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Biopreservation of Poultry Feed with Probiotic Microorganisms

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Fungi are naturally present in poultry feed and unsuitable storage conditions on hatcheries, such as poor ventilation, high temperature or high humidity, can lead to their rapid proliferation. Fungal contamination adversely affects feed quality through physical damage, alteration of its nutritive value or/and toxins production.

The consumption of fungi or toxins by chickens can cause from a loss of performance to mortality, depending on the fungus contaminant. Furthermore, residues of mycotoxins consumed by animals can appear in derived products, thus representing a serious risk on human health.

Chemical preservatives, mainly weak organic acids, have long been used to control fungi growth on animal feed. However, many spoilage moulds have become resistant to some acids used as preservatives, namely, sorbic and benzoic acids (Brul y Coote, 1999) so that the susceptibility of some microorganisms to most currently used preservatives is falling.

Based on the increasing documentation of emergence of resistance and public pressure for reducing the use of chemical additives, legislation has restricted the use and permitted levels of some currently accepted preservatives in foods and is expected that some of them will be restricted in the short term. Therefore, development of viable alternatives to improve feed safety is becoming an urgent need and is currently under active investigation worldwide.

This has promoted the interest in food biopreservation, i.e., extension of the shelf life and improvement of safety of food using microorganisms and/or their metabolites. Lactic acid bacteria (LAB) are particularly interesting as biopreservatives since they have Qualified Presumption of Safety-EU and Generally Recognized as Safe-US status and ability to inhibit spoilage and foodborne pathogens. Many LAB produce antifungal compounds (Schnürer and Magnusson, 2005) and inhibit the production or inactivate mycotoxins (Dalie et al., 2010). Among LAB probiotic, strains are valuable for the food industry because of their positive effect on consumers´ health.

The aim of the work was to develop a poultry feed additive that acts as a biopreservative and contains probiotic microorganisms.

Cheese whey was used as substrate due to its low cost and high availability. Kefir grains, an association of diverse LAB and yeasts included in a polysaccharide and protein matrix, were chosen as starter for their long history of safe human consumption, antimicrobial capacity and beneficial health properties (Ahmed et al., 2013).

The fermentation of whey with kefir grains has been optimized and characterized (Londero et al., 2012a). The fermented product had bactericidal power over bacterial intestinal pathogens, antagonistic effect against Salmonella spp. and immunomodulatory capacity (Londero et al., 2011, 2014a). Preliminary results obtained in our laboratory strongly suggest that the consumption of fermented whey protects chicken against Salmonella enteritidis intestinal colonization (unpublished data).

INNOCUOUSNESS AND EFFECT ON INTESTINAL MICROBIOTA

The innocuousness of fermented whey and its effect on intestinal microbiota were studied in vivo. Twelve broiler chickens (Ross PM3; 14-day-old) were divided in 2 equal groups: i) without treatment, and ii) receiving whey fermented with 10% w/v of kefir grains (1 ml/chicken per day). Excreta moisture, food and water intake and body weight were recorded daily for each chicken during 10 days. At the end of treatment, the Lactobacillus community of the ileum of all chickens was analysed. DNA was extracted from microorganisms present on the surface of 5 cm of the ileum epithelium. A fragment of 430 pb of the 16S DNAr of Lactobacillus sp. was amplified with the primers Lac 1 (5´-AGCAGTAGGGAATCTTCCA-3´) and GC-Lac2 (5´-GCCCGGGGCGCGCCCCGGGCGGCCCGGGGGCACCGGGGGATTYCACCGCTACACATG-3´), and the PCR products were analysed by denaturing gradient gel electrophoresis (DGGE).

We found no significant differences between the control and the treated groups for any of the parameters evaluated; suggesting that whey fermented with kefir grains was innocuous to chickens (Table 1).

Table 1. Parameters indicative of chicken health in response to fermented whey administration.

Treatment	Excreta moisture (%)	Total water intake (L)	Total food intake (kg)	Body weight gain (g/day)	Feed conversion^{1}
None	66.4 ± 7.5 ^{a}	1.80 ± 0.28 ^{a}	1.19 ± 0.12 ^{a}	46.8 ± 4.9 ^{a}	2.83 ± 0.17 ^{a}
Fermented whey	69.3 ± 10.8 ^{a}	2.22 ± 0.29 ^{a}	1.19 ± 0.22 ^{a}	51.1 ± 6.6 ^{a}	2.60 ± 0.46 ^{a}

Values are means ± SD of 6 chickens per treatment. Within a given column equal letters indicate that no statistical differences exist between treatments ($p<0.05$) according to ANOVA test. [1]Feed conversion=food intake/ (final body weight – initial body weigh)

The Lactobacillus community from the ileum epithelium surface of treated chickens analysed by DGGE profiles showed more bands than untreated ones. Profile similarity was higher among chickens from the treated group, thus indicating that the consumption of fermented whey caused variations in Lactobacillus community (Londero, 2012b).

ANTIFUNGAL ACTIVITY

The antifungal activity of fermented whey against Aspergillus flavus, Aspergillus parasiticus, Aspergillus terreus, Aspergillus fumigatus, Penicillium crustosum, Trichoderma longibrachiatum and Rhizopus sp. was assessed by determining its capacity to inhibit conidial germination. Whey fermented with kefir grains showed a high percentage of conidial germination inhibition (≥70 %) on all fungal species evaluated. While pH per se was not the causal agent of the inhibition, organic acids were largely involved in the antifungal effect of fermented whey (Londero et al, 2014b).

The synergic effect between lactic and acetic acids against fungi (León Peláez et al., 2012) could explain the strong antifungal effect of the product. These components are also responsible for the inhibitory power against spoilage and pathogen bacteria of milk and whey fermented with kefir grains (Garrote et al., 2000; Londero et al., 2011).

ADDITION TO POULTRY FEED: EFFECT ON FUNGAL RESISTANCE AND KEFIR MICROORGANISMS SURVIVAL

We have recently described the addition of fermented whey to poultry feed for preventing fungal contamination and for delivering kefir microorganisms (Londero et al., 2014b).

Fermented whey was added to poultry feed Nutrisur® BB (La Plata, Argentina) at 1 ml/g and then dried in a convection oven at 50 °C until achieving a water activity (aw) of 0.5 ± 0.05. Using the same methodology, fresh whey acidified with HCl to pH 3.6 or with organic acids to have the same concentration of the fermented products (8.6 g/l lactic acid and 0.8 g/l acetic acid) were added to poultry feed. Feed added with water and dried by the same procedure was used as control. The survival of kefir microorganisms during storage for 0, 15, and 30 days was determined by viable counts on MRS and YGC agar. Finally, the resistance of the added feed to fungal contamination was analysed, fractionating it on Petri plates (10 g feed/plate) and spraying with 0.1 ml of a conidial (105/ml) suspension. Plates were incubated at 20 °C and checked daily to determine the time (in days) at which moulds became visible in the feed.

Feed added with whey fermented with kefir grains presented a notorious resistance to fungal contamination since, for all the 7 species evaluated, the storage time without fungal growth was 23 to 29 days, thus extending 2 to 4 times the shelf life of the feed.

The delay in fungi growth on feed added with whey acidified with HCl was not significant (P>0.05). Excepting T. longibrachiatum, A. terreus and A. fumigatus, the time without fungal growth of feed containing lactic and acetic acids was not significantly extended, suggesting that organic acids were not the only responsible for the antifungal effect.

In poultry feed added with fermented whey, 64% of LAB and 13% of the yeast supplied survived the drying process. Kefir microorganisms were notoriously resistant to storage at 20°C since the viability decreased 1 log during the first 15 days and thereafter the concentration remained constant up to 30 days. After storage, the product contained 1 x 108 CFU/kg of BAL and 6 x 107 CFU/kg of yeast, values within the range considered as effective dose for other probiotics included in poultry feed (Apata, 2008; Mountzouris et al., 2010).

CONCLUSION

We showed that the addition of fermented whey to poultry feed is safe for chickens and protects food from fungal contamination so that it can be used to replace chemical preservatives. This additive also provides probiotic microorganisms; nevertheless, further studies are needed to deepen the knowledge on its beneficial effects on chickens. Considering that fermented whey production is economical and that the addition method proposed is simple, it is expected that this product can be easily inserted in the market. Further, this work opens perspectives on the application of whey fermented with kefir grains as a biopreservative in other foodstuff.

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