# OLIGOSACCHARIDES PREBIOTIC EFFECT OBTAINED FROM *LUPINUS EXALTATUS* IN PREVENTION OF SALMONELLA IN CHICKEN EMBRYOS

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## ABSTRACT

**Oligosaccharide are utilised as nutritional supplements to promote the growth of beneficial intestinal bifidobacteria (prebiotics) that improve the resistance to gastrointestinal infections caused by *Escherichia coli*, *Clostridium* and *Salmonella* spp. This must to the antagonistic action and the toxic product formation of the fermentation by bifidobacteria. Salmonella is considered to be the main cause of human intestinal infections originating from the consumption of chicken meat and eggs contaminated with this bacterium. In order to provide chicken products that are free from this pathogen, it is common from the poultry industry to administer antibiotic. Nevertheless, the indirect antibiotic consumption by humans causes problems of resistance by the pathogens.**

**Three doses of oligosaccharide isolated from seeds of *Lupinus exaltatus* to chicken embryos infected with salmonella were evaluated in this experiment. Fecal samples of two days hatching were collected to determine the cfu number of *Salmonella enteritidis* and bifidobacterias. The experimental results show that oligosaccharide from *L. exaltatus,* stimulate bifidobacteria development and this obtains a decrease in the level of Salmonella; indicating that it has potential for use as a prebiotic to reduce the incidence of Salmonella infection in poultry.**

## KEY WORDS

oligosaccharides, prebiotics, *Lupinus exaltatus,* salmonella

## INTRODUCTION

Oligosaccharides are a carbohydrate group of chain short indigerible molecules found naturally in some foods like fruits, vegetables, cereals, legumes (Kuo *et al*. 1988), honey and milk (Swallow and Low, 1990).

They are defined as glycosides which have 3-10 units of monosaccharides linked together and are characterised by the type and sequence of these (Bornet and Brouns, 2002).

Most oligosaccarides have a mildly sweet taste. In addition they lend certain other characteristics to food, such as the mouth feel which has attracted the interest of the food industry as a partial substitute for fats and sugars in some foods as well as improved texture. Because of this, more and more of the oligosaccharides in food are synthetically produced.

Recent interest has also been drawn to oligosaccarides from the nutritional community due to its resistance characteristic from the human digestive system finding it difficult to break down many of the carbohydrates. Almost 90% of oligosaccharides escape digestion in the small intestine and reach the colon where they selectively stimulate the growth and/or activity of beneficial bacteria like bifidobacterias and lactobacillus, providing prebiotic properties (Gomes and Malcata, 1999; Stanton *et al*. 2003).

The bacteria that feed on fermentable carbohydrates produce many beneficial substances, including short-chain fatty acids (SCFAs) and certain B-vitamins. Additionally, there is some evidence that they may promote further absorption of some minerals that have escaped the small intestine, including calcium and magnesium. The SCFAs probably provide many benefits, both locally in the colon, and in the rest of the body, although the research in this area is quite new. In particular, butyrate has received attention as possibly being protective of colon tissue from damage, including colon cancer and ulcerative colitis. Other possible benefits include: lower cholesterol, lower triglycerides, improved insulin sensitivity and glucose metabolism and improved immune system function (Niness, 1999).

Bifidobacteria are normal components of the intestinal flora in animal and man. Studies have demonstrated that these bacteria reduce pathogenic

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bacteria like *Salmonella spp*. (Ten Bruggencate *et al.* 2003), *Clostridium diffile* (Hopkins and Macfarlane, 2003) and *Campylobacter* spp. (Stern *et al.* 2000), by diverse mechanisms including competitive exclusion of nutrients or adhesion sites to intestinal mucosa (Stern *et al.* 2000) and fatty acid of short chain production, like propionic and butyric acids (Francis and Brouns, 2002), which modifies the acidic conditions and inhibits the growth of microorganisms that cause infections (Collins and Gibson, 1999).

A significant world-wide problem is the incidence of intestinal infections due to the consumption of food contaminated by pathogenic bacteria, particularly *Salmonella enteritidis*. Poultry foods are frequency more at risk (De Vries, 2003).

The poultry industry have implemented preventive measures against this problem with the application of strict sanitary measures in handling, vaccination and the constant monitoring of the population of broilers (De Vries, 2003; Gast, 2001). In addition, the prophylactic application of antibiotics in food and water of chickens is a common practice, however this application constitutes a risk to the health of both chickens and humans since it can cause the development of antibiotics resistant pathogens (Barton, 2000).

It is important to find alternative measures to replace the indiscriminate use of antibiotics, and the use of prebiotics for reinforcing the intestinal flora of broilers to avoid the development of *S. enteritidis* and other pathogens may offer a practical alternative.

## MATERIALS AND METHODS

Seeds of *Lupinus exaltatus* were collected in CD. Guzmán, Jal. and used for the extraction and purification of oligosaccharides, according to the Muzquiz *et al.* (1999) Technique.

In the prebiotic test, embryos of chickens were selected from the incubator Main Farm A.S. and distributed in groups of 10. The oligosaccharides were administered at two times (12 and 17 days of incubation) and at four dose rates (0, 0.7765, 0.8815 and 1.763 mg/embryo), using solution to ringer as the vehicle, in a 2 x 4 x 10 factorially designed experiment. Treatment applications was done via an air chamber (McReynolds, 2000), utilising an ovumscopic, to drill a hole in the eggshell with a sterilised sting. The oligosaccharides was later administered using an insulin syringe and the orifice was sealed with white glue following the application.

Later, the eggs were placed in a chicken incubator with an automated turnaround of 20° degrees to avoid that the embryo adhering to the eggshell. The incubation conditions were temperature of 36.6 ± 0.5°C and relative humidity of 80.0 ± 5.0%.

Two days after hatching, fecal samples were taken using sterile hyssops that were introduced in to cloacae of chickens to stimulate the evacuation. Later, the hyssops were placed in tubes containing broth MRS (Man Rogosa and Sharpe) with 0.5% of lithium Chloride (Kok, 1996). 500 µL of the box tubes were transferred to petri dishes with Agar MRS added with Lithium Chloride. The samples were incubated in anaerobic conditions of 37ºC for 72 hrs and the count of CUF of bifidobacterias following the technique of Bergey *et al.* (2001) was completed.

With the dose and time optimal for the growth of bifidobacterias, the protective effect of oligosaccharides was evaluated in 45 chicken embryos that were distributed into three groups and each group of 15 animal were subject to one of three treatments, as follow: ringer solution without oligosaccharides (negative control), 0.8815 mg of oligosaccharides, and 40 µg of commercial antibiotic (gentamicina). Embryos were inoculation 12 days of age using the methodology and conditions of incubation as described.

On the first day of birth, chickens were inoculated with *Salmonella enteritidis* to a concentration of $10^7$ CFU in 200 µl of ringer solution. Fecal samples were collected and the salmonella was isolated and was quantified (Bergey *et al.* 2001).

## RESULTS AND DISCUSSION

The oligossacharide administrated at two times showed an increase in the growth of bifidobacterias as compared with the control group, with the largest increase in growth observed at the dose level of 0.8815 mg with 5.6 and 5.28 $Log_{10}$ recorded at 12 and 17 days of age, respectively (Table 1).

The variance of analysis showed that the dose and time of application significantly influenced the growth of bifidobacterias, and was comparable to earlier reports for inulin and oligofructose.

The interaction between dose and time of application was not statistically significant and for this reason the conditions of oligosaccharides application can be considered independent.

**Table 1.** Effect of oligossacharide administration to chicken embryos on the bifidobacteria number/gr of faeces collected from 1 day old.

| Dose | Time administration (days) | |
|---|---|---|
| mg | 12 | 17 |
| 0 | 4.3 ± 0.6 | 4.5 ± 0.3 |
| 0.1763 | 5.2 ± 0.4 | 4.6 ± 0.6 |
| 0.8815 | 5.6 ± 0.3 | 5.3 ± 0.4 |
| 1.763 | 5.1 ± 0.6 | 5.0 ± 0.6 |

Mean ± SD.
N = 10.

The number of bifidobacterias in the group control (4.34 $log_{10}$ CFU) demonstrates that the chickens at birth have these bacteria in their intestinal biota, in agreement with that reported earlier by Lisowski *et al.* (2003), that quantified 4.71 $log_{10}$ CFU of bifidobacterias in chicken.

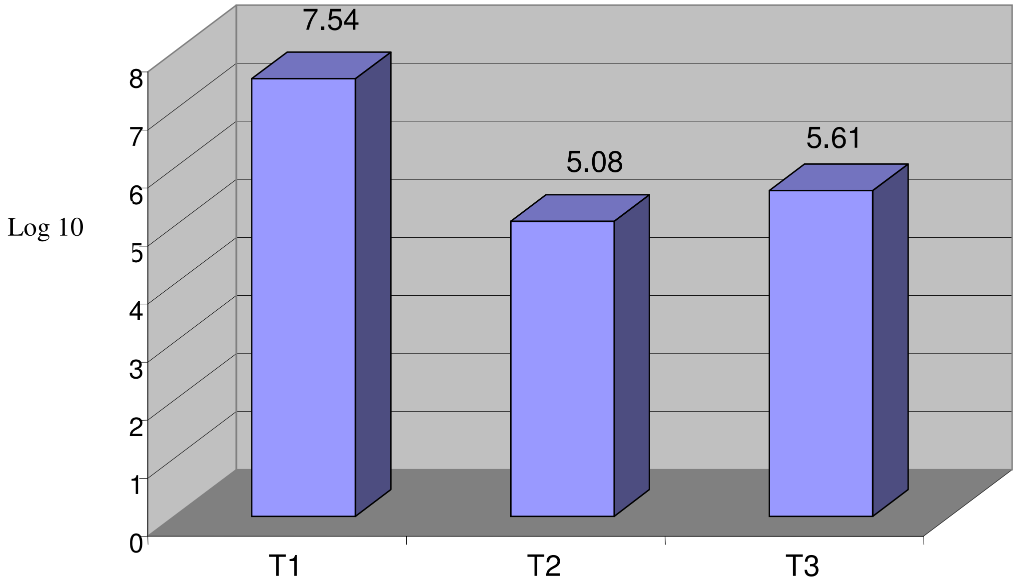


**Fig. 1.** Inhibition of *Salmonella enteritidis* growth in 1 day old chicken from embryos inoculated with oligossacharides.

The protective effect in 1 day old chicken from inoculation is shown in Figure 1, where the level of *Salmonella enteritidis* was reduced to CFU of 5.61 ± 0.14 and 5.08 ± 0.14 $\log_{10}$ as compared with the untreated control level of 7.54 $\log_{10}$ with the administration of antibiotic and the oligosaccharide inoculum, respectively. This represents an inhibition in salmonella growth of 69% for oligosaccharide and 49% for antibiotic administration.

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