ASSAY VALIDATION OF FECAL BIOMARKERS IN PIGLETS: SPECIES-SPECIFIC AND HUMAN REAGENTS

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Introduction

Weaning is a stressful process in swine industry worldwide which contributes to immune system and intestinal disorders and increases mortality in **piglets**.

ZnO has been used to prevent intestinal inflammation and control pathogenic bacteria. In June of 2022, European Union banned the use of ZnO, forcing farms to find different diet supplementation to regulate these alterations.

OBJECTIVE

Developing a rapid and **non-invasive diagnostic method** capable to predict intestinal **inflammatory process** in pigs

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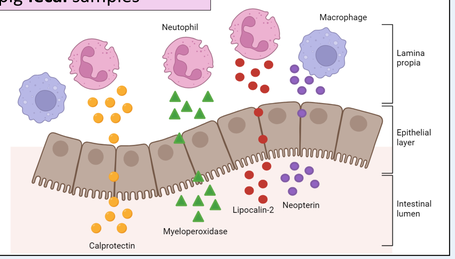
Evaluation and validation of inflammatory **biomarkers** in pig **fecal** samples

Selected biomarkers

- Calprotectin (fCal)
- Immunoglobulin A (IgA)
- Lactoferrin
- Lipocalin-2 (LCN-2)
- Neopterin
- Myeloperoxidase (MPO)
- Alpha-1-antitrypsin (AAT)
- Adenosine deaminase (ADA)



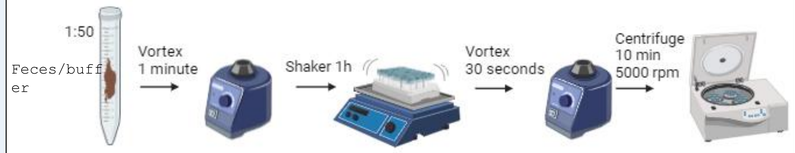
Materials and methods

Selected kits and methods

Biomarker	Method (Brand)	Specie
Calprotectin	Turbidimetric immunoassay (Bühlmann)	Human
IgA	ELISA (Bethyl Laboratories)	Porcine
Lactoferrin	ELISA (TechLab, Immundiagnostik)	Human
Lipocalin-2	ELISA (Biotechne)	Porcine
Neopterin	ELISA (IBL International)	Human
MPO	Enzymatic colorimetric (O-dianisidine method)	Human
AAT	ELISA (LSBio LifeSpan BioScience)	Porcine
ADA	Enzymatic colorimetric (BioSystems)	Human

Except for calprotectin, none of the kits used are specific to fecal samples

Extraction process



Validation

ASVCP guidelines

Precision: is performed with three concentration levels (low, medium, and high) to cover all the analytical range (Table 1)

- Intra-assay → 20 replicates of each sample in the same assay.
- Inter-assay → 5 replicates of each samples during 5 different days using a new aliquot each time.

Accuracy:

- Linearity under dilution → 5 different serial dilutions. Represent graphically the expected vs the observed concentration (Figure 1).
- Recovery → Prepare spike+, spike-, and control assayed in duplicate. Three concentration levels (low, medium, and high) (Table 1).

Acceptance criteria:
- Intra and inter-assay coefficient variation (CV) <20%
- Linearity determination coefficient close to 1
- Recovery 80-120%

Results

Table 1. Validation results. Accuracy and precision assay, CV and recovery measurement of IgA, MPO, LCN-2, fCal and ADA

	Concentration	Intra-assay CV (%)	Inter-assay CV (%)	Recovery (%)
IgA	Low	15,03	14,19	100,38
	Medium	6,79	18,05	105,94
	High	9,78	17,57	99,18
MPO	Low	5,03	11,02	106,16
	Medium	1,10	5,03	101,00
	High	0,83	1,10	102,28
LCN-2	Low	14,76	13,10	95,34
	Medium	3,53	6,80	76,97
	High	6,59	5,98	89,38
fCal	Low	7,4	5,9	95,2
	Medium	2,7	8,3	83,9
	High	2,3	4,4	114,9
ADA	Low	4,91	11,35	95,60
	Medium	2,25	15,65	107,31
	High	1,39	10,22	108,80

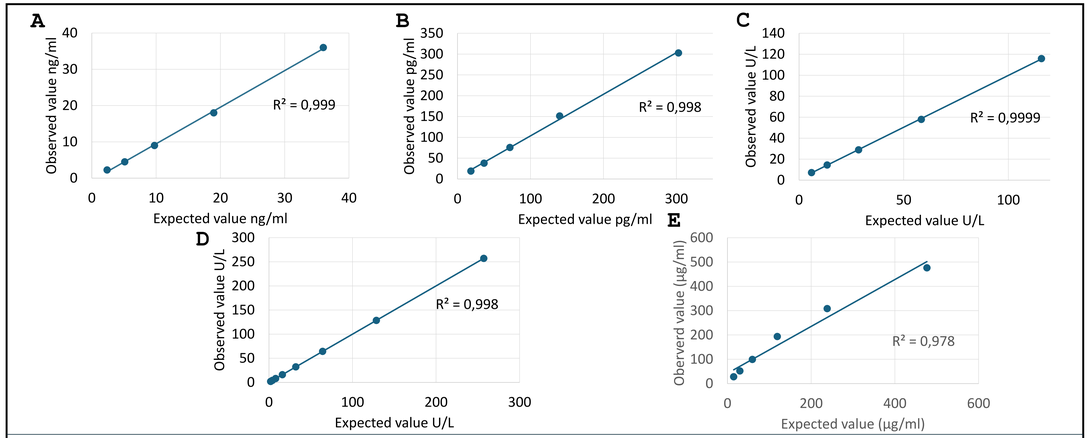


Figure 1: Dilutional linearity for (A) IgA R^2 >0,999, (B) Lipocalin R^2>0,998, (C) MPO R^2>0,999, (D) ADA R^2 >0,998 and (E) Calprotectin R^2 >0,978. Expected concentrations were plotted in X-axis against the observed concentrations in Y-axis.

Conclusions

- IgA, ADA, LCN-2, MPO and calprotectin
  - CV, linearity and recovery in acceptance criteria
- Lactoferrin, neopterin and AAT
  - Undetectable concentration and linearity problems
    - Low concentration samples
    - Unspecific kits for pig faeces

Encouraging outcomes have been achieved with five out of the eight biomarkers. This establishes a methodology for assessing and evaluating them in porcine feces. Ultimately, validation is a previous and mandatory step before to their introduction as non-invasive biomarkers to predict health condition of piglets.

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