

51. Effect of freezing on fermentation activity and fermentation patterns of equine faeces as inoculum in the semi-continuous fermentation technique Caesitec (Wirkung von Gefrierkonservierung auf die Fermentationsaktivität und das Fermentationsverhalten von Pferdekot als Inoculum im *in vitro* Verfahren Caesitec). Ann-Marie Müller*, Diana Gall, Sabine Bremer, Kristin Romanowski and Annette Zeyner – Rostock

Fermentation processes in the caecum of equines can in principal be simulated by help of the semi-continuous fermentation technique Caesitec. In previous studies fresh caecum content and equine faeces, respectively, were successfully used as inocula. The use of preserved inocula may provide numerous advantages like a better reproducibility of results from consecutive trials. The effect of preservation on rumen fluid has always been investigated (1), but studies with faeces are missing. The aim of this study was to compare fresh and frozen equine faeces as inocula in the Caesitec-technique.

Methods: Freshly defecated faeces from 3 mares were collected, homogenised, split and used to prepare 2 inocula for Caesitec-studies. The first part with fresh faeces (F) was immediately used for *in vitro* fermentation. The second part (O) was deeply frozen (-80 °C) for 14 days and thawed immediately prior to incubation. Each inoculum was incubated in 6 fermenter flasks over a period of 9 days. The fermenters were fed 8 grams of ground hay and 2 grams of maize starch from day 1 to day 4 and from day 7 to day 9 as well as 8 grams of hay and 16 grams of starch from day 5 to day 6. Gas production and fermentation patterns within the liquid fermenter content (pH, redox potential, short chain fatty acids [SCFA]) and the gaseous phase (CH₄, H₂) were daily measured.

Results: All fermentation patterns were influenced by the variance factors 'day of incubation' and 'inoculum' ($P < 0.05$, table). Starting with d1, O induced a redox potential (RP) that was continuously below -300 mV (-343±32 mV). With F, the RP reached -300 mV first after 4 days and stayed then close to this level (-305±54 mV). Irrespective of the inoculum, high starch feeding decreased the pH and elevated the GV, H₂ and SCFA. There was a trend towards lower pH and higher SCFA in F vs. O indicating a lower microbial activity in the thawed faeces. However, this effect was not consistent.

Conclusion: Freezing and thawing faeces as inoculum is supposed to reduce microbial fermentative activity. However, significant different effects of fresh and preserved inocula were only visible on individual incubation days. Reactions to high starch feeding were similar for F and O. Further studies are needed to decide finally whether fresh faeces can be displaced by frozen material as inoculum.

Item	(I)	Day of incubation								
		1	2	3	4	5	6	7	8	9
pH	O	6.84 ^a	6.77 ^{bA}	6.72 ^{bcA}	6.71 ^{bcA}	6.74 ^{bcA}	6.60 ^d	6.49 ^e	6.55 ^{de}	6.68 ^c
	F	6.82 ^a	6.65 ^{bB}	6.65 ^{bB}	6.59 ^{bcB}	6.66 ^{bB}	6.57 ^{bcd}	6.48 ^d	6.52 ^{cd}	6.54 ^{cd}
GV	O	848 ^{dA}	1168 ^c	1364 ^{bA}	1306 ^{bcB}	1093 ^{cd}	1596 ^b	1968 ^a	1538 ^b	1318 ^{bc}
	F	718 ^{dB}	1112 ^c	1148 ^{cb}	1530 ^{bA}	1320 ^{bc}	1456 ^b	1905 ^a	1574 ^b	1458 ^b
H ₂	O	33 ^{cA}	51 ^c	333 ^b	360 ^b	80 ^{cB}	360 ^b	642 ^a	272 ^{bc}	72 ^c
	F	166 ^{bcB}	74 ^c	188 ^{bc}	343 ^b	184 ^{bcA}	498 ^a	573 ^a	186 ^{bc}	76 ^c
SCFA	O	14.3 ^c	23.5 ^b	26.6 ^b	28.8 ^{bB}	27.2 ^b	34.1 ^{ab}	43.4 ^a	43.2 ^a	33.8 ^{ab}
	F	13.9 ^e	24.0 ^d	27.4 ^{cd}	34.9 ^{bcA}	30.1 ^{cd}	41.1 ^{ab}	46.3 ^a	44.5 ^{ab}	42.8 ^{ab}

F, fresh faeces; GV, gas volume, in mL; H₂ in µmol/L; (I), inoculum; O, frozen and thawed faeces; SCFA, short chain fatty acids, in mmol/L; ^{abc} $P < 0.001$ for means within a row; ^{ABC} $P < 0.05$ for means within a column; pooled s.d.: pH, ± 0.050; GV, ± 170.6; H₂ ± 125; SCFA, ± 6.53

1) HARVAS et al. (2005): Anim. Feed Sci. Technol. 123: 107-118.

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52. The use of natural n-alkanes as digestibility markers in sows (Zur Nutzung von pflanzlichen n-Alkanen als Verdaulichkeitsmarker bei Sauen). M. Bulang*, Emmrich T., Scheffler K., Kneisel S., H. Kluge and J. Broz – Halle (Saale)/Basel

Alkanes have been used as digestibility marker in several animal species for some time. However, their use in pig digestibility studies, especially in sows, has not been extensively investigated. Commonly, pig diets have a low content of natural n-alkanes. Diets for sows sometimes including low energy components like dried lucerne- or grass meal with higher concentrations of natural alkane and therefore seem to be suitable for digestibility measurements. In the present study, n-alkanes from dried lucerne meal were evaluated for their suitability as marker for estimation of digestibility of nutrients in sows diets compared to the total collection method.

Methods: Two feeding trials were used, with lactating and gestating sows, respectively. Trial one was carried out with 14 lactating sows (live weight: 284 kg SD=33kg) of a hybrid combination [(Large White x Landrace) x Hermitage] which were allotted to two treatments (control diet ('BA-') and 0.5 % of benzoic acid in the diet ('BA+')) and housed in standard lactating pens. The sows were fed *ad libitum* and feed intake was monitored over the lactation period. Total faeces were collected quantitatively over five days in the third week of lactation. The sows were fed a wheat-soybean meal-based diet and included 5% dried lucerne meal [1]. Subsequent, trial 2 was carried out with the same sows, but gestating (live weight: 239 kg SD=29kg) and allotted again to the two treatments 'BA-' and 'BA+' but fed a diet with barley, molasses beet pulp, distiller's grains and soybean meal and additionally 15% dried lucerne meal. Alkane concentrations were analysed according to the established method (2). Digestibilities were calculated by total collection as well as standard marker equation. Faecal alkane recoveries (FAR) were calculated as the proportion of ingested marker which was recovered in the faeces.

Results and Discussion: The analysed concentrations of natural alkanes C27, C29, C31 and C33 in diets for lactating sows were 4.3, 17.2, 18.0 and 2.5 mg/kg DM, and in diets for gestating sows 7.7, 41.9, 75.7 and 6.3 mg/kg DM, respectively. Concentrations for C27 and C33 were smaller than 10 mg

Table: FAR (%) and digestibilities of sows diets (%)

Trial	Treatment	FAR*		EED [#]	tOMD	OMD _M		SEM
		C29	C31			alkane		
						C29	C31	
Lactating	BA-	59	66	85	87.8 ^a	79.0 ^b	81.3 ^b	0.79
	BA+	55	61	85	88.2 ^a	78.3 ^b	80.6 ^b	0.47
Gestating	BA-	87	89	54	79.7 ^a	76.8 ^b	77.5 ^b	0.55
	BA+	91	94	51	78.3 ^a	76.2 ^b	76.9 ^a	0.48

Different letters within trial and treatment indicate significant differences of OMD_M to tOMD (p<0.05, Dunnett); *Faecal alkane recovery; [#]ether extract digestibility

and not more considered. FAR were not different between treatments 'BA+' and 'BA-' within trials (Table). Hence, all treatments within both trials showed significant differences of total organic matter digestibility (tOMD) to marker based organic matter digestibility (OMD_M), except for C31 based digestibility in gestating trial and treatment 'BA+'.

Conclusion: Without correction of marker recovery, calculated digestibility of organic matter based on lucerne alkane C31 showed a good accuracy in case of gestating sow diet (treatment 'BA+'). However, as alkanes are lipid soluble the different digestibility of ether extract (EED) between trials may have been interacting on FAR.

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53. The effect of three betaine sources on *in vitro* fermentation of different carbohydrate and protein combinations using faecal inocula from pigs (Der Effekt von drei Betainquellen auf die *in vitro* Fermentation von verschiedenen Kohlenhydrat-Protein Kombinationen mit mikrobiellen Inokulum beim Schwein). Franziska Rink*, Eva Bauer, Meike Eklund and R. Mosenthin – Stuttgart-Hohenheim

Microbial fermentation of carbohydrates, resulting in the production of short-chain fatty acids (SCFA), is considered to be beneficial for gut health, in contrast to protein fermentation, which delivers detrimental substances including biogenic amines and ammonia, and often is associated with growth of potential pathogens (Williams et al. 2005). To minimize post-weaning associated disorders in piglets, feed additives may be used to modulate intestinal microbial fermentation, and thus to improve gut health. Betaine, the trimethyl derivative of the amino acid glycine, has been shown *in vivo* to exert beneficial effects on e.g. growth performance and carcass quality, but also on digestibility of specific nutrients, such as minerals and amino acids (Eklund et al., 2005). Additionally, improvements in fiber digestibility due to dietary betaine supplementation indicate a possible stimulation of intestinal fermentation (Eklund et al., 2006). This study assessed the influence of three different betaine sources on fermentation characteristics of different carbohydrate-rich feed ingredients combined with two different protein sources.

Methods: The *in vitro* technique of the modified Hohenheimer Futterwerttest (HFT) was used to assess the influence of three different betaine sources (betaine monohydrate, betaine hydroxide and condensed molasses soluble) on fermentation characteristics of different carbohydrate-rich feed ingredients combined with two different protein sources. Carbohydrate-rich feed ingredients were inulin, pectin, corn starch, potato starch and cellulose. These carbohydrates were combined with either a steam heated soybean meal (Soypass®), which is hardly fermentable by microbes, or with easily degradable casein as protein source. Faeces originating from 6 grower pigs fed a standard pig diet were used as microbial inocula. At the end of fermentation (24 h), total gas and ammonia production were measured. The experiment was carried out in a block design (Cyc DesigN 3.0), with eight blocks in two experimental periods resulting in 5 observations per treatment.

Results: Significant differences in gas ($P < 0.0001$) and ammonia production ($P < 0.0001$) were obtained for the different carbohydrates, while for the two proteins significant differences were determined in ammonia production only. Of the different betaine sources, addition of condensed molasses solubles led to significantly higher gas ($P < 0.0001$) and ammonia ($P = 0.0002$) production, while betaine monohydrate and betaine hydroxide had no effect on fermentation. Additionally, no interactions of the different betaine, protein and carbohydrate combination could be found. However, the carbohydrate-rich feed ingredients inulin and pectin as well as corn and potato starch showed high fermentability and a low ammonia production. A particularly efficient reduction in ammonia content was achieved for the heated soybean meal in combination with the readily fermentable corn starch, and for casein in combination with the more slowly fermentable cellulose.

Conclusion: There were no interactions between betaine, protein and carbohydrate combinations. Condensed molasses solubles showed the most pronounced stimulatory effects on gas and ammonia production. Furthermore, the modified HFT can be also used for pigs as a fast and reliable screening method to evaluate *in vitro* fermentation characteristics of potential feed ingredients.

- 1) EKLUND, M., BAUER, E., WAMATU, J., MOSENTHIN, R.: (2005): Nutr. Res. Rev. 18, 31-48
- 2) EKLUND, M., MOSENTHIN, R., PIEPHO, H.P. (2006): Acta Agric. Scand. Section A 56, 83-90
- 3) WILLIAMS, B., BOSCH, M.W., BOER, H., VERSTEGEN, M.W.A., TAMMINGA, S. (2005): Anim. Feed Sci. Technol. 123-124, 445-462

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