ASSESSMENT OF THE PREBIOTIC EFFECT OF QUINOA AND AMARANTH IN THE HUMAN INTESTINAL ECOSYSTEM

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Abstract

Quinoa and amaranth belong to the group of the so called "superfoods" and have a nutritional composition that confer multiple benefits. In this work, we explored the possibility of these foods exhibiting a prebiotic effect. These pseudocereals were subjected to an *in vitro* digestion and used as carbon sources in batch cultures with faecal human inocula. The effect on the microbiota composition and in their metabolic products were determined by assessment of variations in pH, short-chain fatty acid (SCFA) production and changes in the dynamic bacterial populations by fluorescence *in situ* hybridization (FISH). After 48 h of incubation, the total SCFAs were 106.5 mM for quinoa and 108.83 mM for amaranth; in line with the decrease in pH. Considerable differences ($p < 0.05$) were found in certain microbial groups, including *Bifidobacterium* spp., *Lactobacillus–Enterococcus*, *Atopobium*, *Bacteroides–Pretovella*, *Clostridium coccoides–Eubacterium rectale*, *Faecalibacterium prausnitzii* and *Roseburia intestinalis*. Our research suggests that these pseudocereals can have a prebiotic potential and that their intake may improve dysbiosis or maintain the gastrointestinal health through a balanced intestinal microbiota, although additional studies are necessary.

Keywords: Functional foods**,** pseudocereals, Fluorescence *in situ* Hybridization, Short Chain Fatty Acids, gastrointestinal health, colonic microbiota

1. Introduction

The human gut microbiota is a complex microbial ecosystem composed not only by bacteria but also Archaea, yeasts, protists, and viruses.[1] The large intestinal microbiota and its metabolic products interact with the host and play an important role in normal bowel function, thus contributing to the maintenance of host health, through the formation of short chain fatty acids, modulation of immune system reactivity and development of colonization resistance.[2]

It is well known that the occurred modifications of human lifestyle in the developed world involves dietary changes that exert an important selective influence upon gut microbiota composition and thereby health and predisposition to various diseases.[3,4] The impact of diet upon microbiota composition is determined by two mechanisms: competition for substrates and tolerance of gut conditions.[4] The different capability of the microbial species to utilize dietary substrates promote the competition for substrates available in the large intestine and contribute to the prevalence of certain species over others, playing an important role in defining microbial composition.[4]

Under this perspective, driven by the great interest in promoting health by modulating the composition and metabolic function of gut microbiota, the concept of prebiotics was introduced in 1995 by Gibson and Roberfroid who first defined it as 'non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and⁄or activity of one or a limited number of bacteria in the colon, and thus improves host health'[5].

In recent years, a number of prebiotic carbohydrates such as xylooligosaccharides[6,7] arabinoxylans[8], galactoglucomannans[9] and pectic-oligosaccharides[10,11] have been tested to evaluate their impact on the gut microbiota composition and their metabolic products. However, few studies have demonstrated the prebiotic potential of foods rich in carbohydrates with similar structures to the cited above. In this context, Gullón et al. (2015)[12] demonstrated the ability of two legumes (broad beans and lupin seeds) to modulate the intestinal microbiota composition and suggested that these pulses may be used in the development of novel functional foods. Maccaferri et al. (2012)[13], also investigated the impact of four flours (wholegrain rye,

wholegrain wheat, chickpeas and lentils 50:50, and barley milled grains), on the intestinal microbiota composition and metabolomic output.

Quinoa (*Chenopodium quinoa* W.) and amaranth (*Amaranthus caudatus* L.) are dicotyledonous species of the Andean origin, known as pseudocereals. They have been recognized for their phytochemical content and their excellent nutrient profile; hence the consumption of quinoa and amaranth grains has increased substantially across the world in the last decade.[14,15] Attending to their excellent nutritional properties and for being gluten free, they are considered as good candidates for supplementation or substitution of common cereal grains such as rye, oat or wheat.[16] They are characterized by their high content of high quality protein and essential aminoacids such as lysine, cysteine and methionine.[17] They have also been reported as an important source of micronutrients such as minerals (calcium, copper, manganese, zinc and iron) and vitamins (thiamine, riboflavin, folic acid, niacin, retinol, among others) and significant amounts of other bioactive components with health-promoting effects, such as flavonoids, phytosterols, and polyphenols.[18-22] Moreover, in the last few years, certain amaranth and quinoa components with biological activities (anti-cancer, anti-diabetic and anti-inflammatory) have also been evaluated, as well as components related with cardiovascular disease prevention and antihypertensive properties.[20,22,23]

Concerning to the polysaccharide composition, Lamothe et al., (2015)[16] have shown that quinoa and amaranth represent a good source of dietary fibre, with contents of 10 and 11%, respectively, which is much higher than that of rice (0.4%), wheat (2.7%) and corn (1.7%).[18] In particular, quinoa and amaranth dietary insoluble fibres (78%) are mainly made up of homogalacturonans and RG-I with arabinan side chains (55-60%), xyloglucans (30%) and cellulose. However, the quinoa soluble fibre (22%) was mainly composed of homogalacturonans and arabinans, whereas in amaranth soluble fibre, branched xyloglucans (40-60%) and arabinose-rich pectic polysaccharides (34-55%) were the predominant constituents present.[16]

However, to the best of our knowledge, the prebiotic potential of quinoa and amaranth has not been yet investigated. Taking into account their phytochemical content and biological

activities as well as polysaccharide composition and structural features analysis, the potential bio-functional properties of these pseudocereals should be deeper evaluated once some studies performed with xyloglucans and pectic oligosaccharides evidence a favorable effect in the colon microbiota.[6-8,10,11]

Thus, the aim of this study was to evaluate the prebiotic properties of quinoa and amaranth and their effects in the intestinal microbiota ecosystem using a small scale *in vitro* batch culture. The prebiotic potential was assessed by measuring the dynamics of selected microbial populations using the fluorescent *in situ* hybridization technique (FISH), quantification of short-chain fatty acids (SCFAs) and pH evolution.

2. Materials and methods

2.1. Substrates and simulated *in vitro* gastrointestinal digestion

Quinoa and amaranth samples were purchased from a local store in Ourense (Spain). Samples from various packages were homogenized in a single lot before processing.

In first place, the quinoa and amaranth were cooked to mimic the real conditions of consumption. Then, the pseudocereals were digested *in vitro* under appropriate conditions according to Gullón et al. (2015).[12] Digestion was performed by enzymes and acid and basic solutions and absorption was simulated by using a dialysis membrane of 1 kDa molecular weight cut-off to remove the low molecular mass digestion products. The method includes three different stages of digestion: mouth, gastric and small intestine conditions. The samples obtained were stored immediately at −20 °C, before freeze-drying for 72 h and lyophilized. These samples were used for the *in vitro* anaerobic fermentation. High Performance Size Exclusion Chromatography (HPSEC) was performed to monitor the removal of low molecular mass digestion products, thus ensuring that they did not remain in the fermentation stage to better simulate the *in vivo* process. The HPSEC consisted of an Agilent 1200 series HPLC system, two TSKGel G3000PWXL columns (7.8mm i.d. x 30 cm/ column) in series (Tosoh Bioscience, Stuttgart, Germany), in combination with a PWX-guard column (6mm i.d. x 4 cm), operating at 30 ºC with a refractive index (RI) detector (Agilent, Germany). The mobile phase

was distilled water (flow rate, 0.6 mL/min). Dextrans (1000-80,000 g/mol) from Fluka (parent company of Sigma-Aldrich, Barcelona, Spain) were used as calibration standards.

2.2. Prebiotic effect assessment

Samples of quinoa and amaranth grains obtained after *in vitro* digestion were assayed for their *in vitro* fermentability using faecal inocula according the method of Gullón et al. (2011).[24] The concentration of quinoa and amaranth in the fermentation medium was 1% (w/v) and the final volume was 50 mL. A medium without a carbon source was used as a negative control. Samples were collected at 0, 6, 24 and 48 h of fermentation to be evaluated for their microbiota composition by FISH.[26] The analysis of lactic acid and short chain fatty acids (SCFAs) were quantified by HPLC. Faecal samples were obtained from healthy human donors (three females; age 25–35 years old). None of the volunteers had taken antibiotics or probiotics for at least 3 months before of faecal donation. Faeces were collected and kept in an anaerobic cabinet (Model Bactron IV, Shel-Lab, Cornelius, OR, USA) for a maximun of 2 h after collection. The faecal inocula (FI) were prepared as per Gullón et al. (2014)[8]. All additions and inoculations were carried out inside an anaerobic cabinet (5% H_2, 10% CO_2 and 85% N_2). All experiments were performed in compliance with the relevant laws and followed our institutional guidelines. Furthermore, an informed consent was obtained from all the subjects involved in the study as required by the Spanish Bioethics Committee (CBE, España).

2.3. Fermentation metabolites analyses

The fermentation media were centrifuged and the supernatants were harvested for analysis of organic acids by HPLC after filtration using 0.20 μm cellulose acetate membranes. An Agilent 1200 series HPLC system with a RI detector (Agilent, Germany) and Aminex HPX-87H column (BioRad, Hercules, CA, USA) were used to perform the analyses of SCFAs. Other analysis conditions were as follows: mobile phase, 3 mM H_2SO_4 at 0.6 mL min^{-1}, 50 °C.

2.4. Statistical analysis

Statistical analysis was performed using SPSS for Windows version 21.0 (IBM SPSS, Chicago, IL). Univariate analysis of variance (ANOVA) and the post hoc Tukey test were used to determine the differences in bacterial populations and acid concentrations. Differences were considered significant at the 5% level.

3. Results and Discussion

In this study the prebiotic potential of quinoa and amaranth was assessed by *in vitro* assays with human faecal batch cultures. Fermentations were performed in order to evaluate the fermentability of these substrates as well as to assess their effect on the gastrointestinal microbiota composition and the accumulation of SCFAs and lactate and formiate.

3.1. Metabolic activity in faecal cultures: effects on pH and on lactate, formiate and SCFAs generation

Pseudocereals are appropriate substrates to support the intestinal microbiota metabolism as evidenced by the fact that they promoted the output of SCFAs, lactate and formiate along fermentations. Table 1 shows the concentrations of SCFAs (acetate, propionate and butyrate), lactate and formiate as well as the pH shifts and the total contents of SCFAs obtained in the fermentation media containing quinoa and amaranth as carbon sources. In addition, in comparative terms, a control without external carbon source was also added. Culture samples were taken at 0, 6, 24 and 48 h and the cited parameters were analyzed according to the methodology described above.

The evolution of the pH along fermentations is displayed in Table 1. The pH profile for fermentations with pseudocereals was similar to the reported by other studies for pectic oligosaccharides of apple pomace,[24] observing two different stages; a first one that lasts about 24 h, during which the pH decreased significantly, in agreement with the largest increase in total SCFAs, and a second one, from 24 h to 48 h, where the pH almost did not change (coinciding with the scarce variation in the total SCFAs during this period). In the case of the control

cultures without carbon source, the pH remained almost constant throughout the whole fermentation time.

As expected, the increase in total SCFAs, lactate and formiate during the fermentation was higher in the media containing quinoa and amaranth than in the negative control cultures. Lactate was only observed during the first 6 h of fermentation and the concentration profiles were similar for both substrates. Regarding the formiate, the media with quinoa as carbon source had the highest concentrations, reaching at 24 h the maximum value of 14.99 mM, in contrast with the maximum of 11.84 mM obtained with the amaranth. A significant degradation of the formiate was also observed at longer times. Lactate degradation has been previously reported in the bibliography,[8,12,25] where it is explained by the conversion of this compound into secondary metabolites (mainly acetate, butyrate and propionate), since some bacterial species could employ end products of other species as substrate.[26] The lactate accumulation in the organism is not habitual and it would be associated with certain gut disorders.[25]

On the other hand, in contrast with the concentration profiles of lactate and formiate, the SCFA profiles showed a progressive increase with time in both cultures, being this trend more pronounced during the first 24 h. As can be seen in Table 1, acetate was the most abundant SCFA produced during the fermentation of these pseudocereals, with the maximum concentrations reaching up to 63.11 for quinoa and 67.63 mM in the case of amaranth at 48 h. On the contrary, the control fermentation only led to lower concentrations of acetate (30.52 mM at 48 h), thus confirming the stimulating effect of the substrates in this parameter. The predominant production of acetate is in line with the results reported by other authors. Gullón et al. (2015)[12] observed similar acetate productions in faecal fermentations using the seeds and broad beans as carbon source. Connolly et al. (2012)[27] also reported high concentrations of acetic acid when whole grain oat based cereals were fermented.

The content of acetate is followed by the propionate and butyrate, which are present in minor amounts in all samples (see Table 1). Similar findings were also reported during the fermentation of other carbohydrate sources such as pectin from orange peel wastes[11] and Akpan (a traditional yoghurt-like product made from fermented cereal starch).[28] Concerning the

propionate concentrations, both substrates allowed maximum values to be reached (≈24 mM) by 48 h of fermentation, these values being three times higher than the obtained in the medium without carbon source.

On the contrary, some differences were detected in the butyrate profiles obtained during the fermentations. In general, the media made with quinoa resulted in high butyrate concentrations (20.31 mM at 48 h), in comparison with the values obtained with the media prepared with amaranth (16.99 mM) although no significant differences were observed between both substrates. On the other hand, only scarce concentrations of butyrate were found during the control fermentation (6.89 mM at 48 h).

It should be noted that the presence of propionate in the gut is very important since it is considered a gluconeogenerator that contributes to inhibit biosynthesis of cholesterol and fatty acids in liver and it could also help to reduce the cardiovascular disease risk.[29] On the other hand, the butyrate plays an important role as a carbon source for the colonic microbiota and in the maintenance of colonic mucosa health since it exerts strong anti-inflammatory activity and in the last decade it was also linked to a reduced incidence of colon cancer.[30]

The higher contents of propionate and butyrate found in this study would confirm the high fermentability of the media made with quinoa and amaranth as carbon source and the contribution of the consumption of quinoa and amaranth to the gut health.

On the other hand, after 48 h of incubation, considerable amounts of SCFA were observed in both fermentation media, the total SCFA content being slightly higher in the Quinoa media than in the Amaranth one throughout the fermentation process. Their concentrations achieved maximum values of 106.5 and 108.83 mM at 48 h for the cited substrates, respectively. Conversely, it can be noted that when the fermentation was performed without external addition of carbon, only 46.19 mM of total SCFA was detected in the media, which was almost 2.5-fold less than that obtained with the substrates evaluated in this study. According to the bibliography, the production of SCFA in cultures lacking carbon sources is mainly associated to protein degradation carried out by putrefactive bacteria present in the intestinal microbiota. Therefore, low contents of SCFA can be expected in this situation, thus

indicating that the external addition of carbon sources are responsible for SCFA generation in the fermentation media.[28,31]

The acetate to propionate ratio (A/P) is an interesting parameter that has been proposed as an indicator of a potential hypolipidemic effect of prebiotic substrates[32] since propionate has been reported to inhibit fatty acid synthesis *in vitro*, whereas acetate is a lipogenic substrate.[32] The ratio A/P for the two pseudocereals assayed remained almost constant along the fermentation time with a low value (≈2.7); this is due to the fact that their concentration increased in the same proportion from the start of the fermentation and can be indicating that these substrates could modify the lipidic metabolism; this value compared favourably with that reported by Gullón et al. (2015)[12] who indicated values of A/P ratio around 3 for broad beans and lupin seeds fermented with faecal human cultures.

3.2. Modulation of human faecal bacterial populations by quinoa and amaranth fermentation

It is well known that the bacterial gut composition can be modified by different factors including the age, genetics, diseases, and diet.[33] Therefore, it is advisable to introduce known functional food ingredients in our diet that can help modulate the microbiota and improve colon health, as well as evaluate the effects of new promising products in the colonic microbiota. A parameter often used in literature by following *in vitro* fermentations of substrates with prebiotic potential is the evolution of the selected microbial populations in the intestinal tract.[13,34,35] In this study the dynamics of the bacterial groups was assessed by FISH. Figure 1 displays the increases in bacterial microbiota at 6, 24 and 48 h, during *in vitro* batch culture fermentations performed with quinoa and amaranth. For comparative purposes the information obtained for media without carbon source (employed as negative control) has also been included in Figure 1.

As expected, the utilization of quinoa and amaranth as substrates resulted in noticeable changes in bacterial populations with respect to the negative control cultures, thus confirming the prebiotic potential of these pseudocereals. As can be seen in Figure 1, in media containing

quinoa and amaranth the total cell counts (DAPI) increased up to 0.96 and 0.88 log-fold after 48 h of fermentation, respectively, and no significant differences were found between both carbon sources ($p > 0.05$).

In general, the highest growth rate occurred during the 6 first hours of fermentation for all the bacterial groups evaluated, being this tendency less pronounced afterwards.

Concerning *Bifidobacterium* spp. and *Lactobacillus–Enterococcus* group, both substrates showed a similar behavior and no significant differences were found between the tested substrates ($p > 0.05$). After 48 h of fermentation, the highest increase in these bacterial groups corresponded to the cultures carried out with quinoa (0.82 log-fold and 0.71 log-fold, respectively). Gullón et al. (2015)[28] also reported a similar bifidogenic activity in fermentation media formulated with Akpan. In the same way, enhanced bifidobacteria and lactobacilli populations were also observed during *in vitro* fermentations of several carbohydrate sources such as lupin seeds, broad beens,[12] galactooligosaccharides,[36] arabinoxylooligosaccharides[8] or pectic-oligosaccharides.[11] However, to our knowledge the bifidogenic stimulation of quinoa and amaranth has not been studied previously and the results of this study confirm the bifidogenic effect of both substrates.

Regarding *Bacteroides/Prevotella* group no significant differences ($p > 0.05$) were observed during cultures with quinoa and amaranth, although the increase was slightly more pronounced for quinoa. After 48 h of fermentation, the cell numbers increased 0.65 log-fold and 0.59 log-fold in cultures containing quinoa and amaranth, respectively. This bacterial group is known for being acetic and propionic acids producers from several carbon.[37,38]

As shown in Figure 1, no significant differences were found among the quinoa and amaranth for the *Atopobium* cluster ($p > 0.05$), having registered an increase in cell counts of 0.66 log-fold for quinoa and 0.73 log-fold for amaranth. Stimulation of this bacterial group upon fermentation of fructooligosaccharides has been reported[11,39] The growth of *Atopobium* has been correlated with the increase of acetic acid concentration.[24,40]

Clostridium coccoides–Eubacterium rectale group showed a similar pattern to that observed for *Bacteroides* and a higher increased of this group for media containing quinoa was

observed. Concerning *Faecalibacterium prausnitzii*, a significant growth ($p \leq 0.05$) was observed by 6 h of fermentation with amaranth with respect to quinoa (see Figure 1). After 24 and 48 h of incubation no significant changes were recorded for both substrates, although a certain degree of selectivity may be observed for amaranth regarding the growth of *F. prausnitzii* (0.72 and 0.67 log-fold for amaranth and quinoa respectively at 48 h). It should be noted that the *C. coccoides–E. rectale* group and *F. prausnitzii* are considered to be major butyrate producers, which has been shown to exert beneficial effects on gut health[41]. Several authors have previously reported of the growth of *E. rectale* and *F. prausnitzii* in media containing different carbon sources such as pectin and pectic oligosaccharides,[11] mixture of chickpeas and lentils,[13] lupin seeds and broad beans,[12] whereas Sarbini et al. (2011)[25] did not observe increase of these bacterial groups on the fermentation of different dextrans. It is important to highlight that *F. prausnitzii* group has been reported as an indicator of intestinal health.[35]

Clostridium histolyticum group clusters I and II increased mainly during the first 6 h of incubation while scarce differences in their growth was achieved at longer fermentation times. For amaranth, significant increase in the *C. histolyticum* was detected at 6 h of fermentation ($p \leq 0.05$). After 24 and 48 h of incubation no significant differences were observed between amaranth and quinoa. After 48 h, the number of bacteria belonging to this group increased an average of 8% for both substrates. Similar increase was observed in the fermentation of legumes[12] and of several purified galactooligosaccharides.[42] In other studies, highest increases of *C. histolyticum* were reported; so, Gómez et al. (2016)[11] and Gullón et al. (2011)[24] reported values by 16% in cultures containing different pectic oligosaccharides as carbon sources. On the other hand, Oliveira et al. (2012)[43] observed an inhibition of this bacterial group using caprine cheese whey oligosaccharides as carbon source. From the point of view of the health, several authors have considered clostridia as harmful bacteria.[11,34]

Concerning *Roseburia intestinalis*, a butyrate-producing group of bacteria, amaranth showed a higher stimulatory effect on this bacterial group than the quinoa at 24 h of fermentation ($p < 0.05$). After 48 h of incubation, no significant differences between both

substrates were observed ($p > 0.05$). At the same fermentation time, populations of *R. intestinalis* increased by 10% as average for amaranth and quinoa.

It is well known that the intake of prebiotics stimulates the growth of beneficial bacteria in the colon such as *Bifidobacterium* and/or *Lactobacillus* species. However, recent studies have discovered that *F. prausnitzii*, *E. rectale* and *Roseburia* spp. are abundant and also desirable species in healthy microbiota that can produce butyrate from prebiotic fermentation, which plays different roles in processes linked to colonic health, including protection against inflammation and colorectal cancer.[30,44] Therefore the discovery of new prebiotic sources such as quinoa and amaranth, which can help to modulate the presence of these bacterial groups, could have interesting health applications.

In general, the changes observed in this study in the microbial population dynamics (determined by FISH) were in agreement with the profiles of organic acids described in the above section. High acetate concentrations observed for both substrates can be correlated with the increase in the *Bifidobacterium* and *Atopobium* or *Bacteroides*, but the higher population recorded in *Atopobium* population following amaranth faecal fermentation could justify the higher production of acetate in this media with respect to quinoa. In addition, *R. intestinalis* are among the microbial groups that can utilize acetate for production of propionate and butyrate via metabolic cross-feeding mechanism and the favorable shifts in SCFA found in this study could also be attributed to the increase of *R. intestinalis*.

Conclusions

Quinoa and amaranth are healthy foods (belonging to the called group “superfoods”) with high nutritional value and in the last decade researchers began to know their potential. This work is the first that assesses the prebiotic potential of quinoa and amaranth through *in vitro* cultures with human faecal microbiota. The results obtained herein showed that the evaluated pseudocereals contain certain components with potential prebiotic impact displayed by the increase of selected groups present in the intestinal microbiota. Both substrates stimulated in similar proportion the growth of certain numerically predominant bacterial groups in the human

intestinal microbiota. Moreover, as consequence of proliferation of these bacterial groups, an increase in the SCFAs was observed being the major ones acetate, propionate and butyrate. Therefore and although more studies are needed, in addition to the known properties of these pseudocereals, their prebiotic potential could be added as a premise for improving or maintaining gastrointestinal health through the equilibrium of intestinal microbiota.

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Table 1

Concentration (mM) of SCFA (acetate, propionate and butyrate), lactate and formiate, and pH of fermentation media made with quinoa or amaranth as carbon sources at 6, 24, and 48 h.

Carbon source	Time (h)	pH	Lactate	Formiate	Acetate	Propionate	Butyrate	Total SCFA
Quinoa	0	7.2	0.12 (0.05)	0.24 (0.17)	1.34 (0.42)	0.00(0.00)	0.29 (0.18)	1.63(0.14)
	6	6.5	2.18[b] (0.65)	8.67 [b] (2.17)	18.80[b] (0.91)	6.80[b] (0.92)	2.05[b] (1.56)	27.66[b] (1.56)
	24	6.0	0.00[a] (0.00)	14.99[b] (6.53)	58.30[b] (2.94)	20.98[b] (1.34)	18.37[b] (1.32)	97.65[b] (3.23)
	48	5.5	0.00[a] (0.00)	8.15[a] (4.70)	63.11[b] (6.87)	23.07[b] (1.43)	20.31[b] (1.81)	106.50[b] (8.62)
Amaranth	0	7.2	0.20 (0.12)	0.32 (0.10)	1.75 (0.75)	0.48 (0.12)	0.35 (0.14)	2.58 (0.45)
	6	6.3	2.31[b] (0.97)	9.38 [b](1.01)	20.57[b] (1.51)	7.64[b] (1.18)	3.83 [b] (0.63)	32.03[b] (2.73)
	24	5.9	0.00[a] (0.00)	11.84 [b](3.17)	61.35[b] (0.51)	21.18[b] (0.72)	16.99 [b] (1.25)	99.52[b] (2.36)
	48	5.4	0.00[a] (0.00)	5.50[a] (3.35)	67.63[b] (2.36)	24.21[b] (1.38)	16.99 [b] (1.48)	108.83[b] (2.60)
Control	0	7.2	0.10 (0.04)	0.15 (0.08)	1.32 (0.25)	0.14 (0.09)	0.00 (0.00)	1.46 (0.11)
	6	6.8	0.00[a] (0.00)	0.00[a] (0.00)	5.12[a] (0.74)	3.70[a] (0.29)	0.94[a] (0.31)	9.76[a] (1.23)
	24	6.9	0.00[a] (0.00)	0.00[a] (0.00)	21.78[a] (0.79)	7.26[a] (0.11)	5.86[a] (0.06	34.89[a] (0.75)
	48	7.1	0.00[a] (0.00)	0.00[a] (0.00)	30.52[a] (0.86)	8.78[a] (0.32)	6.89[a] (0.64)	46.19[a] (1.70)

Starting concentration of the test substrates were 1% (w/v). Standard deviation is shown in parentheses (n = 3). Different letters indicate significant differences ($p \leq 0.05$) for the same acid. Substrates were compared for three fermentation times (6, 24 and 48 h).

Figure Caption

Figure 1. Viable numbers of selected bacterial populations determined by FISH in fecal cultures using quinoa or amaranth as the carbon source. Controls did not contain added carbohydrates. Error bars indicate standard deviations (n=3). Different letters indicate significant differences between different substrates for each bacterial group. Initial bacterial counts: DAPI, 8.19 ± 0.13 log cells/mL; *Bifidobacterium* (Bif164), 7.53 ± 0.12 log cells/mL; *Lactobacillus/Enterococcus* (Lab158), 7.25 ± 0.15 log cells/mL; *Bacteroides/Prevotella* (Bac303), 7.76 ± 0.08 log cells/mL; *Clostridium histolyticum* clusters I and II (Chis 150), 7.45 ± 0.07 log cells/mL; *Atopobium* (Ato291), 7.32 ± 0.03 log cells/mL; *Clostridium coccoides/Eubacterium rectale* (Erec482), 7.76 ± 0.07 log cells/mL; *Faecalibacterium prausnitzii* (Fpra655), 7.67 ± 0.06 log cells/mL; *Roseburia intestinalis* (Rint623), 7.49 ± 0.02 log cells/mL.

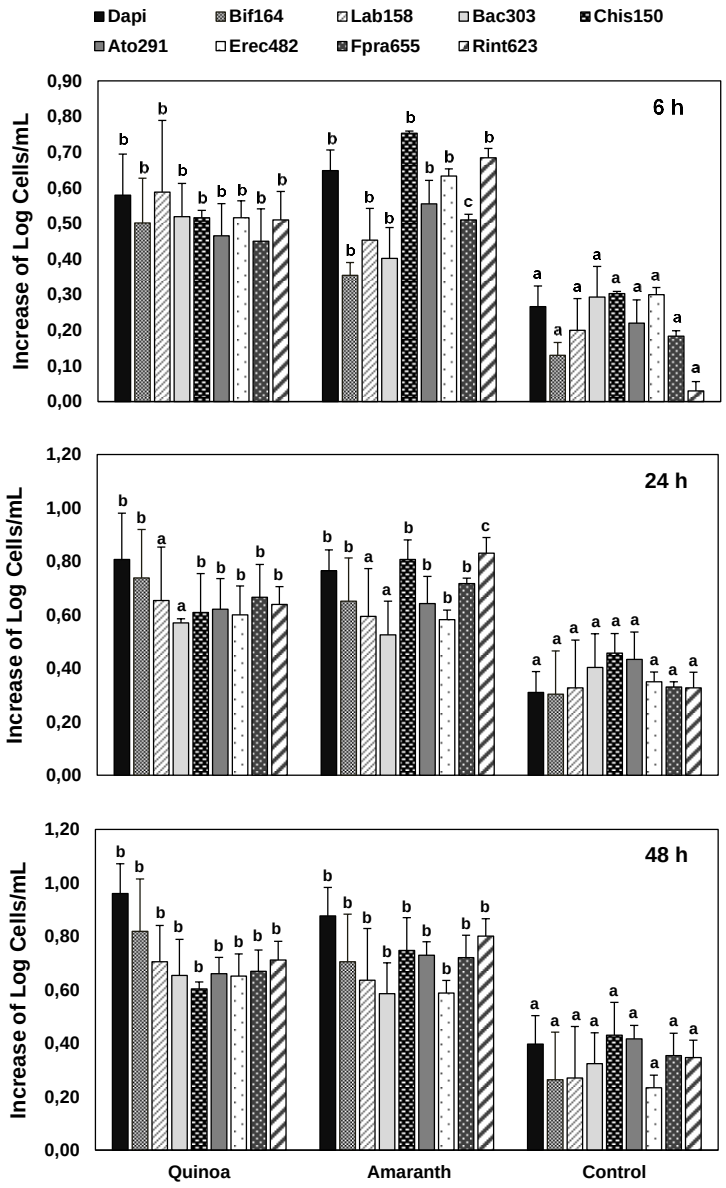
Dapi
Bif164
Lab158
Bac303
Chis150
Ato291
Erec482
Fpra655
Rint623
6 h
24 h
48 h
Increase of Log Cells/mL
Quinoa
Amaranth
Control

Figure 1