DIGESTIBLE FIBRE TO STARCH RATIO AND ANTIBIOTIC TREATMENT TIME IN GROWING RABBITS AFFECTED BY EPIZOOTIC RABBIT ENTEROPATHY

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ABSTRACT

The study aimed to evaluate if the digestible fibre (DF, hemicelluloses+pectins) to starch ratio of the diet and the time of antibiotic treatment after epizootic rabbit enteropathy (ERE) outbreak affect health status, digestive physiology, growth performance, and carcass traits of early weaned rabbits. Two hundred forty Grimaud hybrid rabbits were put in individual cages and controlled from 25 d of age to slaughter (70 d). The rabbits were assigned to four groups according to a 2x2 factorial arrangement (2 DF to starch ratios by 2 antibiotic treatment times). Half of the rabbits fed *ad libitum* diet L, with low (1.0) DF to starch ratio (19.1% DF and 18.9% starch as-fed) and half fed diet H with high (2.5) DF to starch ratio (23.9% DF and 9.6% starch). The diets were characterized by similar protein (16.8%), ADF (19.8%), and ADL (3.9%) concentrations. After the ERE appearance, half of the rabbits within diet were submitted to an early antibiotic treatment (from 38 to 42 d of age), while half of the rabbits to a late treatment (from 45 to 49 d). The antibiotic treatment was realized by an association of oxytetracycline hydrochloride (100 g/100 l) and colistin (24 g/100 l) administered in water. At 43 d of age, 36 rabbits (6 per group) were sacrificed to collect caecal content and ileum mucosa samples. Increasing DF to starch ratio of the diet improved fibre fraction digestibility ($P<0.001$) therefore maintaining a similar nutritive value (digestible energy=10.7 MJ/kg) among diets. Mortality (31.7% vs. 11.5%) and morbidity (38.5% vs. 18.5%) significantly decreased ($P<0.001$) by increasing DF to starch ratio, thus reducing sanitary risk by two thirds. These results were apparently associated to a higher caecal fermentation activity (volatile fatty acids: 49.6 vs. 60.7 mmol/l; $P=0.03$) in rabbits fed the high DF to starch diet, while ileal mucosa morphometry was unaffected. Once the health status was recovered, growth performance and slaughter results were scarcely affected by the dietary treatments. An early antibiotic treatment, administered within one week from the first ERE symptoms, reduced mortality (17.3 vs. 26.0%; $P=0.07$) and improved growth performance and slaughter results in comparison with a later treatment. Even the early antibiotherapy did not avoid that rabbit mortality reached a level unacceptable for a commercial farm, however. The association of a diet with a high DF to starch ratio and an early antibiotic treatment permitted to maintain the mortality at a basic level (5.8%) and provided the best growth performance and carcass quality.

Key words: Digestible fibre, Starch, Epizootic rabbit enteropathy, Growth performance, Health status.

INTRODUCTION

The high risk of severe mortality and morbidity consequent to the rapid outbreak of epizootic rabbit enteropathy (ERE) is the main reason for the frequent use of antibiotic treatments in the current rabbit production (Licois *et al.*, 2005). Feeding strategies can represent an alternative to antibiotherapy, but alone they do not seem capable of preventing the appearance of ERE. Since long time, low starch levels are recommended for both weaning and growing rabbits, but the negative role of starch on digestive troubles has been recently put under discussion (Gidenne and García, 2006). Low-digested fibre fractions (ADF = cellulose and lignin) are known to exert a protective role against enteric pathogens by regulating digestive transit and modulating caecal microflora and volatile fatty acid production. In addition, a positive effect of the most digestible fibre fractions (DF = pectins and hemicelluloses) on gut health has been recently outlined: the gut barrier function was improved and digestive troubles decreased when DF to ADF ratio increased (Gómez-Conde *et al.*, 2004 and 2007;

Xiccato *et al*., 2006; Fragkiadakis *et al*., 2007) or when DF replaced starch or protein in iso-ADF diets (Perez *et al*., 2000; Soler *et al*., 2004).

This study aimed to evaluate if the DF to starch ratio of the diet and the time of antibiotic treatment after ERE appearance can affect health status, digestive efficiency, caecal fermentation, growth performance, and carcass traits of early weaned rabbits.

MATERIALS AND METHODS

Animals and experimental design

Two hundred and forty rabbits of both genders of a Grimaud hybrid line were weaned at 25 d (live weight 486±40 g) and put in individual cages under controlled environmental conditions. They were assigned to four groups according to a 2x2 factorial arrangement (2 DF to starch ratios by 2 antibiotic treatment times). Half of the rabbits had *ad libitum* access to diet L, with low (1.0) DF to starch ratio (19.1% DF and 18.9% starch as-fed basis) and half to diet H, with high (2.5) DF to starch ratio (23.9% DF and 9.6% starch). The DF to starch ratio was increased by a higher inclusion of dried beet pulp, wheat bran and soybean meal at the expenses of barley, alfalfa meal and sunflower meal. The diets had similar energy and protein values as well as similar ADF and ADL concentrations (Table 1).

Table 1: Ingredients and chemical composition and nutritive value of experimental diets

	Diet L DF to starch = 1.0	Diet H DF to starch = 2.5
Ingredients (%):		
Dehydrated alfalfa meal (15,5% CP)	28.0	20.0
Wheat bran	10.0	25.0
Barley (six rows)	35.0	5.0
Dried sugar beet pulp	4.0	31.0
Soybean meal (48% CP)	3.5	11.0
Sunflower meal (30% CP)	15.0	4.0
Soybean oil	1.0	1.0
Cane and sugar beet molasses	1.5	1.5
Amino acids, minerals and vitamin premix	2.0	1.5
Chemical composition (% as fed):		
Dry matter	89.6	88.9
Crude protein	16.6	16.9
Total dietary fibre (TDF)	38.3	44.4
NDF	32.9	36.2
ADF	19.2	20.5
ADL	4.0	3.8
Digestible fibre (TDF-ADF)	19.1	23.9
Starch	18.9	9.6
Digestible energy (DE)[1] (MJ/kg)	10.7	10.7
Digestible protein to DE ratio[1] (g/MJ)	11.7	11.6

[1]Determined by *in vivo* digestibility trial. Average values of two groups with different antibiotic treatments within diet

Seven to 10 days after weaning, severe symptoms of ERE appeared and both *Escherichia coli* and *Clostridium perfringens* were detected. From 38 d of age, soon after the confirmation of ERE diagnosis by microbiological and antibiogram analyses on the first dead animals, half of the rabbits were submitted to a 5-days antibiotic treatment (Early treatment) by providing an association of oxytetracycline hydrochloride (100 g/100 l) and colistin (24 g/100 l) in drinking water. The remaining rabbits received the same therapeutic treatment from 45 d onwards (Late treatment).

Experimental procedures, recording and analyses

Individual live weight and feed intake were recorded three times a week. Health status was controlled daily and rabbits were considered ill when evidencing diarrhoea or a reduction of live weight and/or a 20% decrease of daily feed intake compared to the previous recording. A total of 45 rabbits died during the trial. Dead animals were not considered in the morbidity calculation. The sanitary risk was calculated as the sum of morbidity and mortality.

At 43 d of age (at the end of the early antibiotic treatment), 32 rabbits (8 per group) in apparently healthy condition were sacrificed to sample ileal mucosa and caecal content. At 53 d, a digestibility trial on 48 rabbits (12 per group) was performed as reported by Xiccato *et al*. (2003). At 70 d of age, 100 out of the 163 remaining rabbits were slaughtered in a commercial slaughterhouse. Twenty-four hours after slaughter, carcasses were dissected (Blasco *et al*., 2003) and the ultimate pH and L*a*b* colour of hind leg muscles measured.

Chemical composition of diets, faeces and caecal content were determined as described by Xiccato *et al*. (2003). Digestible fibre was calculated as the difference between total dietary fibre (TDF), determined by gravimetric/enzymatic procedure (AOAC 991.43, 32-07, 32-21, 985.29, 32-05), and ADF (AOAC 973.18). Growth performance of 163 animals and carcass traits of 100 animals were analysed by two-way ANOVA using the GLM procedure (SAS Inst. Inc., Cary, NC). The effect of the interaction DF to starch ratio by antibiotic treatment was not significant. Mortality, morbidity and sanitary risk were analysed by the CATMOD procedure of SAS.

RESULTS AND DISCUSSION

Since a standard definition of DF and a harmonized method to measure it are still lacking, in our study DF was assumed to be composed by hemicelluloses and pectins and calculated by difference between TDF, which contains both digestible and low-digested fibre fractions, and ADF, which contains only low-digested fibre.

Nor the time of antibiotherapy nor the dietary treatment modified digestibility of DM (62.7% on average) and GE (63.5%) (data not reported in table). However, the digestibility of both DF (58.1 vs 66.1%) and ADF (21.1 vs 29.0%) was significantly higher (P<0.001) in diet H than in diet L. This can explain the similar DE concentration found in the two diets despite starch concentration in diet H was 50% lower than in diet L.

Sanitary status was greatly affected by feeding (Table 2): both mortality (31.7% vs. 11.5%) and morbidity (38.5% vs. 18.5%) significantly decreased (P<0.001) by increasing DF to starch ratio, thus reducing sanitary risk by two thirds. Similarly, in a recent study (Fragkiadakis *et al.*, 2007), the best health condition was achieved by rabbits fed a low-starch diet with high DF to ADF ratio (mortality 2.8%, sanitary risk 13.9%), while the worst results by rabbits given a high-starch diet with low DF to ADF ratio (mortality 47.2%; sanitary risk 80.5%). Despite high starch-diets are known to favour digestive troubles, several studies did not show a clear relationship between dietary starch level and mortality (Gidenne and Garcia, 2006).

Table 2: Health status from 25 to 70 d of age

	DF to starch ratio			Antibiotic treatment time		
	1.0 (Diet L)	2.5 (Diet H)	Prob.	Early	Late	Prob.
Mortality (%)	31.7	11.5	<0.001	17.3	26.0	0.07
Morbidity (%)	38.5	18.5	<0.001	22.1	28.9	0.51
Sanitary risk (%)	70.2	24.1	<0.001	39.4	54.8	0.02

The time of antibiotic treatment affected health status at a lower extent than the dietary treatment. Rabbit treated at 45 d showed higher mortality (+50%, P=0.07) and sanitary risk (+39%, P=0.02) than rabbits treated at 38 d. However, even in the group treated soon after the confirmation of ERE, mortality reached a rate unacceptable in a commercial breeding. The best health condition was achieved when rabbits were fed the diet with high DF to starch ratio and were submitted to the early antibiotic treatment (mortality 5.8%, sanitary risk 19.2%), while the worst health status was shown by the rabbits fed the diet with low DF to starch ratio and submitted to the late antibiotic treatment (mortality 34.6%; sanitary risk 80.8%).

Growth performance was influenced by the dietary treatment as a consequence of the effects on the sanitary status (Table 3). Live weight at 46 d of age was significantly higher in rabbit fed diet H (P<0.001), which were in better health conditions at that moment. Final live weight and daily weight gain were not affected, however, due to the recover of good health and the compensatory growth of ill animals fed diet L. On the contrary, the late antibiotic treatment impaired rabbit health and their growth performance with lower daily gain and live weight both at 46 and 70 d of age. Feed intake and conversion index were lower in the late-treated animals.

Table 3: Growth performance from 25 to 70 d of age

	DF to starch ratio			Antibiotic treatment time			RMSE
	1.0 (Diet L)	2.5 (Diet H)	Prob.	Early	Late	Prob.	
Rabbits	71	92		86	77		
Live weight at 25 d (g)	487	485	0.71	488	484	0.58	40
Live weight at 46 d (g)	1278	1525	<0.001	1466	1337	<0.01	262
Live weight at 70 d (g)	2680	2732	0.23	2758	2654	0.02	270
Daily gain (g/d)	48.7	49.9	0.19	50.5	48.2	0.02	5.8
Feed intake (g/d)	124	131	<0.001	132	123	<0.001	16
Feed conversion	2.54	2.62	<0.001	2.61	2.55	<0.001	0.14

Despite the great differences between dietary treatments in the evolution of ERE incidence at the moment of sampling (43 d), caecal traits of all rabbits were typical of healthy animals, even if caecal fermentation pattern of those fed diet H appeared more favourable: increasing the DF to starch ratio increased total VFA production (49.6 vs. 60.7 mmol/l for diet L and H) and the proportion of acetate (78.6 vs. 83.7 mmol/100 mmol VFA) at the expenses of butyrate (16.1 vs. 11.6 mmol/100 mmol VFA). The characteristics of the intestinal mucosa were not significantly affected by the dietary treatment, however. Differently, Gomez-Conde *et al*. (2007) described improved mucosal integrity and functionality with increasing dietary soluble fibre.

The antibiotic treatment did not modify caecal fermentation activity: the higher N-ammonia found in the caecum of early-treated rabbits likely depended on the selection of microflora induced by antibiotherapy. The lower crypt depth of ileal mucosa in rabbits submitted to the late antibiotic treatment (P=0.04) and the similar villi height of ileum mucosa did not support the great differences in the health status between groups.

Table 4: Fermentation activity of caecal content and mucosa morphometry in rabbits at 43 d of age

	DF to starch ratio			Antibiotic treatment time			RMSE
	1.0 (Diet L)	2.5 (Diet H)	Prob.	Early	Late	Prob.	
Rabbits	16	16		16	16		
Gut content weight (% LW)	23.1	25.7	0.01	24.7	24.1	0.56	2.7
Caecal content traits:							
pH	5.80	5.77	0.74	5.78	5.79	0.89	0.22
$N\text{-}NH_3$ (mmol/l)	6.2	8.2	0.17	9.3	5.1	<0.01	3.9
Total VFA (mmol/l)	49.6	60.7	0.03	51.1	59.1	0.11	13.6
- C_2 (mmol/100 mmol VFA)	78.6	83.7	<0.01	81.5	80.8	0.65	4.1
- C_3 (mmol/100 mmol VFA)	4.7	4.3	0.63	3.9	5.1	0.16	2.3
- C_4 (mmol/100 mmol VFA)	16.1	11.6	<0.01	14.1	13.6	0.74	4.5
- C_5 (mmol/100 mmol VFA)	0.6	0.4	<0.001	0.5	0.5	0.90	0.1
Mucosa morphometry:							
Villi height (μm)	424	375	0.14	389	411	0.51	92
Crypt depth (μm)	100	98	0.83	106	91	0.04	19

As usually happens in rabbits, dietary treatment slightly affected slaughter results and meat pH and colour (data not reported). As the only relevant result, dressing percentage decreased from 59.3 to 58.2% (P<0.001) with increasing DF to starch ratio, as a consequence of the higher incidence of gut content (P<0.01), which is usually observed in rabbits fed diets with high sugar beet pulp inclusion levels. The earlier the antibiotic treatment the better the carcass quality as a consequence of the increased live weight: early-treated rabbits showed higher carcass weight (1589 vs. 1511 g, P=0.02) and dressing percentage (59.1 vs. 58.4%, P=0.06) than late-treated animals.

CONCLUSIONS

In conclusion, health condition was greatly improved and sanitary risk reduced by two third by increasing DF to starch ratio. This result was related to a higher caecal fermentation activity, but digestive efficiency and gut mucosa integrity were not affected by dietary treatment. Moreover, once the health status was recovered, final growth performance and slaughter results were not affected by the dietary DF to starch ratio. On the contrary, a late antibiotic treatment against ERE impaired rabbit health and growth performance, but even the early antibiotherapy only partially controlled the evolution of ERE after its outbreak. Feeding a diet high in DF to starch ratio associated to an early antibiotherapy after ERE outbreak maintained rabbit mortality at a minimum level and permitted to obtain the best growth performance and carcass quality.

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