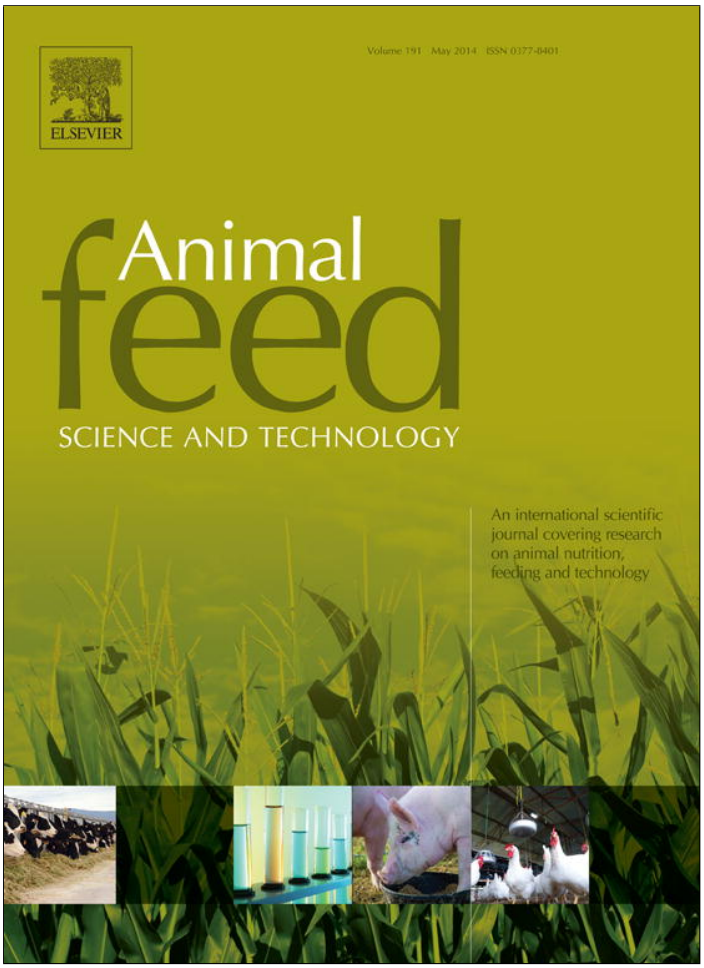

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Fermentation *in vitro* of a mixture of dietary fibers and cane molasses by the cecal microbiota: Application on mineral absorption through the laying hen's colonic epithelium

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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.

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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.

The cecal slurries were incubated at 37 °C inside an anaerobic glove box and samples were removed after 6 h for organic acid analysis by high performance liquid chromatography (HPLC) (Skoog et al., 1998). Samples were acidified with 0.01 mol/L H_2SO_4, centrifuged at 12,000*g* for 15 min at 4 °C to remove particulates and filter-sterilized onto 0.2 μm pore size membrane (Millipore, Massachusetts, USA). Twenty μL of filtered supernatants were injected into an HPLC system (Knauer, Germany) equipped with a smart line pump 100, a refractive index detector (Knauer, K-2301), a smart line auto sampler AS 3800 plus and a BIO-RAD Aminex HPX-87H (300 × 7.8 mm) column. The different components were eluted with 5 mM H_2SO_4 at a flow rate of 0.6 mL/min. Quantification of the samples was carried out by using calibration curves of acetic, propionic, butyric and lactic acids with a sensibility of 11, 8.6, 5.6 and 6.7 mmol/L per area, respectively.

2.3. Ionic absorption assays through intestinal epithelium

Two layers were sacrificed for each assay and the large intestines removed within 3 min after bleeding. The outside and inside surfaces of the isolated intestine were washed several times with ice cold saline solution (NaCl, 154 mmol/L). Segments from the cecum to the cloaca were taken and tissue flaps were prepared by the method described earlier (Awad et al., 2005; Rehman et al., 2006). The intestinal segments were opened longitudinally along the mesenteric border, washed free of intestinal contents several times with Ringer Lavoisier solution (RLS) at 4 °C and then the tissues were placed in cold RLS and gassed with O_2. The composition of RLS used was: NaCl, 135 mM; $NaHCO_3$, 2 mM; KCl, 3 mM; $CaCl_2$, 0.9 mM (Bouglé et al., 2002).

The serosa and muscle layers were removed, and stripped preparations consisting of mucosa and submucosa were mounted onto an Ussing Chamber. It was assembled in our laboratory and exposed a 1 cm^2 circular area of epithelium. The mucosal and serosal sides of the tissue were bathed in 2 mL of RLS containing 5 mMD-glucose and continuously exposed to 100% O_2 to equilibrate the system for 30 min. The medium on both sides was then removed. Two mL of RLS containing 10 mM $CaCl_2$ or 0.1 mM iron as gluconate was added to the mucosal side and 2 mL of RLS was aggregated to the serosal side of the chamber system. After 30 min of incubation, mucosal and serosal solutions were removed to determine the concentration of mineral absorbed. The same procedure, consisting of a 30 min period of equilibration and 30 min of incubation, was replicated three times.

Four SCFA mixtures were prepared to study their effects on mineral absorption. The concentrations of acetic, propionic and butyric acids recorded after 6 h of homogenate cecal fermentation without fiber addition (control) and with molasses, oligofructane or a combination of both were used to prepare the SCFA mixtures. Water content in the cecal slurries was assumed to be 80% (w/v). Hence, the SCFA concentrations measured as mmol/g in each fermentation mixture were expressed as mmol/L when the solutions for ionic absorption assay were prepared. Absorption experiments ($n = 3$) were carried out as mentioned above, but each mixture of organic acids was incorporated into the RLS preparation. This RLS modified solution was used only on the mucosal side of the Ussing chamber where calcium or iron absorptions were assayed. In another trial ($n = 3$), 12, 62 and 125 mmol/L of acetic, propionic or butyric acids were studied using the same methodology in order to establish the contribution of each acid on mineral absorption. These concentrations are within the physiological ranges of SCFA in the intestinal content of poultry (Józefiak et al., 2004) and were consistent with those derived from cecal fermentation in the homogenate without fiber (control) in our study.

The Ca^{2+} and Fe^{2+} concentrations absorbed were measured with an atomic absorption spectrophotometer with flame ionization (Perkin Helmer A Analyst 100, USA) and a calibration curve with a SIGMA standard solution was used. The data were expressed as nmol per min and cm^2 of epithelial area.

2.4. Statistical analyses

All results were expressed as mean ± standard deviation (SD) and were statistically evaluated by the analysis of variance (ANOVA) test (Minitab Release 14 Statistical Software, 2003 Minitab Inc.). The differences were considered significant at $P < 0.05$ with Tukey's test.

3. Results

3.1. Organic acid production by in vitro cecal fermentation of different carbohydrates and their mixtures

Concentrations of the main organic compounds produced after 6 h of *in vitro* fermentation of molasses, oligofructose, polydextrose and arabic gum, individually and mixed, in the layers' cecal homogenates are shown in Table 1.

A negligible amount of lactic acid was produced in the sample without supplements (control). The main SCFA produced was acetic acid and lower concentrations were observed for propionic and butyric acids.

A significant increase in total acids (TA) and SCFA occurred during fermentations of all the individual substrates studied; the highest values were produced with molasses and oligofructose. Ratios of each SCFA were different depending on the energetic substrate utilized by the cecal microbiota. Acetic acid reached a higher amount in the fermentation of molasses than in that of oligofructose, polidextrose and arabic gum. Propionic and butyric acids were significantly higher with oligofructose than with the addition of molasses. The concentrations of these acids were lower in samples with polydextrose and arabic

Table 1
Organic acid production by *in vitro* fermentations of layers'cecal homogenates with different carbohydrates and their mixtures.

Cecal homogenate	Organic acid concentration (mmol/g)					
	Total acids	Lactic acid	SCFA	Acetic acid	Propionic acid	Butyric acid
Control	$0.20^{a} \pm 0.01$	$0.01^{a} \pm 0.00$	$0.19^{a} \pm 0.01$	$0.11^{a} \pm 0.01$	$0.06^{a} \pm 0.01$	$0.02^{a} \pm 0.00$
Supplemented with 10 g/L						
Molasses	$0.74^{b} \pm 0.03$	$0.11^{b} \pm 0.01$	$0.63^{bg} \pm 0.02$	$0.30^{b} \pm 0.01$	$0.26^{b} \pm 0.01$	$0.07^{b} \pm 0.01$
Oligofructose	$0.80^{b} \pm 0.03$	$0.19^{c} \pm 0.01$	$0.61^{b} \pm 0.02$	$0.19^{c} \pm 0.01$	$0.33^{c} \pm 0.02$	$0.09^{c} \pm 0.01$
Polidextrose	$0.41^{c} \pm 0.01$	$0.04^{d} \pm 0.01$	$0.37^{c} \pm 0.01$	$0.16^{d} \pm 0.01$	$0.16^{d} \pm 0.01$	$0.05^{bd} \pm 0.01$
Arabic gum	$0.29^{d} \pm 0.01$	$0.01^{a} \pm 0.00$	$0.28^{d} \pm 0.01$	$0.16^{d} \pm 0.01$	$0.08^{a} \pm 0.01$	$0.04^{ad} \pm 0.00$
Supplemented with 10 g/L of each dietary fiber						
Molasses–oligofructose	$1.27^{e} \pm 0.03$	$0.41^{e} \pm 0.01$	$0.86^{e} \pm 0.02$	$0.28^{e} \pm 0.01$	$0.44^{e} \pm 0.01$	$0.14^{e} \pm 0.01$
Molasses–polidextrose	$0.87^{f} \pm 0.03$	$0.15^{f} \pm 0.01$	$0.72^{f} \pm 0.02$	$0.23^{f} \pm 0.01$	$0.39^{f} \pm 0.01$	$0.10^{c} \pm 0.01$
Molasses–arabic gum	$0.78^{b} \pm 0.03$	$0.10^{b} \pm 0.01$	$0.68^{fg} \pm 0.02$	$0.24^{f} \pm 0.01$	$0.34^{c} \pm 0.01$	$0.10^{c} \pm 0.01$

Results from different trials are expressed as means ± SD ($n = 3$). A SD indicated as 0.00 corresponds to values ≤0.005. Means in each column with different superscript letters are significantly different at $P < 0.05$.

gum, and propionic and butyric acids originated by fermentation of the last substrate were not significantly different with respect to the control.

Substrate mixtures produced a higher concentration of TA and SCFA than individual carbohydrates suggesting a complementary effect. The fermentation of the molasses–oligofructose mixture generated the highest levels of TA and SCFA, consistent with the pH decrease registered at 6 h of incubation (3.48). A higher propionic acid production and a molar ratio of 33:51:16 for acetic:propionic:butyric acids, similar to the ratio achieved with oligofructose alone (31:54:15), was observed when the molasses–oligofructose mixture was added.

3.2. Calcium and iron absorption across colonic mucosa driven by SCFA

Mixtures of pure acetic, propionic and butyric acids that reproduce the concentrations reached after carbohydrate fermentation were used to investigate SCFA driven mineral absorption. Only values obtained with molasses, oligofructose and their combination were assayed as the highest acids levels were observed in these cases.

Calcium and iron absorption driven by mixtures of SCFA are shown in Table 2. The calcium absorbed without SCFA addition was 0.08 nmol/min/cm^2 under the experimental conditions used. In the presence of mixtures of SCFA, an improved Ca^{2+} absorption was observed, independently of the mixture assayed. However, an enhanced absorption was detected as a higher total concentration of SCFA was assayed and no relation between absorption and acid molar ratios was evidenced. The concentrations of the SCFA that resemble the fermentation of the mixture molasses–oligofructose were most efficient in the process of absorption of this ion. Iron epithelial absorption was also favored when mixtures of SCFA were used. As was observed for calcium, a higher level of iron was carried by SCFA concentrations that simulate those originated by molasses–oligofructose fermentation.

Table 3 shows the calcium and iron absorption driven by 12, 62 and 125 mmol/L of acetic, propionic or butyric acids. These concentrations are equivalent to acid concentrations of 0.01, 0.05 and 0.10 mmol/g of wet intestinal content, respectively. The same were selected to establish the contribution of each acid to mineral absorption in concentrations that resemble those derived from *in vitro* cecal fermentation without fiber (Table 1). Ca^{2+} absorption increased with the concentration increment of each acid, but it was more remarkable with butyric acid addition. A behavior similar to that of Ca^{2+} was observed when Fe^{2+} absorption was assayed. However, iron driven transport by acetic acid showed a saturation effect at a concentration of 62 mmol/L.

Table 2
Mineral absorption driven by SCFA mixtures across laying hens' colonic mucosa.

Sample	SCFA (mmol/L)	Acetic:propionic:butyric molar ratio	Mineral absorbed (nmol/min/cm^2)	
			Calcium	Iron
Control	–	–	$4.95^{a} \pm 0.72$	$0.08^{a} \pm 0.00$
$SCFA_{WF}$	238	58:32:10	$17.4^{b} \pm 2.21$	$0.15^{b} \pm 0.02$
$SCFA_{M}$	788	48:41:11	$25.4^{c} \pm 2.06$	$0.33^{c} \pm 0.02$
$SCFA_{O}$	762	31:54:15	$27.4^{c} \pm 2.80$	$0.39^{c} \pm 0.03$
$SCFA_{M-O}$	1075	33:51:16	$41.2^{d} \pm 3.53$	$0.62^{d} \pm 0.03$

Sample: without SCFA (Control); in presence of a SCFA mixture that resembles the concentrations derived from the cecal fermentation: without fibers ($SCFA_{WF}$), with molasses ($SCFA_{M}$), oligofructose ($SCFA_{O}$) and a molasses–oligofructose mix ($SCFA_{M-O}$). Results from different trials are expressed as means ± SD ($n = 3$). A SD indicated as 0.00 corresponds to values ≤0.005. Means in each column with different superscript letters are significantly different at $P < 0.05$.

Table 3
Mineral absorption across laying hens' colonic mucosa in presence of different SCFA concentrations.

Sample	Acid (mmol/L)	Mineral absorbed (nmol/min/cm^2)	
		Calcium	Iron
Control	0	4.95^{a} ± 0.72	0.08^{a} ± 0.00
Acetic acid	12	8.02^{ab} ± 0.74	0.17^{b} ± 0.00
	62	12.4^{c} ± 1.84	0.22^{c} ± 0.02
	125	17.3^{d} ± 2.06	0.24^{c} ± 0.00
Propionic acid	12	9.48^{b} ± 1.62	0.23^{c} ± 0.00
	62	15.4^{e} ± 2.36	0.23^{c} ± 0.00
	125	25.5^{f} ± 1.92	0.37^{d} ± 0.01
Butyric acid	12	10.6^{b} ± 1.47	0.18^{b} ± 0.01
	62	18.0^{e} ± 1.62	0.25^{c} ± 0.00
	125	30.7^{g} ± 2.21	0.45^{e} ± 0.02

Results from different trials are expressed as means ± SD (n = 3). A SD indicated as 0.00 corresponds to values ≤0.005. Results from different trials are expressed as means ± SD (n = 3). A SD indicated as 0.00 corresponds to values ≤0.005. Means in each column with different superscript letters are significantly different at $P < 0.05$.

4. Discussion

Any carbohydrate that reaches the cecum is a potential substrate that may be fermented by the microbiota with the consequent production of SCFA (Alles et al., 1996). The concentrations and the relationship between them differ according to the substrate fermented (Cummings et al., 2001).

In our study, the individual fermentation of molasses and oligofructose produced higher concentrations of acetic, propionic and butyric acids than other carbohydrates (Table 1). In a similar batch culture system, where inulin was fermented, an increase in propionic acid concentration was observed (Rycroft et al., 2001). The lower production of SCFA found in our experiments with polydextrose and gum with respect to oligofructose could be due to its different chemical structure. Oligofructose presents β (2 → 1) fructosyl–fructose glycosidic linear bonds with an average polymerization degree of 4 to 5. In contrast, polydextrose, a water soluble dietary fiber, is a glucose polysaccharide that contains small amounts of sorbitol and citric acid. Unlike the low production of SCFA found in our study, *in vitro* fermentation of gum in human feces resulted in a significant production of SCFA (Titgemeyer et al., 1991), which may be due to differences in the microbiota composition of humans and birds. The amount of available substrates, their chemical composition and physical form impact on cecal fermentation, which depends on the different bacterial populations as well as on their interactions (Macfarlane and Macfarlane, 2003).

Considering substrate mixtures, acetic acid was produced in a lower amount with molasses–oligofructose mixture fermentation than with molasses alone. By contrast (Donalson et al., 2008), an *in vitro* fermentation study of laying hens' cecal homogenates reported that the alfalfa–fructooligosaccharides mixture led to high concentrations of acetic acid. The formation of this acid could be related to some bacterial groups like clostridia, bacteroides and bifidobacteria which may be at a different population level depending on the substrates used (Macfarlane and Macfarlane, 2003). On the other hand, a higher propionic and butyric acid production was obtained with molasses–oligofructose fermentation. According to these results, the molar ratio of acetic:propionic:butyric acids was similar to the ratio achieved with oligofructose alone, but different from the one obtained with molasses alone or without fibers (Table 2) which was close to 68:20:12, a proportion generally found in birds (Breves and Stück, 1995). The presence of propionic acid in fermented homogenates could be associated with a high activity of bacteroides as observed in a study conducted in our laboratory with mouse cecal homogenates (Lorenzo-Pisarello et al., 2010), while butyric acid production seems to be related to clostridia populations as reported by other researches (Olano-Martin et al., 2000). On the other hand, the increase in lactic acid production with the molasses–oligofructose mixture could be related to the stimulation of lactobacilli populations by oligofructose as demonstrated in another study with inulin as the fermentation substrate (Lorenzo-Pisarello et al., 2010).

In our study, the major effect on the production of lactic, propionic and butiric acids was observed by the fermentation of the molasses–oligofructose mixture related to that of individual fibers. Homogenates containing a fiber mixture (final concentration of 10 g/L of each fiber) had double the amount of total substrates for fermentation than homogenates with each individual fiber. However, lactic acid originated from the mixture fermentation was higher than the one expected by the addition of this acid derived from the fermentation of each individual fiber, thereby suggesting a synergic effect of the mixture on lactic acid production. On the other hand, propionic and butyric acids were produced in concentrations that evidenced a complementary but not a complete consumption of both substrates of the mixture. These results suggest that complex interactions between different populations are established selectively promoting the production of some acids depending on the available substrates.

Mixtures of SCFA that simulate the acid concentrations obtained from fermentations of molasses, oligofructose or a mix of both improved mineral transport through the colonic mucosa of laying hens (Table 2). The latter increased the amount of calcium and iron absorption eightfold approximately when compared to absorption in the absence of SCFA. However, this effect was not linked to the SCFA molar proportion. Similar concentrations of total SCFA and different molar ratios among acids led to similar ionic absorption, as observed for a SCFA mixture that resembles the concentrations derived from the

cecal fermentation of molasses ($SCFA_M$) and oligofructose ($SCFA_O$). By contrast, higher SCFA production in the same molar ratio increased ionic transport, as evidenced from the absorption results obtained with $SCFA_O$ and $SCFA_{M-O}$.

Calcium and iron absorption through the colonic mucosa driven by 12, 62 and 125 mmol/L of acetic, propionic and butyric acids was dependent on acid type and concentration (Table 3), butyric acid being the most effective at the highest concentration used. The addition of each individual acid at concentrations between 10 and 100 mmol/L increased calcium absorption in a similar study carried out with rat intestinal epithelium (Mineo et al., 2001). The authors found that the addition of 100 mmol/L acetic, propionic or butyric acids to the luminal side led to calcium absorption of 20, 25 and 28 nmol/min/cm^2, respectively. These results are consistent with those found in the present study when 125 mmol/L of acetic, propionic or butyric acids were added (Table 3). We assumed that the SCFA protonated forms, absorbed by diffusion through the apical membrane, are dissociated inside the cell because their pK_a values are lower (approximately 4.8) than the intracellular pH (between 6 and 7) and hydrogen ions are exchanged with luminal Ca^{2+}, allowing mineral absorption into the cell. This mechanism was previously stated by Fleming et al. (1991) and Trinidad et al. (1996).

The results of Table 3 also allowed us to infer that under physiological conditions, without fiber addition, the main contribution to calcium and iron absorption is due to the acetic and propionic acids. Indeed, a total concentration of 238 mmol/L SCFA, composed of 138, 75 and 25 mmol/L (ratio 58:32:10) and compatible with SCFA production by colonic fermentation in the absence of fibers, led to calcium and iron absorptions of 17.4 and 0.15 nmol/min/cm^2 (Table 2); these absorption results were similar to those obtained when 125 mmol/L of acetic acid or 62 mmol/L of propionic acid were assayed individually to drive mineral transport (Table 3). By contrast, a similar absorption of calcium and iron was obtained with 62 mmol/L of butyric acid, which is not a physiological concentration, in the absence of fermentable supplements. On the other hand, 0.14 mmol/g of butyric acid (equivalent to 112 mmol/L in the wet cecal content) were obtained by molasses–oligofructose fermentation (Table 1). This concentration was close to the highest concentration assayed to drive mineral transport with individual acids, and the best result was obtained with butyric acid. However, its contribution in the cecal environment may be very different depending on the production of other SCFAs. A significantly greater calcium absorption has been reported to occur with the addition of propionic acid than with acetic acid on the mucosal side (Trinidad et al., 1999). Bouglé et al. (2002) and Ohta et al. (1995) have shown that SCFAs, mainly propionic and butyric acids, enhance iron absorption in the proximal colon of rats. It is possible that the formation of stable complexes between ions and propionic and butyric acids is a promoter mechanism for mineral absorption (Ohta et al., 1995; Trinidad et al., 1999; Bouglé et al., 2002). In the present study, the molasses–oligofructose fermentation mixture produced the highest concentration of propionic and butyric acids and enhanced Ca^{2+} and Fe^{2+} absorption.

The improved absorption of Ca^{2+} and Fe^{2+} in the presence of a combination of SCFAs in concentrations that simulate the one produced by molasses–oligofructose mixture fermentation, suggests that trials using a combination of these fibers could be performed *in vivo* to improve dietary ionic bioavailability. This effect could increase calcium serum, consequently leading tobetterment in egg quality and layers' health as shown by Chen and Chen (2004) when the diet of birds was supplemented with inulin and oligofructose.

5. Conclusions

Acetic, propionic and butyric acids improved the absorption of individual minerals in a concentration dependent way. Fermentation of molasses, oligofructose or both by the cecal microbiota of hens resulted in an increase in SCFA whose presence had a positive influence on calcium and iron absorption through the colonic mucosa of laying hens. Nevertheless, the best result of mineral transport was reached with a SCFA mixture similar to the one obtained by molasses–oligofructose fermentation. These results imply that an improvement in intestinal mineral absorption with a positive impact on egg quality and avian health could be achieved when molasses–oligofructose makes up a part of the layers' diet.

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