Phenotypic variation of milk antioxidant activity in Italian Mediterranean buffaloes

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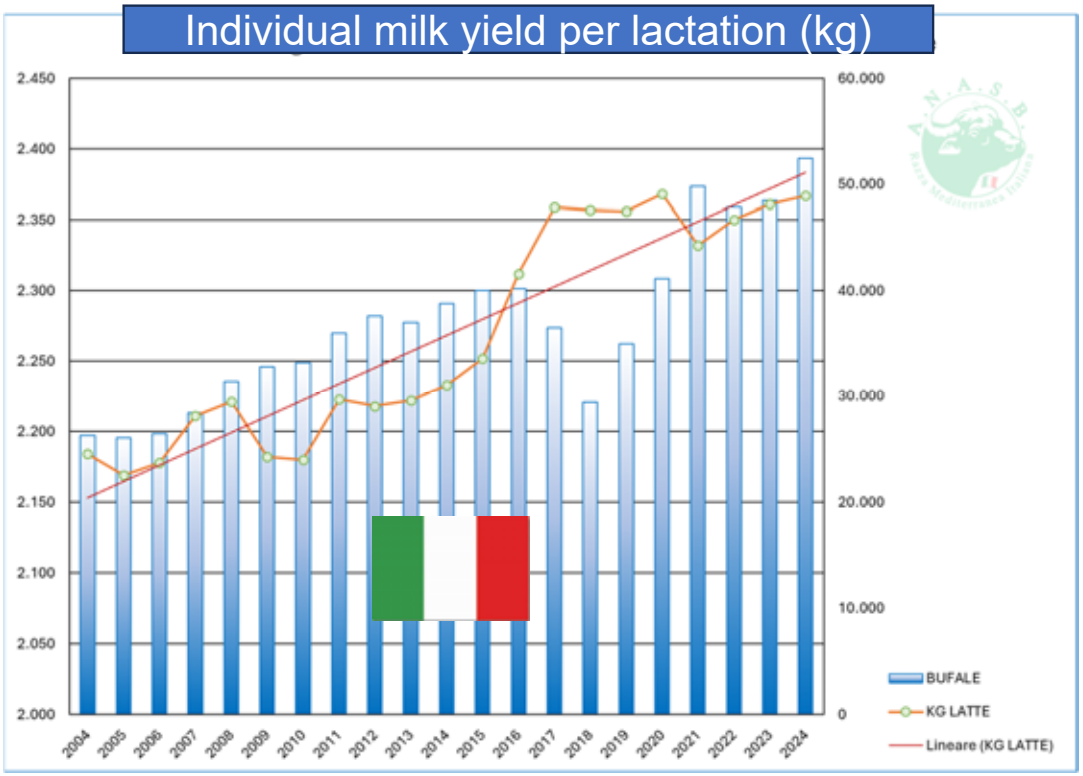
INTRODUCTION

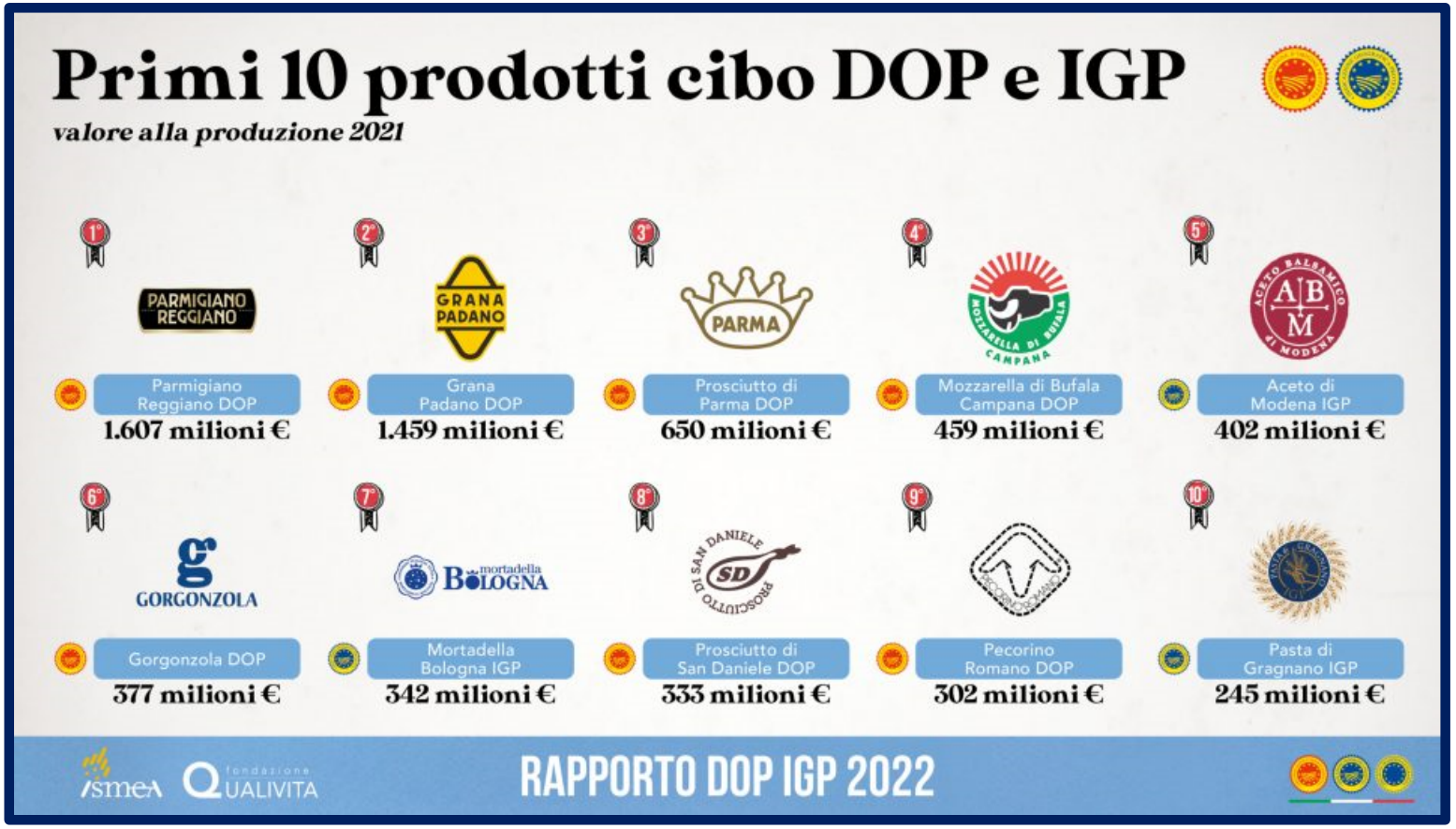


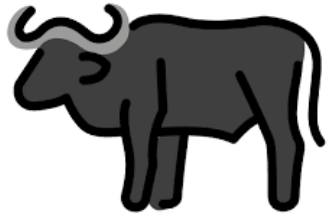
ANASB, 2026. https://www.anasb.it/statistiche/

Milk production in Italian Mediterranean buffaloes has steadily increased over the years.

INTRODUCTION

Approximately **121,000** lactating buffaloes (raised in **650** farms) are monitored annually in Italy (**ANASB, 2026**).





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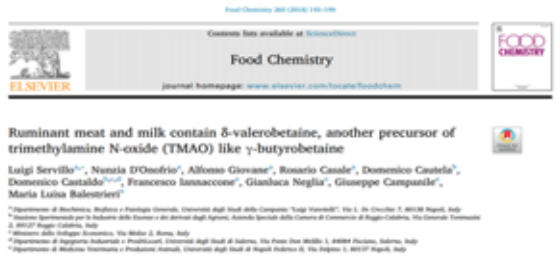
BACKGROUND

Current evidence on antioxidant activity in buffalo milk:


Food Chemistry

Ruminant meat and milk contain δ-valerobetaine, another precursor of trimethylamine N-oxide (TMAO) like γ-butyrobetaine

Luigi Servillo, Nunzia D'Onofrio, Alfonso Giovane, Rosario Casale, Domenico Cautela, Domenico Castaldo, Francesco Iannaccone, Gianluca Neglia, Giuseppe Campanile, Maria Luisa Balestrieri

γ-butyrobetaine, L-carnitine, δ-valerobetaine, etcc…

Bioactive background

- Buffalo milk contains bioactive compounds with potential antioxidant and anti-inflammatory activity.
- L-carnitine derivatives and betaines have been reported in buffalo milk and dairy products.
- These compounds may contribute to the functional value of buffalo milk.

What TAC and FRAP mean

TAC
Total antioxidant capacity

FRAP
Ferric reducing antioxidant power

Both assays are used as complementary indicators of the antioxidant profile of milk.


Journal of Animal Physiology and Animal Nutrition

ORIGINAL ARTICLE | Open Access

Use of former food products in dairy buffalo nutrition: In vitro and in vivo evaluation

Gianluca Neglia, Serena Calabrò, Alessio Cotticelli, Angela Salzano, Roberta Matera, Alessandro Vastolo, Nunzia D'Onofrio, Andrea Giorgino, Elisa Martino … See all authors

First published: 17 May 2023 | https://doi.org/10.1111/jpn.13830 | VIEW METRICS

Gianluca Neglia and Serena Calabrò contributed equally to the work.

Dietary modulation is possible
Green forage or fresh-cut sorghum affected TAC and FRAP (Salzano et al. 2021).


Food Chemistry

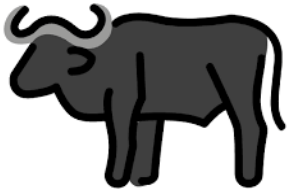
Green feed increases antioxidant and antineoplastic activity of buffalo milk: A globally significant livestock

Angela Salzano, Gianluca Neglia, Nunzia D'Onofrio, Maria Luisa Balestrieri, Antonio Limone, Alessio Cotticelli, Raffaele Marrone, Aniello Anastasio, Michael J. D'Occhio, Giuseppe Campanile

What is still unclear?

The sources of variation in buffalo milk antioxidant activity remain poorly defined, particularly under commercial farm conditions.

- Farm-level and physiological effects may confound dietary signals.
- The contribution of TMR composition and physical structure requires specific evaluation.



Animal-related factors

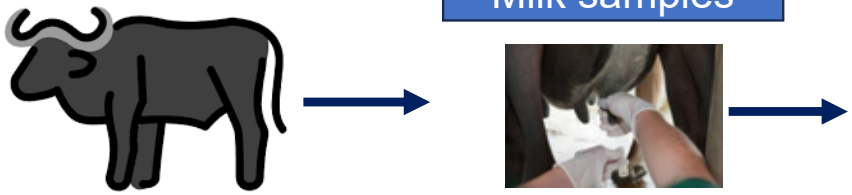
Total mixed ration characteristics

Farm / sampling context

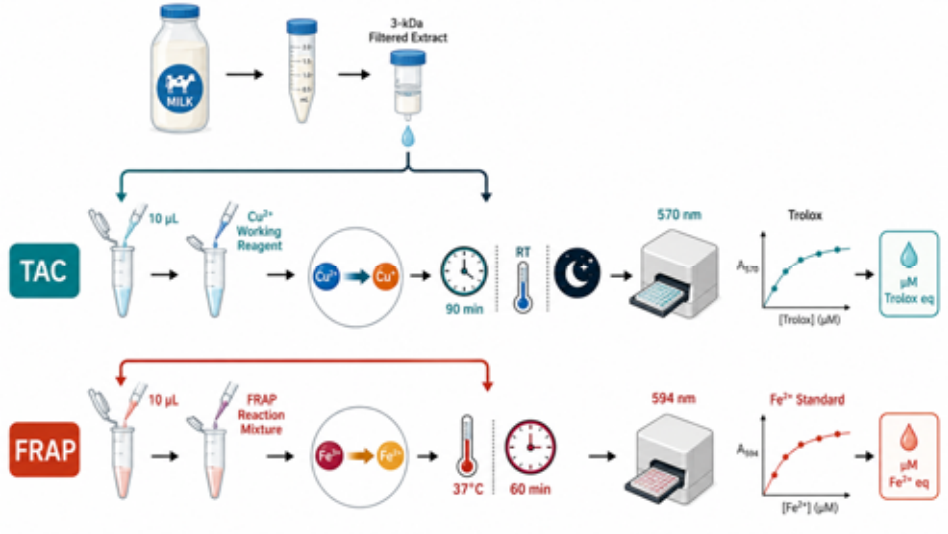
Materials and Methods

433	19	4	2021–2023
Clinically healthy lactating buffaloes	Commercial farms	Regions in central and southern Italy	Sampling period: Dec 2021 to Aug 2023

- Animals were **selected** to balance **days in milk**, **parity** and **sampling season**.
  - A minimum of **five animals** was sampled on each sampling date.



Milk samples



Materials and Methods

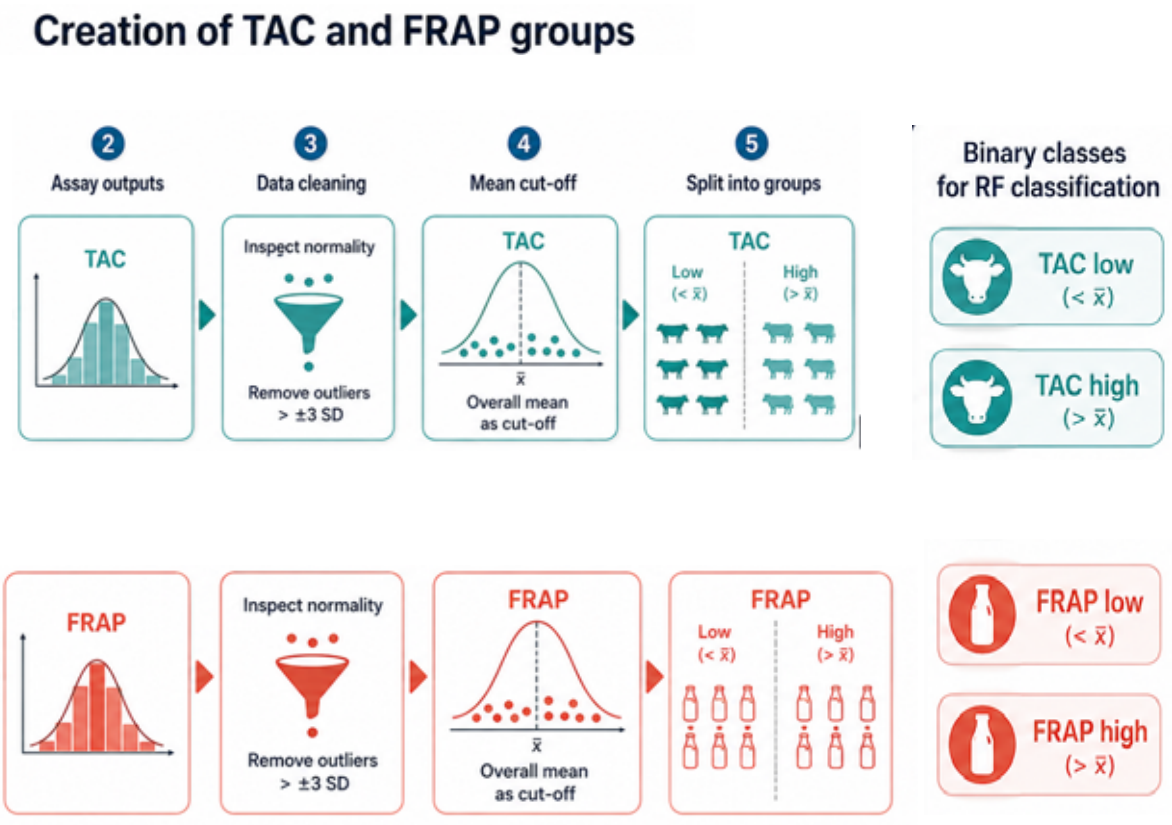
Both indicators were used as complementary measures of milk antioxidant activity.

TAC

Mean ± SD:
150 ± 44.0 µM/L Trolox eq.

FRAP

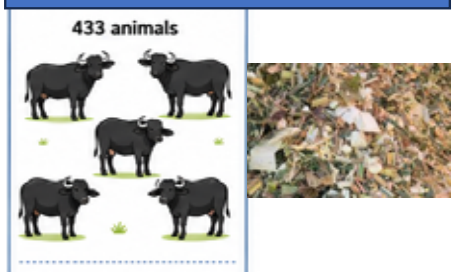
Mean ± SD:
84.2 ± 20.3 µM Fe eq.



Materials and Methods

TMR samples were collected on the same farm and on the same day as milk sampling.

TMR samplings



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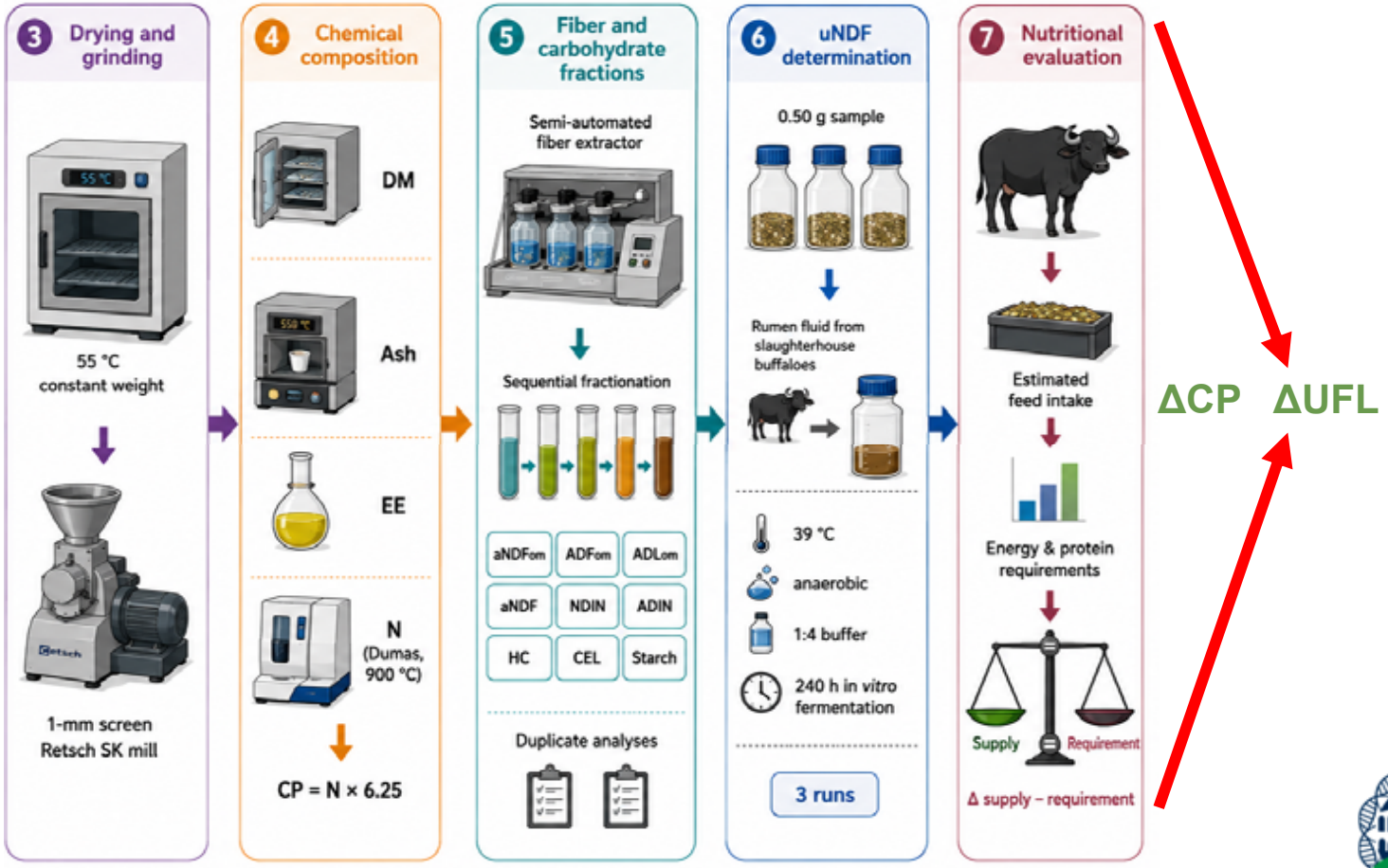

Materials and Methods

R Studio®

Analysis

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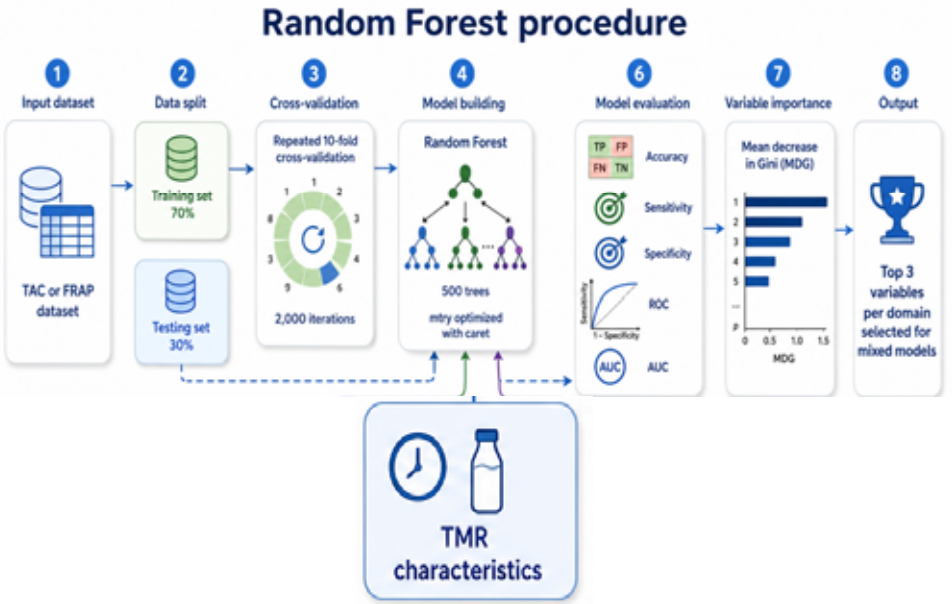
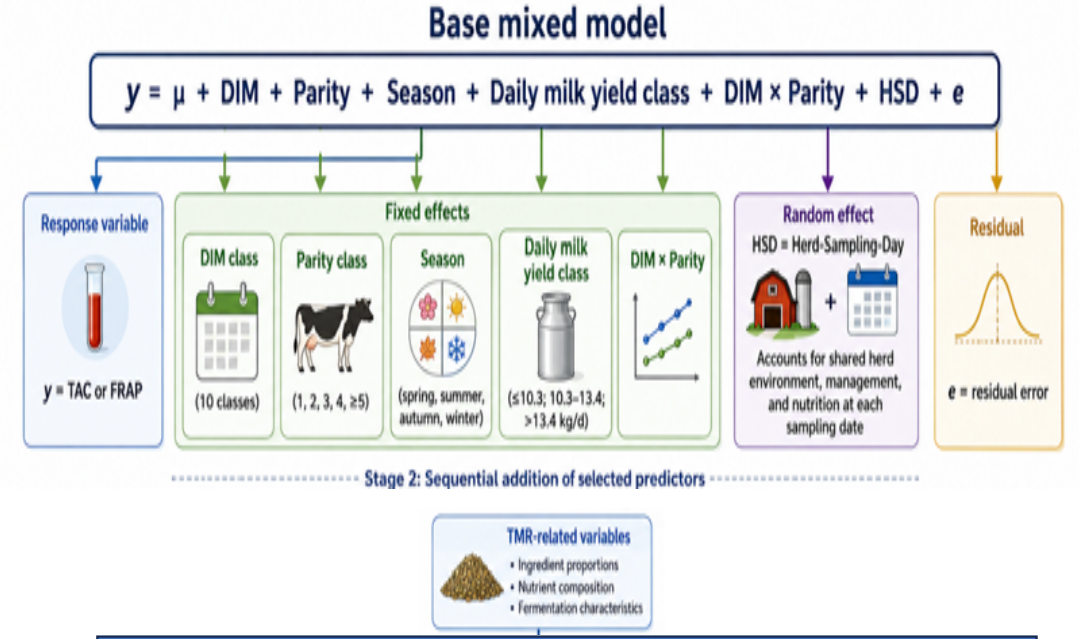




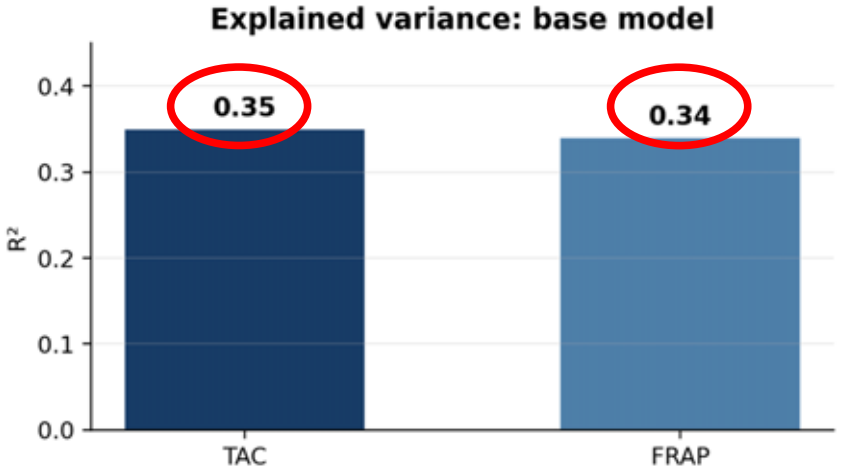
Random Forest models based on TMR-related variables were used to identify the top three variables associated with the classification of TAC and FRAP into high and low classes.

Top two RF-selected TMR variables included as covariates in the mixed model.

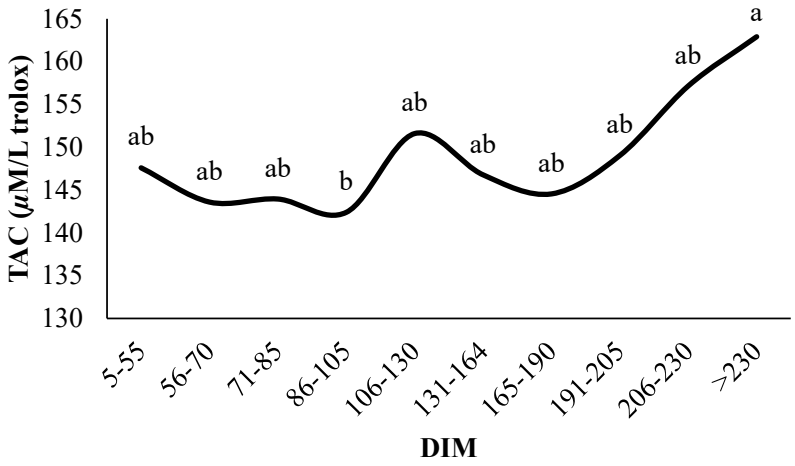
RESULTS

-The base model explained a similar proportion of variance for TAC and FRAP, with **DIM** having the most important effect.

-Animal-related, seasonal and productive effects contributed only moderately to the overall variability.



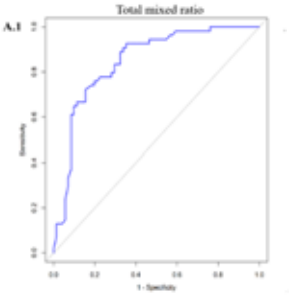
Effect	TAC P-value	FRAP P-value
Parity	0.8503	0.3498
Season	0.8590	0.5867
Milk yield class	0.3873	0.8697

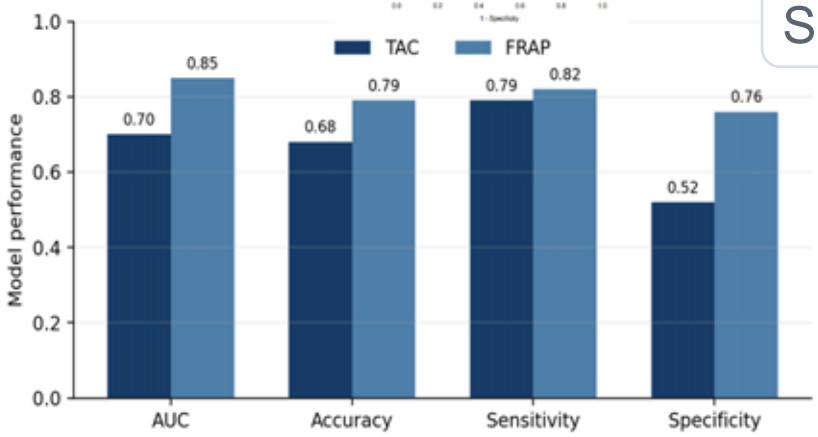


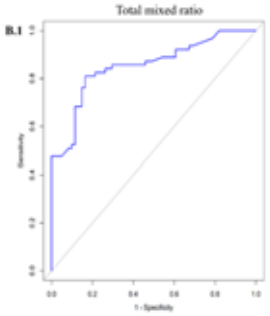
TAC significantly increased progressively across DIM.

FRAP was not significantly affected by DIM.

RESULTS:TMR variables classified antioxidant activity







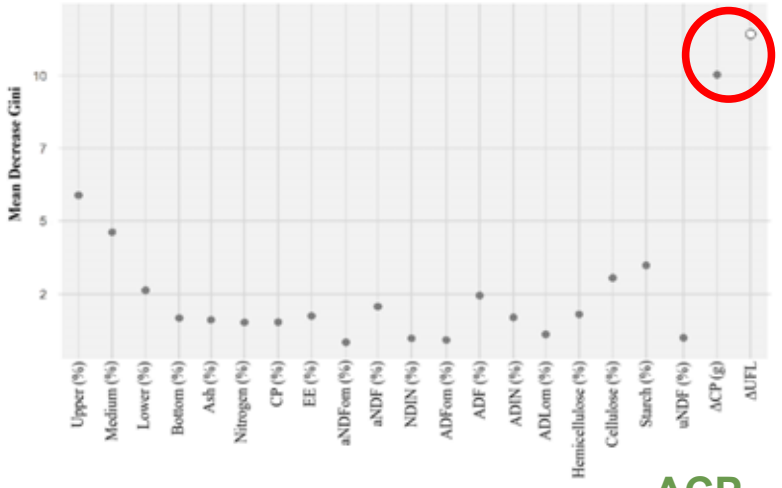
TAC model

AUC = 0.70
Accuracy = 0.68
Sensitivity = 0.79
Specificity = 0.52

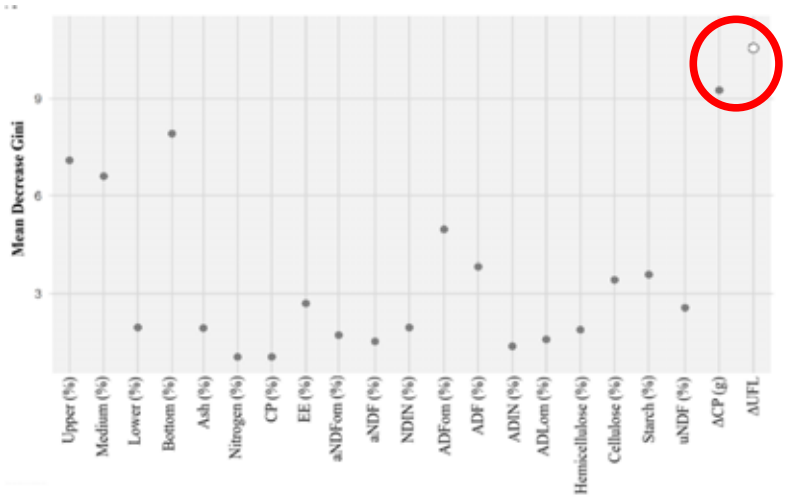
FRAP model

AUC = 0.85
Accuracy = 0.79
Sensitivity = 0.82
Specificity = 0.76

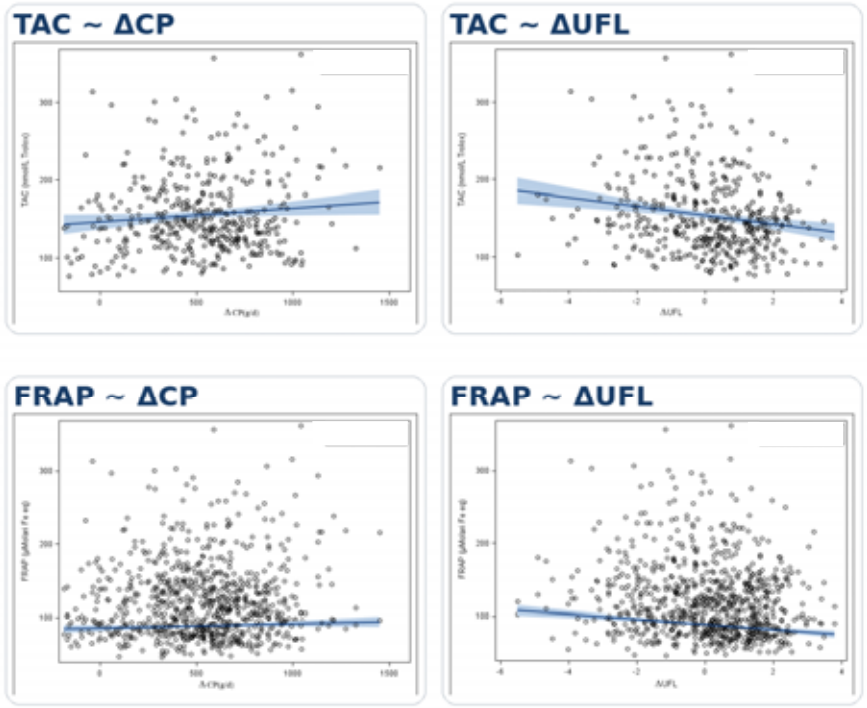
ΔCP ΔUFL



ΔCP ΔUFL



RESULTS: Key TMR predictors and direction of associations



The inclusion of the selected variables in the base model improved model fit, increasing R^2 to **0.66** and **0.67** for TAC and FRAP, respectively.

Positive association

ΔCP with TAC and FRAP

Negative associations

ΔUFL with TAC and FRAP

Greater **protein** supply may support milk antioxidant capacity through **amino acid** availability and related bioactive compounds.

Conversely, the negative association with ΔUFL indicates that an energy surplus, particularly when linked to **starch-rich diets**, may not enhance antioxidant capacity and could reflect shifts in rumen fermentation and metabolic balance.

CONCLUSIONS

Conclusions and take-home message

- **Animal-related** and productive factors had **limited** (34-35%) influence on TAC and FRAP variability.
- TMR variables provided the strongest discriminative information alone, especially for **FRAP**.
- **ΔCP was positively associated with both antioxidant indicators**, whereas **ΔUFL was negatively associated** with both.
- Further studies are needed to confirm the causal role of specific dietary components.

Università degli Studi della Campania *Luigi Vanvitelli*

Dipartimento Medicina Veterinaria Produzioni Animali

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Thank you for your attention.

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RESULTS

TMR variables descriptive statistics

Variables[1]	Mean	STD	Minimum	Maximum
Penn State Particle Size:				
Upper, %	14.7	9.02	1.95	40.2
Medium, %	42.1	9.49	18.4	60.6
Lower, %	36.8	8.09	7.22	58.4
Bottom, %	6.40	5.36	3.04	27.1
Chemical composition:				
DM	48.3	7.80	32.5	64.9
Ash, %DM	7.42	1.20	5.10	9.75
Nitrogen, %DM	2.29	0.39	1.92	2.82
CP, %DM	14.3	2.45	12.0	17.6
EE, %DM	3.86	1.10	1.57	7.42
aNDFom, %DM	38.8	6.73	27.2	50.2
aNDF, %DM	40.9	7.20	29.0	53.7
NDIN, %DM	0.46	0.22	0.16	1.10
ADFom, %DM	23.8	4.20	17.9	32.0
ADF, %DM	24.4	4.60	18.5	33.5
ADIN, %DM	0.16	0.08	0.08	0.42
ADLom, %DM	4.86	1.35	2.76	8.30
Hemicellulose, %DM	14.5	4.02	5.24	22.3
Cellulose, %DM	19.0	3.20	13.5	25.5
Starch, %DM	16.9	5.50	8.10	31.8
uNDF, %DM	14.1	2.64	8.75	18.0
ΔCP, g	528	304	-186	1581
ΔUFL	0.10	1.55	-1.01	2.05

RESULTS

Milk characteristics variables descriptive statistics

Variables[1]	Mean	STD	Minimum	Maximum
Daily milk yield, kg/d	12.1	3.57	2.80	23.6
TAC, µM/l trolox	150	44.0	63.3	362
FRAP, µM Fe eq	84.2	20.3	45.8	155
Fat, %	7.93	1.74	5.01	13.9
Protein, %	4.59	0.43	3.45	6.00
Lactose, %	4.69	0.26	3.80	5.34
Casein, %	3.73	0.41	2.59	5.28
Urea, mg/dL	32.8	13.5	0.28	78.5
Total solids, %	18.5	2.28	12.35	26.0
SNF, %	10.6	0.48	8.83	12.4
DSCC, %	50.1	18.0	7.60	93.7
SCS	3.31	1.63	0.06	4.06
Acetone, µmol/L	0.28	0.27	-0.02	2.11
BHB, µmol/L	0.19	0.16	-0.02	1.88
C14, %	0.81	0.26	0.20	1.77
C16, %	2.16	0.64	0.01	5.12
C18, %	0.69	0.19	0.12	1.43
C18:1, %	4.06	0.19	0.39	5.04
SCFA, %	0.96	0.29	0.22	1.97
MCFA, %	3.15	0.76	1.13	5.92
LCFA, %	3.04	1.02	0.10	6.56
SFA, %	5.27	1.43	1.49	10.3
UFA, %	2.29	0.75	0.40	4.97
MUFA, %	2.19	0.75	0.35	4.75
PUFA, %	0.17	0.07	0.01	0.40
TFA, %	0.14	0.08	0.00	0.42
Cryoscopic point, °C	-0.54	12.5	-0.59	-0.50
pH	6.66	0.20	6.00	7.15