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Fermentation *in vitro* of a mixture of dietary fibers and cane molasses by the cecal microbiota: Application on mineral absorption through the laying hen's colonic epithelium

M.L. Gultemirian[a,b], H.R. Corti[c], A. Perez Chaia[a,b,*], M.C. Apella[a,b,*]

[a] *Centro de Referencia para Lactobacilos (CERELA-CCT-CONICET-Tucumán), Chacabuco 145, T4000ILC San Miguel de Tucumán, Tucumán, Argentina*
[b] *Universidad Nacional de Tucumán, Ayacucho 491, T4000INI San Miguel de Tucumán, Tucumán, Argentina*
[c] *Departamento de Física, Centro Atómico Constituyentes (CAC), Comisión Nacional de Energía Atómica (CNEA), Av. Gral. Paz 1499, 1650 San Martín, Buenos Aires, Argentina*

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ABSTRACT

Short-chain fatty acid (SCFA) production from a mixture of dietary fibers and cane molasses fermentation by laying hens' cecal microbiota and calcium and iron absorption through the colonic mucosa driven by these acids were studied *in vitro*. Oligofructose, polydextrose or arabic gum at concentrations of 10 g/L of cecal suspension, alone or combined with molasses in a 1:1 (w/v) relation were assayed. Fermentation of molasses and oligofructose by hens' cecal microbiota significantly increased SCFA production; a similar effect was also observed with polydextrose and arabic gum, but to a lower extent. The highest level was attained by cecal fermentation of combined molasses–oligofructose, suggesting a complementary effect of these fibers in the mixture. SCFA mixtures with acid levels similar to that derived from the fermentation of molasses, oligofructose or a combination of both had a positive influence on mineral absorption by the colonic mucosa when assayed in an Ussing chamber. The best result was achieved with a SCFA concentration that simulated that of the molasses–oligofructose mix fermentation as the amount of calcium and iron absorbed grew approximately eightfold when compared to the one in the absence of SCFA. Different SCFA, in a range of concentrations similar to those derived from colonic fermentation without fiber addition, increased ionic absorption which was dependent on acid type and concentration used, being more remarkable for butyric acid. The effectiveness in mineral absorption was lesser than the one obtained with SCFA mixtures derived from fiber fermentations as a consequence of lower amounts of acids. The results of this study suggest that molasses–oligofructose as a layers' diet supplement could improve mineral absorption in the intestinal lumen.

1. Introduction

Mineral absorption in the intestinal lumen has an influence on the laying hen's health and nutrition as well as on eggshell formation and egg quality. The major site of calcium absorption is the upper jejunum with some absorption occurring at

Abbreviations: HPLC, high performance liquid chromatography; NSPs, non-starch polysaccharides; RLS, Ringer Lavoisier solution; SCFA, short-chain fatty acid; TA, total acids.

* Corresponding authors at: CERELA-CONICET, Ecofisiología Tecnológica, Chacabuco 145, 4000 San Miguel de Tucumán, Tucumán, Argentina. Tel.: +54 381 431 1720/+54 381 431 0465; fax: +54 381 4005600.
E-mail addresses: apchaia@cerela.org.ar (A.P. Chaia), mapella@cerela.org.ar (M.C. Apella).

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other places in the small and large intestine (Denbow, 2000). This process takes place by transcellular and paracellular transport (Fullmer, 1992; Ballard et al., 1995; Bronner, 1998; Mineo et al., 2002). On the other hand, iron absorption takes place immediately after the arrival of this mineral at the duodenum when the pH is still acidic, thus avoiding insoluble complex formation of Fe^{2+} with some dietary components such as phytates, oxalates and polyphenols. Some compounds such as organic acids in food help to stabilize Fe^{2+} and thereby increase solubility and mineral absorption (Miret et al., 2003).

Increased absorption of several minerals, primarily calcium, iron and magnesium is closely related to cecal production of organic acids by microorganisms that use non-digestible poly and oligosaccarides as a source of energy as seen in rats and birds (Ohta et al., 1995; Scholz-Ahrens et al., 2001; Kruger et al., 2003; Chen and Chen, 2004). The main energy source in laying hens' commercial diet is starch. However, non-starch polysaccharides (NSPs) such as cellulose and non-carbohydrates such as lignin, both derived from plant cells and usually called dietary fiber, are also important. These polymer mixtures are scarcely digested due to the lack of intestinal enzymes that are able to break them down. Intestinal bacteria usually ferment dietary fiber into hydrogen, carbon dioxide, SCFA such as acetic, propionic and butyric acids, and others like lactic acid (Jamroz et al., 2002), thereby favoring mineral solubility and bioavailability by reducing intestinal pH. In addition, undissociated SCFAs absorbed in the intestine contribute to mineral transport from the intestinal lumen to blood (Bar, 2009).

Some authors demonstrated that SCFAs enhance Ca^{2+} absorption in the cecum and colon of rats when applied to the luminal side of the mucosa in an Ussing Chamber (Mineo et al., 2001). Also, Mineo et al. (2002) observed that the presence of anhydrous difructose III and IV and melibiose in the apical region promotes the absorption of Ca^{2+} in the small and large intestine of rats. A low pH in the vicinity of the apical membrane allows SCFA anions to be protonated and form the corresponding acids, which can be absorbed by non-ionic diffusion in the epithelium. Besides, in a study carried out with an Ussing Chamber, the presence of mainly propionic acid on the mucosal side was reported to increase iron absorption in the proximal colon of rats (Bouglé et al., 2002).

Different strategies to enhance mineral absorption in birds by using appropriate dietary supplements have been successfully assayed. When fructans or organic acids were added to the drinking water of laying hens to improve eggshell hardness, egg weight and quality, an increase in serum calcium level was observed (Chen and Chen, 2004; Soltan, 2008). However, the relationship between physiological concentrations of SCFA and egg quality improvement through mineral transport driven by SCFA absorption remains unclear.

Hence, the aim of our investigation was to study SCFA production by the cecal microbiota of laying hens from the fermentation of different dietary supplements and to relate this production with the absorption of minerals that are important for egg quality and avian health. Fermentations of oligofructose, polydextrose, arabic gum and molasses, an inexpensive carbohydrate source from the sugar cane industry, and their mixtures were studied *in vitro* in layers' cecal homogenates. SCFA mixtures that simulate the acid levels resulting from the fermentations of fibers, and SCFAs of different types and concentrations were evaluated *in vitro* for their effects on the intestinal absorption of calcium and iron through the colonic epithelium.

2. Materials and methods

2.1. Cecal slurries preparation

Laying hens used in this investigation ($n = 18$) were obtained from three different flocks of a commercial poultry farm where they received a conventional balanced diet (ingredients in g/kg: corn, 622; soybean, 171; $CaCO_3$, 93.4; meat meal, 54.7; soybean meal, 49.9; NaCl, 2.79; DL-methionine, 2.03; vitamin and trace mineral premix, 5.00; threonine, 0.93; lysine, 0.77; coline, 0.50) with full access to water. For each fermentation trial ($n = 3$), six random animals with a mean weight of 1.75 ± 0.23 kg were sacrificed by stunning followed by cervical dislocation and bleeding. Intestines were immediately carried to the laboratory at 4 °C and the ceca removed and introduced into an anaerobic glove box with a 100% N_2 atmosphere (Anaerobic System model 1024, Forma Scientific, Marietta, USA). The cecal content, obtained by opening the blind gut longitudinally, was weighed and homogenized in a pre-reduced sterile saline solution to obtain a uniform slurry pool. The cecal suspension was then diluted to an adequate volume in the saline solution to get a 10% (w/v) concentration (Argañaraz-Martínez et al., 2013). The suspension was used to study the fermentation of molasses, oligofructose, polydextrose, arabic gum and their mixtures by hen cecal microbiota.

2.2. Fermentation experiments in vitro

Molasses and oligofructose were provided by La Florida sugar industry (Tucumán, Argentina) and SAPORITI S.A. Orafti (Brasil), respectively, while polydextrose and arabic gum were supplied by Gelfix S.A. (Argentina) and used as pure or mixed supplements. The cecal slurry prepared as indicated above was dispensed in sterile flasks and an appropriate volume of sterile saline solution (control) or dietary supplements were added. Pure supplements were assayed at final concentrations of 10 g/L cecal suspension (0.1 g per gram of cecal content wet weight) and combinations with molasses were added in a 1:1 (w/v) relation in a final concentration of 10 g/L each. Three independent trials were carried out under the same conditions with cecal homogenates each obtained from a different flock as described in Section 2.1.