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The effect of *Acacia senegal* as potential prebiotic on obese gut microbiota

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Abstract

Gut microbiota is able to affect body weight by fermenting dried fibers and generating short chain fatty acids (SCFA). *Acacia senegal* is an edible dried which might have potential prebiotic activity. The aim of this study was to investigate the effect of *Acacia senegal* as potential prebiotic on the composition and activity of human colonic microbiota of obese healthy subjects. In order to identify a potential prebiotic effect of *Acacia senegal* on obese individuals, anaerobic, pH-controlled batch cultures system was applied. The growth of different microbes and the concentration of lactic acid and short chain fatty acids were studied at 0, 6, 12, 24 and 36 hrs using fluorescent in situ hybridization (FISH) and HPLC respectively. The results showed that *Acacia senegal* was able to simulate the microbiota population since it significantly increased (P>0.05) the population of *Bifidobacterium* and decreased the population of *Clostridium.* Moreover, the supplement of *Acacia senegal* to the models significantly modulate short-chain fatty acid concentration. These findings clearly identified *Acacia senegal* as an additional contributing factor to the gut microbiota of obese subject and suggest that *Acacia senegal* could be an interesting supplement for obese human which might help to control obesity.

1. Introduction

The prevalence of obesity is escalating in communities worldwide (Nguyen and El-Serag, 2010). It has been proposed that the current obesity epidemic may have been caused by a discrepancy between the physiological mechanisms for maintaining energy balance, and the composition of the current diet (Eaton and Konner 1985). During the last decade, however, diet has altered significantly to food with high energy density that has low dietary fiber, which led to low production of satisfaction and satiety signals (Prentice and Jebb 2003; Popkin *et al*., 2012).

Bodyweight may possibly be stimulated and managed by increasing the consumption of dietary fiber using a specific type of fiber. Several researchers claimed that large consumption of dietary fiber, such as gum arabic (GA) is linked with considerable and favorable impact on metabolism of fat (Ali *et al*., 2009; Slavin, 2003). GA, an edible dried sticky exudate from two types of *Acacia* (*Acacia seyal* and *Acacia senegal*) is rich in non-viscous soluble fiber (Slavin, 2013). In the area of food industry and pharmaceutical, GA has been widely used as an emulsifier and preservative (Ali *et al*., 2009). GA was used in both North Africa and Middle East as oral hygiene material (Tyler *et al.,* 1977). Successfully, dietary fiber showed a great ability to manage weight by promoting satiation and satiety (Chandalia *et al*., 2000). However, prebiotic has the greatest health benefits on human gut where it can modulate the population of salutary bacteria. In general, microbiota plays the main role in gut function (Van Nuenen *et al*., 2004). Generally, *Bifidobacteria* and *Lactobacilli* had significant path in preserving intestinal well-being attributable to their impact on the stability of the immune system (Peran *et al*., 2007; Sartor, 2008; Zeuthen *et al*., 2010) and their inhibiting efficacy on pathogens (Collado *et al*., 2007; Fukuda *et al*., 2011). Furthermore, for the desired health effect, the ingestion of sufficient and effective prebiotic is required (European Food Safety Authority, 2010). Postbiotics are the principle product of the probiotic metabolism of

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nondigestible carbohydrates in the gut and one of these major postbiotics is short chain fatty acids (SCFAs) (Wong *et al*., 2006; Lunn and Buttriss, 2007). The process of bacterial fermentation is an energy harvest system for undigested material, delivering energy that is impassable to be absorbed in the small bowel and is utilized as the main energy source for some species (McNeil, 1984; Popovich *et al*., 2005). Three acids namely (acetate, propionate and butyrate) are the major acids generated by the fermentation of bacteria (Cummings *et al.,* 1987). The different percentage and rate of SCFAs production during fermentation are dependent on the kind of prebiotic and the microbiotas (Brinkworth *et al*., 2009; Murphy *et al*., 2010). Furthermore, Reimer (2012) revealed that adult rat that devoured a weaning regimen supplied with prebiotic was preserved from obesity when compared with a western-style diet high in fat.

Proposed mechanism by which fiber may protect us against obesity is based on the beneficial impact that such kind of metabolites have on host energy balance, e.g. by mediating the secretion of gut hormones involved in the regulation of energy metabolism and food intake (including leptin, peptide YY and glucagon-like peptide-1) (Freeland and Wolever, 2010; Tarini and Wolever, 2010). Thus, high consumption of prebiotic would be a significant way to decrease the hazard of gaining weight (Liu *et al*., 2003; Anderson *et al*., 2009).

After all, SCFAs could only produce 5 to 10% of total dietary energy (Royall *et al.,* 1990). Nevertheless, neoteric study dug in such risk-reduction role. A growing body of evidence illustrated that the concentration of SCFAs varies among the source of microbiota, either from lean or obese individuals. Although it is well known that prebiotic affect obesity and performed in a broad domain of health benefits, further research is needed to fully elucidate the growth of gut microbiota and the production of SCFAs which led to different and numerical effects. However, published research often focuses on positive physiological impacts associated with diet supplementation. Nowadays, there is a number of *in vitro* studies mimicking colon fermentation from various sources of microbiota (lean, obese). Given this background, the present work aimed studying the effect of *Acacia senegal* as potential prebiotic on obesity by using colon model applied with faecal microbiota from obese individuals.

2. Material and methods

2.1 Samples

In this research, the gum arabic samples (*Acacia senegal*) was collected from a Sudanese market at North Kordofan. The positive control (Inulin) was from chicory root (Warcoing, Belgium).

2.2 Faecal inoculation

Four healthy male adult donors between the age of 20 to 30 years old and with BMI 30-35 kg/m was carefully selected. Before the beginning of the study, the donors have been checked for their health status. The donors did not take any antibiotic treatment and did not use any pre or probiotic diet since the last six months before the donation. Fresh faecal sample was assembled in 50 mL plastic sterile at University Putra Malaysia, Bintulu campus on the same day of the fermentation. Faecal inoculums were prepared by diluting the faecal with sterile phosphate buffered saline (PBS) (1:10 w/w) (0.1 M, pH 7.4) and then put in a stomacher at normal speed for 2 min (Stomacher 400, Seward, West Sussex, UK) for homogenization (Ahallil *et al*., 2018).

2.3 In vitro fermentation

The impact of *Acacia senegal* on the growth of microbiota from the gut and postbiotic production was carried out in customised glass vessels of anaerobic pH-controlled batch culture fermentation system. Aseptically 45 mL of pre-sterilized basal nutrient medium was filled in 100mL fermenter vessels (Sarbini and Rastall, 2011). The basal medium was prepared according to Sarbini and Rastall (2011). A day before the fermentation, the basal medium was persistently supplied with oxygen (15 mL/ min) and stirred overnight. The *Acacia senegal* and inulin were added with the concentration of 1% w/w (0.5g in 50 mL). Before the addition of the substrates, 10% of fresh inoculums (fecal slurry) were added. The circulating water bath was set at 37°C for all batch vessels. The pH was controlled at 6.8 using pH controller. The experiments were carried out for 36 hrs and the sampling for FISH and SCFA analysis was at 0, 6, 12, 24 and 36 hrs. Four replicates from four different fecal donors were used to performer this study.

2.4 Bacterial enumeration by fluorescence in situ hybridization (FISH)

Fluorescence *in situ* hybridization was used to enumerate the gut microbiota. Specific types of the 16 S ribosomal ribonucleic acid molecule bonded with synthetic oligonucleotide probes and labelled with the fluorescent dye Cy3 were used to count the different bacterial groups (Table 1). All samples were treated with filtered paraformaldehyde (pH 7.2) in a ratio of 1:3 (v/v) for 4 hrs in 4°C. Phosphate buffered saline with 0.1 m and pH 7.0 was used to wish the sample twice and then centrifuged at 13 000 x *g* for 5 mins. The cells were resuspended in phosphate buffered saline/ ethanol (99%)

Table 1. 16S rRNA oligonucleotide probes used in FISH analysis of bacterial populations.

Probe Code	Target group	Sequence (5'-3')	Reference
Chis150	Most of the *Clostridium histolyticum* group (Clostridium clusters I and II)	TTATGCGGTATTAATCTYCCTTT	Franks *et al.* (1998)
Lab158	Lactobacillus–Enterococcus	GGTATTAGCAYCTGTTTCCA	Harmsen *et al.* (1999)
Bac303	Most *Bacteroidaceae and Prevotellaceae,* some P*orphyromonadaceae*	CCAATGTGGGGGACCTT	Manz *et al.* (1996)
Bif164	*Bifidobacterium spp.*	CATCCGGCATTACCACCC	Langendijk *et al.* (1995)

at 1:1 (v/v) after which the pellets were stored at -20°C. In order to gain a computable number of bacteria in every slide, dilution of the cells was applied by using PBS. For the enumeration, 20 µL of each diluted sample was added to each well of six-well polytetrafluoroethylene/ poly-L-lysine-coated slide. Drying step with 46°C for 15 mins was applied to all slides. Dehydration with various concentration of ethanol (50, 80 and 96%) was also applied to all slide for 3 mins. The ethanol was then evaporated in oven dryer for 2 mins.

Hybridisations were carried out by adding 50 µL hybridisation mixture (which consisting of 45 µL hybridisation buffer) and 5 µL different probe (Chis150, Lab158, Bac303 and Bif164) to each single well and kept to hybridise for 4 hrs in a microarray hybridisation incubator. Next, hybridized slides were washed in washing buffer for 15 mins then followed with dipping in cool water for a few seconds and dried using compressed air. Before covering the slide with coverslip, 5 µL of polyvinyl alcohol was added for each and every well on the slide. The enumeration of each bacteria group was done by taking the average of 15 reading times from 15 different areas using epifluorescence microscope with reflected fluorescence attachment. (Sarbini and Rastall, 2011).

2.5 Analysis of lactic acid and short chain fatty acids

Ion-exclusion HPLC system (SHIMADZU SPD-20A) connected with UV-VIS detector was used to detect the short chain fatty acids namely (acetate, propionate and butyrate) and lactic acid. The column applied in this research was Ionexclusion Rezex ROA-Organic Acid H^+ (8%) column. The eluent utilized in this study was 0.0025 mmol/L of sulphuric acid. The samples were prepared by centrifuging all samples from batch culture vessels for 10 mins at 13 000 x *g* then the supernatant filtered with 0.2 mm polycarbonate filters. A total of 15 µL of the filtered supernatants was immediately injected into an HPLC-UV system. For HPLC, the column was heated to 40°C and the flow rate at 0.5 mL/min. The calculation of the SCFAs concentration was done using calibration curves generated using external standards namely lactate, acetate, propionate, and butyrate.

2.6 Statistical analysis

The data were statistically analyzed using SPSS 23 software with two-way ANOVA and one-way ANOVA. Duncan test was applied to study the significant differences ($p<0.05$) between.

3. Results and discussion

FISH analyses were performed to enumerate different bacterial species in fermented substrates collected from the study group. Statically, there was a significant difference between the samples types and also the fermentation time (Table 2).

Table 2. Mean value of *Bifidobacterium* (Bif) population (log_{10} cells/mL batch culture fluid) in the colon model at 0, 6, 12, 24 and 36 hrs inoculated with feces microbiota from obese.

Bif	Senegal	Inulin	Control
0 hr	7.83 ± 0.21^{ef}	7.83 ± 0.21^{ef}	7.83 ± 0.21^{ef}
6 hrs	8.18 ± 0.13^{de}	8.45 ± 0.49^{bcd}	8.07 ± 0.19^{de}
12 hrs	8.29 ± 0.15^{cd}	8.9 ± 0.3^{a}	8.06 ± 0.18^{de}
24 hrs	8.61 ± 0.13^{abc}	9.02 ± 0.28^{a}	7.85 ± 0.23^{ef}
36 hrs	8.82 ± 0.3^{ab}	8.76 ± 0.15^{ab}	7.59 ± 0.46^{f}

Values are expressed as mean±SD (n = 4). Different superscript letters are significantly different ($P<0.05$).

The growth of *Bifidobacterium* was not significant at the first 6 hrs of fermentation (8.18 log_{10}), but after 12, 24 and up to 36 hrs of *Acacia senegal* fermentation, the growth was significant at 8.29 log_{10}, 8.61 log_{10} and 8.82 log_{10} respectively (Table 2). Inulin showed different contribution by the obese microbiota, a significant increase of *Bifidobacterium* was observed from the first 6 hrs (8.45 log_{10}) of incubation up to 12, furthermore, 36 hrs of fermentation did not produce any significant change. Comparing between samples, after 6 hrs of fermentation no significant difference was observed. At 12 hrs, inulin showed a higher ($p<0.05$) growth compared to *Acacia senegal* and control. Furthermore, up to 36 hrs showed sample produced growth not different from inulin. This shows *Acacia senegal* has the potential to match inulin as prebiotic. In general, the findings of this study were in line with Kalliomaki *et al*. (2008) who stated that; a minimum population of *Bifidobacteria* at childbirth has been linked with gaining weight later in childhood. Furthermore, Collado *et al*.

(2010) claimed that overweight motives procreate neonates that have a lessened population of *Bifidobacteria*, which refer to that obesogenic microbiota is an 'inheritable' trait.

Selectively, the growth of *Bifidobacteria* was spotted to significantly expand in obese individuals after the fermentation of *Acacia senegal* and inulin. As obtained by Korpela *et al*. (2014), at the beginning of an intrusion there was little the abundance of *Bifidobacterium* spp. the high growth was optioned after the administration of prebiotic. Additionally, Rycroft *et al*. (2001) and Roberfroid *et al*. (1998) early noted such inverse relationship for different periodic such as FOS. Inulin also showed a great increase in *Bifidobacteria* (Ahallil *et al*., 2018). Phenomenal growth of *Bifidobacterium spp* has been spotted in obese mice fed with specific diet enhanced with inulin (Cani *et al*., 2009). FISH analyses of the microbiota proved the ability of *Acacia senegal* to modulate the colon.

Bacteroide showed the difference in growth between samples and fermentation (Table 3). There was no significant growth of *Bacteroidaceae* from 0 hr up to 12 hrs of *Acacia senegal* fermentation. The significant growth of *Bacteroidaceae* only observed after 24 hrs of *Acacia senegal* fermentation. Simultaneous, the fermentation of inulin illustrated that *Bacteroidaceae* significantly increase ($P<0.005$) from the first 12 hrs compared to 0 hr (Table 3). However, comparing between samples, there was no significant growth of of Bacteroidaceae at 6 hrs of inoculation. But at 12 hrs, inulin showed a higher ($p<0.05$) growth of *Bacteroidaceae* compared to *Acacia senegal* and control. However, there was no significant difference in the growth of *Bacteroidaceae* between *Acacia senegal* and inulin at 24 hrs and 36 hrs. *Bacteroidetes* showed significant growth in distal colon system when treated with *Acacia senegal*, these finding in agreement with Terpend *et al*. (2013) who illustrated high growth of *Bacteroidetes* in mice when the fed with 10 g of gum arabic per day for 4 weeks. From the result, it can be noted that fermentation of inulin was higher and faster than *Acacia senegal* on promoting the growth of *Bifidobacterium* and *Bacteroidetes*. The fermentation rate of GA was slow as compared with inulin that is because of the molecular weight of the substrates.

Table 3. Mean value of *Bacteroidaceae* (Bac) population (log_{10} cells/mL batch culture fluid) in the colon model at 0, 6, 12, 24 and 36 hrs inoculated with feces microbiota from obese.

Bac	Senegal	Inulin	Control
0 hr	8.28 ± 0.18^{ef}	8.28 ± 0.18^{ef}	8.28 ± 0.18^{ef}
6 hrs	8.38 ± 0.19^{def}	8.57 ± 0.14^{bcde}	8.42 ± 0.16^{cdef}
12 hrs	8.46 ± 0.15^{cdef}	8.87 ± 0.11^{ab}	8.36 ± 0.15^{def}
24 hrs	8.65 ± 0.23^{abcd}	8.91 ± 0.3^{a}	8.17 ± 0.12^{fg}
36 hrs	8.72 ± 0.28^{abc}	8.86 ± 0.3^{ab}	7.95 ± 0.2^{g}

Values are expressed as mean±SD (n = 4). Different superscript letters are significantly different ($P<0.05$).

The growth of lactobacilli during the fermentation of *Acacia senegal* and inulin showed in Table 4. Comparing to 0 hr, lactobacilli did not grow with *Acacia senegal*. However, comparing between samples, the growth of lactobacilli was not significantly different at 6 hrs, 12 hrs and 24 hrs. But at 36 hrs, it was significantly high in growth with *Acacia senegal* and inulin. There was significantly low growth of lactobacilli which only noted 36 hrs of fermentation. As observed by Sarbini *et al.* (2014), oligosaccharides with lower molecular-weight might be quickly metabolized than the oligosaccharides with high molecular-weight. This may be due to non-reducing ends of the carbohydrate which preferred by lactobacilli (Gibson *et al*., 2004; Sarbini and Rastall, 2011).

There was a slight increase in *Clostridium* growth at first 6 hrs of *Acacia senegal* fermentation compared to 0 hr, and after 12 hrs of fermentation the growth dramatically decreased and showed no significant different with 0 hr (Table 5). Moreover, the growth of *Clostridium* during the fermentation of inulin was significant at 12 hrs and 24 hrs compared to 0 hr, but at 36 hrs showed no significant difference compared to 0 hr. However comparing between samples, there was no significant difference during all fermentation times. These findings are also in line with the results from previous in vivo studies (Wyatt *et al*., 1986; Cherbut *et*

Table 4. Mean value of lactobacilli (Lab) population (log_{10} cells/mL batch culture fluid) in the colon model at 0, 6, 12, 24 and 36 hrs inoculated with feces microbiota from obese.

Lab	Senegal	Inulin	Control
0 hr	7.95 ± 0.3^{cd}	7.95 ± 0.3^{cd}	7.95 ± 0.3^{cd}
6 hrs	8.02 ± 0.27^{bcd}	8.26 ± 0.36^{abc}	8.11 ± 031^{abcd}
12 hrs	8.24 ± 0.22^{abc}	8.45 ± 0.27^{ab}	8.09 ± 0.24^{bcd}
24 hrs	8.32 ± 0.16^{abc}	8.57 ± 0.35^{a}	7.93 ± 0.28^{cd}
36 hrs	8.36 ± 0.19^{abc}	8.36 ± 0.29^{abc}	7.74 ± 0.28^{d}

Values are expressed as mean±SD (n = 4). Different superscript letters are significantly different ($P<0.05$).

Table 5. Mean value of *Clostridium* (Chis) population (log_{10} cells/mL batch culture fluid) in the colon model at 0, 6, 12, 24 and 36 hrs inoculated with feces microbiota from obese.

Chis	Senegal	Inulin	Control
0 hr	7.33 ± 0.12^{de}	7.33 ± 0.12^{de}	7.33 ± 0.12^{de}
6 hrs	7.76 ± 0.13^{a}	7.58 ± 0.12^{abcd}	7.5 ± 0.2^{abcd}
12 hrs	7.59 ± 0.14^{abcd}	7.73 ± 0.29^{ab}	7.52 ± 0.03^{abcd}
24 hrs	7.48 ± 0.18^{bcde}	7.68 ± 0.2^{abc}	7.41 ± 0.03^{de}
36 hrs	7.42 ± 0.22^{cde}	7.59 ± 0.17^{abcd}	7.22 ± 0.05^{e}

Values are expressed as mean±SD (n = 4). Different superscript letters are significantly different ($P<0.05$).

Table 6. Mean value of lactate concentration (mM) in the colon model at 0, 6, 12, 24 and 36 hrs inoculated with feces microbiota from obese individuals.

Lactate	Senegal	Inulin	Control
0 hr	76.3 ± 6.7^{a}	76.3 ± 6.7^{a}	76.3 ± 6.7^{a}
6 hrs	2.78 ± 1.5^{bc}	15.8 ± 6.7^{bc}	2.75 ± 1.17^{bc}
12 hrs	1.69 ± 0.9^{bc}	6.68 ± 3.03^{bc}	7.08 ± 6.06^{bc}
24 hrs	2.03 ± 1.6^{bc}	14.59 ± 4.62^{bc}	2.36 ± 0.2^{bc}
36 hrs	1.16 ± 0.6^{c}	13.87 ± 3.7^{bc}	1.03 ± 0.57^{c}

Values are expressed as mean±SD (n = 4). Different superscript letters are significantly different (P<0.05).

Table 7. Mean value of acetate concentration (mM) in the colon model at 0, 6, 12, 24 and 36 hrs inoculated with feces microbiota from obese individuals.

Acetate	Senegal	Inulin	Control
0 hr	11.05 ± 2.77^{g}	11.05 ± 2.77^{g}	11.05 ± 2.77^{g}
6 hrs	50.7 ± 4.06^{defg}	73.3 ± 18.3^{bcd}	35.14 ± 6.8^{efg}
12 hrs	58.19 ± 3.13^{cdef}	114.2 ± 18.5^{ab}	32.5 ± 6.7^{bc}
24 hrs	84.01 ± 5.2^{bcd}	133.3 ± 28.9^{a}	18.9 ± 4.5^{fg}
36 hrs	98.6 ± 19.1^{abc}	110 ± 26.5^{ab}	19.5 ± 3.5^{fg}

Values are expressed as mean±SD (n = 4). Different superscript letters are significantly different (P<0.05).

al., 2003; Calame *et al*., 2008). *C. histolyticum* group of bacteria has occasionally been associated with inflammation and large bowel disease (Gibson and Roberfroid, 2008; Hughes, 2008).

3.1 Changes in SCFA concentrations

The production of lactic acid as the final postbiotic product of the fermentation of both *Acacia senegal* or inulin shown in Table 6. Table 6 shows that during the fermentation time from 0 to 6 hrs lactate concentration significantly reduced (p<0.05) for all samples. However, the fermentation time from 6 to 36 hrs did not produce any significant changes in lactate concentration for all samples. Comparing between all samples for each fermentation time showed no significant difference.

The concentration of acetate in the fermentation of *Acacia senegal* did not significantly increase at the first 6 and 12 hrs compared to 0 hr (Table 7). An increase in acetate concentrations in the fermentation of *Acacia senegal* was observed only after 12 hrs. In the fermentation of inulin, acetate showed a significant increase (P<0.05) at the first 6 hrs (73.3 mmol/L) of inoculation. At 6 hrs of fermentation, no significant difference observed for acetate concentration between sample and inulin. Inulin was significantly high (P<0.05) than *Acacia senegal* in the production of acetate after 12 and 24 hrs of fermentation, whereas at 36 hrs there were no significant differences in acetate concentration among both samples. As can be seen from the results, the main production of *Acacia senegal* and inulin fermentation was acetate and followed by propionate. Recently it has been proved by Fukuda *et al.* (2011), that the production of acetate enhanced intestinal defence mediated by epithelial cell, preventing the host from the infection of enteropathogenic.

Propionate concentration in the colon model fed with either *Acacia senegal* or inulin at 0, 12, 24 hrs showed no significant difference compared to 0 hr (Table 8). At 36 hrs, *Acacia senegal* showed significantly high production of Propionate. Comparing between samples, there was no significant difference in Propionate production at 0, 6, 12 hrs of fermentation. However, at 24 hrs, the concentration of Propionate in both *Acacia senegal* and inulin were significantly higher than control. But at 36 hrs, *Acacia senegal* significantly had the highest concentration of Propionate.

Table 8. Mean value of propionate concentration (mM) in the colon model at 0, 6, 12, 24 and 36 hrs inoculated with feces microbiota from obese individuals.

Propionate	Senegal	Inulin	Control
0 hr	23.2 ± 7.4^{bcd}	23.2 ± 7.4^{bcd}	23.2 ± 7.4^{bcd}
6 hrs	23.4 ± 4.1^{bcd}	22.4 ± 4.4^{bcd}	17.5 ± 2.5^{cd}
12 hrs	24.7 ± 3.9^{bcd}	38 ± 7.2^{bc}	19.04 ± 3.9^{cd}
24 hrs	39.4 ± 4.4^{bc}	45.5 ± 13.8^{ab}	13.2 ± 4.7^{d}
36 hrs	64.5 ± 9.1^{a}	39.3 ± 10.6^{bc}	10.3 ± 2.7^{d}

Values are expressed as mean±SD (n = 4). Different superscript letters are significantly different (P<0.05).

In spite of that majority of researches about the anti-inflammatory concept of SCFA centered on the impact of acetate (Al-Lahham *et al*., 2010; Roelofsen *et al*., 2010), while there is significant prove presenting propionate as a metabolite with a dynamic path against inflammation (Al-Lahham *et al*., 2012). On the one hand, propionate plays as a ligand of G-protein-couple receptors (GPCR) 41 and 43 (Brown *et al*., 2003). These receptors when stimulated or activated, lead to significant production of GLP-1 (which could simulate satiety and decrease the gastric emptying) and PYY (be able to control the absorption and digestion of food). Moreover, Maslowaski *et al*. (2009) found that the drop or lost of GPCR led to serious inflammation. Furthermore, the reduction in plasma levels was noted with the increasing of propionate (Al-Lahham *et al*., 2010).

The rise in propionate percentage in the fermentation of arabinogalactan treated with obese microbiota proposed that such metabolite of arabinogalactan could prevent inflammation and enhance satiety in obese individuals (Aguirre *et al*., 2016). So, the increase in propionate and acetate from the fermentation of *Acacia senegal* may help controlling obesity. Likewise, the chemical structure and composition of gum arabic were probably accountable for SCFA production. Gum arabic,

singularly *Acacia senegal*, be able to modify propionate ratios, and this finding is in agreement with the finding from previous researchers (Walter *et al.,* 1988; May *et al*., 1994; Annison *et al*., 1995). Together with observations from Annison (1995) results assumed that the increases in propionate production most probably relate to galactose content.

As can be seen from the result, *Acacia senegal* had no effect on the production of butyrate. Butyrate showed no appreciable production in the inoculation of both *Acacia senegal* or inulin gum (Table 9). This may be owed to the declined growth of *Clostridium* in the fermentation of both *Acacia senegal* and inulin as *Clostridium* is known to contribute a major fraction of butyrate production (Louis *et al*., 2010). Butyrate served as the major driving source for the intestinal epithelial cells and plays a significant role in the regulation of cell proliferation and differentiation (Topping and Clifton, 2001).

Table 9. Mean value of butyrate concentration (mM) in the colon model at 0, 6, 12, 24 and 36 hrs inoculated with feces microbiota from obese individuals.

Butyrate	Senegal	Inulin	Control
0 hr	24.14±2.3[a]	24.14±2.3[a]	24.14±2.3[a]
6 hrs	29.15±10.3[a]	34.2±7.0[a]	21.9±8.03[a]
12 hrs	19.8±8.6[a]	29.61±10.6[a]	25.5±17.9[a]
24 hrs	24.06±8.7[a]	26.12±7.9[a]	17.6±6.3[a]
36 hrs	14.4±2.3[a]	28.3±6.9[a]	16.05±5.2[a]

Values are expressed as mean±SD (n = 4). Different superscript letters are significantly different (P<0.05).

Based on Figure 1 acetate: propionate (A/P) rates were noted to be decreased at 24 hrs and 36 hrs during the fermentation of *Acacia senegal*. Acetate and propionate play a significant rule in stimulating lipid synthesis (Delzenne and Cani, 2011). As acetate concentrations are higher than those of propionate it is speculative that the ratio of SCFA produced by the obese microbiota is in a sense not "protective" against excess lipid production.

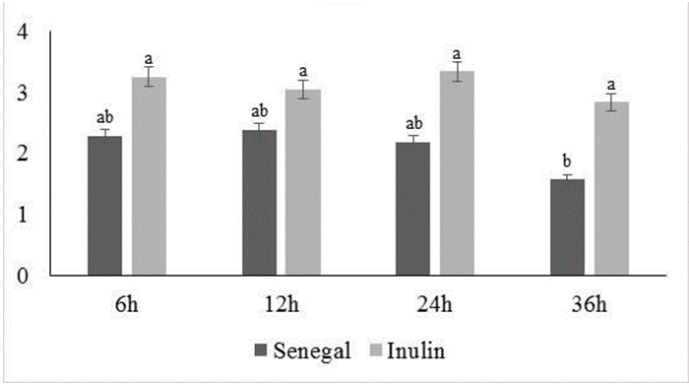

Figure 1. Acetate: propionate rates (mmol) in pH-controlled batch cultures at 0 ,6, 12, 24 and 36 hrs (n = 4). Samples with different letters are statistically different (p<0.05).

In vitro fermentation of obese microbiotas fed with *Acacia senegal* showed no significant increase in total SCFAs during the first 6 h for all samples (Figure 2). For both *Acacia senegal* and inulin compared to 0 hr, increasing the fermentation time up to 36 hrs resulted in a significantly higher (p<0.05) total SCFA. Thus, higher fermentation time resulted in higher total SCFA production. Interestingly, total SCFA produced in both fermentation (*Acacia senegal* and inulin) at 24 and 36 hrs did not show any significant difference among both substrates.

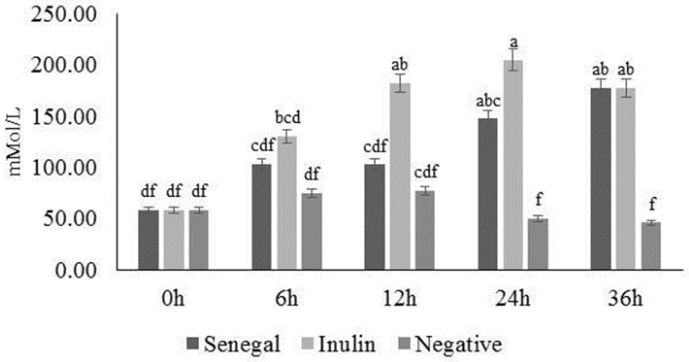

Figure 2. Sum (total) of SCFA obtained after 36 hrs fermentation experiments of *Acacia senegal* and inulin using obese microbiota. Samples with different letters are statistically different (p<0.05).

It can also be observed that there is a slow production of SCFAs during the fermentation of *Acacia senegal* whereas inulin illustrated much higher production of SCFAs. This might be linked to the growth of bacteria which was slow during the early hours of fermentation and then started to increase. By looking at the molecular-weight, gum arabic has complex mixture of heteropolysaccharide with high molecular-weight, whereas inulin is lower in molecular-weight which showed differences in the production of SCFA. However, this observation is in line with the finding of Sarbini et al. (2014), where the oligosaccharides with small molecular-weight are much faster fermented than oligosaccharides with bigger molecular-weight.

4. Conclusion

This work suggests that *Acacia senegal* exert an interesting complementary effect. In fact, *Acacia senegal* changed the colon gut microbiota of obese individuals. *Acacia senegal* showed increased in bifidobacteria growth in obese individuals. It not only stimulates the bifidobacteria growth but also increases their acidifying activity. However, the growth of probiotic forms SCFAs which in turn have actions at the gut system and a beneficial effect on obesity. The results indicate that *Acacia senegal* could be a potential novel prebiotic to control obesity.

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