

Duodenal flow of the chlorophyll metabolite phytanic acid in beef steers offered grass and red clover silages. By M.R.F. LEE, J.K.S. TWEED, R.J. DEWHURST and N.D. SCOLLAN, *Institute of Grassland and Environmental Research, Aberystwyth, UK, SY23 3EB*

Phytanic acid is a chlorophyll metabolite produced via the bacterial hydrogenation of phytol. It is associated with retinal changes of the eye and Refsum disease in man with a gene mutation resulting in a deficiency of the enzyme phytanoyl-CoA hydroxylase which is required for the α -oxidation of phytanic acid (Mihalik *et al.* 1995). However, in healthy individuals without this gene mutation, numerous potential health benefits of phytanic acid have been discovered in animal models. These are: induction of white and brown adipocyte differentiation; prevention of vitamin A teratogenesis; the ability to activate peroxisomal proliferator-activated receptor- γ agonists in a similar way to conjugated linoleic acid as a potential treatment and prevention of diabetes (McCarty, 2001). As it is a by-product of chlorophyll breakdown in the rumen, the main mode of intake in human consumers is through ruminant products from forage-based systems. However there is little information in the literature regarding the flow of this potential nutraceutical to the small intestine in ruminants fed on forage diets. The present study sought to quantify the flow of phytanic acid to the duodenum in beef steers fed a range of forage silages. The present study is part of a larger investigation into lipid metabolism of high-sugar grass and red clover silages reported by Lee *et al.* (2004).

Six Hereford \times Friesian steers, 163 (SE 5.9) kg, prepared with rumen and duodenal cannulae were allocated at random to receive one of five silage diets *ad libitum*: high-sugar grass (HG); control grass (CG); HG and red clover (50:50 DM basis); HGR; CG and red clover (50:50 DM basis); CGR and red clover (R). The experiment was conducted as a 5 \times 5 incomplete Latin square with an additional randomly repeated sequence. There were four experimental periods each lasting 24 d, with a 14 d adaptation period to the diets, followed by a 10 d measurement period. Digesta flow at the duodenum was estimated using a dual-phase marker system with Yb(CH₃COO)₃ and Cr EDTA as particulate- and liquid-phase markers, respectively. On days 20 and 21 of each period duodenal digesta was collected every 3 h over a 24 h period. Lipids were extracted by direct hydrolysis, with added internal standard (C₂₁:o), in 5 M-KOH made up in methanol. Fatty acids were methylated using HCl in methanol at 80 °C and analysed by GC. Statistical analysis was undertaken using an unbalanced ANOVA, blocking according to period (Genstat 5; Lawes Agricultural Trust, 1997).

The principal chemical components of the silages were (g/kg DM): DM, 277, 258, 289, 276, 309 g/kg; neutral-detergent fibre, 551, 587, 459, 482, 397; total N, 24.5, 25.4, 29.4, 29.2, 32.3; chlorophyll, 2.51, 2.06, 2.64, 2.45, 2.64; water soluble carbohydrate, 90.5, 55.3, 66.5, 43.8, 29.2 for HG, CG, HGR, CGR and R respectively. Silage pH ranged from 4.06 to 4.14 with a predominantly lactate fermentation. The control grass silage (CG) had a significantly lower DM intake than the other four silage treatments, with a consequently lower duodenal flow.

	HG	CG	HGR	CGR	R	SE _D	P
DM intake (kg/d)	4.44 ^b	3.55 ^a	4.70 ^b	4.57 ^b	4.51 ^b	0.42	0.05
Duodenal flow							
DM (kg/d)	2.27 ^b	1.80 ^a	2.36 ^b	2.31 ^b	2.16 ^b	0.201	0.05
Phytanic acid (g/d)	4.30 ^c	2.73 ^b	2.79 ^b	2.76 ^b	1.37 ^a	0.384	0.01

a,b,c Mean values within a row with unlike superscript letters were significantly different.

The greater duodenal flow of phytanic acid for animals fed HG was due to the combined effects of higher DM intake and chlorophyll content of silage HG compared with silage CG. The lower duodenal flows of phytanic acid on silage R are more difficult to explain. It may be related to the enzyme polyphenol oxidase in red clover inhibiting the hydrolysis of chlorophyll or phytol in the rumen in a similar way to the postulated method for the reduced biohydrogenation of 18:3n-3 on red clover diets (Lee *et al.* 2004).

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Comparison between an essential oil blend and oregano whole herb on growth performance in weaners. By G.M. JONES, H. KOLLER, F. WAXENECKER and S. PASTEINER, *Biomim Research and Development, Industriezstrasse 21, 3130 Herzogenburg, Austria*

Oregano used either as a whole herb (Hofmann *et al.* 2002) or as essential oil (Kyriakis *et al.* 1998) in pig diets has been shown to have a positive effect on growth performance in weaners. However less information is available on direct comparisons between the effect of essential oil of oregano and the whole herb in the diet. In the present experiment a blend of essential oils containing a minimum 80% oregano oil was tested against ground dried whole herb oregano at equal concentrations of oregano oil in weaner diets for the effect on growth performance.

Sixty-two weaner pigs were split into two groups at weaning. One group received the blend of essential oils consisting of oregano, citrus and anise in the diet (EO blend) and the other group received oregano as the dried whole herb at 1920 g/tonne diet (oregano). Both diets were formulated to contain the same amount of oregano essential oil (75 g/tonne feed), based on the analytical findings of oregano oil in the whole herb, which was 3.9%. Analytical findings for carvacrol content were 54% in the EO blend and 92% in the oregano herb, resulting in 41 g carvacrol/tonne diet for the EO blend group and 69 g carvacrol/tonne diet for the oregano group. Diets were fed until day 21 post-weaning and piglets were weighed at day 12 and 21 post-weaning. Feed intake was measured daily on a per pen basis.

	EO blend		Oregano		P value
	Mean	SD	Mean	SD	
No. of animals	31		31		
Weaning weight (kg)	7.65	0.70	7.67	0.71	0.878
Wt day 12 post-weaning (kg) unadjusted	9.48	1.20	9.20	1.03	0.321
Wt day 12 post-weaning (kg) adjusted*	9.50	1.20	9.18	1.03	0.078
ADWG weaning-day 12 (g/d)	153	61.5	127	57.3	0.092
Wt day 21 post-weaning (kg) unadjusted	12.01	1.62	11.44	1.29	0.131
Wt day 21 post-weaning (kg) adjusted*	12.03	1.62	11.42	1.29	0.033
ADWG day 12-day 21 (g/d)	281	53.5	249	58.5	0.051
ADWG weaning-day 21 (g/d)	208	58.5	180	47.7	0.040

ADWG, average daily wt gain.

* Piglet weaning weight covariate.

Average weaning weight of piglets was 7.66 (SD 0.697) kg and similar for both groups. Overall piglet growth rates post-weaning were significantly ($P < 0.05$) higher in the EO blend group than in the oregano group. Overall feed intake was 303 g/d for the EO blend group and 297 g/d for the oregano group resulting in improved feed conversion rates for the EO blend group, 1.46 v. 1.65 respectively.

These findings indicate that despite similar oregano essential oil intake and a higher carvacrol intake in piglets fed the whole oregano herb, nutrient utilisation in pigs was inferior compared with pigs fed the essential oil blend containing oregano essential oil. This could be due to the active substances of the oregano plant being more readily available when processed to essential oil before feeding or the increased intake of dried plant material having a negative effect on digestibility of the diet. Further studies are required comparing the effect of oregano dried plant material and oregano essential oil from the same plant variety on piglet performance and digestibility.

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Studies on the fermentation patterns of wheat samples, differing in their chemical characteristics, using ileal microflora from chicks. By S. SHANNUGAVELU¹, V.R. PIRGOZLIEV¹, J.D. BROOKER², T. ACAMOVIC¹ and S.P. ROSE³, ¹Avian Sciences Research Centre, SAC, Ayr, UK, KA6 5HW, ²University of Adelaide, Australia and ³Harper Adams University College, Newport, Shropshire, UK, TF10 8NB

The content of wheat in poultry diets in Europe is about 600 g/kg or more. Wheat contains variable amounts of NSP, mainly arabinoxylans, which have been associated with enteric problems. The problems associated with dietary NSP include increased digesta viscosity, elevation of microbial activity, alteration in microbial profiles, and reduction in performance. Austin *et al.* (1999) evaluated twelve wheat samples with soluble NSP between 15.2–23.5 g/kg DM and did not observe any relationship between soluble NSP and the apparent metabolisable energy. Choct *et al.* (1996) attributed the increase in fermentation in the small intestine to the presence of viscous NSP. The aim of the present study was to evaluate the microbial fermentation patterns of extracts of ten wheat samples of known chemical characteristics.

Ileal contents from two 3-week-old female broilers fed an enzyme- and antibiotic-free wheat-based diet were collected and diluted 1:10 (w/v) in minimal media, thoroughly mixed and filtered through 8 micron glass wool filter to separate the feed particles. A sample (0.5 ml) of this inoculum was added to 5 ml of minimal media with 0.2 ml of extracts of ten wheat samples (Prigozliev *et al.* 2003) varying in their content of soluble NSP (16–30 g/kg DM), into 15 ml Hungate tubes, under anaerobic conditions. Extraction was based on the methods of Bedford & Classen (1993). The control was minimal media with only the inoculum. All the tubes were randomly allocated with four replicates per treatment. The tubes were placed in a water-bath at 39 °C and microbial fermentation monitored by gas accumulation with an electronic pressure transducer every 2 h for 10 h (Shannugavelu *et al.* 2004). The viscosity of the extract was measured immediately after extraction (Brookfield DV III viscosimeter at 23 °C, at 12 rev/min).

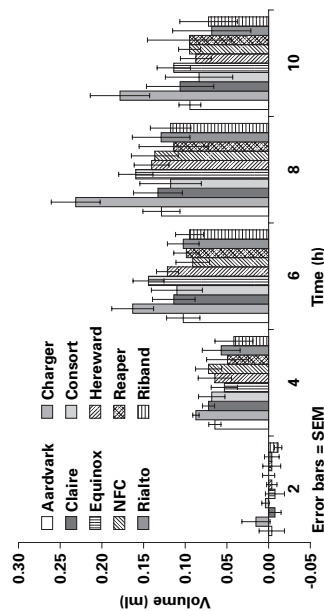


Fig. 1. *In vitro* fermentative activity.

The viscosities of the extracts of the ten wheat samples were low and varied from 4.6 to 12.8 centipoise. The extract viscosity correlated well (r^2 0.55; $P < 0.01$) with the viscosity of the digesta from birds fed diets containing these wheat samples (Prigozliev *et al.* 2003). The fermentative activity at each time of measurement was deducted from the mean of the control values to remove basal fermentation from the substrates present in the ileal contents. There was no difference in *in vitro* fermentative activity between the wheat samples (Fig. 1) despite a three-fold difference in viscosity. Similarly, there was no relationship (from regression analysis) between *in vitro* fermentation and the composition of the wheat samples. Despite the differences in viscosity and NSP content of the wheat samples it appears that *in vitro* microbial fermentative activity was not affected. The results may be confounded by the quantity of starch in the extracts but suggest that viscosity *per se* may not be well correlated with fermentative microbial activity *in vivo*.

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Dietary Quillaja saponin during gestation does not affect colostrum immune profile but decreases stillbirth incidence at farrowing. By S.E. ILSLEY and H.M. MILLER, *School of Biology, University of Leeds, Leeds, UK, LS2 9JT*

Saponins extracted from the plant *Quillaja saponaria* are known to have immunostimulatory activity (Ilsley *et al.* 2004). Work by Krakowski *et al.* (2002) found that when sows were immunostimulated in gestation, increases in colostrum antibodies were observed which correlated with improved piglet weaning weight. The aim of the present study was to determine whether feeding Quillaja saponins to sows during the period of colostrogenesis would stimulate colostrum immunoglobulin concentrations and improve piglet growth and viability before weaning.

Forty-four hybrid sows were allocated to one of two dietary treatments on day 72 of gestation on the basis of parity, fatness and past performance. Treatments were control (C; reproductive sow pellets) or supplemented (QS; 2.5 g Quillaja saponin/d as a top dress). Sows received 2.5 kg feed/d throughout gestation. Treatment diets were fed between days 72 and 93 of gestation. After day 93, all sows were managed in an identical manner. Colostrum was sampled at farrowing and milk on day 21 of lactation. Piglets were weighed at birth and 21 d of age and any mortality in this period noted. Colostrum and milk were analysed for Ig G and Ig A concentrations using ELISA. Day 21 milk samples were analysed for protein content by Direct Laboratories (Wolverhampton, UK). Data were analysed in the general linear model of Minitab 12.2.

	C	QS	SE	P
Litter size (birth)	11.88	10.81	0.64	
% Stillborn/litter	13.25	7.67	1.93	<0.05
No. died/litter, birth–day 21	1.12	0.64	0.30	
Piglet average daily gain (g/kg per d) day 0–21	200	179	5.5	<0.05
Ig G (mg/ml)	85.59	72.45	7.18	
Day 21 milk	8.24	8.08	1.33	
Colostrum	56.62	70.74	6.21	
Ig A (mg/ml)	0.61	0.53	0.10	
Day 21 milk	5.16	4.66	0.19	<0.1

Quillaja saponins did not stimulate an increase in the concentrations of immunoglobulins in the colostrum and milk when fed to sows between days 72 and 93 of gestation, contrary to our hypothesis. It is possible that an extended period of supplementation or different dose rate is needed to elicit an effect. However a negative effect on piglet pre-weaning growth was observed which may be linked with reduced milk protein levels. The cause of the reduction in milk protein is unclear. Pre-weaning mortality was unaffected by sow treatment; however, stillbirth incidence at farrowing was lower for supplemented sows. This effect has previously been observed when sows were fed crude Quillaja extract during the week before farrowing (Ilsley *et al.* 2003), and indicates long-acting activity of the saponin. Possible modes of action include an increase in the O₂-carrying capacity of maternal blood resulting in improved piglet oxygenation.

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