IMPACT OF A DIETARY FIBER DEFICIENCY ON THE CÆCAL ECOSYSTEM OF THE YOUNG RABBIT. MODULATION BY YEAST PROBIOTICS.

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ABSTRACT

The effect of a fiber deficiency combined with a live yeast dietary supplementation (*Saccharomyces cerevisiae*) was analyzed on the young rabbit cæcal ecosystem from 28 (weaning) to 58 days of age. A bifactorial experimental design (2x2) was used: fiber concentration in diet (fiber deficient "FD" = 9% ADF *vs* 19% ADF for control diet), and yeast supplementation (10g/kg Actisaf® yeast, i.e 10^7CFU/gDM) or not. At 36 days of age, the cæcal pH was higher in FD groups (+0.3 units) than control. Ammonia (NH_3) level in cæcum at 58 days increased from 6.3 for control to 14.8 mmol/l for FD groups. Cæcal redox potential (E_h), diversity and structure of bacterial communities (fingerprint method) were not affected by the dietary fiber level. The yeast supplementation had no significant effect on biotope (pH, E_h, NH_3) and cæcal bacterial biocenosis (structure, diversity). However, the yeast supplementation tended to increase the bacterial biodiversity by 9% (P=0.06) compared to the control.

Key words: Probiotics, yeast, rabbit, biotope, biocenosis, ecosystem

INTRODUCTION

The rabbit cæcal ecosystem is influenced by biotic factors like probiotic, commensally or pathogenic microorganisms and abiotic factors like nutrients or feeding strategy. For instance, when the level of fiber in the diet is sharply reduced from 19 to 10% ADF, rabbit cæcal VFA decreased by 20% and pH and NH_3 increased, whereas the frequency of post-weaning digestive troubles is higher (Gidenne *et al*., 2008). Respect to microbial community, the density of cellulolytic bacteria were lower with a low-fiber diet, and the concentration of *Ruminococcus albus* tended to decrease (Bennegadi *et al*., 2003), without change in the total bacteria density. Effect of probiotics, such as live yeast on performance and digestive ecosystem were studied in several species. On dairy cow fed a starchy diet, a live yeast supplementation increased rumen pH (Marden, 2007), while in horse (Medina *et al.*, 2002), it increased the cæcal pH and reduced lactic acid concentration. In the growing rabbit, live yeast effects were only reported on growth performances and health after weaning (Falcao-e-Cunha *et al.*, 2007). But, no data are available on the effects of the live yeast on rabbit cæcal ecosystem.

Thus we aimed to evaluate the effect of live yeast supplementation on the young rabbit cæcal ecosystem (biotope and biocenosis), in response to a fiber deficiency.

MATERIALS AND METHODS

Experimental design and animal

A 2x2 factorial design was used with two levels of fiber deficient diet FD and control diet C (table 1), and yeast supplementation or not. Live yeast supplementation corresponded to 10 g/kg Actisaf® (*Saccharomyces cerevisiae NCYC Sc 47,* 10^7 CFU/g of dry matter, coated with saccharides, Lesaffre Feed Additives, Marquette-Lez-Lille, France). Consequently, four groups were constituted: FD0 and C0 and FD10 and C10. One hundred and forty four (144) young rabbits (INRA hybrid line), weaned at 28 days old, were caged collectively (four rabbits per cage) and allotted to the 4 groups.

Diets chemicals analyses

Diets were analyzed according the European Group on Rabbit Nutrition recommendations (E.G.R.A.N., 2001). Fibrous fractions (NDF, ADF and ADL) according to the sequential method of Van Soest et *al*. (1991) with an amylolytic pre-treatment, and crude protein (CP) was determined according to DUMAS combustion method using Leco auto-analyser (model FP-428, Leco Corporation, St Joseph, MI, USA).

Sampling procedure and physic–chemical parameters of cæcum

Cæcal samplings were made at 36 and 58 days of age. Ten healthy rabbits of each group were randomly selected and anesthetized using intra-muscular injection of ketamine, then cæcal pH and E_h were measured according to Kimsé *et al*. (2009). Then, rabbits were euthanized with intra-cardiac injection of embutramide, and cæcal content was sampled for further analyses of fermentation pattern (VFA, NH_3) and microbial community structure and diversity.

Analysis of cæcal bacteria community

DNA was extracted using QIAamp™ DNA Stool Mini kit from about 200 mg of fresh cæcal content. Bacterial community was analyzed after PCR amplification of the 16S rRNA gene V3 region using the W49-W104 primer pairs and according to Michelland *et al.* (2010). The cæcal bacterial community structure and diversity were realized using a fingerprint approach by single strand conformation polymorphism (SSCP), as described by (Michelland *et al.*, 2009). Data processing was computed with StatFingerprints software (Michelland *et al*., 2009), to calculate the Simpson diversity index (Michelland *et al.*, 2010).

Statistical analysis

The pairwise Euclidean distances of the 56 CE-SSCP profiles were calculated to test similarities among them (Michelland et al., 2010). To explore this distance matrix, non-metric Multidimensional Scaling (nMDS) was performed using 10000 random starts (nMDS is a two-dimensional display where each CE-SSCP profile is represented by a single point. Analysis of similarity (ANOSIM) was performed on the distance matrix using 10000 Monte Carlo permutations. Global ANOSIM was performed to test the fixed effects of fiber and yeast level. Pairwise ANOSIM was used to determine which level differed within a significant fixed effect. The factor tested was considered to be not significant if $P>0.05$, whatever the value of ANOSIM R. The factor tested was considered to be significant when $P<0.05$ and ANOSIM $R>0.25$. And relationships between bacterial communities, chemical and physical parameters were also tested using redundancy analysis (RDA). Diversity index and biotope parameters (pH, E_h, NH3) were subjected to analysis of variance using the GLM procedure of SAS.

Table 1. Ingredient and chemical composition of experimental diets

Ingredients	C (g/kg)	FD (g/kg)
Wheat bran	280	45
Barley	125	546
Lucerne meal	340	70
Wheat straw	31	20
Soya bean hulls	69	180
Sunflower meal	52	62
Sugar beet pulpe	80	20
Vegetable oil	10	-
Sugar	-	30
Mineral and premix[a]	13	27
Chemical composition, g/kg		
Starch	162	364
Crude protein	185	222
Crude fiber	171	73
Neutral-detergent fiber	374	201
Acid-detergent fiber	187	87
Acid-detergent lignin	47	21

C: control fiber diet; FD: deficient fiber diet; [a]: Vitamins: A: 1,500,000 UI/kg; D3: 200,000 UI/kg; E: 3000 mg/kg; B1: 200 mg/kg; K3: 50 mg/kg and oligo elements: Cu^{2+}: 800 mg/kg; Fe^{2+}: 8000mg/kg; Zn^{2+}: 20,000 mg/kg; Mn^{2+}: 4000 mg/kg and coccidiostatic: robenidine. b: calculated values.

RESULTS AND DISCUSSION

No interaction was found between live yeasts and dietary fiber factor on the cæcal biotope parameters: E_h, pH, DM and NH_3 (Table 2).

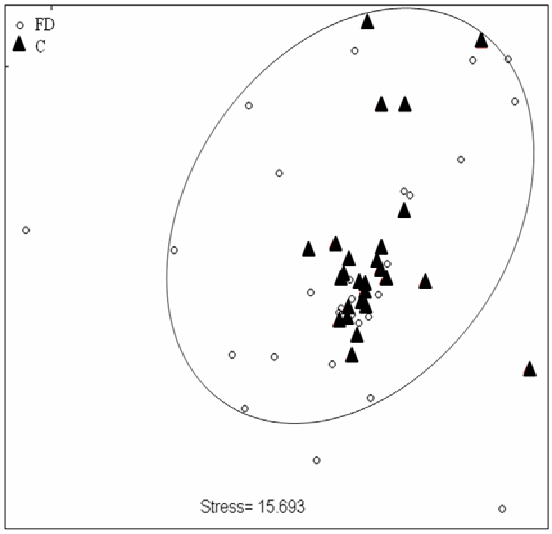
In optimal breeding condition, there were no significant effects of live yeast on growing rabbit performances or health status (Onifade *et al*., 1999). In this work, we lowered the dietary fiber level to increase the risk of digestive disorder and to potentially unbalanced the commensal bacterial community in the cæcum. Decreasing the fiber level led to an increase in pH and in DM and NH_3, +6% ($P<0.05$) and +135% ($P<0.01$) at 58 d respectively (Table 2). But, other biotope and biocenosis parameters were no significantly affected by the fiber level. The cæcal bacteria community was similar in FD and control (ANOSIM-R DF *vs* C = -0.01, P=0.1, Figure 1). The cæcal pH increased with a FD diet, but only one week after diet change, then the cæcal ecosystem seemed to be adapted to the low fiber intake (7 vs 15g ADF/d). Nevertheless, 30 days after weaning, the cæcal NH_3 doubled for rabbits fed a FD diet and DM increased by 8% ($P<0.01$). Cæcal bacteria community structure was not significantly separated and overlapped with yeast addition (ANOSIM-R = -0.1, P=0.3, Figure 2). However, the yeast supplementation tended to increase the bacterial diversity by 9% at 58 d (4.9 *vs* 4.5, P=0.07, table 2). In healthy rabbit, the cæcal biotope and biocenosis was not greatly affected by live yeast supplementation. The biotope parameters, notably pH and NH_3 concentration, were similar to those reported previously (Gidenne *et al*., 2007). The potential redox (E_h) was similar on each treatment. In this work, E_h was slightly higher (+ 30mV) than that reported by Kimsé *et al*. (2011) and Kimsé *et al*. (2009), but remained in the same range and indicated a strongly anaerobic medium. However, the cæcal bacterial diversity tended to increase with live yeast addition. Besides, Gidenne *et al* (2006) observed an increase in one fibrolytic bacteria proportion with live yeast supplementation. For instance, *Ruminococcus albus* rate tended to doubled in the young rabbit cæcum. In horse fed with high starch/low fiber diet, yeast supplementation modified the pH, and the fermentation pattern in the cæcum (Medina *et al*., 2002).

Table 2: Effect of diet fiber and yeast on healthy rabbit cæcal ecosystem and growth

	Fiber		Yeast		*RMSE*	*P*		
	C	FD	Y-	Y+	.	*Fiber*	*Yeast*	*Fiber* x *Yeast*
N	20	20	20	20				
36 days of age								
E_h (mV)	-196	-188	-194	-190	*23*	*0.3*	*0.5*	*0.3*
pH	5.72	6.03	5.905	5.85	*0.4*	*0.02*	*0.8*	*0.7*
DM (%)	23.4	23.9	23.6	23.7	*1.3*	*0.4*	*0.9*	*0.2*
NH_3 (mmol/l)	13.5	11.8	12.4	12.9	*4.0*	*0.3*	*0.8*	*0.4*
Boby weight (g)	1063.5	950.8	1005.5	995.7	*128.3*	*<0.01*	*0.65*	*<0.01*
ADF intake from 28 to 36d (g/d)	14.9	6.9	13.3	9.2	*2.7*	*<0.01*	*0.26*	*<0.01*
58 days of age								
E_h (mV)	-186	-185	-190	-182	*24*	*0.9*	*0.3*	*0.8*
pH	5.86	5.95	5.88	5.92	*0.3*	*0.4*	*0.8*	*0.4*
DM (%)	22.1	23.5	23.8	21.8	*2.3*	*0.05*	*<0.01*	*0.5*
NH_3 (mmol/l)	6.3	14.8	8.4	12.2	*6.2*	*<0.01*	*0.15*	*0.6*
Diversity*	4.88	4.54	4.89	4.51	*0.96*	*0.30*	*0.06*	*0.35*
Body weight (g)	2145.9	1794.6	1933.4	1927.3	*246.6*	*<0.01*	*0.92*	*<0.01*
ADF intake from 37 to 58d (g/d)	26.5	9.1	14.4	10.9	*5.9*	*<0.01*	*0.19*	*<0.01*

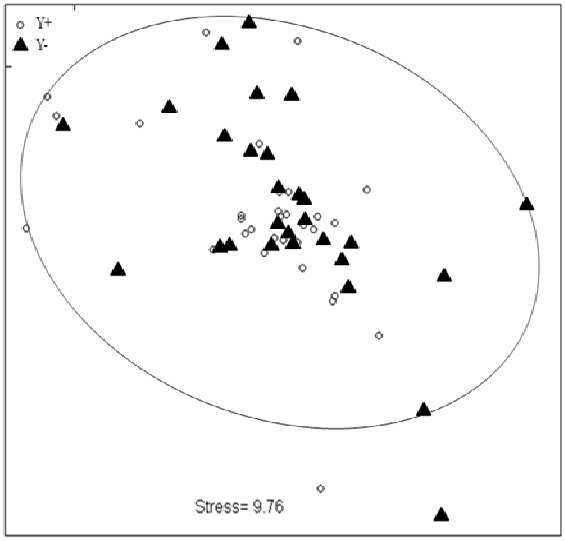
*: Simpson diversity, C : Control fiber diet, FD : fiber deficiency diet, Y- (C0+FD0) : 0% of Actisaf® live yeast in diet, Y+ (C10+FD10) : 1% of Actisaf® live yeast in diet, N: rabbit number, RMSE: root mean square error

Figure 1. Effect of dietary fiber level on the bacteria community structure approached by two-dimensional nMDS plot of the 56 CE-SSCP profiles.



C : Control fiber diet, FD : fiber deficiency diet

Figure 2. Effect of yeast probiotic (*S. cerevisiae*) on the bacteria community structure approached by two-dimensional nMDS plot of the 56 CE-SSCP profiles.



C : Control fiber diet, FD : fiber deficiency diet, Y- (C0+FD0) : 0% of Actisaf® live yeast in diet, Y+ (C10+FD10) : 1% of Actisaf® live yeast in diet

However, the fingerprint approach characterizes the major bacterial populations, and is limited to account 30 to 40 phylotypes. Since, the digestive microbiota would correspond to several hundred species of bacteria, some of them would not be detected here. Furthermore, CE-SSCP gives a semi-quantitative view of the cæcal colonization, and minor changes might be missed.

No significant correlations were found between biotope parameters (pH, E_h, DM, NH_3) and biocenosis (Simpson diversty index, microbial community structure). No correlation was found between biotope parameters. However, pH and NH_3 were slightly correlated with DM and R-squares were 0.30 and 0.12 respectively ($P<0.01$).

CONCLUSION

A dietary fiber deficiency in growing rabbit modified the cæcal biotope (DM, pH and NH3), but without a great impact on the microbiota structure or diversity as approached through a fingerprint analysis. A live yeast supplementation did not greatly modify the cæcal biotope and microbiota, except a tendency to increase the bacterial diversity (+ 9%). The present study performed in healthy rabbits failed to show a major impact of a fiber deficiency or yeast addition on the cæcal ecosystem, using the fingerprint approach.

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