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Research Letter

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Cytotoxic damage of soybean agglutinin on intestinal epithelial cells of broiler chicks: *in vitro* protection by *Bifidobacterium infantis* CRL1395

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One sentence summary: The cytotoxicity of soybean agglutinin on intestinal epithelial cells of broiler chicks is partially avoided by its capture by *Bifidobacterium infantis* CRL1395.

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ABSTRACT

Plant lectins, which are proteins/glycoproteins present in a wide range of vegetables, fruits, cereals and beans, are resistant to digestive enzymes and food cooking temperatures. They bind reversibly to specific glycosidic residues expressed on the membrane of intestinal epithelial cells (IEC) and cause anti-nutritional effects in humans and animals. Soybean lectin (SBA) has been detected in poultry diets, and its ability to bind to the intestinal epithelium has been reported. The development of new methods for removing SBA from feeds or to prevent interaction with the intestinal mucosa is of interest. In this study, the *in vitro* cytotoxicity of SBA on IEC of chicks was demonstrated for the first time. The LD_{50}, assessed after 2 h exposure of IEC to SBA, was 6.13 μg mL^{-1}. The ability of *Bifidobacterium infantis* CRL1395 to bind SBA on the bacterial envelope was confirmed, and prevention of IEC cytotoxicity by lectin removal was demonstrated. Safety of *B. infantis* CRL1395, resistance to gastrointestinal stress and adhesion were also determined. It was concluded that the early administration of *B. infantis* CRL1395 to chicks would effectively reduce the toxicity of SBA. Besides, it would favour the colonization of the gut with a beneficial microbiota.

Keywords: soybean agglutinin; cytotoxicity; intestinal epithelial cells; broiler chicks; *Bifidobacterium*; probiotics

INTRODUCTION

Chicks acquire their intestinal microbiota from chickens or from the eggshell surface, but in modern farming they hatch in a disinfected environment that leads to the late settlement of desirable microbiota in the gastrointestinal tract or to failure of this process (Sterzo *et al.* 2005). Intestinal colonization with desirable microorganisms directly after birth may act as a natural barrier against the colonization of pathogens (Wardwell, Huttenhower and Garrett 2011) and may also protect the animals from the anti-nutritional effects of some constituents of grains included in the diet (Shetty and Jespersen 2006).

Protein supplements are the second major component in poultry feeds, with most dietary protein requirements fulfilled by vegetal protein, mainly soybean meal, which contains

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Table 1. Specificity and organism source of FITC-labelled dietary lectins tested.

Lectin	Source	Specificity
Con A	*Canavalia ensiformis* (jack bean)	α−D-mannose, α−D-glucose
DBA	*Dolichos biflorus* (horse gram)	GalNAc-α-1,3-GalNAc
PHA-P	*Phaseolus vulgaris* (kidney bean)	Gal-β-1,4-GalNAc-β-1,2-Man
PNA	*Arachis hypogaea* (peanut)	Gal-β-1,3-GalNac
UEA-I	*Ulex europaeus* (furze)	α-L-Fucose
WGA	Wheat germ agglutinin (wheat)	GlcNAc-β-1,4-GlcNAc, NeuNAc

49%–54% raw protein (Ortega *et al.* 2016; Rossi, Newcomb and Gatlin 2016) but also several anti-nutritional factors such as lectins. Lectins are proteins/glycoproteins of non-immune origin that have at least one non-catalytic domain that binds reversibly to specific monosaccharides or oligosaccharides (Singh, Kaur and Kanwar 2016). Lectins are widely distributed among plants and throughout nature (Vojdani 2015), and thus most plant-based feeds include appreciable amounts. The high percentage of soybean in poultry feeds results in concentrations of soybean lectin (SBA) of 0.08–2.4 mg g^{-1}, amounts high enough to produce detrimental effects on chick nutrition (Casaubon-Huguenin *et al.* 2004). The membrane of chicken intestinal epithelial cells (IEC) contains N-acetyl-D-galactosamine and galactose (Zhou, Deng and Ding 1995, Kitagawa *et al.* 2000), receptors specifically recognized by SBA. In the small intestine, SBA binds to the membrane of IEC and is internalized by pinocytosis. This process alters microvilli development, brush border enzymes expression, apical membrane transport mechanisms and cellular viability (Maenz, Irish and Classen 1999), leading to malabsorption of nutrients, lower animal weight gain and diminished capability to trigger cell-mediated immune responses (Casaubon-Huguenin *et al.* 2004). Heat treatment of soybean is of great importance to increase protein digestibility and inactivate anti-nutritional factors. Nevertheless, the SBA concentration drops significantly only with high-temperature treatments, which destroy other components and raise production costs. Thus, the development of new approaches to inactivate dietary lectins is of interest. Zárate and Perez Chaia (2009) observed that some lactic acid bacteria can diminish the cytotoxic effects of dietary lectins on mice IEC by capturing them through interaction with carbohydrates on the bacterial surface. Thus, the aim of this study was to characterize the probiotic potential of *Bifidobacterium infantis* CRL1395 and its ability to protect IEC of chicks from the cytotoxic effects of SBA.

MATERIALS AND METHODS

Bacterial strains and culture conditions

Bifidobacterium infantis CRL1395 was activated by three successive transfers for 24 h at 37°C in MRS broth (Rogosa, Mitchell and Wiseman 1951) supplemented with 4% cysteine (Guowei *et al.* 2012). *Propionibacterium avidum* LET106 was also activated by three successive transfers for 24 h at 37°C in LAPTg broth (Raibaud *et al.* 1973).

Simulated gastrointestinal digestion

The resistance of *B. infantis* CRL1395 to gastrointestinal digestion was assessed as previously described by Babot *et al.* (2014).

Ex vivo adhesion to intestinal mucosa

Fourteen-day-old broiler chicks were slaughtered, and the ability of *B. infantis* CRL1395 to adhere to jejunum mucosa was assessed as previously described (Argañaraz-Martínez *et al.* 2013). Homogenized tissue samples with adhered bacteria were plated onto MRS agar and incubated for 5 days at 37°C under anaerobic conditions generated by Anaerocult A (Merck, Germany). The number of CFU $(mm^2)^{-1}$ of tissue was determined after incubation. Experimental procedures were approved by The Committee of Ethics for Animal Studies (CERELA-CONICET).

Strain safety

Bacterial suspensions (3×10^8 CFU mL^{-1}) were inoculated on MRS and LSM agar plates (Klare *et al.* 2005) using sterile cotton swabs. Antibiotic discs (Britania, Argentina) listed in Table 2 were placed on the surface of the plates, which were incubated for 24 h at 37°C under microaerophilic conditions, and the inhibition zones diameters were measured. *Bifidobacterium infantis* CRL1395 was classified as sensitive (S), with intermediate resistance (IR) or resistant (R) according to Charteris *et al.* (1998).

Haemagglutination assays were performed according to Izumi *et al.* (2005) with modifications. Briefly, active cultures were washed twice with sterile PBS pH 7.40 (10 000 × *g*, 10 min, 4°C), and 10^9 CFU mL^{-1} bacterial suspensions were incubated with 2% human red cells for 1 h in a microtiter plate. A lattice coating the wells indicated a positive reaction. SBA was used as a positive control. Gelatinase test was performed as previously described (Eaton and Gasson 2001), and cytolysin (haemolysin) was detected as reported by Elsner *et al.* (2000). *Propionibacterium avidum* LET 106 was the positive control for both tests.

Assessment of surface carbohydrates

Active cultures of the strain were washed twice (10 000 × *g*, 10 min, 4°C) with a lectin buffer designed by Leathem and Brooks (1997) (Tris 0.05 M, NaCl 0.15 M, $MgCl_2$ 0.002 M, $CaCl_2$ 0.001 M, pH 7.6), resuspended in the same buffer containing 20 μg mL^{-1} of each FITC-labelled lectin (Table 1, Sigma-Aldrich, Germany) and incubated in the dark for 1 h at 25°C. The suspensions were washed three times with lectin buffer (10 000 × *g*, 8 min, 4°C) and suspended in equal volume of the same buffer. The lectin binding pattern of *B. infantis* CRL1395 was assessed through observation of the bacterial suspension with a conventional fluorescence microscope (Carl Zeiss Axio Scope A1) fitted with the appropriate filter.

Quantification of lectin capture

Active cultures of *B. infantis* CRL1395 were washed twice (10 000 × *g*, 10 min, 4°C) and diluted in lectin buffer to obtain 1 × 10^8 bacteria mL^{-1} suspensions. Cell concentrations were confirmed by phase-contrast microscopy according to Lorenzo-Pisarello *et al.* (2010). PNA or SBA was added to cell suspensions in a final concentration of 50 $\mu g\ mL^{-1}$, and following incubation for 1 h at 25°C, the supernatants were collected after centrifugation (10 000 × *g*, 10 min, 4°C). The amounts of lectin bound by the bacteria were calculated considering the concentration of lectin, as measured by the technique of Bradford (1976), before and after incubation with the bacteria by constructing calibration curves using known concentrations of each lectin.

Exopolysaccharides production

The production of exopolysaccharides by *B. infantis* CRL1395 was evaluated according to Mozzi, Torino and de Valdez (2001).

Effect of SBA on adhesion of *B. infantis* CRL1395 to IEC

The assay was carried out as described by Zarate *et al.* (2002) with modifications. Briefly, 14-day-old broiler chicks were slaughtered, the jejuna were extracted, cut lengthwise and washed with cold PBS pH 7.40 supplemented with 1% FBS (PBS/FBS). IEC from the distal portion of the jejunum were gently scraped off with the edge of a microscope slide and were suspended and washed twice (800 × *g*, 5 min, 4°C) with PBS/FBS. Then, they were incubated with 0.25% trypsin-EDTA (Gibco, USA) at 37°C for 5 min before cold PBS/FBS was added to inactivate the enzyme. The cells were collected (800 × *g*, 5 min, 4°C), and their concentration was adjusted to 1 × 10^6 cells mL^{-1} in RPMI 1640 medium supplemented with 1% FBS (RPMI/FBS). Cells were counted in a Neubauer chamber in a conventional light microscope. Suspensions of 1 × 10^8 CFU ml^{-1} of *B. infantis* CRL1395 and recently obtained IEC were mixed (1:4) and incubated for 1 h at 41.5°C ± 0.5°C under 5% CO_2 (Nuaire Co., USA). The suspensions were washed twice with RPMI (120 × *g*, 5 min, 4°C) and suspended in the initial volume of RPMI/FBS containing 50 $\mu g\ mL^{-1}$ of SBA. After 2 h of incubation at 41.5°C ± 0.5°C under 5% CO_2 atmosphere, cell adhesion to IEC was examined under phase-contrast microscopy by counting adhered bacteria in 30 IEC. Cell adhesion to IEC before incubation with SBA was also assessed. Results are expressed as the percentage of IEC with adhered bacteria (adhesion percentage).

SBA cytotoxicity on IEC

Jejuna IEC from 14-day-old broiler chicks were obtained and adjusted to 1 × 10^6 cells mL^{-1} in RPMI/FBS as described above. IEC suspensions of 5 × 10^5 cells mL^{-1} were incubated in RPMI/FBS with different concentrations of SBA (0, 12.5, 25, 50, 100, 150 and 200 $\mu g\ mL^{-1}$) for 1 and 2 h at 41.5°C ± 0.5°C under 5% CO_2 atmosphere. Suspensions of 100 μL were stained with 10 μL of a freshly prepared mixture of 2.5 μg of PI + 7.5 μg of FDA in 10 μL of PBS pH 7.40 for 10 min in an ice bath in the dark. Finally, the suspensions were centrifuged (800 × *g*, 5 min, 4°C), resuspended in cold PBS/FBS, and the viable (green) and non-viable (red) IEC were counted with a conventional fluorescence microscope to determine the percentage of dead cells. The percentage of dead cells versus SBA concentration (C in $\mu g\ mL^{-1}$) is represented by the data after 1 and 2 h of incubation; the maximum death ($\%_{max}$) and the SBA concentration responsible for half of this value (LD_{50}) were determined for each condition using the mathematical expression of a model adapted for toxic chemicals in experimental bioassays (Sánchez-Bayo and Goka 2007) as follows:

$$\text{Death}(\%) = \frac{\text{Maximum death}(\%_{max}) \times C}{LD_{50} + C}.$$

SBA cytotoxicity protection by *B. infantis* CRL1395

The protective effect of *B. infantis* CRL1395 was studied through two different protocols, one considering bacteria adhering to IEC and the other considering free bacteria in the supernatant. Active cultures of 1 × 10^8 CFU mL^{-1} *B. infantis* CRL1395 were washed three times with sterile PBS pH 7.40 (10 000 × *g*, 10 min, 4°C), resuspended in RPMI/BFS and stored at 4°C until use.

IEC from 14-day-old broiler chicks obtained as described above were adjusted to 5 × 10^5 cells mL^{-1} in cold RPMI/BFS and incubated with the bacterial suspension (4:1) for 30 min at 41.5°C ± 0.5°C under 5% CO_2 atmosphere to allow the adhesion of bacteria to IEC. Then, the suspensions were washed twice with sterile PBS pH 7.40 (120 × *g*, 5 min, 4°C) to eliminate non-adhering bacteria, and the sediments were resuspended in 50 $\mu g\ mL^{-1}$ SBA and incubated for 2 h at 41.5°C ± 0.5°C under 5% CO_2 atmosphere. Suspensions of 100 μL were stained with PI and FDA, and viable and non-viable cells were counted with a conventional fluorescence microscope as described above.

Separately, bacterial suspensions were incubated with 50 $\mu g\ mL^{-1}$ SBA for 1 h at 41.5°C ± 0.5°C to allow the attachment of lectin to the bacterial surface. Then, the suspensions were centrifuged (10 000 × *g*, 10 min, 4°C), and the supernatants containing the remaining lectin were collected and stored at 4°C until use. Simultaneously, IEC of 14-day-old broiler chicks were adjusted to 5 × 10^5 cells mL^{-1} in cold RPMI/BFS and incubated with the solutions containing the remaining SBA. Suspensions of 100 μL were stained with PI and FDA, and viable and non-viable cells were counted with a conventional fluorescence microscope.

RESULTS AND DISCUSSION

Many studies of probiotics support the theory of host specificity (Lin *et al.* 2012; Nader-Macias and Juarez Tomas 2015). Nevertheless, it has been suggested that allochthonous bacteria are more appropriate as probiotic organisms than autochthonous bacteria (Dogi and Perdigon 2006). *Bifidobacterium* strains have been widely studied as putative probiotics for poultry (Seifert *et al.* 2011; Baffoni *et al.* 2012; Giannenas *et al.* 2014). We analysed several probiotic properties of *B. longum* subsp. *infantis* CRL1395, which was previously isolated from intestine of a healthy infant (Reuter 1971).

A putative probiotic bacterium must reach the large or small intestine in a viable state (Uriot *et al.* 2016). Tolerance to low stomach pH and intestinal bile salts suggests the potential to overcome gastrointestinal digestion (Sahadeva *et al.* 2011). During their transit through the gastrointestinal tract, bacteria are exposed to stressful conditions in a sequential way that affects their overall viability. In this study, bacteria were subjected to artificial digestion under conditions that simulated the gastrointestinal tract of the chicken. *Bifidobacterium infantis* CRL1395 showed acceptable endurance, with a decrease in viable mL^{-1} counts of 1.50 log after testing. In a similar trial that was adjusted to the conditions of the human gastrointestinal tract, Faye

Table 2. Antibiotic sensibility profile of *B. infantis* CRL1395.

Culture medium	Antibiotic						
	AM	CLI	CMP	ERY	STR	TET	VAN
MRS	S	S	S	S	R	S	S
LSM	S	S	S	S	R	S	S

AM: ampicillin (10 μg); CLI: clindamycin (2 μg); CMP: chloramphenicol (30 μg); ERY: erythromycin (15 μg); STR: streptomycin (300 μg); TET: tetracycline (30 μg); VAN: vancomycin (30 μg). S: sensible, R: resistant.

et al. (2012) reported similar endurance for *Lactobacillus* strains (viability losses between 0.8 and 2.3 log CFU mL^{-1}).

Bacteria that remain viable in the intestine and adhere to the intestinal epithelium have a high probability of persisting in this ecosystem (Puniya *et al.* 2016). Furthermore, bacterial adhesion is one mechanism of probiotics that helps to reinforce the mucous barrier structure by blocking pathogen adhesion and translocation to tissues and organs. The adhesion property has therefore been proposed as a selectivity criterion for probiotics (FAO/WHO 2002). This property has been evaluated by several direct methods, including adhesion to tumour cell lines and immobilized intestinal mucus (Celebioglu *et al.* 2016). Nevertheless, different molecules are expressed on the surface of tumour and regular cells, which can lead to misinterpretations when cell lines are used for adhesion assays. The efficiency of immobilized mucus as support for adhesion tests depends on the method used for its extraction and purification. Thus, to assess the adhesion properties of *B. infantis* CRL1395, we used tissue explants from jejuna. This strain showed an adherent phenotype, binding 850 ± 42.4 CFU $(mm^2)^{-1}$ of tissue. The adhesion of *Bifidobacterium* sp. to jejunum mucosa in chicken has not been extensively studied and is a strain-specific property (Perez *et al.* 1998). Nevertheless, Ma, Xu and You (2004) reported the adhesion of a *B. bifidum* strain to mucus glycoproteins of different parts of chicken intestine.

Although *Bifidobacterium* sp. has 'generally regarded as safe' status, the safety of putative probiotics must be carefully evaluated. Antibiotic resistance and the potential for virulence are the main safety aspects requiring study (Borriello *et al.* 2003). Currently, because there are no standardized protocols for the study of antibiotic resistance of bifidobacteria, different culture media were used for these tests: Müller-Hinton and Iso-Sensitest (Citar *et al.* 2015), MRS (El Sayed, Badran and Hamed 2014), or a mixture of Iso-Sensitest and MRS with or without the addition of L-cysteine (Klare *et al.* 2005). The methodology described by Bauer *et al.* (1966) using either LSM or MRS was followed (Table 2). Although differences between inhibition zones around the antibiotic discs in both culture media were observed, no discrepancies in the interpretation of the sensitivity to the same antibiotic were observed. This agrees with results of Huys, D'Haene and Swings (2002), who used MRS and Iso-Sensitest and reported significant differences in the size of the inhibition zones. *Bifidobacterium infantis* CRL1395 showed resistance only to streptomycin. Nevertheless, intrinsic resistance to this antibiotic in the genus *Bifidobacterium* has previously been suggested (Charteris *et al.* 1998; Wei *et al.* 2012). Antibiotic resistance of a putative probiotic strain is a risk factor when it can be transferred to potential pathogens. Thus, the intrinsic resistance of *B. infantis* CRL1395 to streptomycin should not be of concern. Moreover, we found the absence of haemagglutinating, gelatinase and haemolysin activities in this strain. This agrees with the work of Bennedsen *et al.* (2011), who screened 6 *Bifidobacterium* sp. strains (including one *B. infantis*) for 408 known toxin and virulence factor genes and found them absent in all strains.

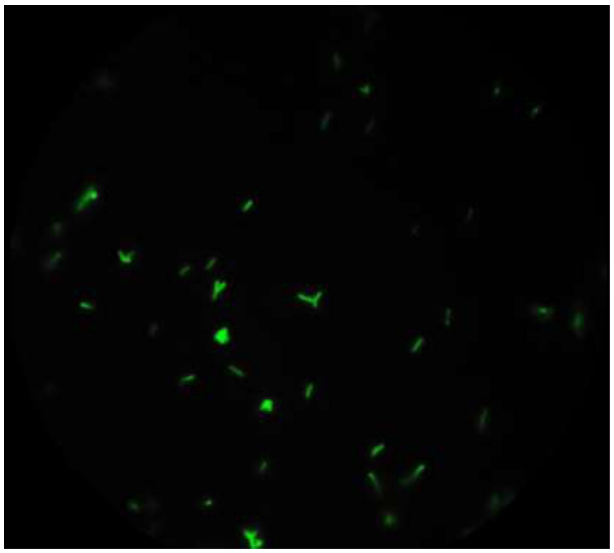

Figure 1. Microscopic field showing the capture of FITC labelled-PNA by *B. infantis* CRL1395 cells.

Carbohydrates are among the main constituents of the bacterial cell wall. Bifidobacteria are gram-positive microorganisms and thus have a thick peptidoglycan layer constituted by units of N-acetyl-muramic acid and N-acetyl-glucosamine (Schleifer and Kandler 1972). Carbohydrates are also produced as capsular and mucilaginous exopolysaccharides on the bacterial surface (Ruas-Madiedo, Salazar and Clar 2009). *Bifidobacterium infantis* CRL1395 bounds only PNA (6.23 ± 0.10 μg of PNA removed in a 50-μg mL^{-1} solution) and SBA (44.45 ± 0.14 μg of SBA removed in a 50-μg mL^{-1} solution), which suggests that galactose-β-1,3-N-acetyl-galactosamine is expressed on its surface. Bacteria capturing FITC-labelled PNA showed fluorescence all over the cells, which suggests that this carbohydrate is uniformly distributed on their surface (Fig. 1). No exopolysaccharide production was detected for *B. infantis* CRL1395, although this property has been described for another strain of this species (Prasanna *et al.* 2012).

SBA showed cytotoxicity on IEC of the 14-day-old broiler chicks. As indicated in Fig. 2, the hyperbolic shape of the graph of the percentage of dead cells versus lectin concentration showed a tendency for maximum effect at high lectin concentrations. Double-reciprocal plots were used to determine $\%_{max}$ and LD_{50} at each incubation time (Purich and Allison 2000). Results were 53.3 and 6.13 μg mL^{-1} for LD_{50} and 80.0% and 79.4% for $\%_{max}$ at 1 and 2 h of exposure, respectively. SBA cytotoxicity on different cell types has been reported by Ohba and Bakalova (2003), who demonstrated viability losses of 90%–100% on cell lines derived from leukemic tumours after incubation with 36 μg mL^{-1} of SBA. The binding of SBA to enterocytes of chicken caecal tonsils has already been reported (Zhou, Deng and Ding 1995; Kitagawa *et al.* 2000). To the best of our knowlede, this is the first time that its toxicity on chicken IEC has been shown.

Lectins capture by gram-positive bacteria was previously reported. Zárate and Perez Chaia (2009) showed binding of Con A, PNA and AIL by different strains of the genus *Propionibacterium* and by *B. longum*. Babot *et al.* (2014) demonstrated capture of Con A by one strain of *L. reuteri*. After binding to IEC, *B. infantis* CRL1395 significantly reduced (4.7%, $P \leq 0.05$) the cytotoxic effect of SBA on IEC (Fig. 3). Although statistically significant,

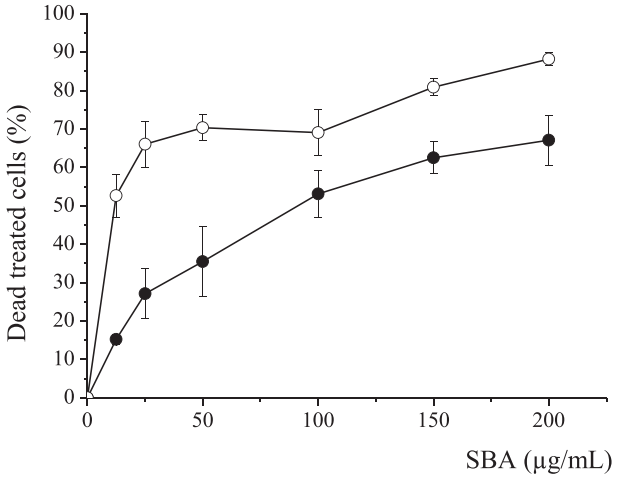

Figure 2. SBA cytotoxicity on IEC of 14-day-old broiler chicks after 1 (filled circle) and 2 h (open circle) of exposure. Results are expressed as mean values ± standard deviations (SD).

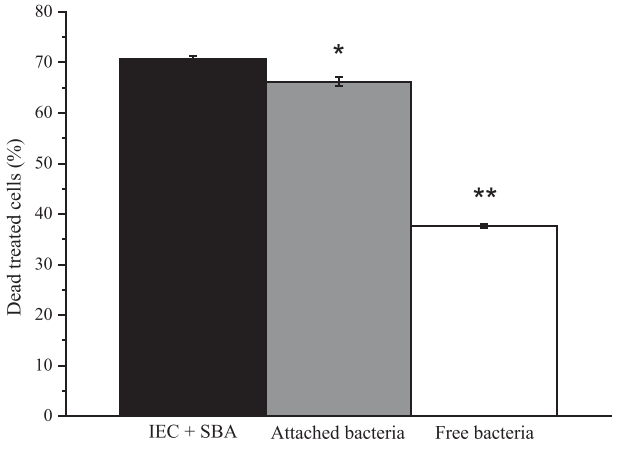

Figure 3. Protection of SBA cytotoxicity by *B. infantis* CRL1395. Percentage of dead cells after IEC incubation with SBA (filled black bar), after IEC incubation with bacteria and then with SBA (filled grey bar), and after SBA incubation with bacteria and then with IEC (open bar). Significant differences between each protocol and the control are indicated with one ($P \leq 0.05$) or two ($P \leq 0.01$) asterisks.

this protective effect would be biologically negligible. Due to the small size of bacteria in comparison to IEC, a significant share of the IEC surface would remain exposed and could interact with SBA even after bacterial adhesion. Moreover, attached bacteria captured ~22 μg ml^{-1} of SBA, as can be seen by interpolation in Fig. 2. In contrast, a remarkably higher protective effect (33.2% decrease in dead cells, $P \leq 0.01$) was observed when IEC were exposed to the lectin after co-incubation of SBA with the bacteria (Fig. 3). When free, all bacteria can bind SBA—and over their entire surface—whereas the number of adhered bacteria able to bind lectin is lower. Thus, the amount of SBA captured by free *B. infantis* CRL1395 (~44 μg ml^{-1} of SBA), and the concomitant protective effect, was higher than that by attached bacteria. In a similar trial, Zárate and Perez Chaia (2009) observed a reduction in the percentage of necrotic colonic cells from 33% to 9% and 5% after the exposure of these cells to supernatants obtained after the incubation of the lectin Con A with *P. acidipropionici* and *P. freudenreichii*, respectively.

Adhesion of *B. infantis* strains to human IEC has been extensively studied (Bernet *et al.* 1993; Chichlowski *et al.* 2012; Kavanaugh *et al.* 2013); nevertheless, there are no reports of their attachment to chick IEC. Zhou, Deng and Ding (1995) suggested that glycoconjugates play an important role in the adherence of bacteria to IEC. In the present study, the percentage of enterocytes with at least one bacterium adhered significantly decreased from 50% to 17% after incubation with the lectin (Fig. 4). Thus, *B. infantis* CRL1395 would bind SBA in the intestinal lumen and, by losing part of its ability to adhere to IEC, would be eliminated, carrying the lectin attached to its surface along with the normal transit of digesta.

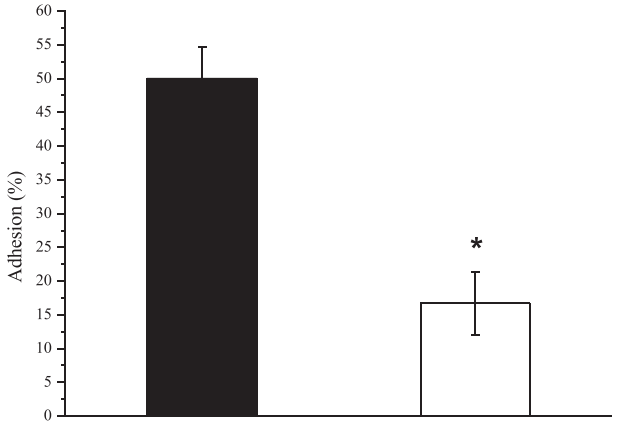

Figure 4. Influence of SBA on the adhesion of *B. infantis* CRL1395 to IEC. Results are represented as mean values ± SD. An asterisk indicates significant differences ($P \leq 0.05$) between adhesion (%) before (filled black bar) and after (open bar) incubation with the lectin.

Several approaches to minimize the toxicity of lectins have been proposed. A reduction in the load of lectins in feeds can be obtained by cooking them (Kalpanadevi and Mohan 2013). However, the cost of feed production increases, and even after cooking, certain toxicity remains (Noah *et al.* 1980). Following the same principle as that in the present work, Ramadass *et al.* (2010) assayed the co-administration to rats of the sugar complementary to the lectin present in the feed; although positive effects were observed, the administration of high doses of sugars could have undesirable changes on intestinal fermentation. However, the early administration of *B. infantis* CRL1395, a strain expressing N-acetyl-D-galactosamine on its surface, to chicks would effectively reduce the toxicity of SBA and also provide the birds with putative probiotic bacteria in the first days after hatch. Moreover, colonization of the gut with a beneficial microbiota would be favoured.

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Conflict of interest. None declared.

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