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***In vitro* evaluation of MOS supplementation in diets for dog**

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Introduction: Several active substances (i.e. minerals, vitamins, proteins, pre and probiotics, etc.) are used in feed and food industry¹. These substances are often very sensitive to high temperature, moisture or oxygen. Several attempts to preserve their activity during production and storage or to keep their effect in specific portion of digestive tract have been made, including their encapsulation into various carriers². Mannano-oligosaccharides (MOS) are considered prebiotic, for various species, including dogs³. The efficiency of encapsulation in preserving MOS during the extrusion process and into the first part of digestive tract was studied by an *in vitro* assay of digestibility and fermentation.

Material and methods: A standard diet (CP: 18; EE: 15, CF: 1.2% a.f., ME 15.73 MJ/kg) was used as control (CTR). The CTR diet was supplemented with 1% of MOS, encapsulated (EN) or in powder (PO), before and after the extrusion process (EX). The five diets were tested *in vitro* by "two consecutive steps": 1st - evaluation of *in vitro* enzymatic digestion (ED)⁴, using in sequence 1% pepsin (pH 2; 2h at 39°C) and 1% pancreatin (pH 6.8; 4h at 39°C); 2nd - evaluation of fermentation characteristics, incubating the diets at 39°C under anaerobic conditions for 48h using the *in vitro* gas production technique (IVGPT)⁵, with a bacterial inoculum composed by buffered dog faeces. The gas produced (OMCV) by the fermenting substrates was recorded 11 times using a manual pressure transducer. At the end of each step the organic matter digestibility (OMD) was determined. In order to evaluate the ability of the envelopment to release MOS into the large intestine, four aliquots for each diet were also tested only by IVGPT. Short chain fatty acids (SCFA) were determined by chromatography⁶. Data were statistically analysed by ANOVA using SAS software (SAS Institute, NC, USA).

Results: Considering the enzymatic digestibility, no differences were observed between encapsulated and powder MOS, despite both the extruded substrates showed significantly ($P<0.01$) higher OMD also compared to the CTR diet, indicating a significant effect of temperature on the supplement digestibility. On the other hand, both extruded substrates seem significantly ($P<0.01$) less digestible and fermentable in the more distal intestinal tract (IVGPT results). When ED and IVGPT were used in sequence, no significant differences were detected for OMD, while diet MOS_EN and MOS_POEX showed significantly ($P<0.01$) higher gas production than the other substrates. Regarding end-product obtained at the end of two step evaluation significant differences ($P<0.01$) were registered for propionate, branched chain fatty acid (BCFA) and SCFA: propionate production and total SCFA were higher into MOS_EN, CTR and MOS_PO, while both the extruded substrates showed the higher amount of branched chain fatty acid (data not showed).

Table 1: *In vitro* digestibility and fermentation parameters

Diet	OMD	OMD	OMCV	OMD	OMCV
	ED	ED	IVGPT	ED + IVGPT	ED + IVGPT
	%	%	ml/g	%	ml/g
CTR	76.86B	88.96A	36.53A	93.52	17.08C
MOS_PO	76.53B	90.17A	29.16B	94.72	26.48C
MOS_EN	75.97B	88.61A	29.87B	95.05	54.19A
MOS_POEX	86.55A	73.84B	16.38C	93.17	38.02B
MOS_ENEX	85.13A	70.87C	16.27C	90.23	26.52C
RMSE	4.97	1.146	2.036	0.874	3.314

CTR: control diet; MOS_PO: MOS in powder post-extrusion; MOS_EN: MOS encapsulated post-extrusion; MOS_POEX: MOS in powder pre-extrusion; MOS_ENEX: encapsulated pre-extrusion. OMD: organic matter degradability; OMCV: cumulative volume of gas. Letters C in the same column indicate $P<0.01$. RMSE: root mean square error

Discussion and conclusion: The encapsulation seems only partially resistant to the extrusion process but unable to preserve MOS from the enzymatic attack. Indeed, when encapsulated MOS were supplemented after thermic treatment, the fermentation process was more intense, while MOS_ENEX showed low OMD and OMCV values.

References: ¹Corbo et al., 2014 *Compr. Rev. Food Sci. F.* 13: 1192-1206; ²Gao et al., 2011 *J. Antibiot.* 64: 625-634; ³Chen et al., 2012 *Indian. J. Anim. Sci.* 82 (1): 81-86; ⁴Horvath et al., 2007 *J. Anim. Phys. Anim. Nutr.* 91(5-6): 205-9; ⁵Calabrò et al., 2013 *Ital. J. Anim. Sci.* vol.12:94. 21-27.