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EFFECT OF THE RATIO LIGNIN TO CELLULOSE (ADF-ADL) ON CAECAL FERMENTATION, GUT MORPHOLOGY AND PERFORMANCE OF RABBITS AROUND WEANING

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ABSTRACT

The effect of the lignin:cellulose ratio (LCR) on intestinal morphology and microbial activity should be described. A pelleted feed was given ad libitum (CF 17.0 %; CP 15.4%; CL 3.4% DM). Supplementation of Arbocel® (23.2% ADL and 71.1% ADF in DM) and Vitacel® (0.1% ADL and 80.5% ADF in DM; Rettenmaier GmbH. Germany) in alternating parts (0-2-4-6-8% each) was practiced. Therefore the amount of ADF increased slightly in the 5 feeds, while the lignin level (as a result of Arbocel® addition) increased from 3.5 to 5.7 % in DM, and the LCR increased (0.26/ 0.30/ 0.32/ 0.34/ 0.40). Fifty females were divided in five feeding groups with 10 mothers and 8.1 suckling animals per doe. After the 28 day suckling period 200 weaned rabbits were divided into five groups, following the feeding group of their mothers. At day 12 after weaning caecum samples from 20 healthy animals of each group were taken and histological parameters resp. volatile fatty acids (VFA) were analyzed.

Neither mortality or morbidity measured from 29 to 40 d old differed among the five groups, while the feed intake and growth was reduced. When the LCR increased the total concentration of VFA in caecum linearly decreased, while the VFA molar proportions were slightly modified. For an increased LCR we detected a decreased in caecal crypt depth (222/ 236/ 192/ 150/ 142 µm). To describe the physiological potential in a complete feed the lignin and cellulose content seemed not sufficient. If the lignin content was 5% (DM) in feed and LCR over 0.4 the microbial activity would decreased and the caecal mucosa would be reduced.

Key words: Rabbit, weaning, lignin, lignocellulose, caecum, volatile fatty acids, morbidity

INTRODUCTION

The fibre fractions are one of the most important nutritional factors for the digestive physiology of the rabbit (Gidenne 1997; Pinheiro and Gidenne 1999; De Blas *et al.* 1999), and the use of the Van-Soest detergent method (NDF, ADF, ADL residue) has improve the fibre recommendations to reduce the digestive diseases in the growing rabbit (Gidenne 2000; Gidenne 2003; Bennegadi et al. 2001; Carabaño et al. 2006). Increasing the dietary lignin (ADL) levels reduces the health risk in weaned rabbits (Perez *et al.* 1994) while increasing cellulose (ADF-ADL) also reduces the mortality and morbidity from diarrhoea (Perez *et al.* 1996). Moreover, increasing the lignin to cellulose ratio (LCR) in the feed led to a decrease of digestive troubles and diarrhoea (Gidenne *et al.*, 2011). Microbial activity in the caecum changes rapidly between 3rd and 5th living week (Debray et al. 2003). Health in weaned rabbits principally depends on lignocellulose (ADF) content in feed, but various investigations result uncertain morbidity with the same ADF content (Gidenne and Perez 2000; Gidenne *et al.*, 2004;

Gidenne and Garcia 2006). The effect of feed on the caecal microbial activity remains to be clarified (Combes *et al.* 2011) and also its effect on morphological parameters of the caecal mucosa.
Products rich in crude fibre like dried and milled wooden material from spruce and pine could be used in animal feeding to supply lignin and cellulose. Arbocel® is a wooden lignocellulose with high lignin content, while Vitacel® contains only cellulose due to a chemical processed extraction of milled wood. Earlier results on rabbit performances with 3% Arbocel® (Krieg *et al.* 2008) suggested that increasing the LCR would reduce the digestible fibre and decrease the microbial fermentation rate. We thus aimed to analyse the influence of high LCR level in the diet, with a constant ADF level, on rabbits performances around weaning and on caecal morphology and microbial activity.

MATERIALS AND METHODS

Animals and feeds

Fifty pregnant multiparous females were divided into five groups of 10 animals. At birth the litters were equalized to 8 suckling rabbits per female, and one female in each group has 9 suckling rabbits. At weaning (d 28) 40 rabbits of each group (20 males /20 females) were allocated in randomized block design and fed freely (water ad libitum) with the feed of their mother group. Ten replicates in every feeding group were realized (4 animals/ cage). Animals were housed in a research farm with wire net cages. A pelleted feed was used (28% alfalfa meal, 27% wheat bran, 16% sugar beet pulp, 6% sunflower meal, 5% soya bean meal, 3.5% oat, 2% linseed meal, 2% molasses, 1% premix, 1% Calcium carbonate, 0.5% Sunflower oil) and supplemented with portions of Arbocel RC fine or Vitacel R 200 (0-2-4-6-8 % Arbocel resp. 8-6-4-2-0 % Vitacel). Technical addition of Arbocel® and Vitacel® was after hammer mill in homogenizer, before pelleting. Arbocel and Vitacel are two fibre products of Rettenmaier GmbH, Germany. The calculated composition of basic feed in all groups was the same (87 % DM; 17.0 % Crude fibre; 15.4 % Crude protein; 33.1 % NDF; 20.5% ADF) except the lignin level (ADL). With increased Arbocel content (0-2-4-6-8 %) the ADL level increased stepwise 3.7; 4.4; 4.8; 5.2 and 5.7 % resp. (analytical results with Van Soest procedure). The fibre fractions of the Arbocel are (92.1 % DM) : 23.2% ADL (DM basis); 71.1 % ADF; 85.0% NDF; and for Vitacel : 93.1 % DM; 0.1% ADL; 80.5 ADF; 88.5% NDF (in DM).

Health status, gut morphology and microbial activity measurements

After weaning daily the health situation of every animal was observed for diarrhoea and/or constipation. Twenty healthy animals of each group were selected and euthanized at 40d old (12d after weaning). The caecum was withdrawn for analyses of mucosa morphology and chemical parameters. Chymus from stomach, ileum, caecum and colon was sampled in sterile plastic tubes and frozen urgently. Gut wall material from Caecum was individually sampled from 8 animals each group and fixed in Bouin solution. After tissue preparation the morphometry was analyzed with a Microscope Nikon Eclipse LV 100 and Software Elements BR.

Statistics

The normal distribution and homogeneity of variance was firstly tested for each groups with the Kolmogorov-Smirnov and Levene- Test resp.(Statistica TM, release 5.5. Statsoft Inc.), then a monofactorial variance analysis was performed and the Tuckey-HSD-Test was used to compare the means.

RESULTS AND DISCUSSION

The lignin (ADL) concentrations increased from 3.7 to 5.7% (Table 1) while the cellulose level remained similar (meanly 14.6%) among the feeds. Accordingly the LCR evolved from 0.26 to 0.40. With increased lignin level in the feed, without changing the cellulose content, the intake and growth of weaned rabbits linearly decreased (Table 2). This disagree with previous studies (Gidenne *et al.*, 2001, Gidenne, 2003), but here the period of measurements was only of 11 days after weaning. Accordingly the feed efficiency remained similar between groups, as well the number of rabbit dead or morbids.

Table 1: Analytical results for lignin (ADL) and cellulose (ADF-ADL) in experimental feeds.

Arbocel %/ Vitacel %	0/8	2/6	4/4	6/2	8/0
Lignin g/kg DM	37	44	48	52	57
Cellulose g/kg DM	139	148	147	150	144
Lignin:Cellulose- Ratio (LCR)	0.26	0.30	0.32	0.34	0.40

Table 2: Performances of weaned rabbits from 29th to 40th living day.

Arbocel %/ Vitacel %	0/8	2/6	4/4	6/2	8/0	
Lignin:Cellulose Ratio	0.26	0.30	0.32	0.34	0.40	*P level*
Performances from 29 to 40 d old						
Weight gain, g	514 ± 67 ^{a}	506 ± 85 ^{ab}	489 ± 83 ^{ab}	484 ± 109 ^{ab}	453 ± 120 ^{b}	*0.042*
Feed intake, g	1214^{a} ± 45	1119 ± 12 ^{ab}	1114 ± 109 ^{ab}	1117 ± 137 ^{ab}	996 ^{b} ± 129	*<0.001*
Feed efficiency	2.36 ± 0.17	2.21 ± 0.12	2.28 ± 0.27	2.31 ± 0.14	2.18 ± 0.25	*n.s.*
Morbidity*	9/40	8 / 40	8 / 40	8 / 40	14 /40	*n.s.*
Mortality*	0 /40	1 /40	2 /40	1 /40	3 / 40	*n.s.*

(Mean value ± Standard deviation for 40 rabbits per group. * : number of cases.

Increasing LCR decreased linearly the caecal VFA concentration (Table 3). The VFA concentration in the 0.40 LCR group was 57% less than in the 0.26 LCR group. The fermentation pattern showed some changes and did not evolved linearly with the LCR. The proportion in propionate was high for a LCR of 0.30, while the butyrate was low for a LCR of 0.26. Butyrate is an energy producing compound in the gut wall of caecum and colon and regulates the apoptose and mitose in the colon (Entschel, 2004). The decreased butyrate level may be a central focus in intestinal health of weaned rabbits.

Table 3: Influence of various Lignin: Cellulose Ratio on production on volatile fatty acids in caecum of weaned rabbits (Mean value ± Standard deviation).

Arbocel %/ Vitacel %	0/8	2/6	4/4	6/2	8/0	
Lignin:Cellulose Ratio	0.26	0.30	0.32	0.34	0.40	*P level*
Volatile Fatty Acids (VFA) mmol/l	109.2 ± 9.5^{a}	80.6±9.3 ^{bc}	69.9 ±11.2^{b}	66.0 ±14.3 ^{b}	62.3±12.2 ^{bd}	*<0.001*
Acetate % VFA	85.0 ±8.2	83.3±9.2	82.3 ±9.1	81.5 ±10.9	82.5 ±11.4	*n.s.*
Propionate % VFA	3.5±0.3 ^{ab}	3.8 ±1.1^{a}	3.1±0.5 ^{b}	3.6 ±0.7 ^{ab}	3.6 ±0.2 ^{ab}	*<0.001*
n- Butyrate % VFA	10.8 ±2.6^{a}	12.1 ±1.6^{ab}	13.3±2.0 ^{b}	13.7±3.0^{b}	12.2±1.6^{ab}	*0.012*
i- Butyrate % VFA	0.0 ±0.0	0.1 ±0.0	0.1±0.0	0.0 ±0.0	0.1±0.1	*n.s.*
i- Valerate % VFA	0.5±0.2	0.4±0.1	0.6±0.2	0.6±0.1	0.7±0.3	*n.s.*
n- Valerate % VFA	0.2±0.1	0.2±0.1	0.1±0.1	0.1±0.0	0.2±0.4	*n.s.*
Propionate:Butyrate	0.32±0.07	0.31±0.11	0.23±0.04	0.26±0.06	0.29±0.08	*n.s.*

a,b: means with a letter in common did not differ at the level P=0.05

The activity of microflora was clearly influenced by the lignin level. Earlier results have shown an increased growth of cellulolytic flora in the caecum (+ 1 $\log_{10}$) when cellulose was increased from 11 up to 17 % (Boulahrouf, 1991).

Gut Morphology

Similar to the VFA levels, the caecal crypts depth and width decreased linearly with the increase of the LCR from 0.30 to 0.40 (table 4), while the values were lower for the 0.26 LCR level. A maximum lignin content of 4.4% with a LCR ranging from 0.26 to 0.30 should be optimal for the growth of caecum crypts.

Table 4: Crypt depth and width in the caecum wall of weaned rabbits fed different lignin levels

Arbocel/ Vitacel % / %	0/8	2/6	4/4	6/2	8/0	
Lignin: Cellulose Ratio	0.26	0.30	0.32	0.34	0.40	*P level*
Crypt depth, µm	221±57[a]	236±66[a]	192±48[a]	150±35[b]	142±50[b]	*<0.01*
Crypt width, µm	36.1±22.3[a]	52.9±35.0[a]	40.4±29.0[a]	38.8±26.8[a]	14.2±8.1[b]	*<0.001*

The effect if purified lignin and cellulose on caecum wall morphology was described by Chiou *et al.* (1994), who found that 12 % lignin in the feed the caecal crypt depth was 207 µm and with 12% cellulose 108 µm resp. The authors linked the morphology of the caecal mucosa wall indirectly with the fermentation products. The caecal VFA level in these trials was the lowest in group with 12% lignin, and confirmed our results in the group 0.4 LCR. In opposite, the crypt depth in our investigations was the lowest in the group 0.4 LCR. Yu and Chiou (1997) reported a significant influence of feeding and dietary fibre content on intestinal mucosal damages. Feed with 14.4 % crude fibre showed a more damaged caecal mucosa cells in comparison with 11.5% or lower. Our results indicated also a reduction of caecal crypt morphometry, but similar results in VFA production.

CONCLUSION

Rabbits fed a high amount of lignin (0.4 LCR) showed a lower weight gain and intake. Increasing LCR led to lower caecal VFA levels, without changes in the fermentation pattern. This lower microbial activity may be linked to a reduction in the crypt development in caecum, that might be linked also to the energy metabolism in the mucosa and butyrate concentration.

The level of lignin and cellulose in pelleted feed and recommendations in minimal lignin level independent of the ratio lignin:cellulose is not advisable for feed calculation. More investigations are necessary to study the fermentation of non-starch polysaccharides like inulin in relationship to health management of weaned rabbits.

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