	55	51	SEM		
29-39d						
Live weight 28 d, g/d	494	522	516			
Feed intake, g/d	98	100	98	2.12	<0.001	0.62
Weight gain, g/d	54.6	53.2	53.4	1.15	<0.001	0.65
Feed efficiency, g/g	0.560	0.529	0.546	0.011	0.004	0.13
Mortality[2], %	4.00	3.90	6.00	0.61	-	0.73
39-59d						
Live weight 38 d, g/d	1116	1101	1103	12.75	<0.001	0.65
Feed intake, g/d	145	155	148	3.54	<0.001	0.12
Weight gain, g/d	47.6	49.5	47.8	1.04	0.19	0.38
Feed efficiency, g/g	0.334	0.332	0.330	0.006	<0.001	0.85
Mortality[2], %	11.9	11.9	17.4	-	-	0.57
29-59d						
Live weight 63 d, g/d	2069	2101	2085	28.33	<0.001	0.72
Feed intake, g/d	160	158	154	2.62	<0.001	0.24
Weight gain, g/d	50.5	51.2	50.2	0.75	<0.001	0.64
Feed efficiency, g/g	0.316	0.325	0.325	0.004	0.87	0.26
Mortality[2], %	17.9	16.4	24.6	-	-	0.44

[1]C: control diet. BG2: diet 200 ppm β-glucans. BG4: diet 400 ppm β-glucans. OT2: diet 200 ppm chito-oligosaccharides. OT4: diet 400 ppm chito-oligosaccharides. [2]N= number of animales finished the growth traits about the 77 which started the trait per treatment. For the mortality N = 82.[3]Live weight at weaning (LW29d).

Table.10: Effect of β-glucan and chito-oligosaccharides supplementation on digestive parameters in rabbits between 19 and 38d of age (Experiment 2).

Age	**19d**	**27d**			**38d**			**SEM**	**P-value**		
Diets		**C**	**BG4**	**OT4**	**C**	**BG4**	**OT4**		**Age**	**Diet**	**Age x Diet**
N	10	10	10	10	10	10	10				
Weight, g											
Body weight	328	531	492	476	1032	1013	966	37.39	<0.001	0.28	0.92
Digestive tract	47	113	112	112	272	260	239	12.3	<0.001	0.38	0.41
Stomach	24.9	55.2	55.2	50.5	70.7	74.1	64.1	4.3	<0.001	0.21	0.82
Ceacum	6.67	26.3	25.5	28.5	98.4	95.8	96.4	4.34	<0.001	0.89	0.89
Relative weight, %BW											
Digestive tract	14.5	21.4	22.9	23.8	26.6	25.7	25.2	1.2	<0.001	0.91	0.30
Stomach	7.64	10.4	11.1	10.7	6.81	7.24	6.68	0.44	<0.001	0.40	0.91
Ceacum	2.03	5.02	5.35	6.12	9.73	9.40	10.2	0.55	<0.001	0.28	0.80

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