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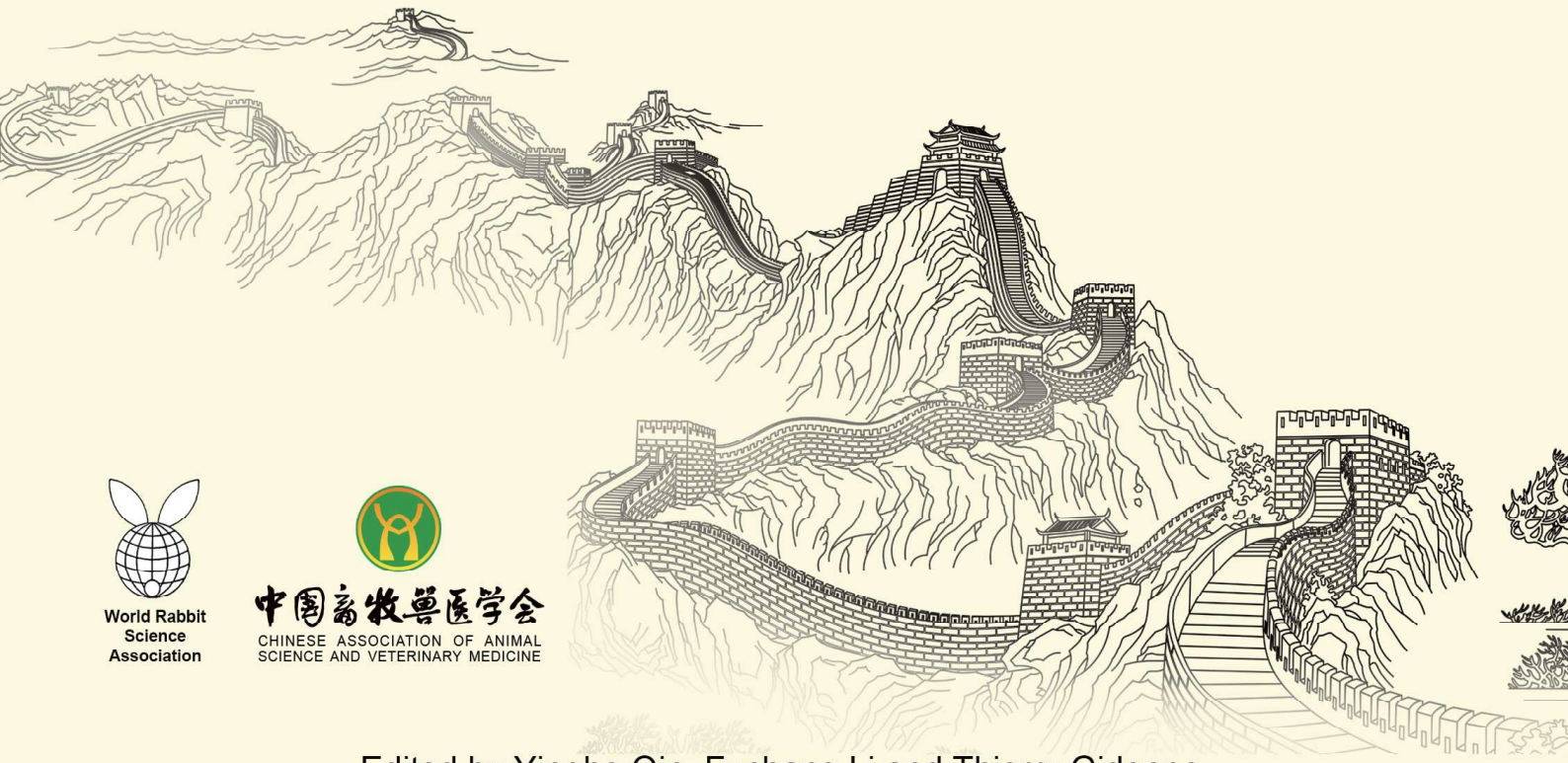

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IMPACT OF ANTIBIOTHERAPY AND RAPIDLY FERMENTABLE FIBER IN THE DIET OF YOUNG RABBIT ASSESSED BY TRANSCRIPTOMIC APPROACHES: PRELIMINARY RESULTS

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ABSTRACT

The aim of this study was to compare effects of diets with rapidly fermentable fiber or antibiotics on the immune response and the cecal microbiota of growing rabbits. A standard feed with antibiotics used to fight the epizootic rabbit enteropathy (AB) was compared to a diet containing rapidly fermentable fiber (RFF). A standard diet was used as a control group (C). A transcriptome profile was carried out on blood and ileal tissue, using microarrays, well-annotated for rabbit immune studies (n= 48 rabbits for blood and n= 24 rabbits for ileum). In blood, only 9 genes were differentially expressed (DE) according to diets at 29 d while no difference were observed at 45 d. At the ileum no difference were observed. On the opposite, age related change in gene profile expression was significantly different according to diet. In blood between 29 d and 45 d, sets of 1657, 438, and 0 DE genes were detected for C, RFF, and AB group, respectively. In the ileum, the gene expression was greater for RFF with 128 DE genes, followed by C (73 DE genes) and AB (36 DE genes). Preliminary analysis seem to indicate that diet affected the age related change of gene expression profile with a higher extend in blood than in ileum. On-going analysis will help to better describe their patterns of expression and interactions.

Key words: Rabbit, transcriptome, diet, rapidly fermentable fiber, antibiotics, blood, ileum

INTRODUCTION

In the last 20 years in rabbit production, the importance of nutrition has increased significantly as feed costs, pathological conditions associated with energy and nutrient deficiencies, and considerations of product quality have become limiting factors to economic output from a unit. At the same time, there has been an increase of digestive disorders such as the epizootic rabbit enterocolitis (ERE), which generates losses of around 25 to 30%. Antibiotic treatments are available (such as aminoglycosides or pleuromutilines) and manage to control this disease, although the exact cause of ERE is not yet known. Moreover, cases of resistance to these antibiotics were detected across different countries in the world (Sayah et al., 2004). But despite this, very few studies have focused on the immune profile of rabbits in breeding conditions, especially with diets containing high-digestible fiber. The aim of this study was to evaluate effect of diet on gene expression at two distinct level (blood and ileal tissues), using an updated and well-annotated microarray.

MATERIALS AND METHODS

Animals and experimental design

The experiment was carried out according to the European Union recommendations on the protection of animals used for scientific purposes (2010) at the PECTOUL Experimental Unit (INRA, Toulouse, France). A total of 398 rabbits (INRA breed) of both sexes were used for this trial. Animals were born from 42 litters, and adjusted between 9 and 10 pups per litter at birth. The 2 days-old pups were

allotted in three groups (n = 14 litters per group) and fed one of the experimental diet (n = 137 for RFF, n = 135 for C+AB, and n = 126 for C) from 16 d of age. Does and litters were housed in specific wire cages (width: 61 x length: 68 x height: 35 cm) containing a nest box for pups (width: 39 x length: 27 x height: 35 cm) with a 16 hour light schedule (0600 to 2200 h). Cages were organized to feed the does and pups different diets. Rabbits were weaned at 28 d and kept in their cages until 45 d.

There were 30 does who received a standard diet, and 15 does fed a medicated feed (oxytetracycline 400 mg/kg and colistine 2.4 m UI/kg). From 15 days of age, pups of medicated does received a standard diet with a relatively high level of low-digested fiber (ADF) and hemicelluloses, according to the fiber requirements of the growing rabbit (Gidenne et al., 2010). For pups of control does, the first half of animals were fed a standard diet enriched with antibiotics from 15 d of age (group C+AB). The medicated feed (C+AB) contained antibiotics : tiamulin (100 ppm) and apramycin (32.5 ppm). Both antibiotics are usually used to prevent ERE around weaning. The second half of pups received a diet with a high level of rapidly fermentable fiber (RFF), formulated to stimulate the microbial activity through a high supply of water soluble fiber and pectins from beet pulp and apple pomace. These diets were similar in terms of CP and NDF concentration. The RFF diet contained nearly 6% more RFF than C+AB and C diets, while the starch level was about 7% lower. The NDSF (Neutral detergent-soluble fiber; Hall et al., 1997) level was about 5% greater in the RFF diet than in the C diet. The feeds were distributed ad libitum at an early age, from 16 to 70 d of age. Water was freely available throughout the study.

Sampling procedures and transcriptomic analyzes

The slaughter of rabbits was carried out by electrical stunning and exsanguinations. Blood was collected at 15 and 29 d with PreAnalytiX PAXgene Blood RNA tubes (Qiagen, Valencia, CA, USA). Ileal tissues were sampled at, 29 d, and 45 d and were immediately frozen by direct plunging into liquid nitrogen. All samples were stored at – 80 degrees Celsius until RNA extraction. RNA extraction from whole blood was carried out per the manufacturer's instructions using the PreAnalytiX PAXgene Blood RNA Kit, Version 2 (Qiagen, Valencia, CA, USA). For ileal tissues, total RNA was extracted using the RNeasy Mini Kit (Qiagen, Valencia, CA, USA) and residual contaminating genomic DNA was cleaned by using RNase-free DNase I (Qiagen, Valencia, CA, USA). Transcriptional profiling was performed using a custom-designed 60 K Agilent microarray, well-annotated for immune studies in the rabbit (Jacquier et al., 2015). A total of 48 samples for blood were processed by the CRB GADIE facility (INRA Jouy-en-Josas, France, http://crb-gadie.inra.fr/). The 24 samples of ileal tissues were managed by the GeT-TRiX facility (INRA ToxAlim UMR1331, Toulouse, France, http://get.genotoul.fr/index.php?id=176). Both trials have strictly followed the same microarray protocol, as described in Jacquier et al. (2015).

Statistical Analysis

Microarray data were analyzed using the R/Bioconductor software package Limma (Linear Models for Microarray Data; Smyth et al., 2005). Log 2-transformed data were normalized by quantile normalization. After averaging probes targeting the same genes, a linear model was fitted for each gene given a series of arrays using lmFit function. Differential expressed (DE) genes were identified based on contrasts for the (stimulation × time levels) interactions. As we had a low number of biological replicates, we also used the empirical Bayes approach to compute moderated t-statistics and log-odds of differential expression. A principal component analysis (PCA) and a hierarchical clustering analysis (HCA) were performed. In the results and discussion, the FC value was transformed into linear value for a better biological understanding. Venn diagrams were produced using the Limma package (Smyth et al., 2005). Finally, the significant DE genes were analyzed with Ingenuity Pathways Analysis (IPA; Ingenuity Systems, Mountain View, CA; www.ingenuity.com) to obtain the top biological functions and relevant networks.

RESULTS AND DISCUSSION

The first step of the analysis was conducted intra-age (29 d and 45 d). At 29 d, only 9 genes were under-expressed with AB diet compared to control in blood. The most down-regulated gene was the serine peptidase inhibitor kazal type 4 (SPINK4, FC value = -1.92), followed by DCHS1 (FC value = -1.37), a member of the cadherin superfamily whose members encode calcium-dependent cell-cell adhesion molecules. At 45 d, there was no difference in gene expression between AB and C diets in blood. The RFF diet did not induce DE genes when compared with C group whatever the age (29 d or 45 d). The two experimental diets (AB and RFF) did not induce significant difference of expression in the ileum. These observations lead to conclude that diets didn't modify gene expression in circulating system and tissues.

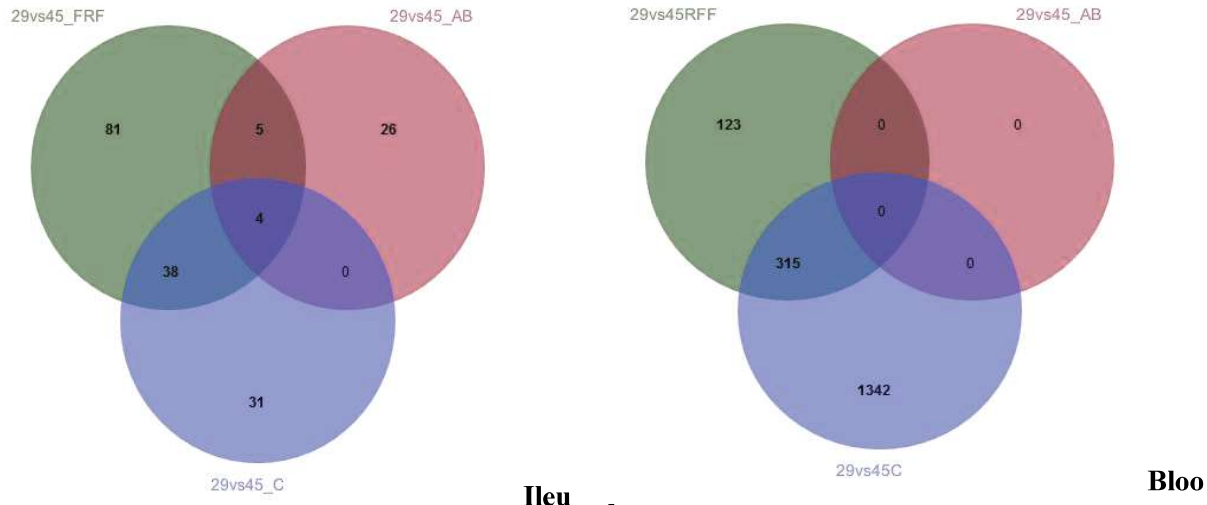

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Figure 1: Number of differential expression of genes between 29 d and 45 d for each diet (RFF: Rapidly fermentable fiber diet, C: control diet and AB: control diet supplemented with antibiotics), left side ileum and right side blood

The second step focused on the differential expression between 45 d and 29 d for each diet (C, FRF, and AB). Thus in blood, sets of 1657, 438, and 0 DE genes were detected for C, RFF, and AB, respectively. For the C group, the differential expression was mainly characterized by an up-regulation, because we recorded a total of 1,146 up-regulated genes against only 511 down-regulated genes, with FC values ranging from -2.12 to 2.51. The same trend was observed for RFF, with a slightly lower level of expression (337 up-regulated and 101 down-regulated genes ; FC values comprised between -1.25 and 2.26). No differential expression was observed for AB group between 45 d and 29 d. For both RFF and C, we had a significant up-regulation of genes involved in immune response with similar range of FC values, such as chemokine 5 (CCL5, FC = 2.26 and 2.09, respectively) and chemokine 4 (CCL4, FC = 1.35 and 1.25, respectively), the interferon regulatory factory 7 (IRF, FC = 1.79 and 2.14, respectively), the interleukin 1 receptor (IL1R, FC = 1.31 and 1.19, respectively), and the toll-like receptor 4 (TLR4, FC = 1.36 and 1.10, respectively) and 2 (TLR2, FC = 1.24 and 0.99, respectively. Moreover, the genes RLA-A1 (FC = 1.49 and 1.74, respectively) and RLA-A2 (FC = 1.62 and 1.73, respectively) were among the top DE genes and coding for the MHC class I region. For C diet, we recorded a down-regulation of the nuclear transcription factor PKNOX2 (FC = -2.12), followed by the dachsous cadherin-related 1 gene (DCHS1, FC = -2.05) and the meiosis specific with OB domains gene (MEIOB, FC = -1.84), all involved in cell proliferation. The top genes inhibited by the diet enriched in RFF between 29 d and 45 d were the NFKB inhibitor interacting ras-Like 2 gene (NKIRAS2, FC = -1.16), the peptidylprolyl isomerase (cyclophilin)-like 6 (PPIL6, FC = -1.09), and the centromere protein A gene (CENPA, FC = -1.07).

The gene expression in ileal tissues was greater for RFF with 128 DE genes, followed by C (73 DE genes) and AB (36 DE genes). With the RFF diet, the number of down-regulated genes (n=64) was equivalent to over-expressed genes (n=64), with FC values ranging from -4.65 to 2.68. But for AB and C groups, the down-regulation was lower with only 11 and 5 under-expressed genes, respectively, whereas activated genes were as numerous as RFF group (62 and 61, respectively). FC values were higher with AB diet compared to C (up to 4.23 vs. 2.93, respectively), but equivalent for inhibited genes (-4.30 vs. -4.06, respectively).

The two most age related up-regulated genes in RFF group were OASL (FC = 2.67) and MX1 (FC = 2.48). The 2'-5'-oligoadenylate synthetase-like gene (OASL) and the MX dynmin-like GTPase 1 (MX1) were both linked with antiviral activities. At the opposite, the Aldo-Keto Reductase Family 1, Member D1 gene (AKR1D1) and the 3-Hydroxy-3-Methylglutaryl-CoA Synthase 2 (HMGCS2) were the most down-regulated genes by RFF (FC = -4.65 and -4.40, respectively), and were involved in hepatic metabolism. AKR1D was also the most down-regulated gene for the AB group (FC = -4.30), followed, to a lesser extent, by the folate receptor 1 gene (FOLR1, FC = -1.16) and the thyroid hormone receptor alpha gene (THRA, FC = -0.75). Age related genes activated by the medicated feed AB were the granzyme B (granzyme 2, cytotoxic T-lymphocyte-associated serine esterase 1) gene (GZMB, FC = 3.63), the perforin 1 gene (PRF1, FC = 3.29), and the chemokine (C-C Motif) ligand 5 (CCL5, FC = 2.72). Others genes involved in the immune response were up-regulated, such as CXCR6 (FC = 2.14), CD96 (FC = 1.89), CD48 (FC = 1.55), CD8A (FC = 0.98), and IL15 (FC = 1.31). As for two other groups RFF and AB, AKR1D1 was the most down-regulated gene between 29 d and 45 d for C group (FC = -4.06), followed by the Deiodinase iodothyronine

CONCLUSIONS

Preliminary results of this study showed a higher gene expression in blood, compared to ileum. Moreover, the diet has also modified the level of gene expression, especially with the comparison 29d versus 45d. This observation suggests that age-related change in gene profile expression were significantly impacted by the diet, with a greater effect at the ileal level with RFF. In blood, our results showed that RFF allowed stabilizing the gene expression compared to C group. In both tissues, antibiotics treatments led to the lowest age related DE gene number. Ongoing analysis will allow to describe more specifically gene expression interaction and to focus on diet effect on age related change of the immune gene expression in order to help in rabbit health preservation.

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