Exploring Mortality and Culling Causes of Breeding Does through Gut Microbiome Analysis

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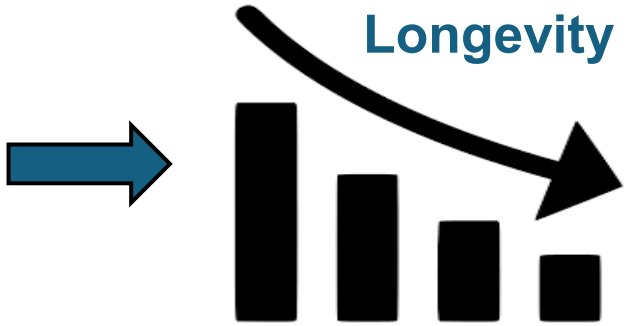
Introduction

Selection for **high litter size at weaning** in maternal lines has been **successful**



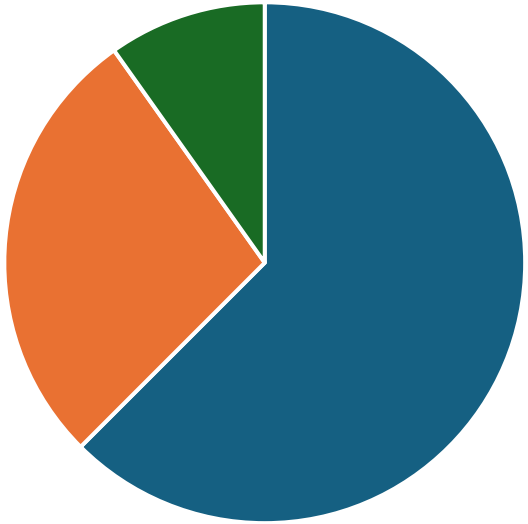
In Spain, adult female rabbits faced a **monthly mortality risk of 3.2%,** accompanied by a **culling risk of between 5.5 and 7%** (Rosell and González, 2009).

- High replacement rates
- Half of the breeders are culled in the first three parities.

Introduction

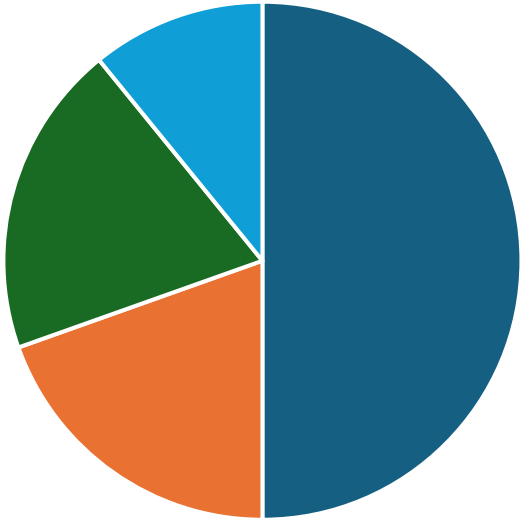
The **main mortality** and **culling causes** are as follows:

Mortality



- Respiratory disease
- Digestive disorders
- Mucoid enteropathy

Culling



- Low productivity
- Mastitis
- Poor body conditions
- Sore hocks

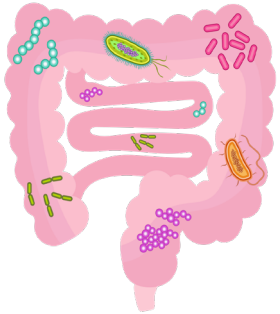
Introduction

Understanding the **causes of mortality** and **culling** is important from an **economic**, **health** and **welfare** perspective

- Improve the longevity and productivity of the females and their litters
- Reduce economic losses in commercial farms
- Better animal health and welfare

Rosell and De la Fuente 2016

Introduction



- Gut microbiota is often associated with **immune system**, **disease**, and **mortality** (Fortun Lamothe and Boullier, 2007)

- Recent studies focused on the link between the **gut microbiome** in rabbits and their **life expectancy** (Funosas et al. 2021 and Biada et al. 2024)

- However, the relationship between **microbiota** and the **causes of culling** and **mortality** in rabbits remains unexplored

Objective

Compare the gut microbiome composition between different culling causes and mortality in female maternal lines

- This could guide **selection** against microbiomes that are associated with adverse outcomes
- Enable **early detection** of at-risk animals

Material and methods

Samples collection

Number of parities

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20

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Maternal lines selected for **litter size at weaning**.

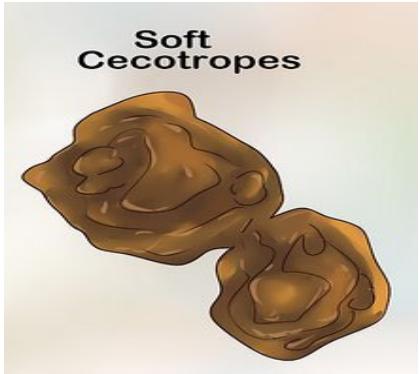
Material and methods

Samples collection

Number of parities

1 2 3 4

Mortality / culling cause



Material and methods

Samples collection

Number of parities

1 2 3 4 5 6 7 8 9 10 11 12

Mortality / culling cause

Material and methods

Samples collection

The recorded **mortality and culling causes** for each female were organized in three categories:

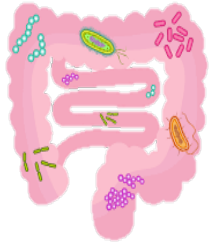
CR: Culled for reproductive reasons or infertility (n= 17)

CH: Culled for health reasons (n= 12)

D: Deceased (n= 15)

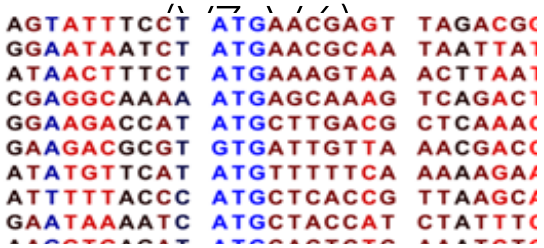
Material and methods

Gut microbiota (Faeces)



DNA extraction

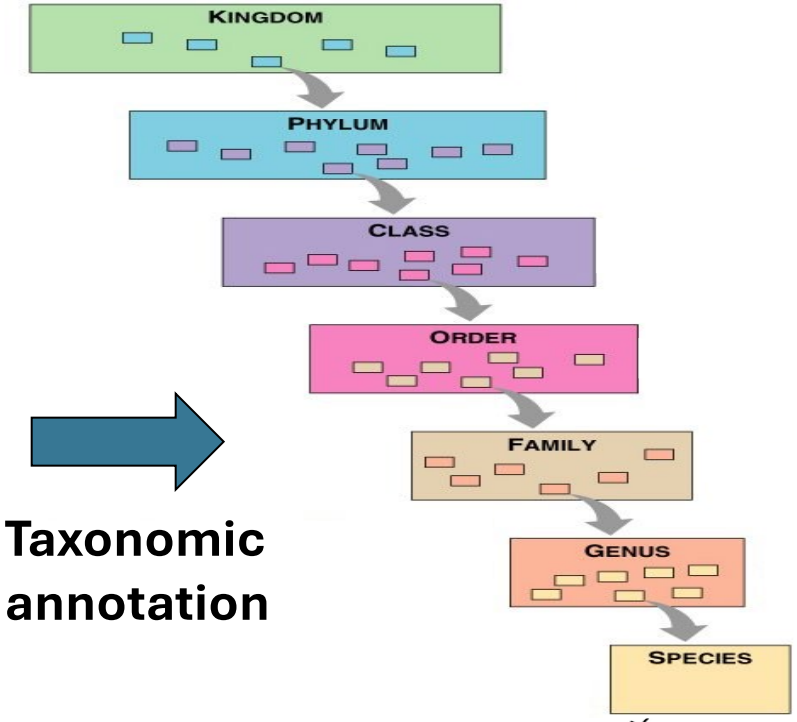
16S rRNA sequencing



DADA2

Amplicon Sequence Variants **(ASV)**

Taxonomic annotation

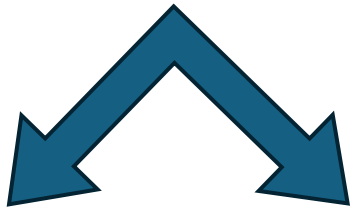


silva
high quality ribosomal RNA databases

Material and methods

Core microbiome

1. **Occurrence** of at least **80%** across all samples
2. Minimum **relative abundance** of **0.1%**



The core microbiome of the entire dataset

The core microbiome specific to each group of mortality and culling cause

Material and methods

Partial Least Square Discriminant Analysis (PLS-DA)

- Removed variables present in less than 20% of samples
- Added a pseudo count to datasets to handle zeros

↳ Centered log-ratio (CLR) transformation

$$CLR(\mathrm{j}|\mu) = log\left(\frac{x_\mathrm{j}}{\mu}\right) = \log(x_\mathrm{j}) - \log(\mu)$$

Material and methods

Partial Least Square Discriminant Analysis (PLS-DA)

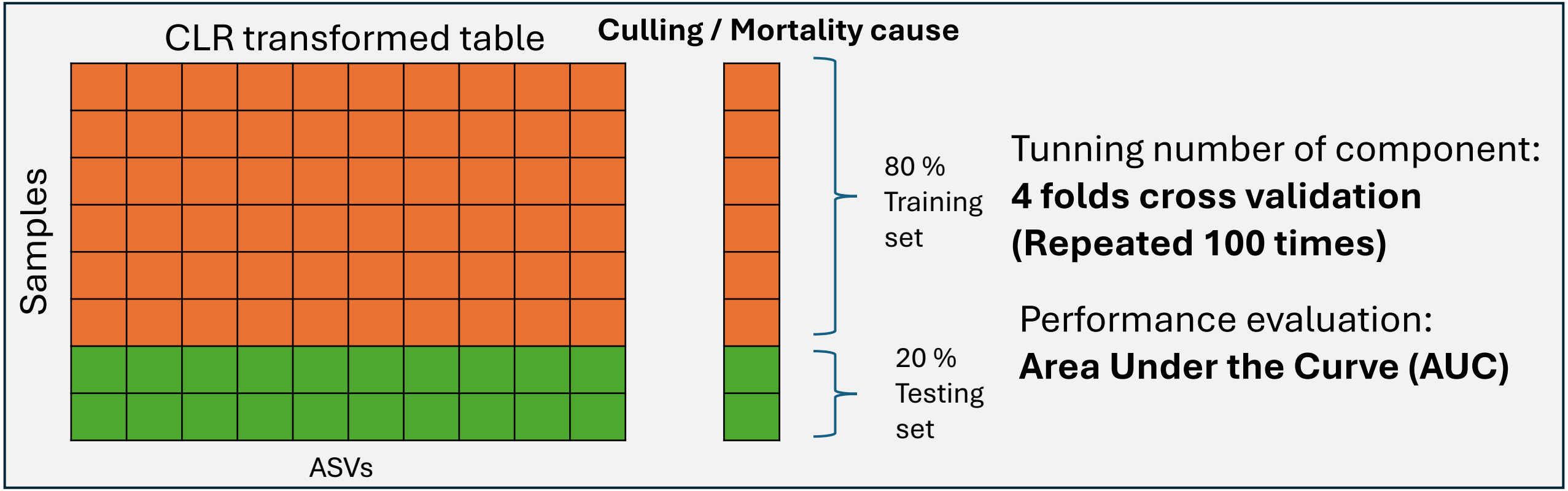
Samples were collected from animals ranging from **26** to **172 weeks of age**

The microbiome is **dynamic**, and the **age** of the animal is one of the main factors influencing microbial communities.

Correction for age effect using the **“removeBatchEffect”** function from the limma package in R

Material and methods

Partial Least Square Discriminant Analysis (PLS-DA)



Variable selection: ASVs with Variable importance prediction (VIP) lower than 1 were iteratively eliminated until the model reached the lowest error.

Results

Core microbiome

Whole dataset core microbiome of **98 ASVs**, and when collapsed to the genus level, the core microbiome was represented by **23 genera.**

Table 1. Core microbiome of each cull and mortality group for amplicon sequence variants (ASVs)

	Core ASVs	Unique ASVs
All dataset	98	0
Culling for reproductive causes (CR)	87	14 (16%)
Culling for health causes (CH)	110	14 (13 %)
Deceased (D)	124	27 (22%)

Results

Core microbiome

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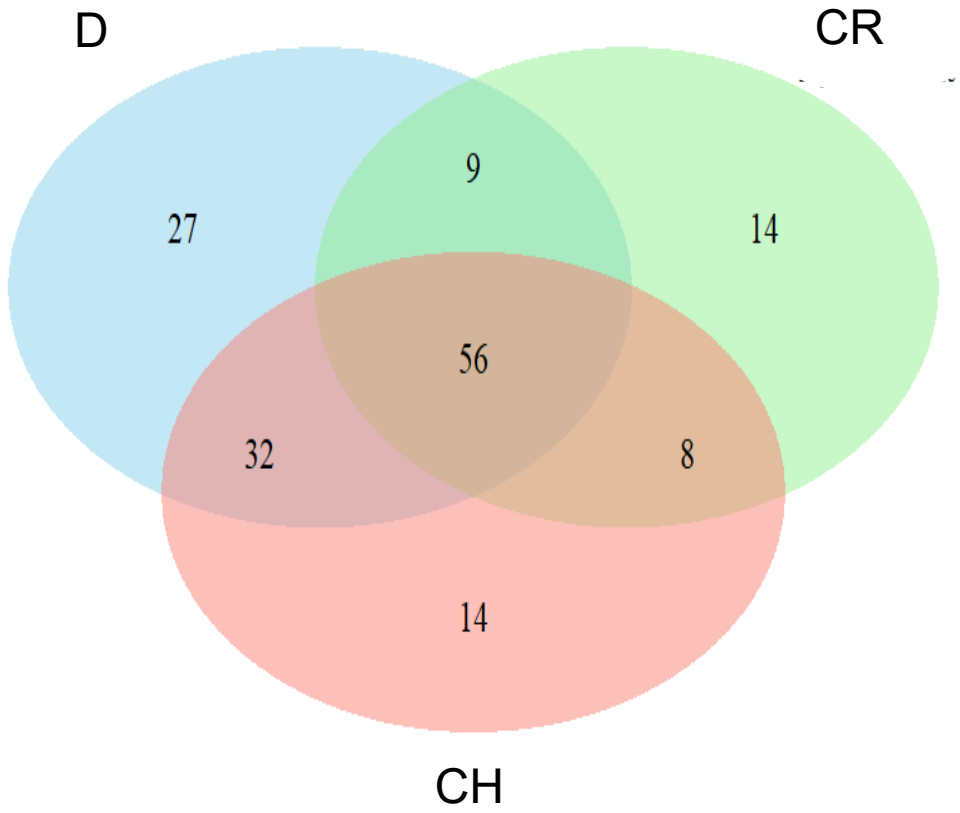

Results

Core microbiome

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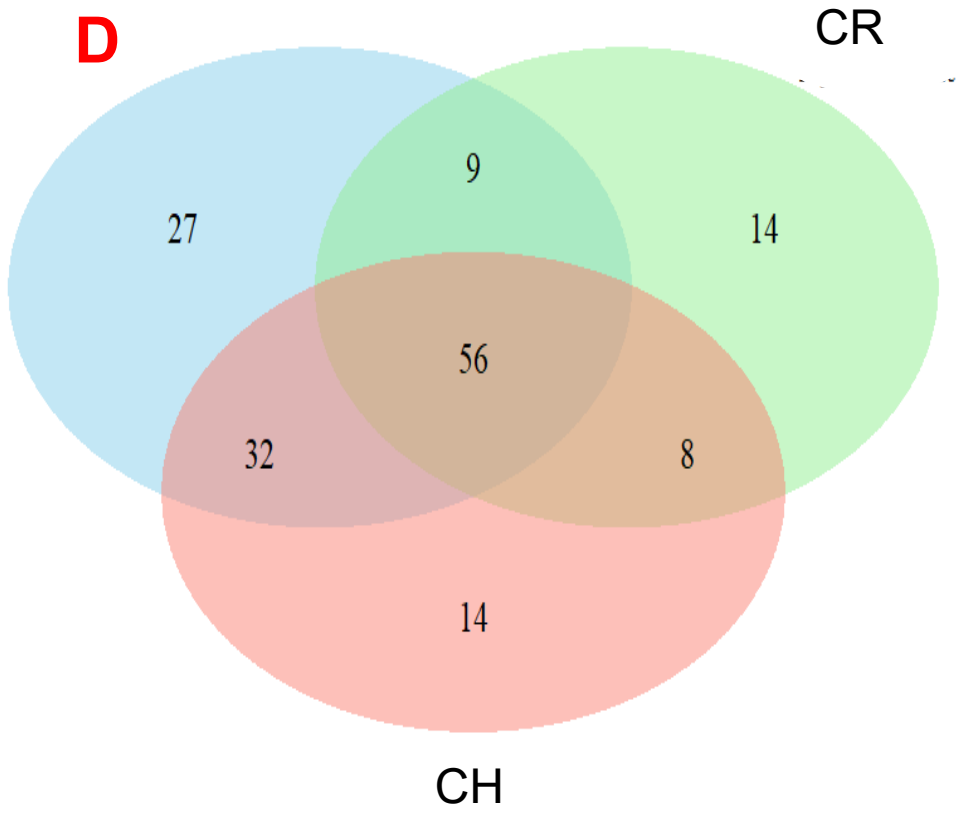
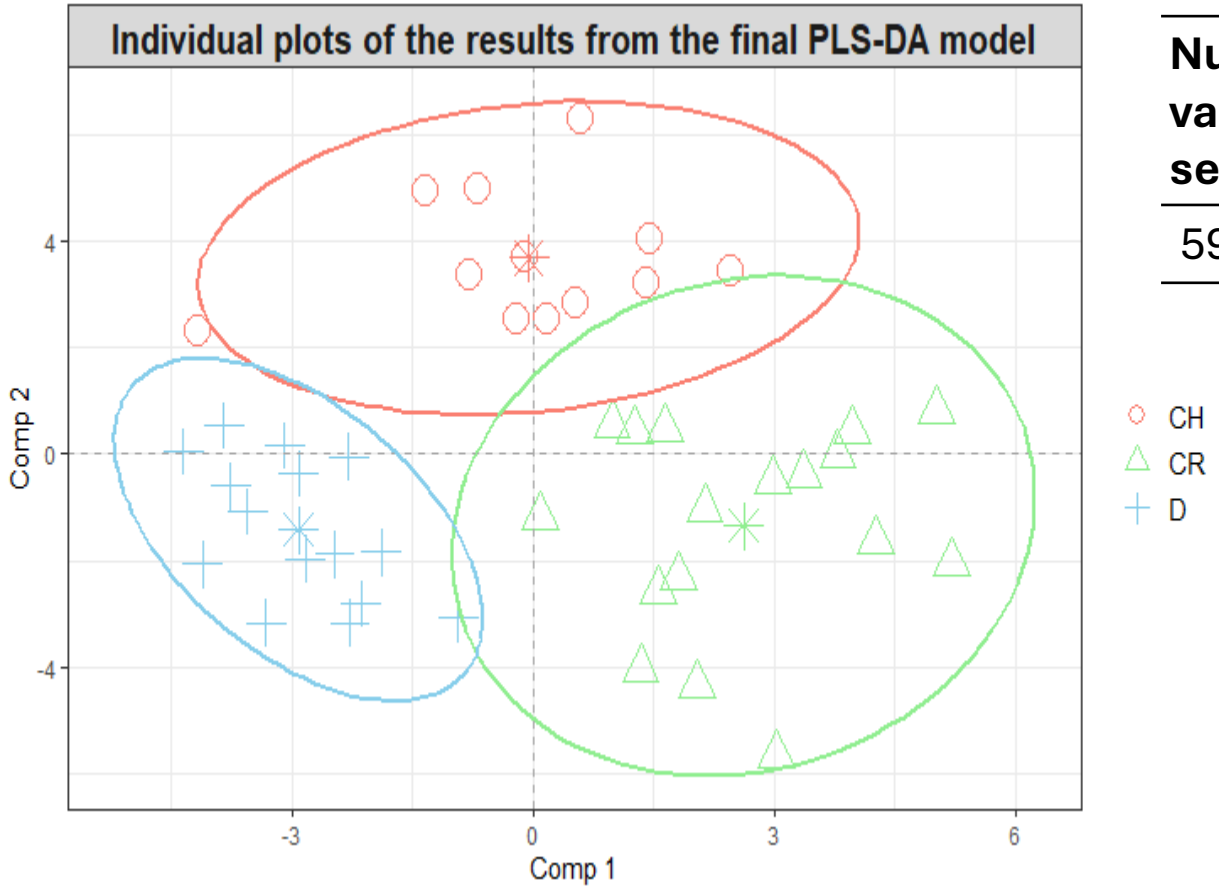


Results

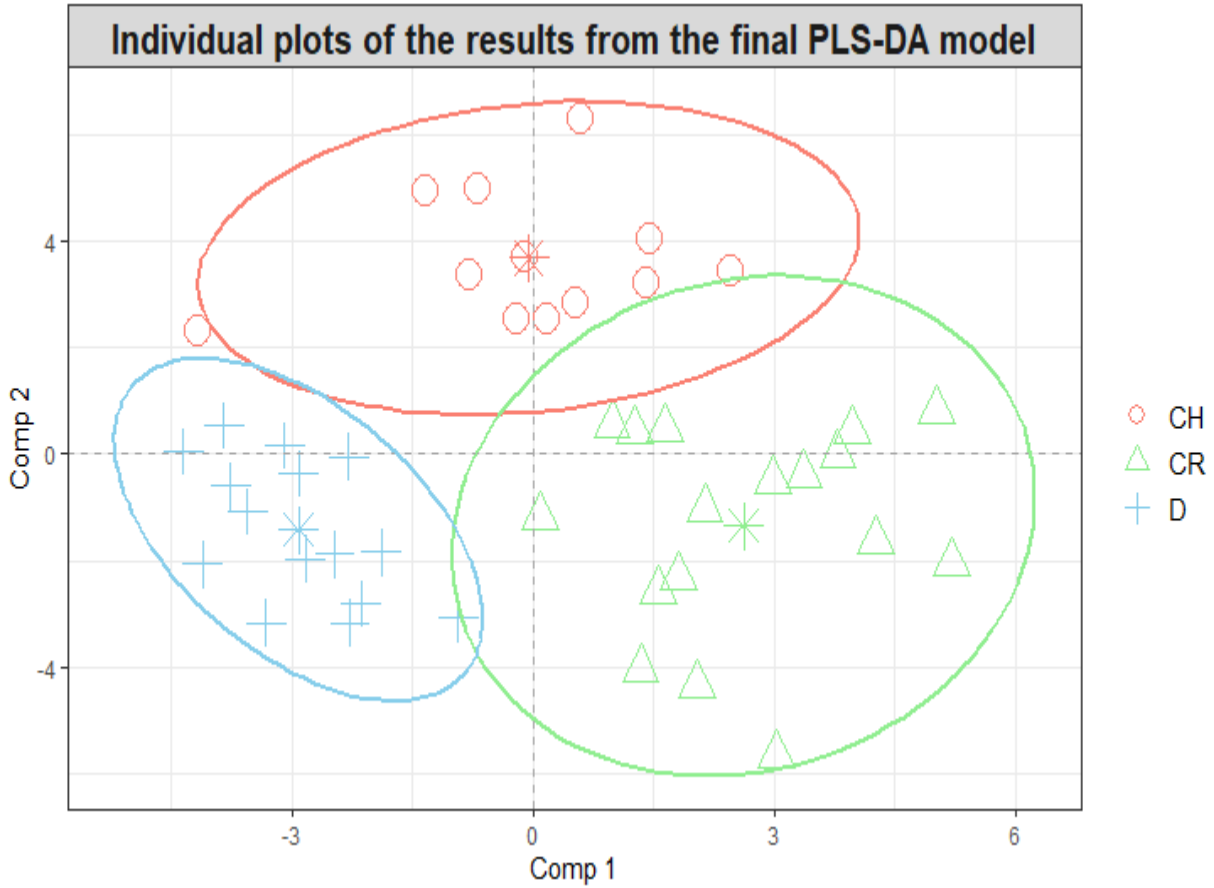
PLS-DA



Number of variables selected	Number of components	Mean AUC	Standard deviation of AUC
59	2	0.97	0.04

Results

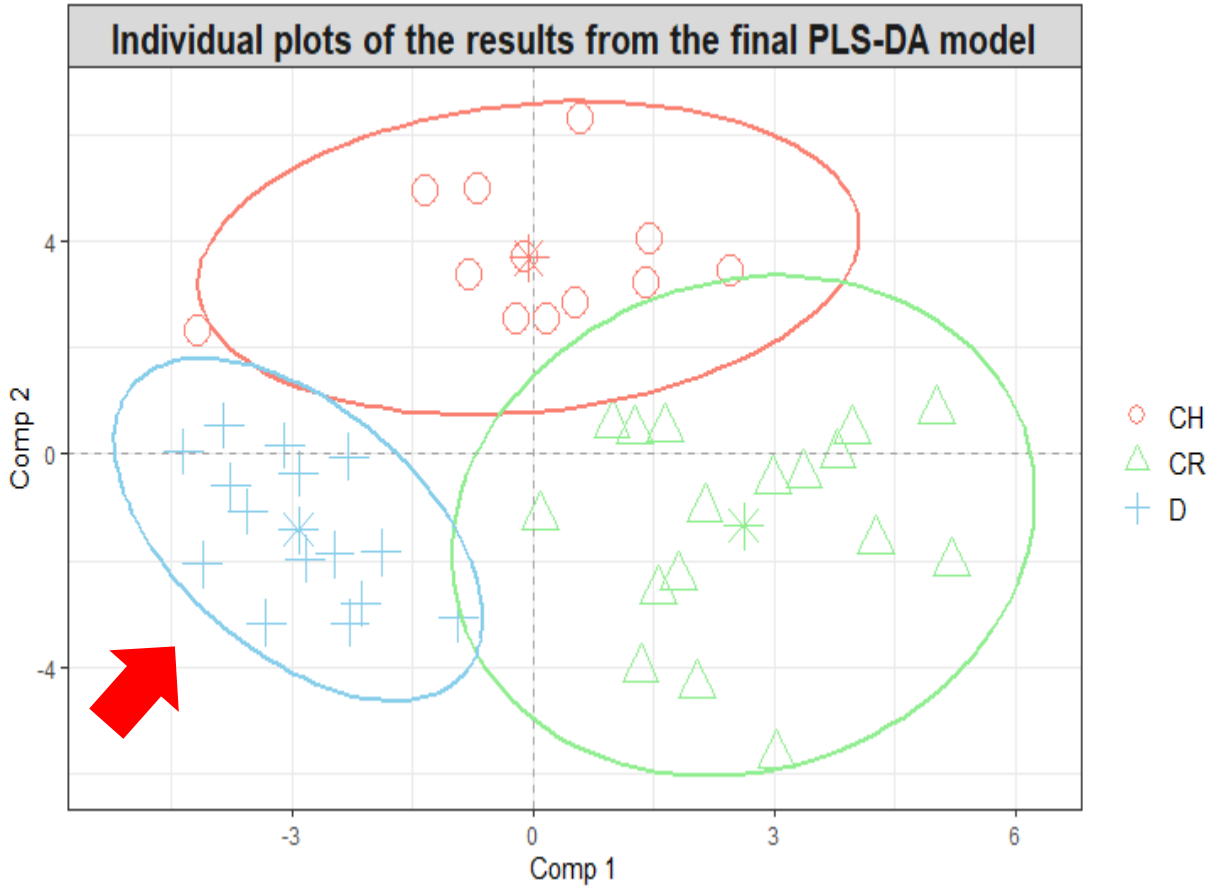
PLS-DA



Confusion matrix	Predicted as		
	D	CH	CR
Deceased (D)	96	0	4
Culling for health causes (CH)	8	90	2
Culling for reproductive causes (CR)	6	11	83

Results

PLS-DA

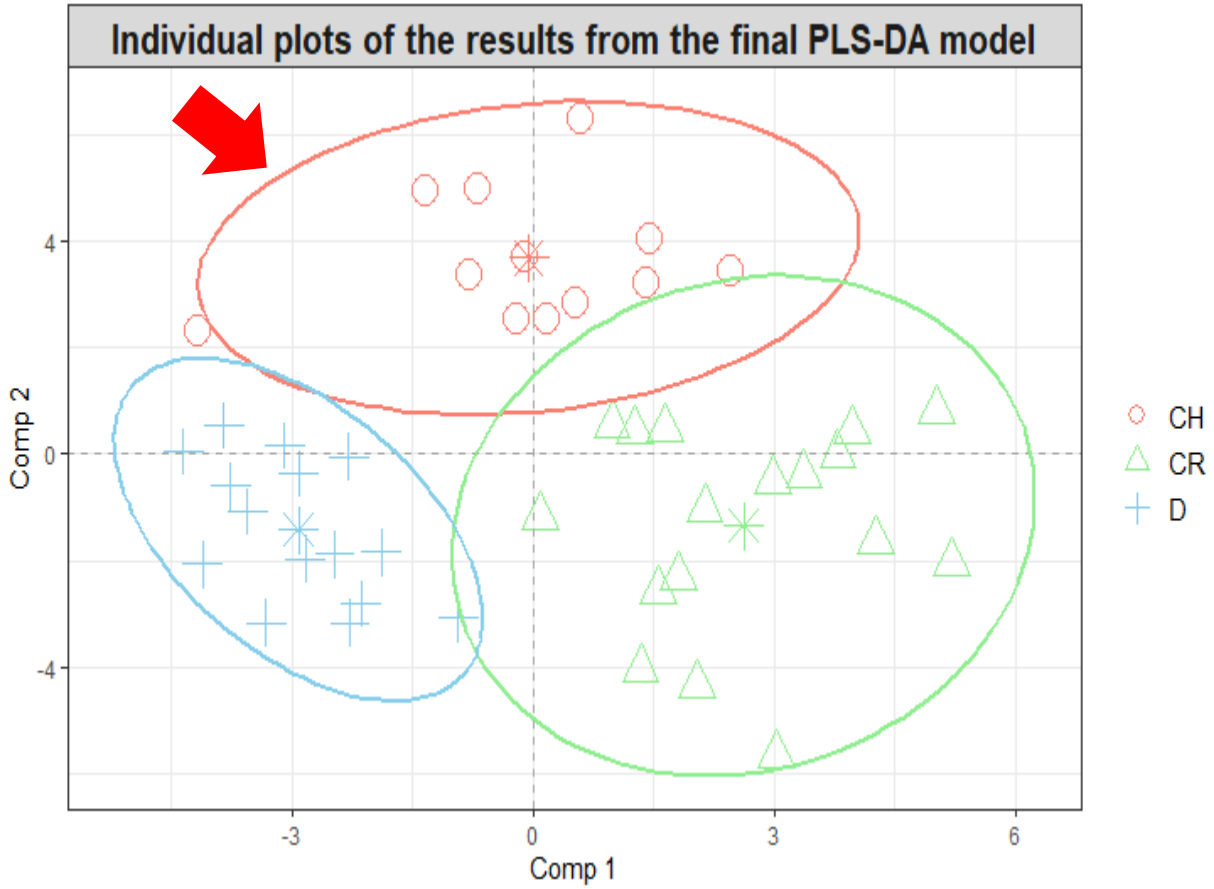


Confusion matrix	Predicted as		
	D	CH	CR
Deceased (D)	**96**	0	4
Culling for health causes (CH)	8	90	2
Culling for reproductive causes (CR)	6	11	83

Very high accuracy for predicting **D (96%)**, indicating strong performance, with only a small proportion is misclassified as CR (4%).

Results

PLS-DA



Confusion matrix	Predicted as		
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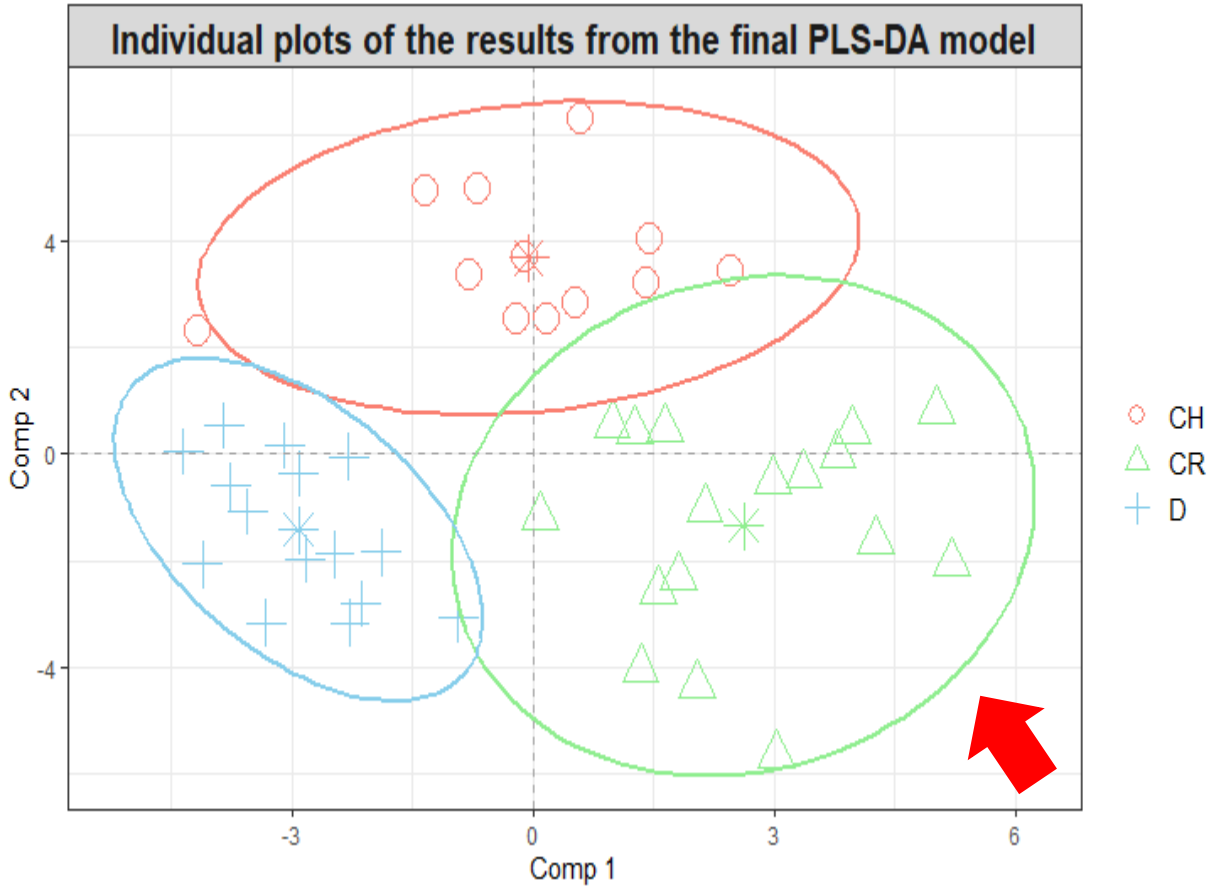
Deceased (D)	96	0	4
Culling for health causes (CH)	8	**90**	2
Culling for reproductive causes (CR)	6	11	83

The model performs **very well** in predicting **CH (90%)**, with some misclassification into D (8%) and CR (2%).

Results

PLS-DA



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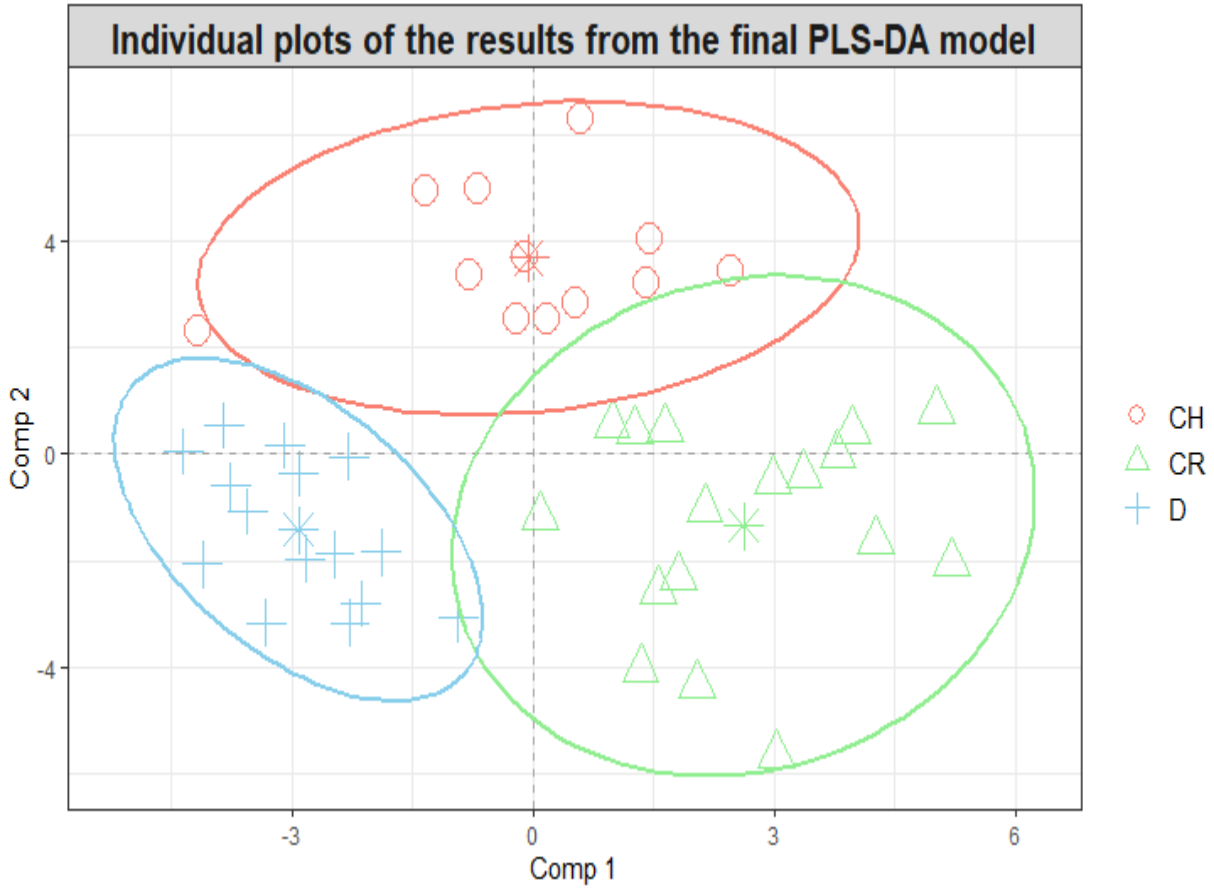
Deceased (D)	96	0	4
Culling for health causes (CH)	8	90	2
Culling for reproductive causes (CR)	6	11	**83**

The model performs **relatively well** in CR but has some misclassification into **D (6%)** and **CH (11%).**

Results

PLS-DA



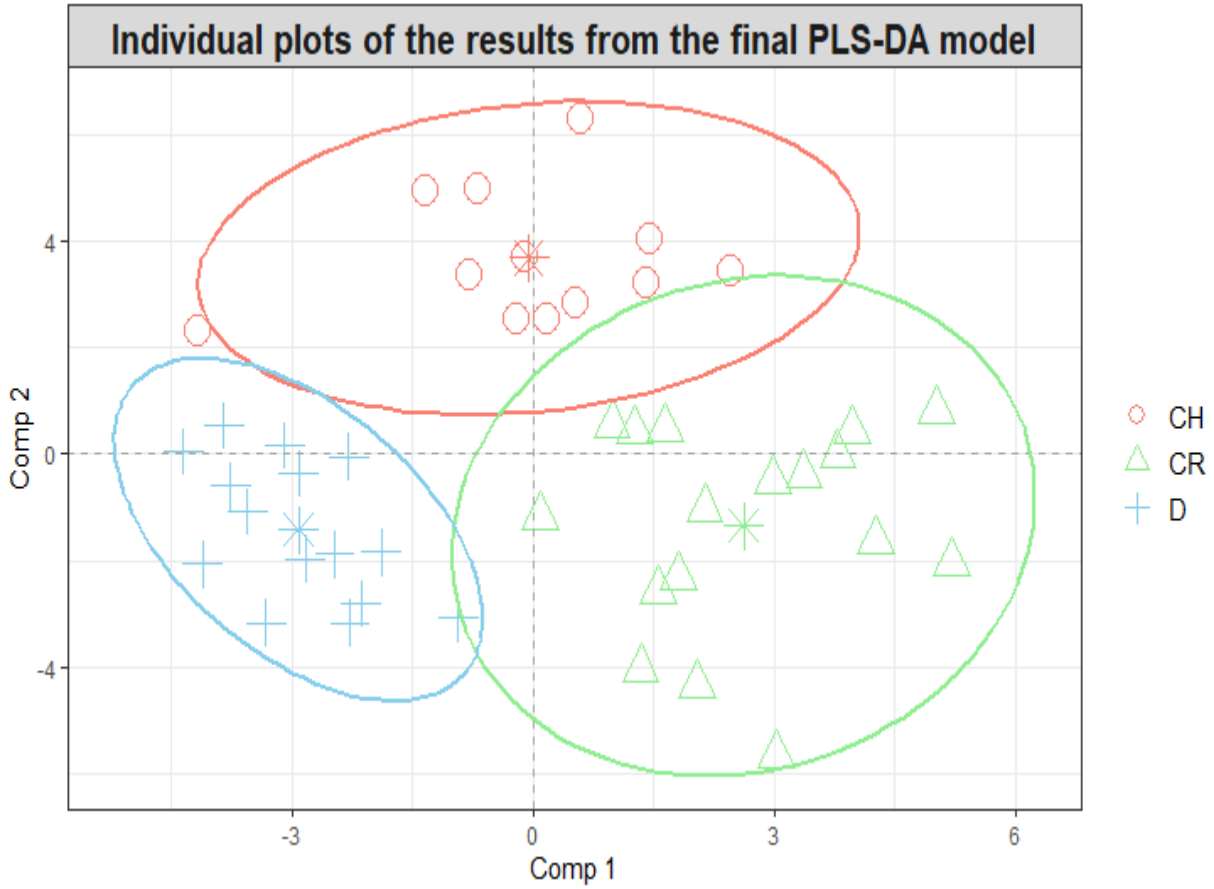
Permutation matrix	Predicted as		
	D	CH	CR
Deceased (D)	33	30	37
Culling for health causes (CH)	32.5	30	37.5
Culling for reproductive causes (CR)	33	30	37

Permutation test (n=10000):

- P-value: 0.0009
- 95% CI: [0.14, 0.57]

Results

PLS-DA

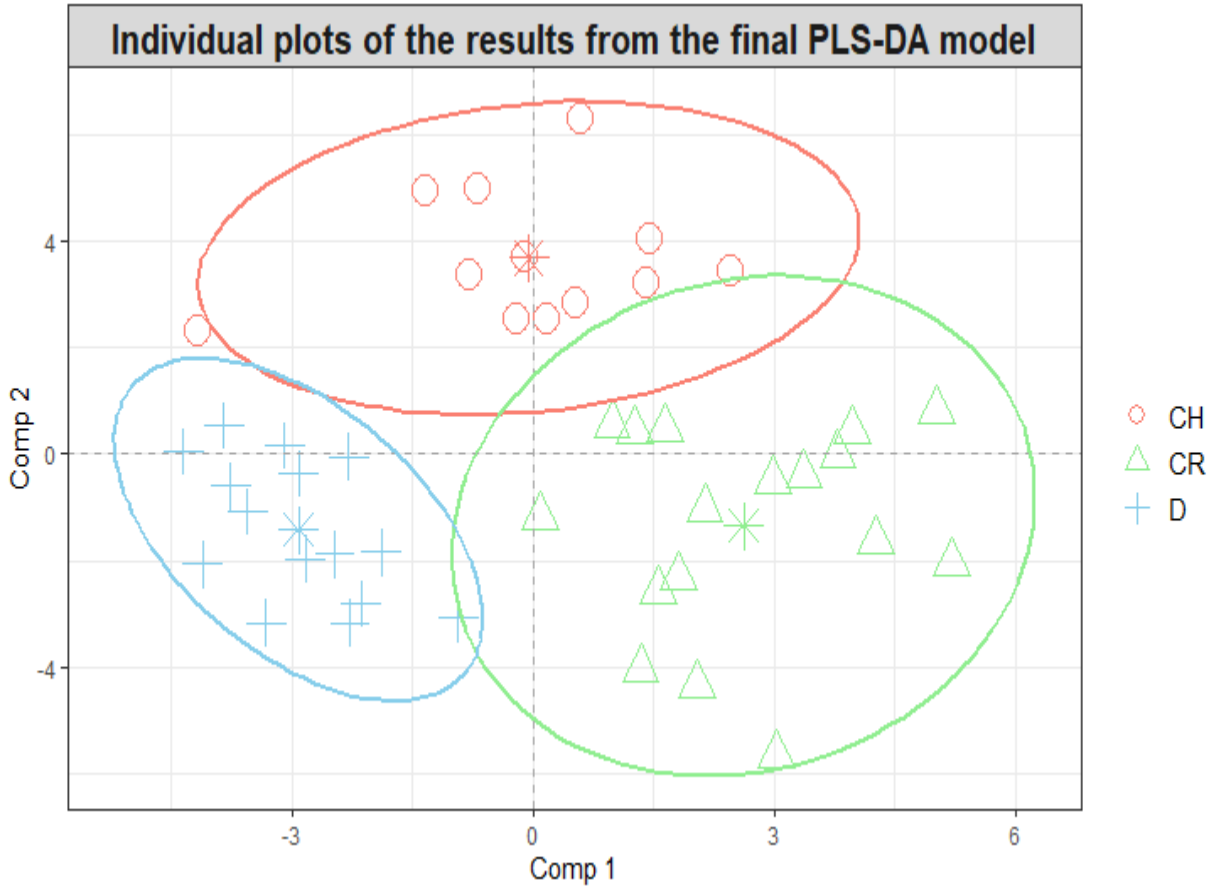


Permutation matrix	Predicted as		
	D	CH	CR
Deceased (D)	**33**	30	37
Culling for health causes (CH)	32.5	**30**	37.5
Culling for reproductive causes (CR)	33	30	**37**

The permutation matrix indicates a performance close to **random chance.**

Results

PLS-DA



Variables selected

Were associated with **low longevity:**

- *Clostridia_UCG-014*
- *Clostridia_vadinBB60_group*
- *Christensenellaceae_R-7_group*
- *Akkermansia*
- *Tyzzerella*
- *UCG_005*

Biada et al. 2024

Results

PLS-DA

In agreement with the **core microbiome results**, the group with the highest predictive accuracy was group **D**, which consisted of **females that had died.**

Alterations and potential **dysbiosis** in their gut microbiota

Conclusions

- The microbiome of female rabbits was found to be **different** depending on **the causes of culling** and **mortality**.

- The **deceased group** had the most **unique core microbiome** and the **highest prediction accuracy**.

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