

ASSESSING THE IMPACT OF DIETARY ADDITION OF LIGNIN ON GROWTH PERFORMANCE, NITROGEN BALANCE AND FAECAL MICROORGANISMS IN GRAIN-FED VEAL CALVES

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ABSTRACT

A study was conducted with ALCELL^R - lignin (A-lignin) to evaluate its impact on performance of grain-fed veal calves. Thirty two male calves (avg. initial wt, 190 kg) were fed for 13 weeks grain corn and a protein supplement with added A-lignin to supply 0, 1.25%, 2.5% or 5.0% of diet. Eight other calves were used to study nitrogen balance, and grab samples of faeces were analyzed for anaerobic, aerobic and coliform bacteria. A-lignin at 1.25% of diet increased ($p < .01$) weight gain by 12%. Addition of A-lignin also decreased ($p < .01$) organic matter digestion, increased ($p < .01$) faecal N excretion but did not significantly alter N balance. A-lignin reduced ($p < .01$) faecal ammonia concentration but increased ($p < .01$) DM content of grab samples of faeces; there were no significant effects on populations of faecal microorganisms. The study revealed a beneficial effect of A-lignin on calf growth but the reason for the response was not uncovered.

KEYWORDS

Calves, growth rate, nitrogen balance, lignin, faecal microorganisms

INTRODUCTION

Natural feed additives may be obtained as byproducts of manufacturing and their use in animal production can minimize environmental damage associated with waste disposal. The Alcell^R process for manufacturing paper pulps yields pure lignin (Pye, 1996), a co-product that appears to have application in animal feeding and nutrition (Stitch, 1984; Kloner, 1985). The addition of lignin (1.25 to 5%) from an organosolv pulping process to the diet of pigs and calves has been shown to improve growth performance and reduce the incidence of diarrhea (Kloner, 1985; Stitch, 1984). Studies cited by Jung and Fahey (1983) also indicate that addition of purified lignin to the diets of chicks led to improvements in performance as well as alterations in VFA patterns and bacterial populations in the hindgut. Based on studies with rats, Nelson *et al.* (1994) suggested that addition of A-lignin to the diet may reduce translocation of enteric bacteria from the gut following burn injury. This research assesses the impact of adding isolated A-lignin on growth performance and nitrogen balance in grain fed veal calves and aims to increase understanding of the role of isolated lignin in gastrointestinal ecology and feed utilisation in ruminants.

METHODS

Growth performance and nitrogen balance: A 91d growth trial was conducted with 32 Holstein male calves (avg. initial wt. 190 ± 16.2 kg). Each of the four calves within a pen was assigned, according to a randomized complete block design, one of four diets consisting of dried shelled corn (70% of ration) and a pelleted protein supplement (34% CP). A-lignin (REPAP Technologies Inc., Valley Forge, PA) was incorporated into the supplements (prior to pelleting) such that when the supplement was mixed with corn the levels of added A-lignin were 0, 1.25%, 2.5% or 5.0% of the total diet. The calves were allowed free access to water and offered their respective diets, twice daily (0830 h and 1500 h), in quantities sufficient to ensure 10% feed refusal. The animals were weighed every two weeks, daily feed consumption was recorded and daily samples of the diet were composited each week for chemical analyses of ash, total N and ADF and minerals (AOAC, 1990; Isaac and Johnson, 1985). A nitrogen balance trial was conducted as a 4 x 4 double Latin square in which eight male calves (avg. initial wt. 215

± 5.9 kg) were offered, twice daily, (0900 h and 1600 h) the four diets used in the growth trial for four 21 d periods. The calves were fed *ad libitum* for 11d after which they were restricted on feed to 85% of *ad libitum* of consumption. Daily output of faeces and urine was collected during the last six days of each period. On the last day of excreta collection, blood samples were collected from the jugular vein. The samples were collected into heparinized evacuated tubes 20 min before and 2 h after feeding; the samples were maintained in an ice bath, then centrifuged at 2000 g for 15 min for analysis of plasma urea (Sigma kit no. 67-UV). Composite samples of faeces were freeze dried and analyzed for ash and total N (AOAC, 1990). Composite samples of the acidified urine were analyzed for total N (AOAC, 1990) and for urea, ammonia and creatinine using Sigma diagnostic kits (no. 67-UV, no. 171-UV and no. 555, respectively).

Microbiological and Chemical characterization of faeces: Faecal grab samples were aseptically collected from each calf 30 min prior to the morning and evening on feeding days 1, 3, and 5 of each collection period. Populations of anaerobic and aerobic microorganisms were enumerated. Faecal coliforms were enumerated on mFC agar plates incubated at $44.5 \pm 0.5^\circ\text{C}$ for 24 ± 2 h (HPB - MFLP-55, 1989). Enumeration of *Yersinia enterocolitica* was accomplished by homogenising 10 g of faeces with 90 ml of phosphate-buffered saline containing 2% sorbitol and 0.15% bile salts; additional dilutions were made in the PSB-sorbitol-bile salts fluid. All flasks were incubated for 3 h at $22-25^\circ\text{C}$ and one drop of culture from each dilution was removed and spread over the surface of Cefsulodin-Irgasan-Novobiocin (CIN) agar. The inoculated plates were incubated for 18-28 h at 29°C (HPB - MFLP-48, 1989).

To enumerate *Campylobacter jejuni* one dilution was prepared in 0.1% peptone and pipetted into tubes containing 9 ml of Preston broth. The tubes were incubated at 42°C for 24 h after which time samples were removed and spread over the surfaces of modified Skirrow medium and Hogan and Harris medium. The plates were incubated for 3-4 d at 43°C (Myers *et al.*, 1984).

To enumerate *Escherichia coli* 0157: H7: one ml aliquots of the 0.1% peptone dilutions were surface plated on Macconkey Sorbitol Agar (Difco). The plates were incubated at 35°C for 18 h (Hitchins *et al.*, 1992).

To determine microbial population shifts, approximately 100 microbial isolates from faeces samples collected from calves fed control and lignin-containing diets were screened using the Biolog⁷ system (Biolog Inc. Hayward, CA). Dry matter content of grab samples faeces was determined by drying samples to constant weight. The pH of faeces was determined and ammonium N content was measured with an ammonia electrode (model 95-10, Orion Instrument, Cambridge, MA). All data were analyzed statistically using the GLM procedure of SAS (1987). Differences among treatments were assessed using preplanned comparisons.

RESULTS and DISCUSSION

Growth Performance and N Balance: The A-lignin product contained 96% DM, 7% ADF and only trace amounts of ash (0.04%) and protein

(0.09% CP). Since cellulose was removed from the A-lignin product (Pye, 1996), the results would imply that there was very little lignin in A-lignin. A more plausible explanation for the low ADF is that the estimate is an artifact of the analytical procedure. During the ALCELL^R process macromolecular lignin is broken down to "lignin fragments" (number average MW less than 9000; Pye, 1996) that may have been solubilized and removed during the ADF procedure. Results of elemental and chemical group analysis as well as impurities analysis indicate that A-lignin is greater than 95% pure and normally contains less than 0.5% sugars (Lora *et al.* 1993).

Calves fed the control diet (0% lignin) exhibited high growth rates (Table 1). All calves were implanted with the growth promotant Ralgro[®] so the estimates of live weight gain (LWG) are not unexpected. Incorporation of A-lignin at 1.25% of the diet increased ($p < .01$) LWG by 12%; higher levels of A-lignin had no effect.

The positive effect of A-lignin on LWG was due to an improvement in feed efficiency ($p < .10$) because feed consumption was not ($p > 0.05$) affected. The beneficial effects of lignin on calf performance are consistent with growth enhancing effects of lignin in studies with pigs and calves (Stitch 1984; Kloner, 1985;) but the optimum level seems to vary with species. The addition of A-lignin at the level of 1.25% had no significant effect on organic matter digestion (OMD) (Table 1); higher levels of lignin significantly reduced OMD and increased faecal excretion of N. A-lignin did not significantly alter nitrogen balance but coefficients of variation for estimates of urinary N and N retention were high. There were no significant effects of A-lignin on plasma urea or on concentrations of ammonia and urea levels in urine (Table 1).

Microbiological and Chemical evaluation of Faeces: Feeding varying amounts of A-lignin to calves had no effect on the numbers of anaerobic, aerobic and coliform microorganisms in the faeces (Figure 1). In addition, there were no marked shifts in the type of microorganisms isolated from the faeces. *Yersinia enterocolitica* was not isolated from any of the grab samples of faeces and *Campylobacter jejuni* was isolated with the same frequency (ca. 15%) from faeces of both control and lignin fed calves. The frequency of isolation of these pathogenic organisms is in agreement with the findings of Myers *et al.* (1984).

E. coli isolations were quite transient in that the different faecal samples collected from one animal during the same test period were either positive or negative for the organism. Presumptive *Escherichia coli* 0157:H7 was isolated sporadically from the faeces (ca. 1.0%) of both control and lignin fed calves.

The isolation of *E. coli* 0157:H7 in the faeces of the calves was not surprising as the organism has been previously isolated from the faeces of dairy calves and heifers (Orskov *et al.*, 1987). The DM content of faeces from calves fed diets containing 2.5% and 5.0% lignin was significantly ($P < 0.01$) higher than that of faeces from control calves (Fig. 1) and are consistent with the findings of Stich (1984) and of Kloner (1985). The latter author has suggested that the reduction in feed efficiency caused by diarrhoea could be avoided by feeding organosolv lignin to the animals. We were unable to corroborate this idea as none of the calves showed symptoms of diarrhoea.

There was no significant effect of A-lignin on the pH of faeces (Fig. 1). At all levels of dietary inclusion, A-lignin reduced ($P < 0.01$) the ammonia content of grab samples of faeces (Fig. 1). As there were no differences in the microbial numbers or types of organisms in the faeces of animals fed control or lignin containing diets, we have no good explanation for the changes in faecal ammonia. Detailed metabolic studies may be required to provide further insights into nitrogen dynamics and gut

ecology following lignin feeding.

In conclusion, the addition of isolated lignin at the level of 1.25% to a grain diet stimulated growth rate of calves. Addition of A-lignin also altered faecal excretion of N and reduced ammonia content of faeces without affecting faecal populations of bacteria. The reason for the beneficial effect of lignin was not uncovered.

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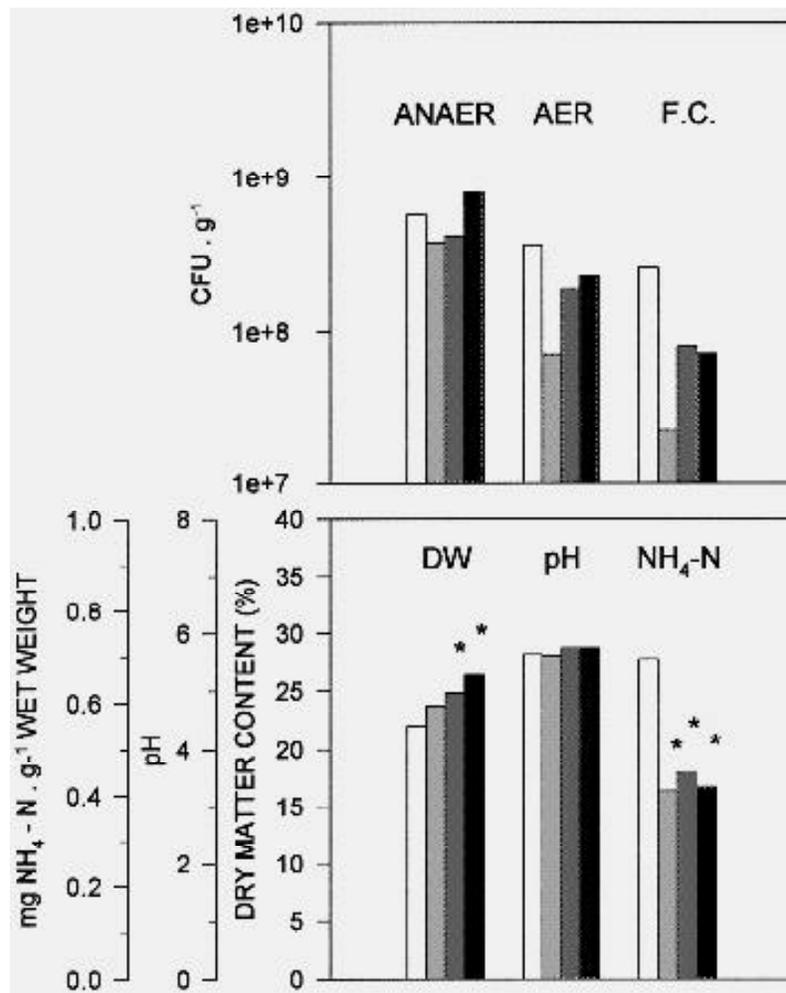
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Figure 1

Changes in anaerobic (ANAER), Aerobic (AER), fecal coliform (F.C) microbial populations, dry weight, pH, and NH₄-N (L.S. mean) in the faeces of calves fed diets containing 0% , 1.25% , 2.5% , or 5%  ALCELL lignin.



*significantly different from control (p<0.01)

Table 1

Growth performance, nitrogen balance and concentration of urea and ammonia in bl and urine of calves fed grain diets with added ALCELL ligning (A-lignin).

Item	Control (C)	1.25% A-lignin	2.5% A-lignin	5.0% A-lignin	S.E. ^Z	Contrasts		
						C vs 1.25% A-lignin	C vs 2.5% A-lignin	C vs 5% A-lignin
Initial wt., kg	183	190	196	196	4.4	ns	ns	ns
ADG, kg	1.62	1.82	1.56	1.57	0.044	**	ns	ns
Organic matter intake, 2/kg/BW/d	21.4	21.3	21.2	21.7	0.065	ns	ns	ns
Feed:gain	3.96	3.65	4.16	4.21	0.12	†	ns	ns
Organic matter digestibility,%	83.2	81.2	78.5	76.5	0.86	ns	**	**
N intake, g/d	133.8	135.0	141.1	142.0	5.85	ns	ns	ns
Faecal N, g/d	33.9	37.8	43.5	43.4	2.39	ns	**	**
Urinary N, g/d	43.2	43.2	37.7	41.7	5.24	ns	ns	ns
N retained, g/kg DOMI ^Y	13.8	13.2	14.5	13.8	0.95	ns	ns	ns
plasma urea, mM	3.37	3.13	2.89	3.15	0.165	ns	ns	n
Urinary urea, mmol/mmol creatinine	20.1	21.8	21.6	21.0	2.25	ns	ns	ns
Urinary ammonia, mmol/mmol creatinine	4.3	2.5	3.3	7.2	3.25	ns	ns	ns

^Z SE = standard error of means

^Y DOMI= digestible organic matter intake

**:, p,0.01; †: p< 0.10; ns= not significant, p > 0.10

