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**PREDICTION OF GROWTH AND FEED EFFICIENCY PERFORMANCES
IN GROWING RABBITS FROM THEIR GUT MICROBIOTA**

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PREDICTION OF GROWTH AND FEED EFFICIENCY PERFORMANCES IN GROWING RABBITS FROM THEIR GUT MICROBIOTA

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ABSTRACT

Selecting for feed efficiency (FE) is challenging in rabbit breeding programs since individual feed intake (FI) of animals is not usually available. The current study raises the possibility that rabbits' cecal microbiota could be considered to predict FE and its component traits, i.e., growth and FI. Our dataset comprised the individual average daily gain (ADG) and cage FI records of 425 kits raised in two farms and fed with the same diet supplemented or not with antibiotics but under different feeding regimes. A 16S rRNA gene amplicons MiSeq sequencing assessment was conducted on cecal samples collected from those kits at 66 days. Paired-end sequences were processed with QIIME2 software resulting in a final table of 2,638 sequence variants for 424 samples. We run cross-validations fitting a sparse partial least squares regression (sPLSR) model with a microbial effect to assess its predictive ability on phenotypic ADG of animals fed V (ADG_V) and on animals fed R (ADG_R), and on their residuals after correction by management factors. For traits ADG, FI and FE, we run cross-validations to compare two models differing by including or not the cecal microbial information. Our sPLSR model showed some predictive capacity for phenotypic ADG_V (0.40) and ADG_R (0.09), but this capacity becomes null for the prediction of the residuals of these traits. Although cecal microbiota explained more than 50% of the variation of ADG, fitting the microbial effect in the model did not improve the predictive accuracy of the recorded values. Cecal microbiota explained 51 and 59% of the variation of FI and FCR, respectively. Unlike growth, models that considered the microbial information improved the predictive accuracy for FI and FCR recorded performance values in 3 and 10%, respectively.

Key words: feed efficiency, growth, gut microbiota, predictive models, rabbit.

INTRODUCTION

Given that food expenses often suppose up to 70% of the production costs, feed efficiency (FE) is an essential trait in rabbit breeding. However, most programs do not perform a direct selection for this trait since it is difficult to measure the individual animals' feed intake (FI). A common alternative to improve FE has been to conduct an indirect selection for average daily gain (ADG) or body weight (BW) at the end of the growing period (Estany *et al.*, 1992). However, the genetic correlation between growth traits and FE may not be high enough to result in an optimal selection response (Piles *et al.*, 2004).

Microbial populations that inhabit the gastrointestinal tract (GIT) of animals constitute a complex ecosystem whose members constantly interact between them and with their host. Those interactions ensure homeostatic balance maintenance since GIT components are involved in many physiological and immunological processes. The cecum is the main organ harboring the microbial fermentation processes in

the domestic meat rabbit, *Oryctolagus cuniculus*, and hosts the richest and the most diverse microbial community of the GIT. That explains why cecum has been the organ of choice for the rabbit gut microbiota assessment (Velasco-Galilea *et al.*, 2018). The rapid development of next-generation sequencing (NGS) technologies and their swiftly decreasing costs allows deeper comprehension of microbial composition and diversity differences found between animals shaped by the production environment.

In the field of livestock production, certain studies have gone one step further and hypothesized that the rabbit gut microbiota could be associated with BW (Zeng *et al.*, 2015) or FE (Drouilhet *et al.*, 2016). However, there is not any study in rabbits reporting the potential association of cecal microbiota with growth and FE performances.

The aim of this study was to assess the value added by the cecal microbiota for the prediction of individual ADG and collective FE performances of growing rabbits raised under two different feeding regimes.

MATERIALS AND METHODS

Animals and experimental design

The biological samples used in this study were collected from animals of a sire line (Caldes) raised in different periods and in two different farms (from July 2012 to July 2014 in farm A, and from April to June 2016 in farm B). For the present study, 425 kits were randomly selected from five batches. All animals were raised under the same management conditions and fed with a standard pellet diet supplemented with antibiotics (except 23 raised in farm B that received a free-antibiotic diet). Immediately after weaning (32 days of age), these kits were randomly assigned to a feeding regime (FR): *ad libitum* (V) or restricted (R) to 75% of the V intake. The rabbits were housed in collective cages during the growing period. Their body weights (BW) and the cage total FI were weekly recorded. ADG was computed as the slope of the regression line after fitting a linear model with all BW measurements recorded during the growing period. Cage-recorded traits of animals fed V were computed as the total FI of the cage divided by the number of days and rabbits raised in the cage (FI) and as the ratio of the average cage FI to ADG (FCR).

DNA extraction, libraries generation and sequencing

Cecal samples were immediately collected after animals slaughtering, kept cold (4°C), and stored at -80°C. Genomic DNA was extracted from 250 mg of each cecal sample with ZR Soil Microbe DNA MiniPrep™ kit (ZymoResearch, Freiburg, Germany) following the manufacturer's instructions. Integrity and purity of DNA extracts were measured using a NanoDrop ND-1000 spectrophotometer equipment (NanoDrop products; Wilmington, DE, United States). A fragment containing V4-V5 hypervariable regions of the 16 rRNA gene was amplified with the pair of primers F515Y/R926 (Parada *et al.*, 2016) and re-amplified in a limited-cycle PCR to add sequencing adaptors and 8 nt dual-indexed barcodes of multiplex Nextera® XT kit (Illumina, Inc., San Diego CA, United States) following the manufacturer's instructions. Final libraries were paired-end sequenced in parallel in a MiSeq Illumina 2x250 platform at the Autonomous University of Barcelona.

Bioinformatics

Sequence processing was performed using QIIME2 software (version 2018.6; Bolyen *et al.*, 2018). The raw sequence data with quality information were imported from Casava 1.8 paired-end demultiplexed FASTQ formatted files into a QIIME2 artifact. The sequence quality control to detect and correct Illumina amplicon sequencing errors, as well as the removal of the chimeras, were performed in a single step with

the DADA2 pipeline (Callahan *et al.*, 2016), implemented through the q2-dada2 plugin. The output table containing for each sample the counts of unique sequences, i.e., 100% amplicon sequence variants (ASVs), was clustered into ASVs with 99% similarity. The ASV table was filtered at: (1) sample level by discarding samples with less than 5,000 final sequence counts and at (2) ASV level by removing the doubleton ones.

Statistical analysis

To unravel whether microbial information can be used to predict growth performance when it is corrected by management factors whose capacity to shape the rabbits' cecal microbiota is known, a sparse partial least squares regression (sPLSR) model with a microbial effect (based in a microbial relationship matrix that was represented by the weighted Unifrac phylogenetic distance matrix) was fitted for phenotypic ADG of animals fed V and R (ADG_V and ADG_R, respectively) and for their residuals after correction by those factors. Cross-validations were replicated 5 times. In each of them, the dataset was randomly split into 4 folds, 3 of which were used to train the models and the remaining to test them. The correlation coefficient between the observed and the predicted data was used to assess the predictive ability of models. Univariate microbial mixed models were fitted for ADG_V, ADG_R and cage-recorded traits FI and FCR of animals fed V. Random effects included in the model were the additive genetic, the litter, the cage, the microbial, and the error. The model included the batch, the presence or the absence of antibiotics in the food, the BW at weaning, and the regression on the age of the rabbit as systematic effects. To deal with the fact that microbial information was not available for some of the rabbits within a cage, an expansion of the microbial relationship matrix was performed by adding an identity matrix for those animals. The residual maximal likelihood (REML) variance components estimation was carried out using the REMLF90 program (Misztal, 2002). For each trait, two cross-validations were compared to assess whether including microbial information in the model improves its predictive ability: one fitting the mixed model above and another fitting the same model but without the microbial effect. Cross-validations were replicated 20 times. In each of them, the dataset was randomly split into 5 folds, 4 of which were used to train the models and the remaining to test them. The correlation coefficient was used as the performance criterion.

RESULTS AND DISCUSSION

The filtered 99% ASV table contained the sequence counts of 2,638 ASVs for 424 samples. The sPLSR model fitting the microbial effect exhibited moderate and low predictive abilities for phenotypic ADG_V and ADG_R, respectively. However, this model could not predict same traits corrected by environmental factors related with management. (Table 1).

Table 1: Means across 5 cross-validation replicates for regression parameters and correlation coefficients between the observed and the predicted values for phenotypic and residuals ADG_V and ADG_R.

	ADG _V PHE	ADG _V RES	ADG _R PHE	ADG _R RES
Intercept	18.53 (11.73)	0.03 (0.60)	32.43 (9.62)	-0.01 (0.81)
Slope	0.63 (0.23)	0.01 (0.15)	0.17 (0.24)	0.06 (0.21)
Corr. coef.	0.40 (0.11)	0.00 (0.09)	0.09 (0.12)	0.03 (0.10)

While Buitenhuis *et al.* (2019) found that the proportion of variance explained by the rumen microbiota (m^2) was generally smaller than that of the genetic component (h^2) for milk fatty acid composition of Holstein cattle, our results suggest that cecal microbiota explains a large percentage of the total phenotypic variance of ADG_V (0.52) and ADG_R (0.59). However, the models fitting the microbial effect did not exhibit a better predictive ability of growth-related traits than the models that did not consider such information (Table 2). In the case of average cage traits, our m^2 estimates (0.51 and 0.59 for FI and FCR, respectively) were also higher than the h^2 estimates (0.22 and 0.28 for FI and FCR, respectively). For these

traits, the models that fitted the microbial effect showed a higher predictive ability of the performance values than the models that ignored that effect (Table 2). Delgado *et al.* (2019) also found a certain capacity of rumen microbiota to predict FI and FE in cattle. Remarkably, microbial information improves by 3 and 10 percent the predictive ability of the mixed model for FI and FCR, respectively.

Table 2: Means across 20 cross-validation replicates for ratios of phenotypic variance and correlation coefficients between the observed and the predicted values for ADG_V, ADG_R, FI and FCR.

	ADG _V Model 1	ADG _V Model 2	ADG _R Model 1	ADG _R Model 2	FI Model 1	FI Model 2	FCR Model 1	FCR Model 2
h ²	0.11 (0.04)	0.10 (0.04)	0.04 (0.03)	0.05 (0.02)	0.19 (0.08)	0.22 (0.08)	0.31 (0.15)	0.28 (0.10)
m ²	-	0.52 (0.07)	-	0.59 (0.05)	-	0.51 (0.11)	-	0.59 (0.09)
Corr. coef.	0.20 (0.07)	0.21 (0.07)	0.16 (0.11)	0.16 (0.10)	0.79 (0.10)	0.81 (0.10)	0.61 (0.15)	0.67 (0.14)

Model 2 includes the microbial effect. Model 1 is identical to model 2 but without the microbial effect.

h²: heritability. Fraction of the phenotypic variance of the traits explained by the additive genetic effect.

m²: microbiability. Fraction of the phenotypic variance of the traits explained by the microbial effect.

CONCLUSIONS

The main finding of this study is that, although cecal microbiota appears to explain a significant percentage of the phenotypic variance of ADG_V and ADG_R, the consideration of microbial information does not improve the predictive ability of the models when management factors are accounted. Therefore, cecal microbiota and growth are both affected by animal management at growing. However, microbiota does not directly explain the variations in rabbit growth. In contrast to the individually recorded growth, adding this information in the models that aim to predict collectively measured traits related to FE is important since it significantly increases their predictive ability.

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IRTA

Prediction of growth and feed efficiency performances in growing rabbits from their gut microbiota

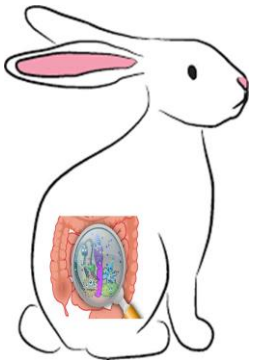
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BACKGROUND & OBJECTIVE

- **Feed intake** can represent **up to 70%** of the production **costs** in rabbit breeding.
- **Difficulties** in **measuring individual intake** impair direct selection for feed efficiency.
- It is worth exploring **new traits/factors** allowing alternative considerations of feed efficiency in breeding programs.

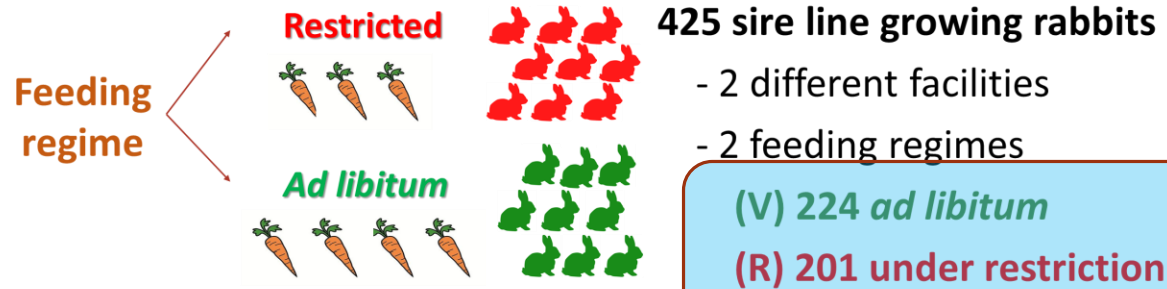


GUT MICROBIOTA a **NEW TRAIT/FACTOR** influencing **FEED EFFICIENCY**

To assess the **value** added by **microbial information** for the **prediction** of **individual growth** and **collective feed efficiency** performances.

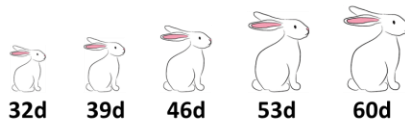
MATERIALS & METHODS

Animals



Traits

Average daily gain (ADG)

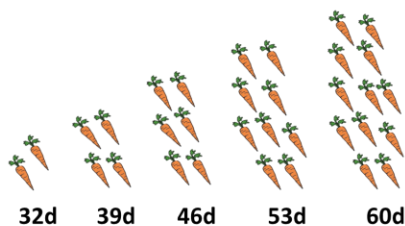


Individual body weight (BW_d)
weekly recorded

$$ADG_R = (BW_{60} - BW_{32}) / 28$$

$$ADG_V = (BW_{60} - BW_{32}) / 28$$

Feed intake (FI)



Cage feed intake (FI_w)
weekly recorded

$$\overline{FI}_V = \sum FI_w / (28 \text{ d} * 8 \text{ rabbits})$$

$$FCR_V = \overline{FI}_V / \overline{ADG}_V$$

Sample processing & sequencing

Collection of cecal samples at day 66



DNA extraction



Library generation

**Amplification V4-V5
16S rRNA gene
(primers 515Y/926R)**

**MiSeq
sequencing**



Bioinformatics for OTU calling

- Filtering sequences & removing quimeric contigs
- Clusterization of **final contigs** into **OTUs**
- Filtering & CSS normalization of **OTU table**
 1. At sample level (< 5,000 reads)
 2. At OTU level (< 0.01% counts)

Final OTU table: 425 samples & 963 OTUs

ANALYSIS OF INDIVIDUAL GROWTH

Variance Components Estimation

M1 $y = X\beta + Z_A a + Z_L l + Z_C c + e$

vs

M2 $y = X\beta + Z_A a + Z_L l + Z_C c + Z_M m + e$

$a \sim NMV(0, A\sigma_A^2)$

$l \sim NMV(0, I\sigma_L^2)$

$c \sim NMV(0, I\sigma_C^2)$

$m \sim NMV(0, M\sigma_M^2)$

$M = OO'$ & O = row-normalized CSS OTU count matrix

Parameter	Model	ADG _V	ADG _R
h^2	M1	0.21 (0.14)	0.21 (0.14)
m^2	M1	-	-
h^2	M2	0.07 (0.07)	0.13 (0.09)
m^2	M2	0.67 (0.15)	0.56 (0.12)

Predictive Ability by Cross Validation

Average (SD) across 100 replicates

Correlation observed – predicted in Validation subset

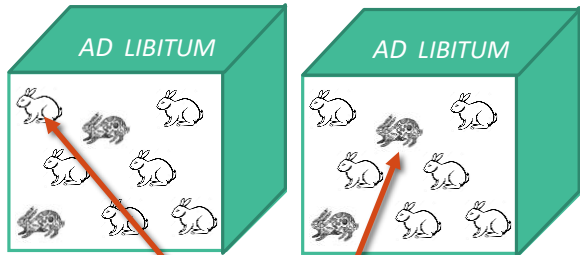
Model	ADG _V	ADG _R
M1	0.30 (0.15)	0.39 (0.13)
M2	0.36 (0.13)	0.56 (0.11)

ANALYSIS OF COLLECTIVE FE TRAITS

Challenges:

- 1.- Traits recorded as cage-averages.
- 2.- Microbiota information recorded individually.
- 3.- No all the cage mates have microbiota information.

$$\overline{FI}_{jmn} = B_j + S_m + \sum_{k=1}^{N_n} \frac{1}{N_n} l_{ok} + \sum_{k=1}^{N_n} \frac{1}{N_n} a_{ik} + \sum_{k=1}^{N_n} \frac{1}{N_n} b_{pk} + e_{jmn}$$



$$\mathbf{B}_{exp} = \begin{bmatrix} \mathbf{I} & \mathbf{0} \\ \mathbf{0} & \mathbf{M} \end{bmatrix} \quad \mathbf{b} \sim \text{NMV}(0, \mathbf{B}_{exp} \text{and} \sigma^2_B)$$

Variance Components Estimation

Parameter	Model	FI_V	RFI_V	FCR_V
h^2	M1	0.26 (0.18)	0.49 (0.20)	0.34 (0.20)
h^2	M2- \mathbf{B}_{exp}	0.19 (0.13)	0.33 (0.15)	0.22 (0.14)
m^2	M2- \mathbf{B}_{exp}	0.48 (0.18)	0.38 (0.17)	0.47 (0.18)

Predictive Ability by Cross Validation

Model	FI_V	RFI_V	FCR_V
M1	0.79 (0.11)	0.42 (0.21)	0.61 (0.16)
M2- \mathbf{B}_{exp}	0.83 (0.08)	0.50 (0.19)	0.69 (0.12)

CONCLUSIONS

- A novel modeling approach to consider **individual microbial** information for **collective performances** even when microbiota was not assessed in all individuals of the cage.
- The consideration of **microbial information** led to **improvements** in the **prediction** of individual growth and feed efficiency traits.
- These improvements dependent of the microbial relationship matrix used.

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OPEN

The value of gut microbiota to predict feed efficiency and growth of rabbits under different feeding regimes

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Gut microbiota plays an important role in nutrient absorption and could impact rabbit feed efficiency. This study aims at investigating such impact by evaluating the value added by microbial information for predicting individual growth and cage phenotypes related to feed efficiency. The dataset comprised individual average daily gain and cage-average daily feed intake from 425 meat rabbits, in which cecal microbiota was assessed, and their cage mates. Despite microbiota was not measured in all animals, consideration of pedigree relationships with mixed models allowed the study of cage-average traits. The inclusion of microbial information into certain mixed models increased their predictive ability up to 20% and 46% for cage-average feed efficiency and individual growth traits, respectively. These gains were associated with large microbiability estimates and with reductions in the heritability estimates. However, large microbiability estimates were also obtained with certain models but without any improvement in their predictive ability. A large proportion of OTUs seems to be responsible for the prediction improvement in growth and feed efficiency traits, although specific OTUs taxonomically assigned to 5 different phyla have a higher weight. Rabbit growth and feed efficiency are influenced by host cecal microbiota, thus considering microbial information in models improves the prediction of these complex phenotypes.

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Feed-a-Gene



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