

# Hay milk – chemical markers in milk for the detection of silage addition in feed of dairy cows

Lösch M. D.<sup>1</sup>, Fava F.<sup>1</sup>, Kaneppele E.<sup>1</sup>, Peratoner G.<sup>1</sup>, Robatscher P.<sup>1</sup>, Matteazzi A.<sup>1</sup>, Scampicchio M. M.<sup>2</sup>, Österreicher A.<sup>3</sup>, Volgger S.<sup>4</sup>, Eisenstecken D.<sup>1</sup>, Venir E.<sup>1</sup>

<sup>1</sup>Laimburg Research Centre, Laimburg, Italy; <sup>2</sup>Free University of Bozen-Bolzano, Bolzano, Italy; <sup>3</sup>Sennereiverband Südtirol, Italy. <sup>4</sup>Beratungsring Berglandwirtschaft, Italy.

**Introduction:** In hay milk production, cows are fed exclusively on fresh grass or hay and a limited amount (max 25%) of concentrated feed in the ration [1]. The feeding of fermented fodder is not allowed. At present, there are no analytical methods for milk that permit to detect the presence of silage in the animal diet. The HEUMILCH project aims at finding an analytical method to discriminate hay-milk from standard milk. The method is based on the detection of a cyclopropane fatty acid (CPFA), as this fatty acid is a component of the cell membrane of microbes that are present during feed fermentation. It is ingested by the dairy cows with the feed and can thus get into the milk.

## Methods

Fat has been extracted from raw milk samples following a method based on Feng et al. [2]. It was then trans-esterified according to the norm ISO 15884:2002 [3]. A GC-MS (QP2010 SE Shimadzu, Kyoto, Japan) was used and the chromatographic separation was carried out using a SLB-5ms (30 m x 0.25 mm x 0.25 µm) capillary GC-Column (Sigma Aldrich, St. Louis, Missouri, USA), with chromatographic conditions based on Caligiani et al., [4]. Mass spectrometer was operated in full scan and SIM mode to detect CPFA. The method has a sensitivity (LOQ) of 25ppm (mg/Kg of fat).



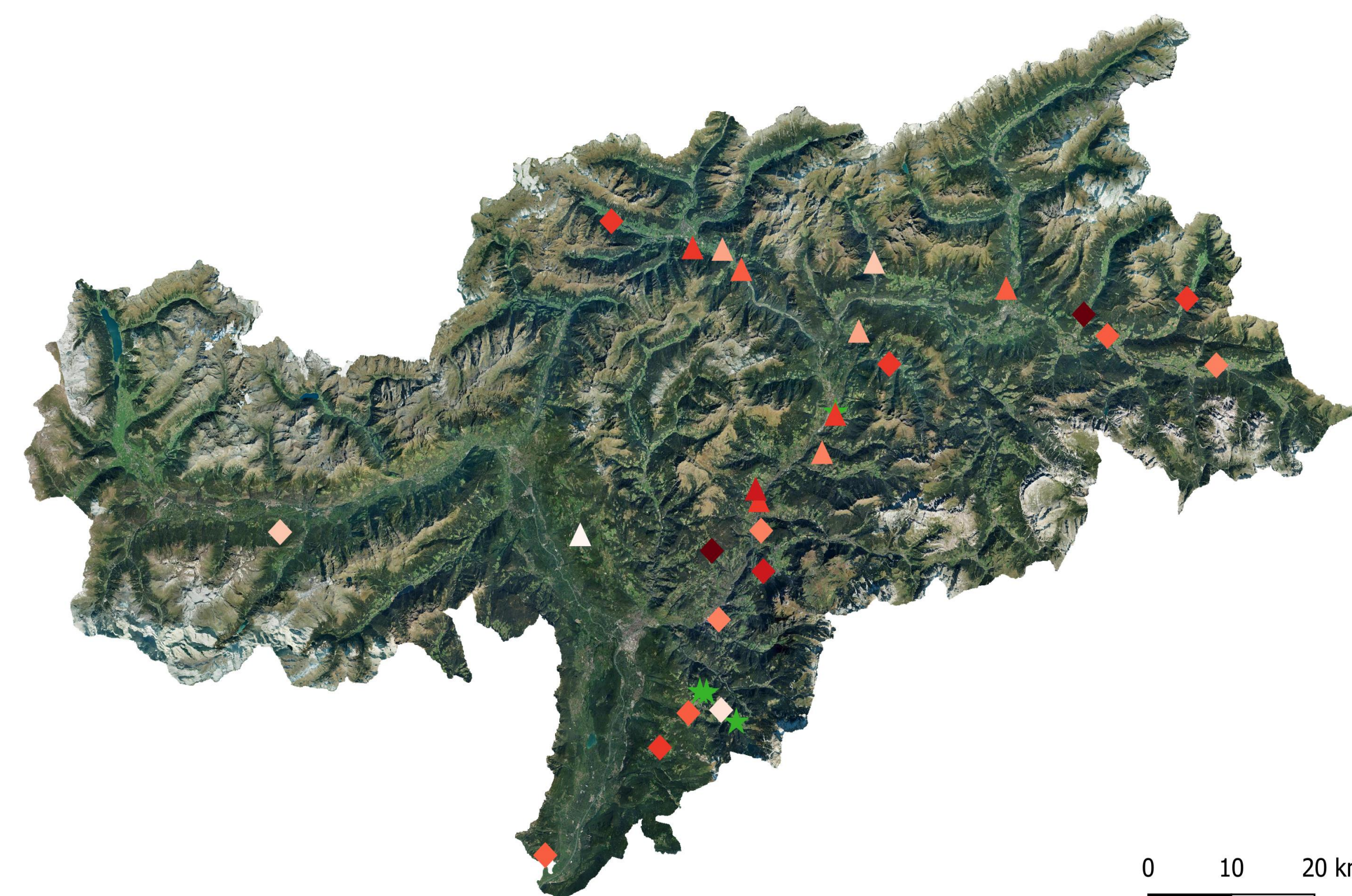
Shimadzu GC MS  
QP2010 SE

**Sampling:** Milk samples were taken from 30 different farms in South Tyrol (Italy). Four farms fed according to the hay milk guidelines, while the other farms fed different levels of maize silage or grass silage. The farms were sampled over two years, both during summer and winter period. In each sampling phase the sampling was repeated three times at intervals of one week.

## Map with the locations of all farms sampled in the project

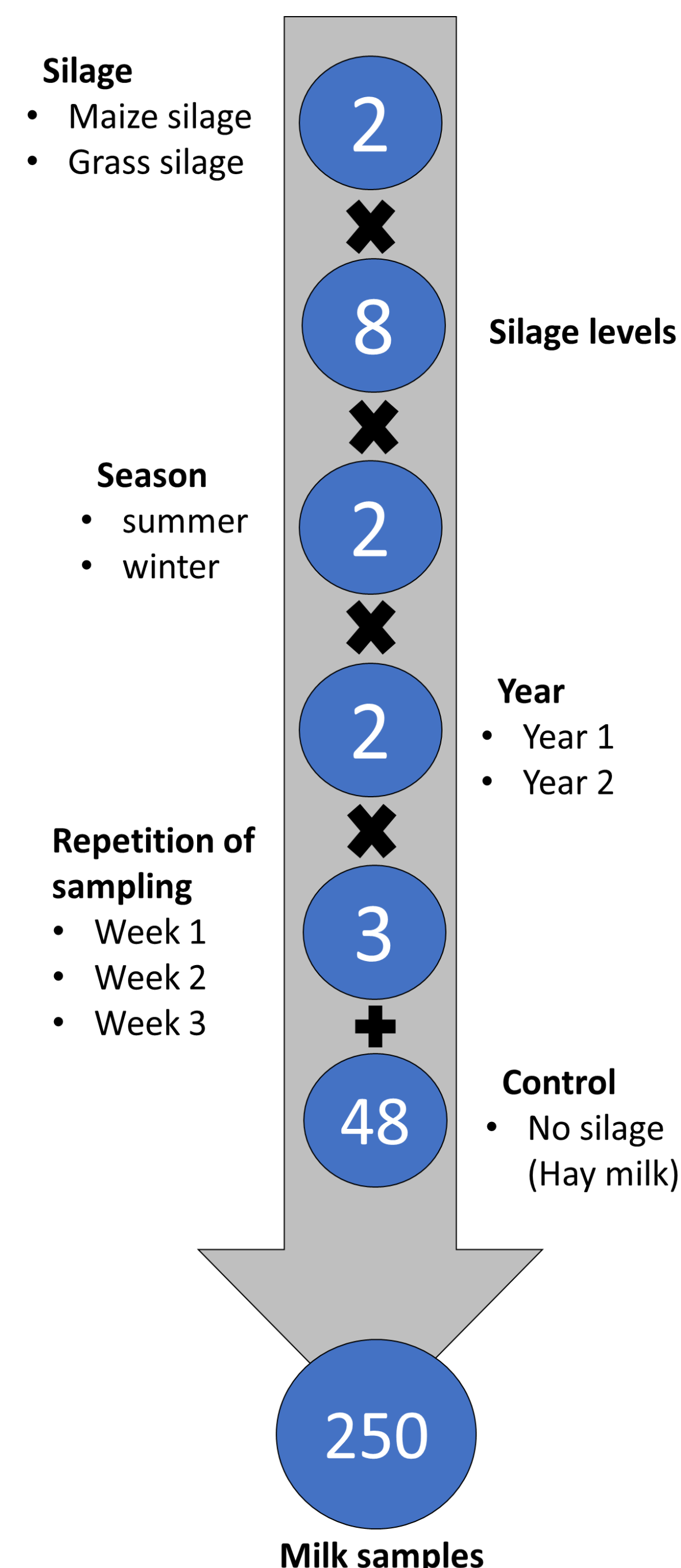
Silage level (% dry matter of the total ration)

- Maize silage
  - 8 - 12
  - 12 - 16
  - 16 - 21
  - 21 - 25
  - 25 - 29
  - 29 - 34
  - 34 - 38
  - 38 - 43
  - 43 - 47
- Grass silage
  - 12 - 16
  - 16 - 21
  - 21 - 25
  - 25 - 29
  - 29 - 34
  - 34 - 38
  - 38 - 43
  - 43 - 47
  - 47 - 51
- Hay milk



0 10 20 km

## Sampling plan

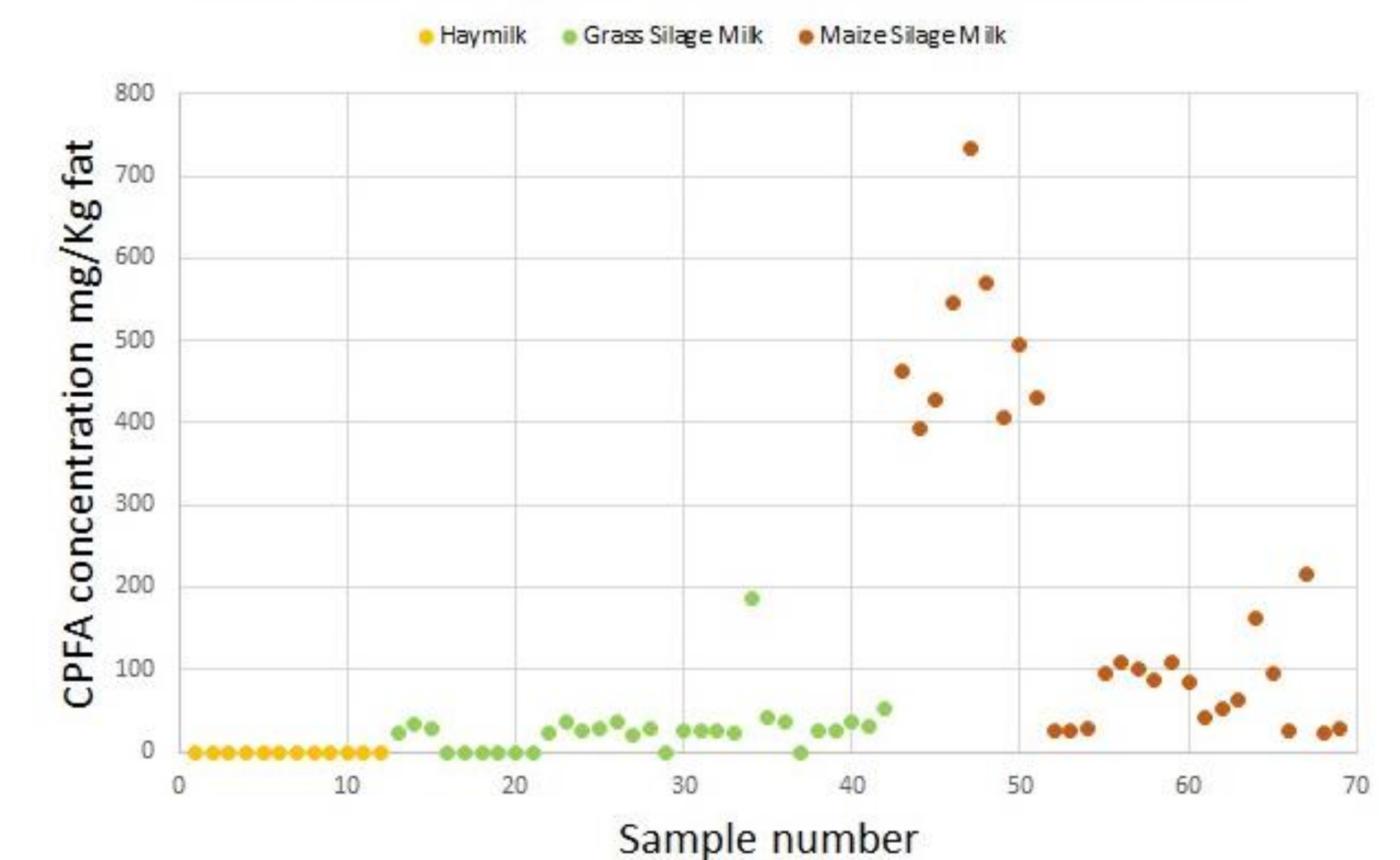


## Results

For the first 71 samples corresponding to the first season of sampling:

- No CPFA could be found in any of the hay-milk samples.
- CPFA could not be quantified in some of the samples in which grass silage was fed, since its concentration was below LOQ.
- CPFA was always detected and quantified in the maize silage milk samples.

## CPFA concentration over different milk types



## Conclusions

A quarter of the collected samples have been analyzed so far and the results shown here are partial results. No CPFA could be found in milk produced under the hay-milk regulatory. The marker could not always be detected in milk samples from animals fed diets including grass silage. The marker was found in all milk samples obtained from cows with diets including maize silage.