

BOVINE FAECAL BIOMARKERS OF INTESTINAL INFLAMMATORY PROCESS: CALPROTECTIN AND LACTOFERRIN, A COMPARATIVE STUDY.

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IRTA

Introduction

Dairy calves and dairy-beef production are exposed to a wide number of factors that might affect gastrointestinal functionality. Inflammation of the gastrointestinal tract impairs gastrointestinal functionality causing diarrheas that compromise calves' health and growth.

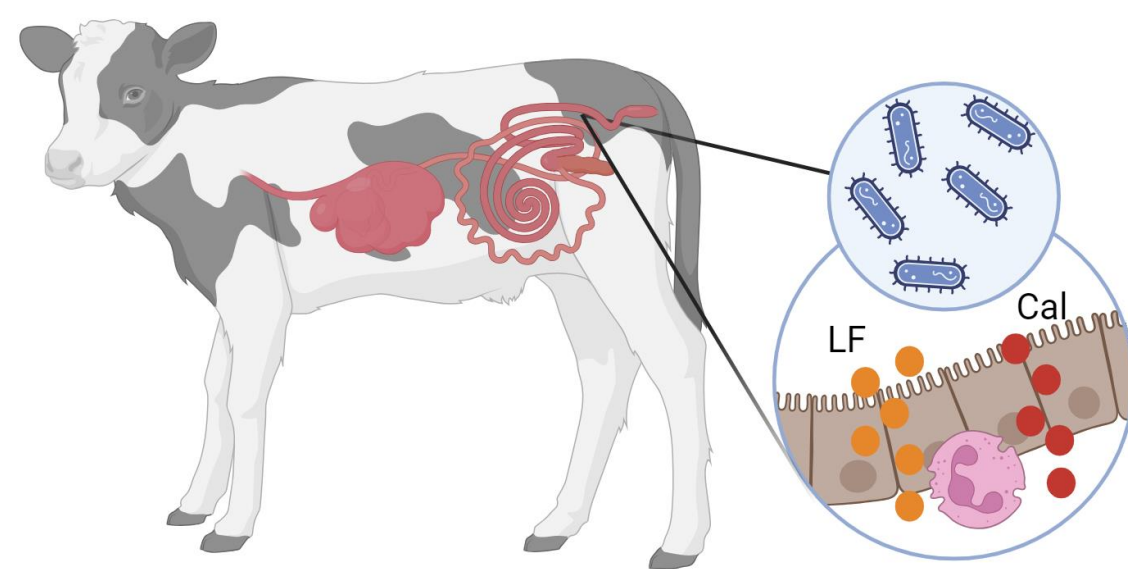
In inflammatory conditions, calprotectin and lactoferrin are released to the intestinal lumen and their determination in faeces is used as biomarkers of inflammation in the gastrointestinal tract.

Calprotectin → calcium-bound protein released by neutrophils, monocytes, activated macrophages and dendritic cells that are accumulated at sites of inflammation.

Lactoferrin → iron-binding glycoprotein secreted by neutrophils and epithelial cells at inflammation sites. One of its main activities is to limit iron uptake by pathogenic organisms.

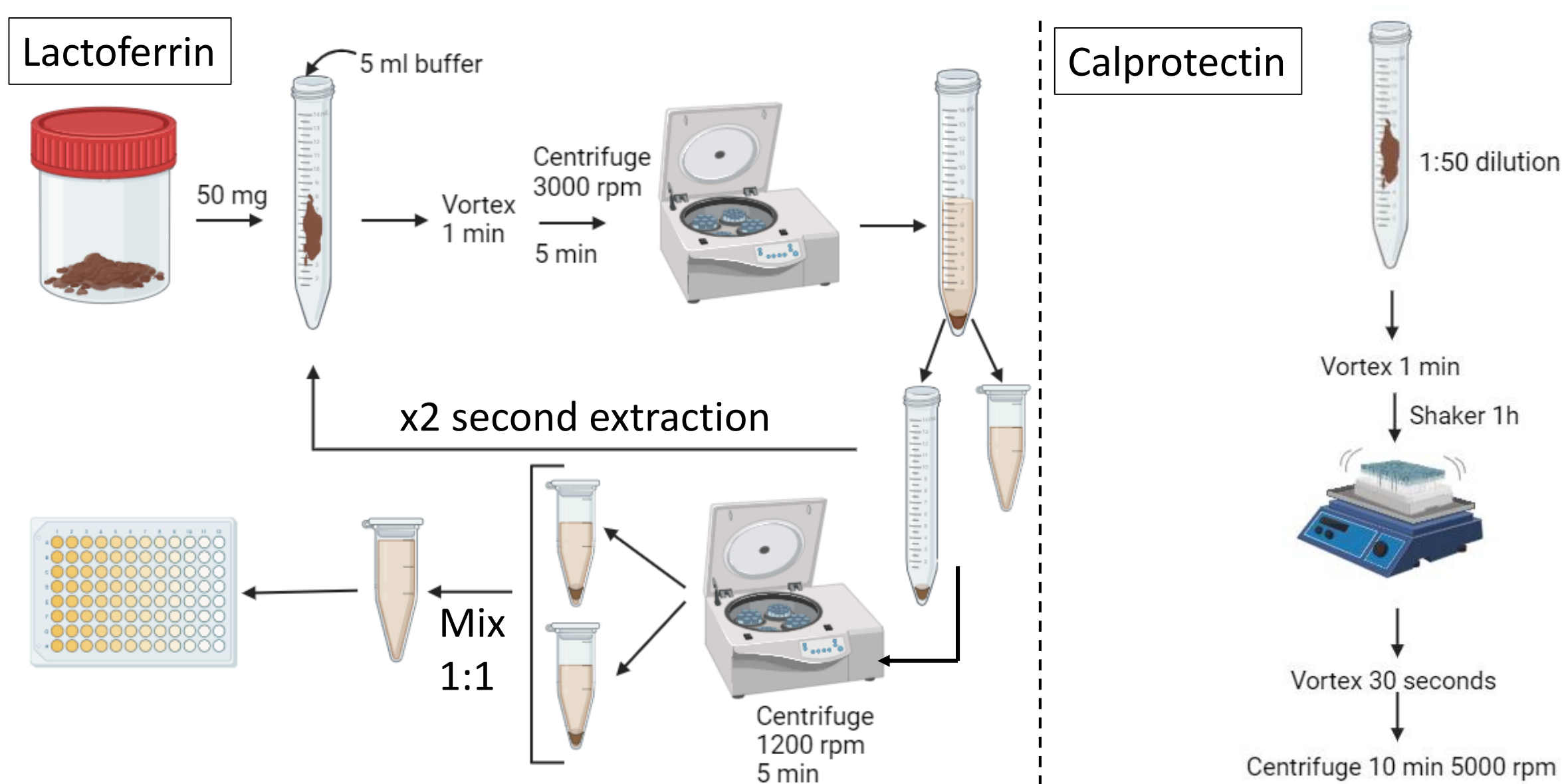
OBJECTIVE

- 1 Validation of milk lactoferrin ELISA assay in calves' faeces
- 2 Influence of water content of faeces in fecal calprotectin (fCal) and faecal lactoferrin (fLF)
- 3 fCal and fLF correlation



Materials and methods

Extraction method



Validation

ASVCP guidelines

Precision: is performed with three concentration levels, low, medium, and high

- ↳ 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 → five different levels of dilution. Represent graphically the expected vs the observed concentration.
- ↳ Recovery → three concentration levels. Prepare spike+, spike-, and control assayed in duplicate

Acceptance criteria:

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

Results

Validation of the milk lactoferrin ELISA kit for its use with calves faecal extracts

Table 1. Intra-assay and inter-assay coefficients of variation of commercial Bovine Lactoferrin ELISA kit, with faecal extracts.

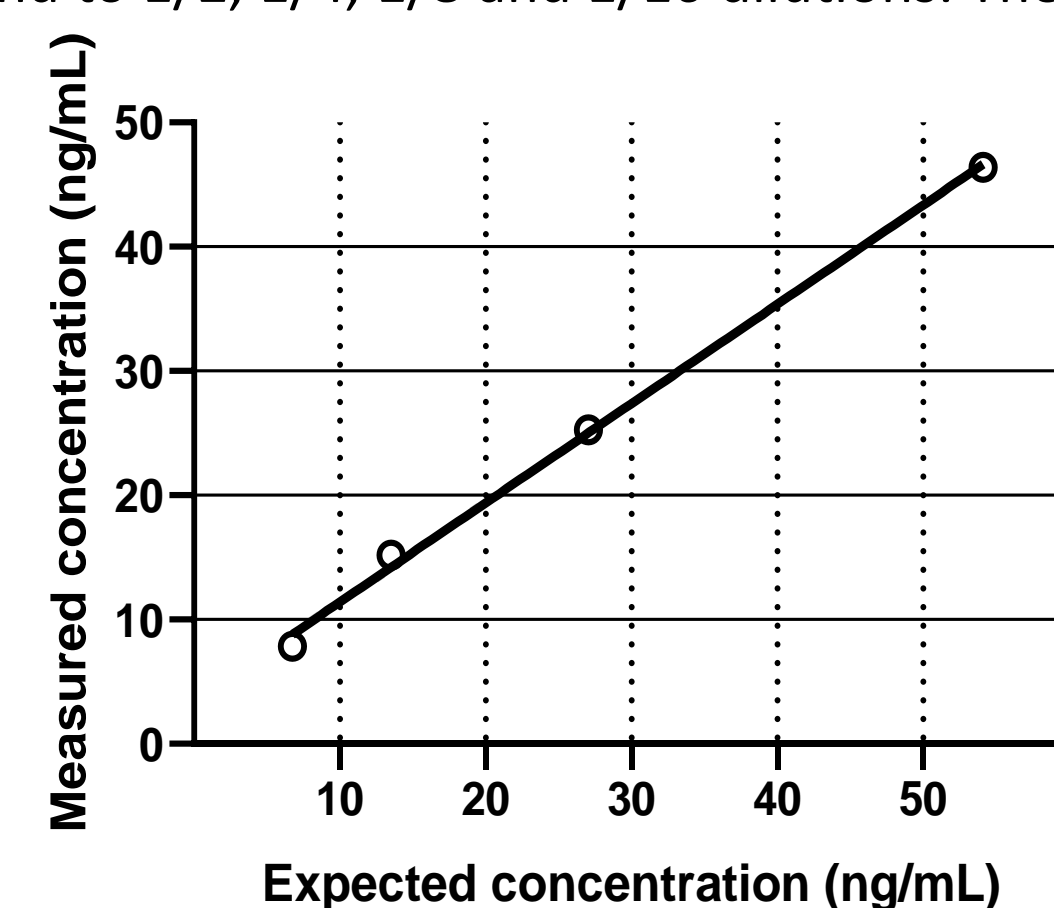
Faecal extracts		Mean (ng/mL)	SD	CV (%)
Intra-assay	Low	56.3	2.9	5.2
	High	392.2	25.2	6.4
Inter-assay	Low	45.7	4.3	9.5
	High	406.5	23.1	5.7

Table 2. Recovery of the Bovine Lactoferrin milk ELISA Kit in faecal samples. Three samples of different concentrations (high, medium, and low) were used.

Lactoferrin Concentration	Recovery (%)
Low (75 ng/mL)	95.9
Medium (241 ng/mL)	94.9
High (466 ng/mL)	86.7

SD Standard deviation
CV Coefficient of variation

Figure 1. Linearity test of Bovine Lactoferrin ELISA kit, when used with faecal samples. Concentrations correspond to 1/2, 1/4, 1/8 and 1/16 dilutions. The linearity was excellent ($r^2 = 0.9976$).



The influence of faecal water content on lactoferrin and calprotectin measurement and correlation of both biomarkers. Spearman's correlation coefficient.

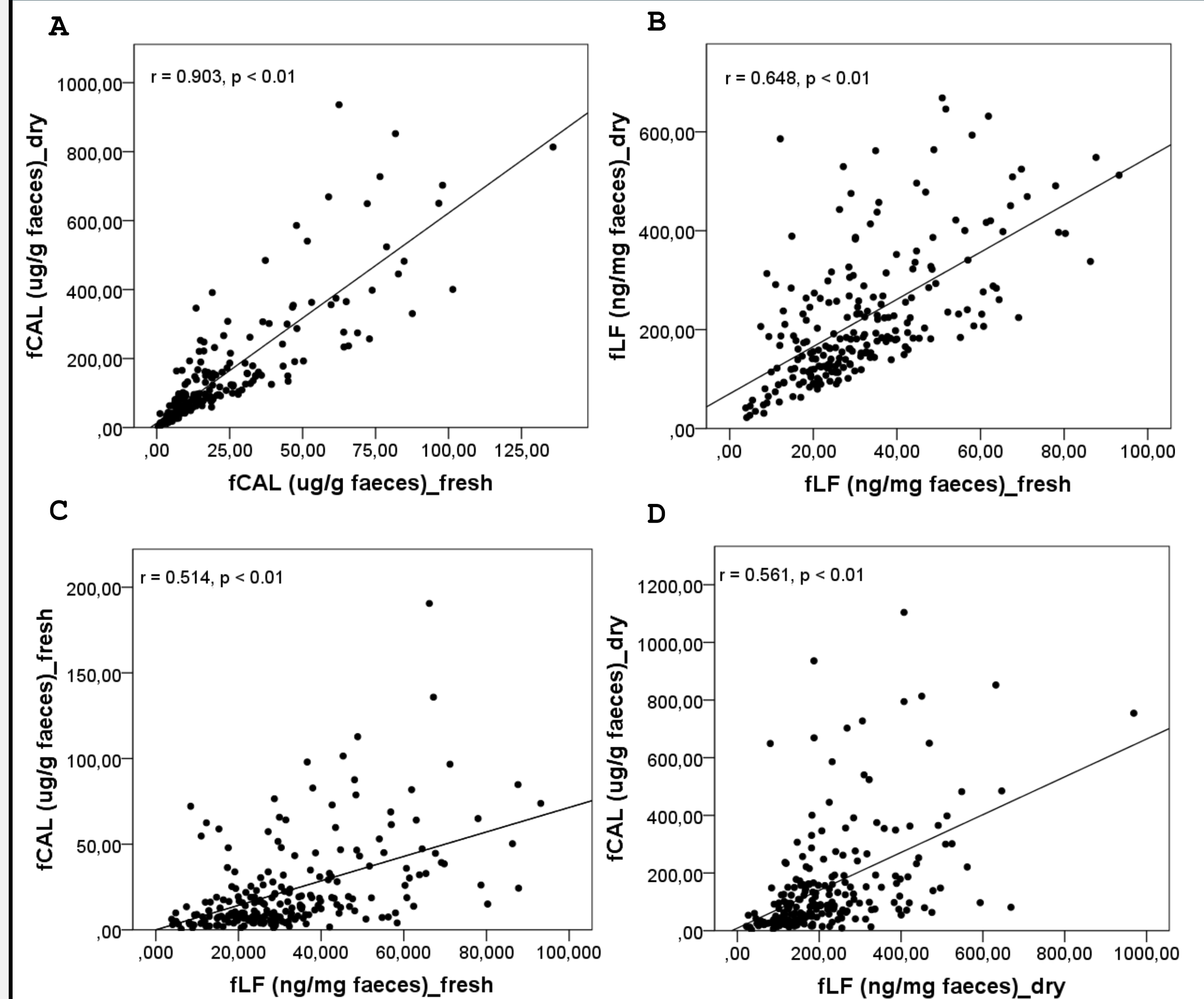


Figure 2. A) strong correlation between faecal calprotectin concentration in fresh faeces and in faecal dry matter ($r = 0.903$, $p < 0.01$). B) moderate lactoferrin correlation in fresh faeces and faecal dry matter ($r = 0.648$, $p < 0.01$). C) fresh fCAL and fLF showed moderate correlation ($r = 0.514$, $p < 0.01$). D) dry fCAL and fLF showed moderate correlation ($r = 0.561$, $p < 0.01$).

Conclusions

- 1 The milk lactoferrin ELISA kit is valid for its use with calves' faecal samples. Recovery results showed that detection of faecal lactoferrin was not affected by the biological matrix of the sample. Linearity under dilution was demonstrated with a coefficient of determination (r^2) close to 1. Repeated individual measures of lactoferrin showed a good precision since the coefficient of variation (CV) of both within-run and between-run precision did not exceed 10%.
- 2 fLF concentration is more influenced by faeces moisture than fCAL concentration
- 3 fCAL and fLF show a moderate correlation taking into account that the extraction carried out for each biomarker candidate is different.

Both proteins can be proposed as good biomarkers when measured in stools and they can be good candidates for point of care development to identify intestinal inflammation