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Aloe arborescens supplementation in cat diet: evaluation of effects by *in vitro* gas production technique

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ABSTRACT

The aim of the present study was to evaluate the effects of *Aloe arborescens* on organic matter digestibility (OMD), cumulative gas (OMCV) and short chain fatty acids (SCFA) production, using the *in vitro* gas production technique (IVGPT). Three adult cats were fed with a commercial diet (CP 31.21; EE 16.64% as fed) for 20 days before the collection of their faeces used as *inoculum*. The same diet, used as substrate, was incubated *in vitro* supplemented with different amounts (0, 0.7, 1.6 and 3.2%) of lyophilised *Aloe arborescens*. OMD, OMCV and SCFA significantly decreased with the increase of Aloe addition; an increase of L-lactic acid production was detected, even if pH was within physiological range. A potential prebiotic role of the *Aloe arborescens* carbohydrates was hypothesised in cats, but it needs further investigations. As a whole, our results show that IVGPT can represent a useful tool for nutritional evaluation of novel ingredient and/or additive also in cats.

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Aloe arborescens; digestibility; IVGPT; L-lactic acid; short chain fatty acids

Introduction

Since many years, the *in vitro* gas production technique (IVGPT) is commonly used to assess the effects of diet changes on fermentation pathways in ruminants, whereas it has been used in carnivores to study fibre source in digestive processes (Sunvold et al. 1995a). Aloe plant contains anthraquinone, glycosides, mucilages, resinous materials, sugars, mucopolysaccharides, fatty acids, glycoproteins, enzymes, vitamins and minerals (Vogler & Ernst 1999; Eshun & He 2010), thus, being particularly rich in carbohydrates, IVGPT studies can provide useful information for an *in vitro* screening of carbohydrates fermentability, as already shown in dogs (Cutrignelli et al. 2007; Bosch et al. 2008). Indeed, the healing properties of Aloe are due to the mucilaginous polysaccharides contained in the gel pulp, but other beneficial effects of Aloe may be due to other carbohydrates and, as a consequence to their fermentability.

Different properties of *Aloe* spp., including wound healing, anti-parasitic, anti-viral, anti-fungal and anti-bacterial, have widely been reported (Boudreau & Beland 2006). Immune-stimulating (Valle-Paraso et al. 2005; Infascelli et al. 2010), anti-proliferative (Di Luccia et al. 2013) and cholesterol-lowering (Tizard et al. 1989) activities have also been shown.

As seen, most of the health benefits associated with *Aloe* spp. have been attributed to the polysaccharides contained in the gel of the leaf and many of its effects may derive from its activity on intestinal function, thus influencing absorption and availability of key substances (Sharma et al. 2014). However, natural products, originating from plants, have an immensely diverse array of structures, which may serve as possible lead compounds for further development into therapeutic treatments and they may be considered as an alternative to the routine pharmaceutical approach (Yarnell 2007; Eloff & Mcgaw 2008).

Proximate analysis, including total dietary fiber (TDF) evaluation (de-Oliveira et al. 2011), gives useful information on the feedstuff and diet characteristics in terms of amount of potential nutrients, but does not provide any information about their utilisation by the microorganisms of the gastrointestinal tract. Therefore, the combination of chemical composition parameters with *in vitro* fermentation characteristics can provide complementary information about the carbohydrates utilisation, in terms of extent and kinetics (Williams et al. 2001; Cutrignelli et al. 2009).

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Therefore, the aim of this study was to evaluate the effects of *Aloe arborescens* supplementation on the fermentation pathway of a commercial diet for adult cat using the IVGPT.

Materials and methods

An extruded diet alone or with addition of three different levels (0.7, 1.6 and 3.2%) of *Aloe arborescens* powder (whole lyophilised plant; HDR Company, Capriati al Volturno, Caserta, Italy) was analysed for crude protein, ether extract and ash (AOAC 2005), starch (Martillotti et al. 1987), the reserve carbohydrates were calculated from difference: [100 – (water – CP – EE – TDF – Ash)]. TDF, soluble dietary fibre (SDF) and insoluble dietary fibre (IDF) contents (Prosky 1990; Lee & Prosky 1995).

The supplementation ratios (0.7, 1.6 and 3.2%) were calculated halving and doubling the oral dosage suggested by the producer for adult cats with 3.5 kg body weight.

The four diets were tested by *in vitro* gas production using cat faeces as *inoculum* (Sunvold et al. 1995b).

Three European adult neutered cats (3–4 years old) were used as faeces donors. All animals were healthy; a clinical visit including stool examination was performed before the onset of the experiment. The cats were progressively adapted to the standard diet [100 kcal of Metabolisable Energy/kg of Metabolic Weight ($kg^{0.67}$)]. After 20 days, faeces were collected into thermostated boxes (39 °C) under anaerobic condition. The four diets were weighed (0.5 ± 0.001 g) in 120 ml serum bottles containing a buffer solution (Bauer et al. 2001). A pool of faecal samples was diluted with NaCl solution (1:10), homogenised, filtered and added to the serum flasks (5 ml). The bottles were incubated at 39 °C under anaerobic condition for 48 h. Each substrate was incubated in quadruplicate. Four bottles without substrate were used as blank.

Gas production was recorded every 2 h using a manual pressure transducer (Cole and Parmer Instrument Co, Vernon Hills, IL). After 48 h of incubation, the fermentation was stopped by cooling, and the fermenting liquor was analysed for pH (Alessandrini Instrument glass electrode; Jenway, Dunmow, UK; model 3030), short chain fatty acids (SCFA) and L-lactic acid. For SCFA determination, the sample was centrifuged twice at 12,000 *g* for 10 min at 4 °C and 1 ml of supernatant was taken and mixed with 1 ml of oxalic acid (0.06 mol). The SCFA were measured by gas chromatography (ThermoQuest Italia SpA, Rodano, Milan, Italy; model. Focus, fused silica capillary column 30 m × 0.25 mm × 0.25 µm film thickness) comparing samples peaks area of each SCFA with the corresponding of an external standard composed by acetate, propionate, butyrate, iso-butyrate, valerate and iso-valerate (Calabrò et al. 2013a). L-Lactic acid was determined by spectrophotometer using a colorimetric kit (L-Lactic acid – UV method – Boehringer Mannheim/R-Biopharm. Enzymatic BioAnalysis/Food Analysis).

Organic matter digestibility (OMD) was determined by filtering under vacuum on pre-weighed glass crucibles (Scott Duran, #2) the fermentation residues, which was dried at 103 °C and burned at 550 °C. Gas volumes recorded during the fermentation were related to the quantity of incubated OM (organic matter cumulative volume, OMCV).

All statistical analyses were performed by SAS (SAS, 2000): the influence of Aloe addition on the *in vitro* parameters was tested by ANOVA, using the PROC GLM; the correlation between chemical composition data and fermentative parameters were analysed by PROC COR of the same software.

Table 1. Chemical composition, % of tested diets.

	Diet 0	Diet 0.7	Diet 1.6	Diet 3.2
CP	31.21 ± 3.74	31.09 ± 4.35	30.95 ± 4.02	30.69 ± 4.91
EE	16.74 ± 1.51	16.59 ± 1.16	16.45 ± 0.99	16.20 ± 1.62
Starch	30.99 ± 3.72	30.77 ± 4.92	30.63 ± 0.65	30.00 ± 4.50
Ash	7.00 ± 0.78	7.15 ± 0.88	7.21 ± 0.89	7.34 ± 1.06
NFE	8.28 ± 0.91	7.85 ± 0.97	7.59 ± 0.94	7.45 ± 1.02
TDF	29.61 ± 5.12	29.65 ± 5.43	29.72 ± 4.95	29.84 ± 4.37
IDF	21.98 ± 2.06	22.05 ± 2.67	22.06 ± 1.93	22.17 ± 3.19
SDF	7.58 ± 0.91	7.62 ± 1.17	7.64 ± 1.07	7.81 ± 0.84

CP: crude protein; EE: ether extract; NFE: nitrogen-free-extract; TDF: total dietary fiber; IDF: insoluble dietary fiber; SDF: soluble dietary fiber.

Results

The chemical composition of *Aloe arborescens* (CP: 15.30, EE: 1.08, Ash: 14.4, CF: 11.50% as fed) and diets (Table 1) confirm the high concentration of both structural and reserve carbohydrates. On the other hand, no differences among diets were detected for the fibre fractions with all the doses of Aloe tested.

Mean values of the *in vitro* organic matter disappearance, cumulative gas production and SCFA are reported in Table 2.

The supplementation significantly decreased OMD and SCFA ($p < 0.01$) as well as OMCV ($p < 0.05$). Comparing diet 0 with diet 3.2, reductions of 27, 14 and 27% were observed for OMD, OMCV and total SCFA, respectively. As concerns SCFA, 3.2% supplementation reduced by 40% the acetate production compared to diet 0. By contrary, a significant ($p < 0.01$) increase, up to 50% in the diet 3.2, was detected for iso-valerate. Propionate, iso-butyrate, butyrate and

Table 2. *In vitro* fermentation parameters and end-products after 48 h of incubation.

		Diet 0	Diet 0.7	Diet 1.6	Diet 3.2
OMD	%	83.98 ± 0.56A	62.89 ± 0.86Ba	61.96 ± 1.20Ba	60.44 ± 0.28Bb
OMCV	ml/g iOM	90.39 ± 10.08a	85.87 ± 4.85ab	77.32 ± 4.84b	78.95 ± 8.52b
pH		6.88 ± 0.03A	6.69 ± 0.02B	6.74 ± 0.02B	6.78 ± 0.01B
Acetate	Mmol/iOM	10.94 ± 0.86Aa	8.49 ± 2.45ABb	7.31 ± 0.7B	6.63 ± 0.61B
Propionate	Mmol/iOM	3.33 ± 0.27	3.15 ± 0.9	3.05 ± 0.35	3.68 ± 0.39
Iso-butyrate	Mmol/iOM	0.135 ± 0.08	0.110 ± 0.28	0.094 ± 0.36	0.115 ± 0.05
Butyrate	Mmol/iOM	1.28 ± 0.02	1.16 ± 0.01	1.22 ± 0.01	1.08 ± 0.01
Iso-valerate	Mmol/iOM	0.036 ± 0.001B	0.055 ± 0.01AB	0.060 ± 0.01A	0.072 ± 0.01A
Valerate	Mmol/iOM	1.37 ± 0.49	1.30 ± 0.06	1.35 ± 0.44	0.99 ± 0.2
SCFA	Mmol/iOM	17.01 ± 0.85Aa	14.41 ± 3.62Ab	13.08 ± 1.70Ba	12.43 ± 0.85Bb
L-Lactic acid	Mmol/iOM	0.08 ± 0.03c	0.21 ± 0.04a	0.17 ± 0.03b	0.19 ± 0.05ab

OMD: organic matter digestibility; iOM: incubated organic matter; OMCV: organic matter cumulative volume; SCFA: short chain fatty acid.
A, B = $p < 0.01$; a, b, c = 7 $p < 0.05$.

valerate production were not significantly affected by Aloe supplementation.

A significant ($p < 0.05$) increase of L-lactic acid was detected after Aloe supplementation. The pH values ranged from 6.69 to 6.88 and significantly ($p < 0.01$) decreased after Aloe addition. Significant correlations ($p < 0.01$) were detected between L-lactic acid and CP ($r = -0.835$), EE ($r = -0.842$) and NFE ($r = -0.834$).

Discussion

Aloe arborescens showed a carbohydrate composition (TDF 29.62, IDF 21.98, SDF 7.58% as fed), which could explain the different results, obtained by IVGPT. The Aloe addition significantly ($p < 0.01$) reduced the OMD. Such result is probably due to the Aloe unfermentable carbohydrates content that probably affected the cumulative volume of gas produced (Sunvold et al. 1995b). This effect should be responsible for the laxative effect (Wenk 2001) of Aloe thus suggesting that the assessment of the suitable dose may be critical when using Aloe as a nutritional additive in cats. In any event, digestibility showed acceptable levels (60% OM) even after Aloe supplementation at the doses used in this trial.

The changes detected for OMD and OMCV were confirmed by a linear decrease of SCFA (Sunvold et al. 1995b), mainly due to a decrease of acetate. In contrast, a significant increase of iso-valerate, the less representative SCFA, was detected. These results may be due to changes in bacterial activity induced by Aloe supplementation. Anyway, butyrate (important energy source for the colon epithelium and regulator of cell growth and differentiation; Salminen et al. 1998) was not affected by Aloe supplementation and SCFA were always represented by more than 80% of acetate plus propionate. Both these results suggest a physiologic trend of gut fermentation (Williams et al. 2001).

A significant increase of L-lactic acid was detected after Aloe supplementation, thus suggesting a possible flattening of the other fermentation pathways. On the whole, these results are suggestive of a higher proliferation of lactic acid bacteria (LAB), which have been indicated as the main probiotic genera for healthy intestine of mammals (Maskell & Johnson 1993). The ability of Aloe in improving LAB activity during digestive processes may explain many of its beneficial properties (Salminen et al. 1998), but further studies focalised on the identification of gut microbiota are necessary.

The diet 3.2 showed the highest values of L-lactic acid and the lowest pH, while the lowest lactic acid concentration and the highest pH values were obtained incubating diet 0. In any event, the pH values were included in the range (5.5–7.5) indicated by Younes et al. (2001) as the physiological pH of colonic contents and resulting faeces in several animal species, including feline and canine.

The present data are in discordance with those obtained in a previous study, where the effect of Aloe supplementation was tested *in vitro* using rumen liquor as *inoculum* and diets for ruminant as substrates (Calabrò et al. 2013b). These differences are clearly due to the differences in microorganism population, thus suggesting that the *IVGPT* can be a useful tool to investigate gut microbiota activity also in carnivores.

Conclusions

The study of fermentation characteristics is important to understand the functional activity of innovative diets and/or feeds. The *IVGPT* seems particularly useful to this aim, since it allows the evaluation of both degradation extent and end-products of microorganism activities.

This study suggests that *Aloe arborescens* modifies fermentation pathways in cat hindgut, but the assessment of the right dosage seems to be critical to ensure a healthy effect. In conclusion, Aloe supplementation

did not reveal beneficial effects in cats, but the literature in carnivores is still poor and merits further studies focalised on the identification of gut microbiota and their relation with the Aloe supplementation.

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Disclosure statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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