Analysis of antioxidant activity in bovine milk to valorize its nutraceutical properties

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GenetoCheese

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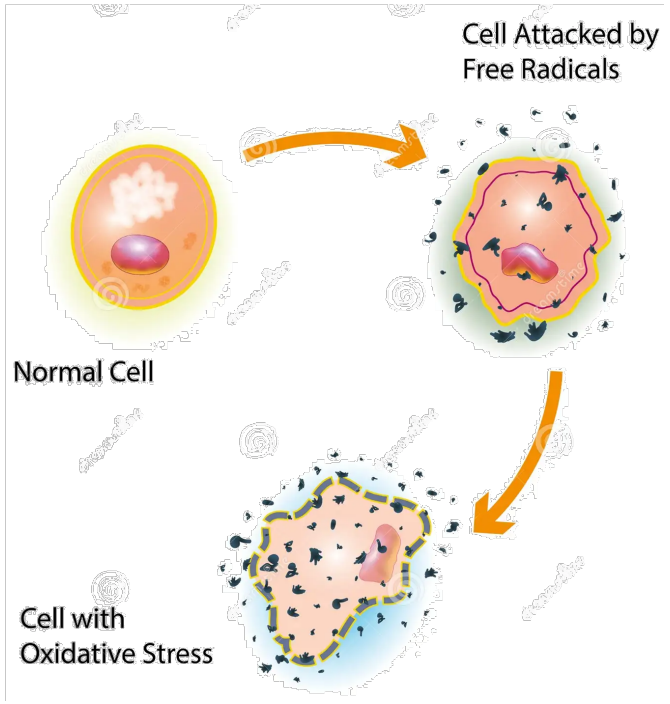
State of the art of the antioxidant activity



The antioxidant activity is:

- Enzymatic
- Non-enzymatic

Non-enzymatic defence systems include:

- scavenger antioxidants (vitamin C, E and coenzyme Q10)
- proteins that act as anti-oxidants by binding Reacting Oxygen Species (ROS) and Reactive Nitrogen Species (RNS)

GAP *Knowledge gap*

1. Several methods of analysis
2. Few samples
3. How environmental and animal related factors affect the variability of milk antioxidant activity

Aim of the study

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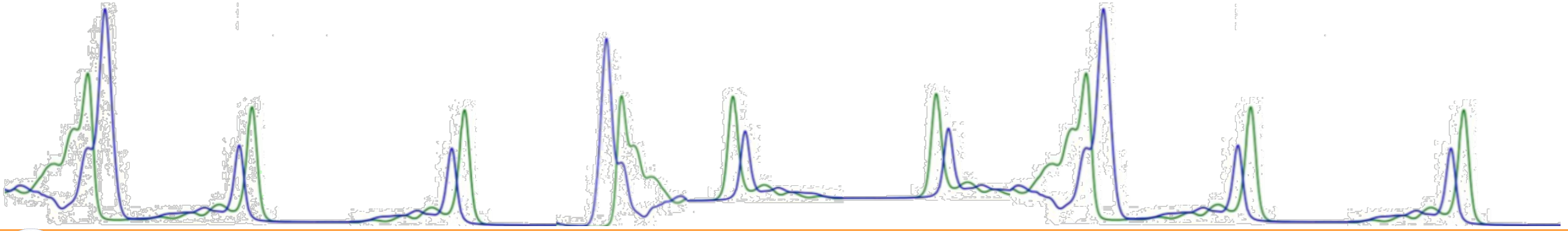
Analyze environmental and animal factors affecting the variability of the non-enzymatic antioxidant activity



Materials and Methods

The GeneToCheese project:

- Area: Emilia-Romagna, Lombardy, Tuscany (Italy)
- Dataset cows: 1,060 Brown Swiss cows
- Herds: 53 farms (20 cows/herd)

Data:

Sampling period: February 2021 to April 2022

ENVIRONMENTAL FACTORS

- Altitude: Mountain, hill, plain
- THI: Temperature and humidity
- Ventilation: Yes/No
- Feeding: →

Total Mixed Ration | Dry TMR | Conventional

ANIMAL FACTORS

DIM: Days in milk

Parity

DMY: Daily Milk Yield

Materials and Methods

GenetoCheese

Non-enzymatic antioxidant activity by FRAP and DPPH essays

(Spectrophotometer, Helios alpha)

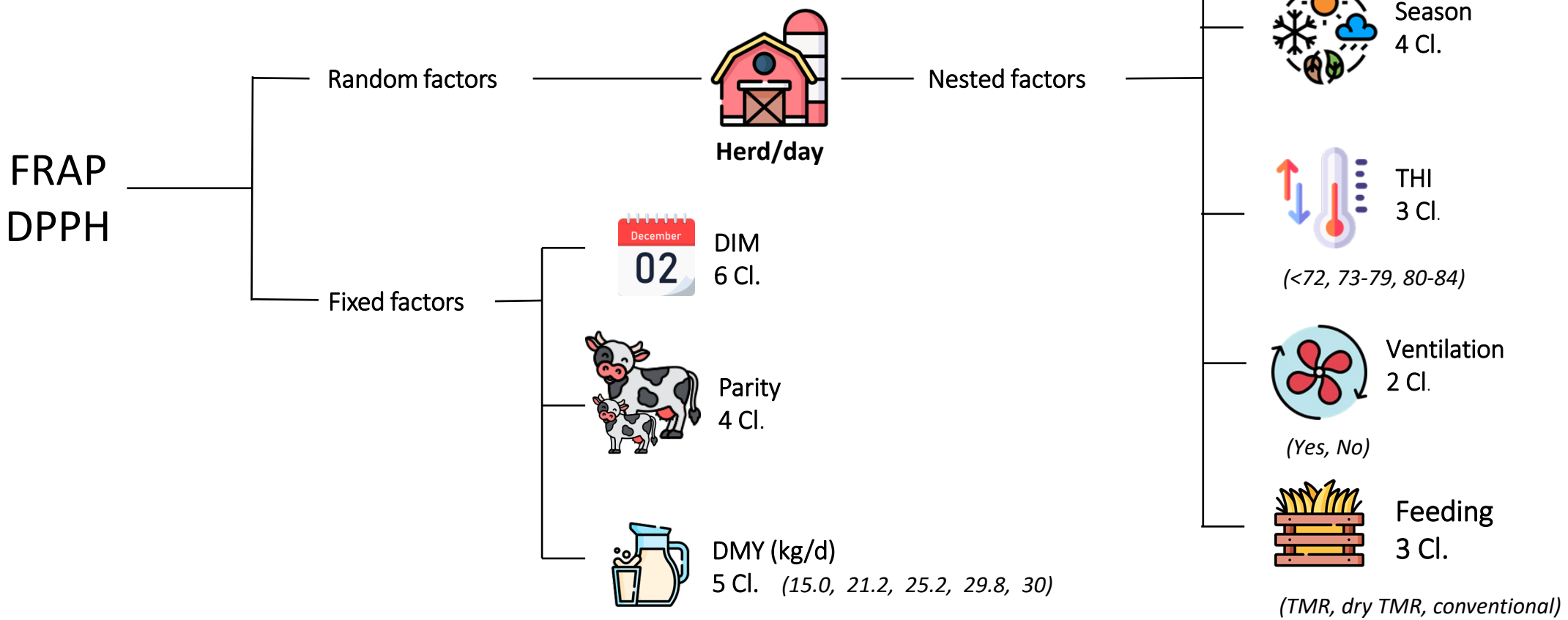
FRAP (*Ferric reducing antioxidant power*) tests:

- Reduction of Fe3+ to Fe2+ (from yellow to blue) for Fe3+ and Fe2+
- Absorbance was measured at 593 nm
- Unit of measurement: µM eq. Ascorbic acid/ mL of milk

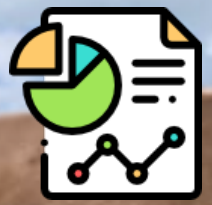
DPPH (*2,2-diphenyl-1-picryl-hydrazyl-hydrate*) tests:

- Reaction is the transfer of an H/ electron with the disappearance of the radical (DPPH deep purple color)
- Absorbance was measured at 515 nm
- Unit of measurement: % inhibition calculated from a blank sample

Statistical model



RESULTS



Descriptive statistics and ANOVA

	Mean±SD	CV, %
FRAP	2.2±0.48	22
DPPH	59.7±2.6	4

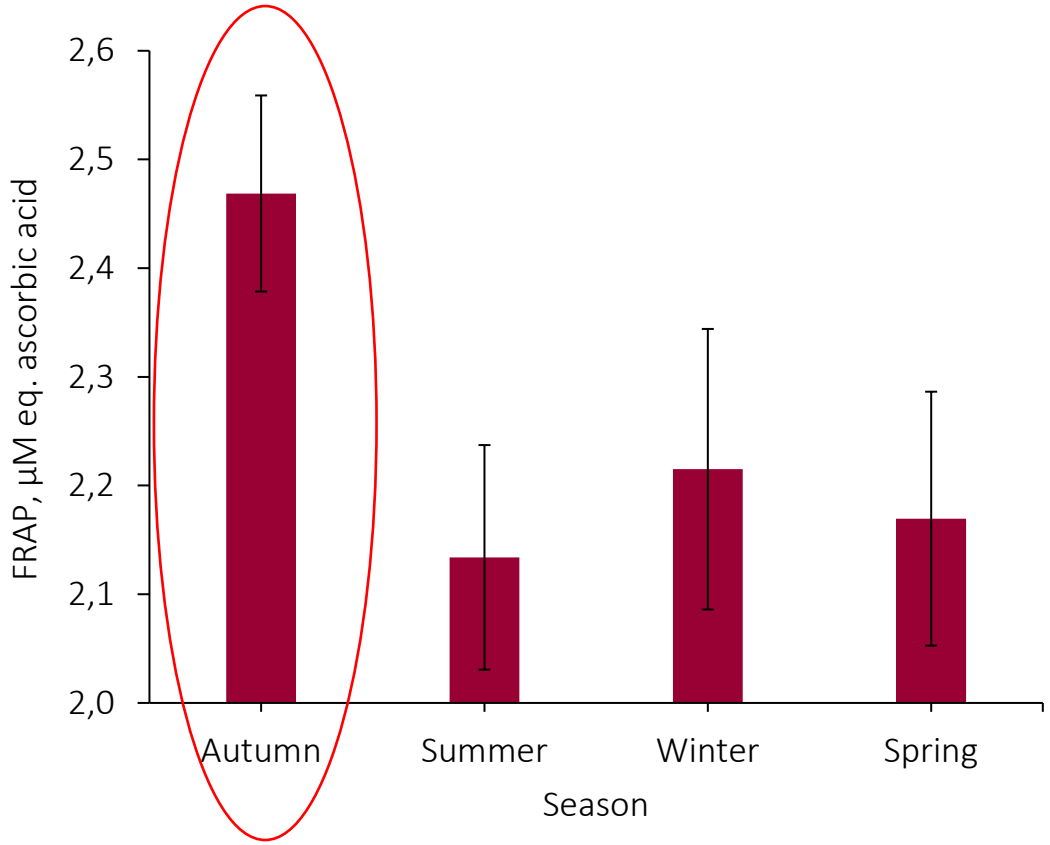
FRAP: expressed in µM eq. Ascorbic acid/mL of milk
DPPH: expressed in %inhibition

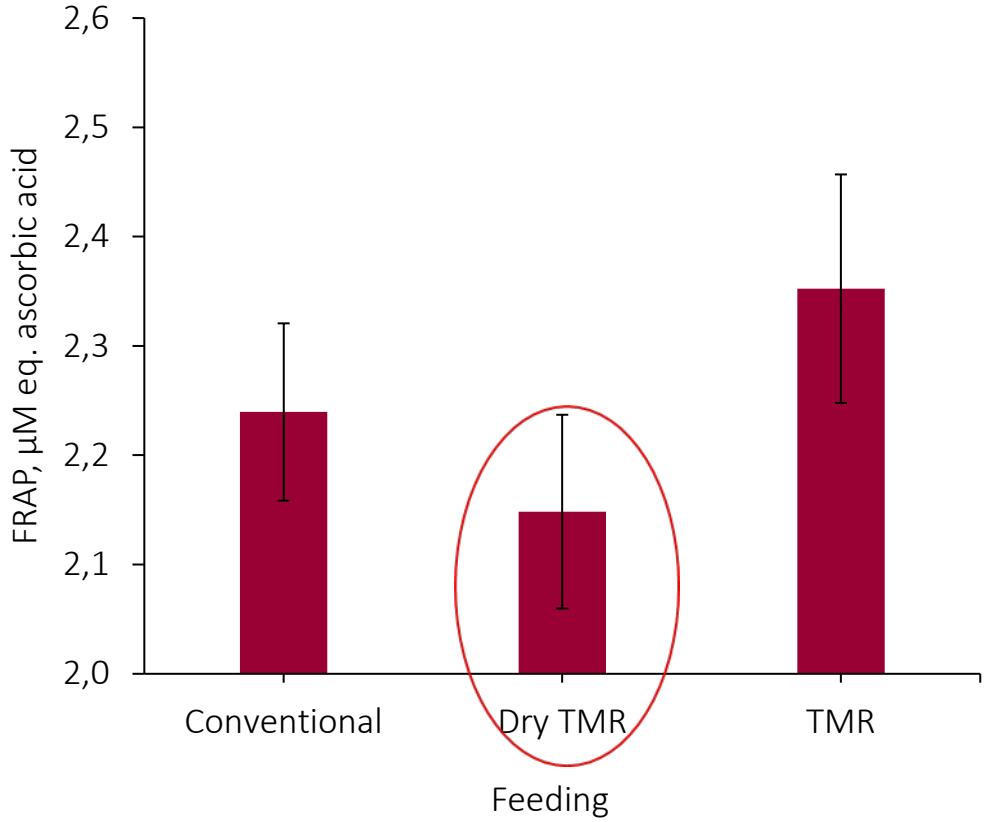
Analysis of Variance

	Environmental effects					Animal effects		
	THI	Altitude	Season	Feeding	Ventilation	DIM	Parity	DMY
FRAP	0.19	2.02	5.37**	2.52 ·	0.81	1.69	2.91*	5.32***
DPPH	1.07	1.41	1.76	1.94	4.05 ·	2.32*	3.15*	0.31

*P <0.05; ** P <0.01; *** P <0.001; .P<0.05

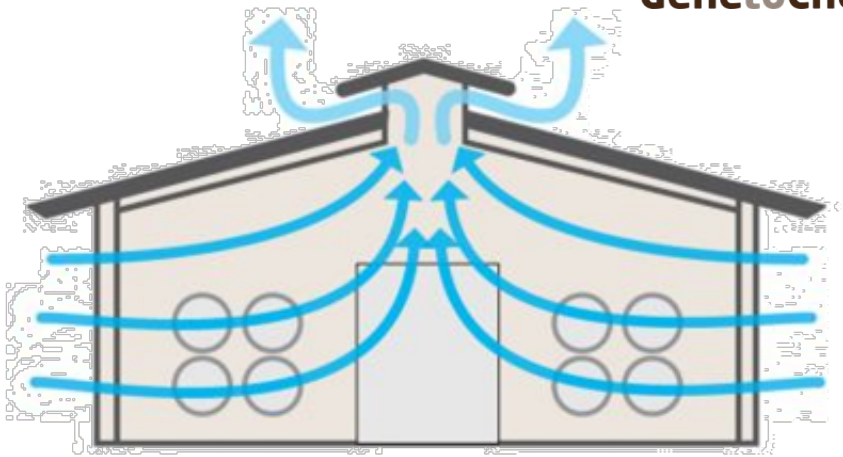
Environmental effects: Season and Feeding

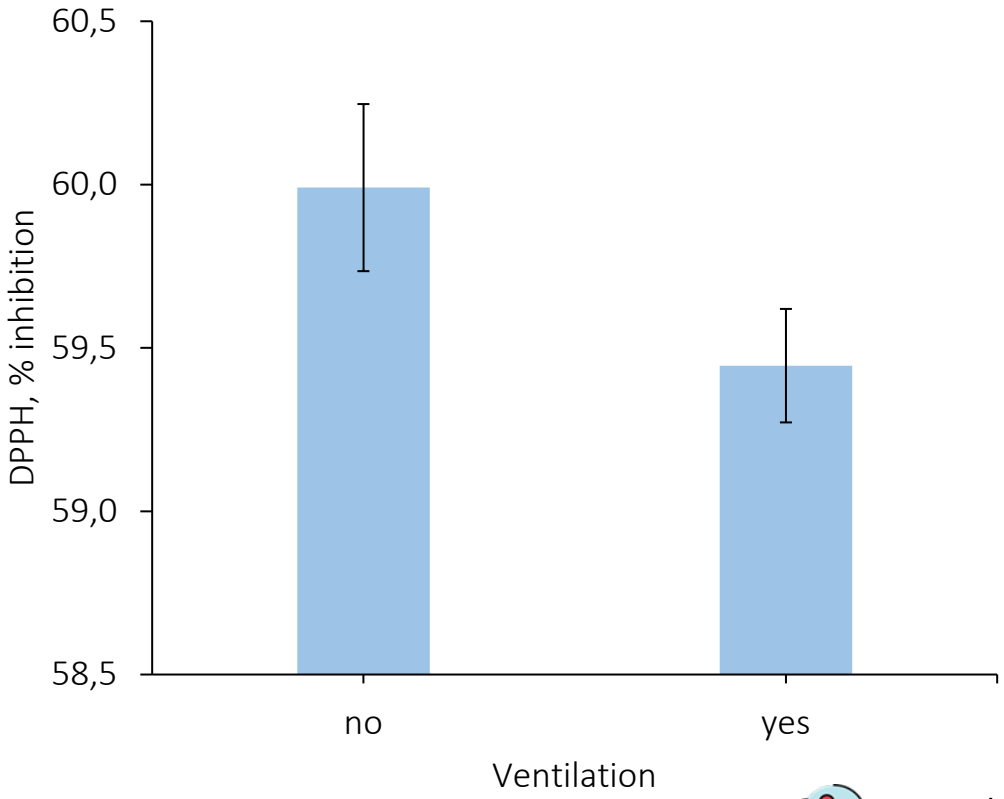




Environmental effect: Ventilation

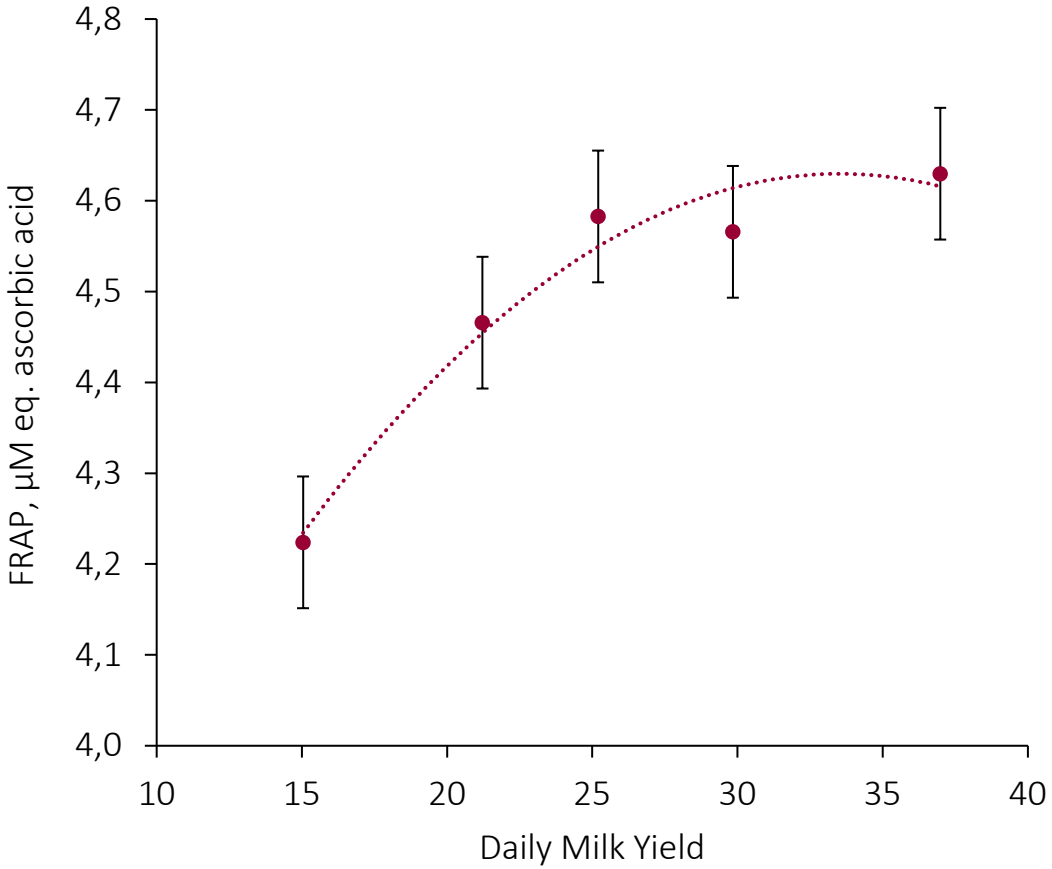
Natural ventilation

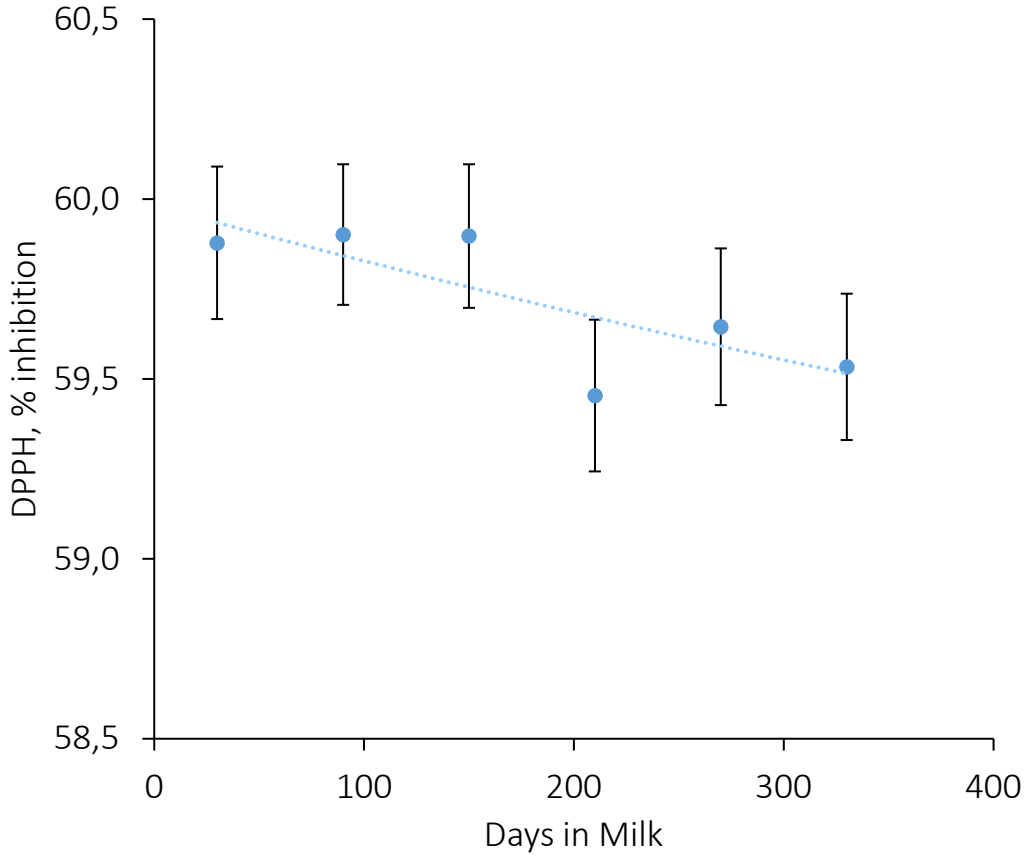




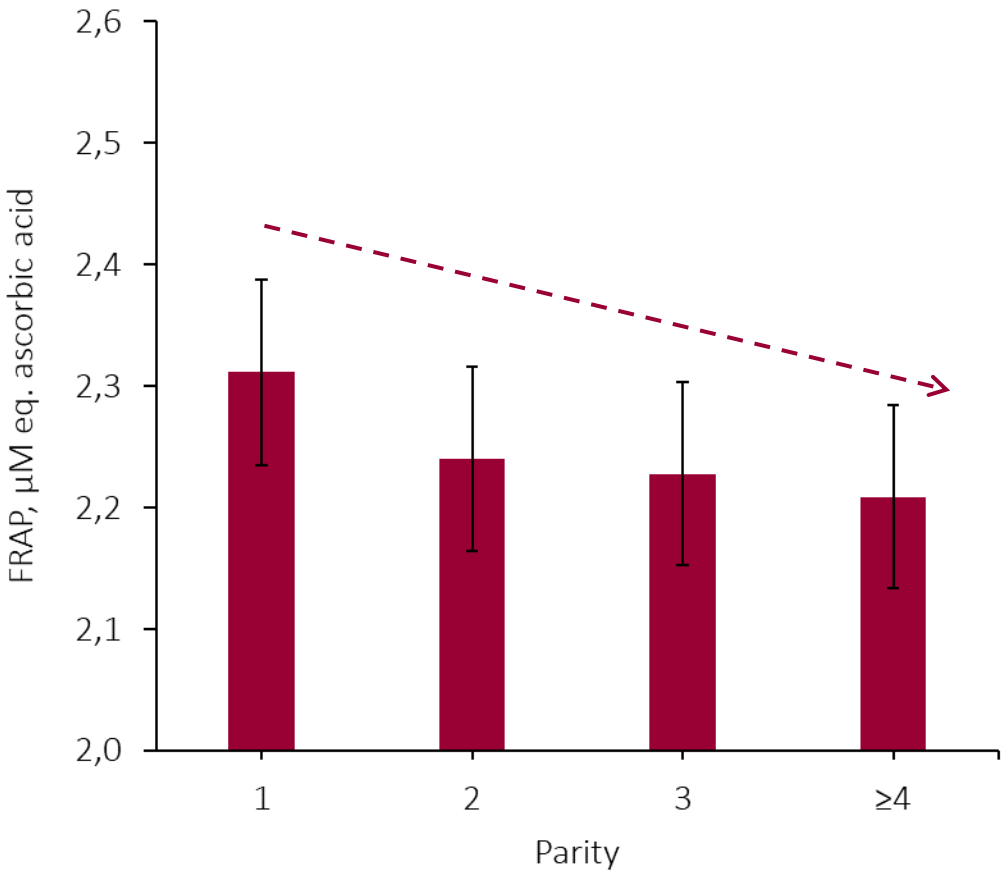
Forced ventilation

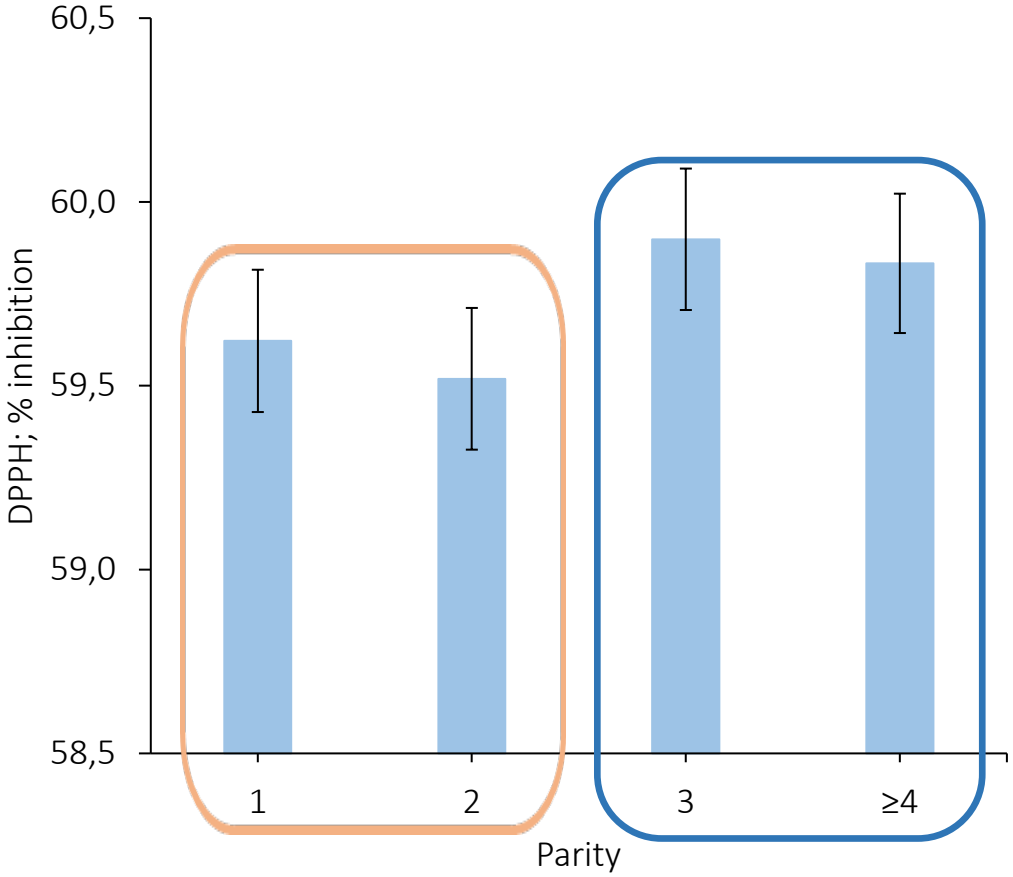
Animal effects: Daily Milk Yield and Days in Milk





Animal effect: Parity

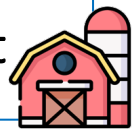




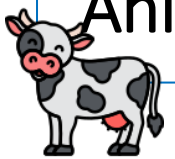
Conclusions

Use of complementary methods

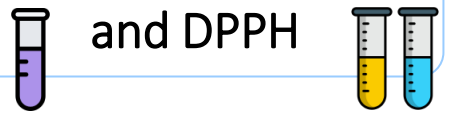
Environment



Animal factors



Affect differently FRAP and DPPH



...& Prospects

- Milk valorization
- Integration of new methods to investigate the variability of antioxidant activity

Thank you for your attention!

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Temperature Humidity Index (THI)

The following formula is used to calculate the THI index:

$$THI = (0.8 * Ta) + [Ur * (Ta-14.4)] + 46.4$$

Ta= air temperature (°C)

Ur=relative humidity (%)

Temperature		% Relative Humidity																		
°F	°C	0	5	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80	85	90
72	22.0	64	65	65	65	66	66	67	67	67	68	68	69	69	69	70	70	70	71	71
73	23.0	65	65	66	66	66	67	67	68	68	68	69	69	70	70	71	71	71	72	72
74	23.5	65	66	66	67	67	67	68	68	69	69	70	70	70	71	71	72	72	73	73
75	24.0	66	66	67	67	68	68	68	69	69	70	70	71	71	72	72	73	73	74	74
76	24.5	66	67	67	68	68	69	69	70	70	71	71	72	72	73	73	74	74	75	75
77	25.0	67	67	68	68	69	69	70	70	71	71	72	72	73	73	74	74	75	75	76
78	25.5	67	68	68	69	69	70	70	71	71	72	73	73	74	74	75	75	76	76	77
79	26.0	67	68	69	69	70	70	71	71	72	73	73	74	74	75	76	76	77	77	78
80	26.5	68	69	69	70	70	71	72	72	73	73	74	75	75	76	76	77	78	78	79
81	27.0	68	69	70	70	71	72	72	73	73	74	75	75	76	77	77	78	78	79	80
82	28.0	69	69	70	71	71	72	73	73	74	75	75	76	77	77	78	79	79	80	81
83	28.5	69	70	71	71	72	73	73	74	75	75	76	77	78	78	79	80	80	81	82
84	29.0	70	70	71	72	73	73	74	75	75	76	77	78	78	79	80	80	81	82	83
85	29.5	70	71	72	72	73	74	75	75	76	77	78	78	79	80	81	81	82	83	84
86	30.0	71	71	72	73	74	74	75	76	77	78	78	79	80	81	81	82	83	84	84
87	30.5	71	72	73	73	74	75	76	77	77	78	79	80	81	81	82	83	84	85	85
88	31.0	72	72	73	74	75	76	76	77	78	79	80	81	81	82	83	84	85	86	86
89	31.5	72	73	74	75	75	76	77	78	79	80	80	81	82	83	84	85	86	86	87
90	32.0	72	73	74	75	76	77	78	79	79	80	81	82	83	84	85	86	86	87	88
91	33.0	73	74	75	76	76	77	78	79	80	81	82	83	84	85	86	86	87	88	89
92	33.5	73	74	75	76	77	78	79	80	81	82	83	84	85	85	86	87	88	89	90
93	34.0	74	75	76	77	78	79	80	80	81	82	83	85	85	86	87	88	89	90	91
94	34.5	74	75	76	77	78	79	80	81	82	83	84	86	86	87	88	89	90	91	92
95	35.0	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93
96	35.5	75	76	77	78	79	80	81	82	83	85	86	87	88	89	90	91	92	93	94
97	36.0	76	77	78	79	80	81	82	83	84	85	86	87	88	89	91	92	93	94	95
98	36.5	76	77	78	80	80	82	83	83	85	86	87	88	89	90	91	92	93	94	95
99	37.0	76	78	79	80	81	82	83	84	85	87	88	89	90	91	92	93	94	95	96
100	38.0	77	78	79	81	82	83	84	85	86	87	88	90	91	92	93	94	95	96	98
101	38.5	77	79	80	81	82	83	84	86	87	88	89	90	92	93	94	95	96	98	99
102	39.0	78	79	80	82	83	84	85	86	87	89	90	91	92	94	95	96	97	98	100
103	39.5	78	79	81	82	83	84	86	87	88	89	91	92	93	94	96	97	98	99	101
104	40.0	79	80	81	83	84	85	86	88	89	90	91	93	94	95	96	98	99	100	101
105	40.5		80	82	83	84	86	87	88	89	91	92	93	95	96	97	99	100	101	102
106	41.0	80	81	82	84	85	87	88	89	90	91	93	94	95	97	98	99	101	102	103
107	41.5	80	81	83	84	85	87	88	89	91	92	94	95	96	98	99	100	102	103	104