# Effects of a nutritional supplement in Italian Mediterranean Buffalo exposed to heat stress

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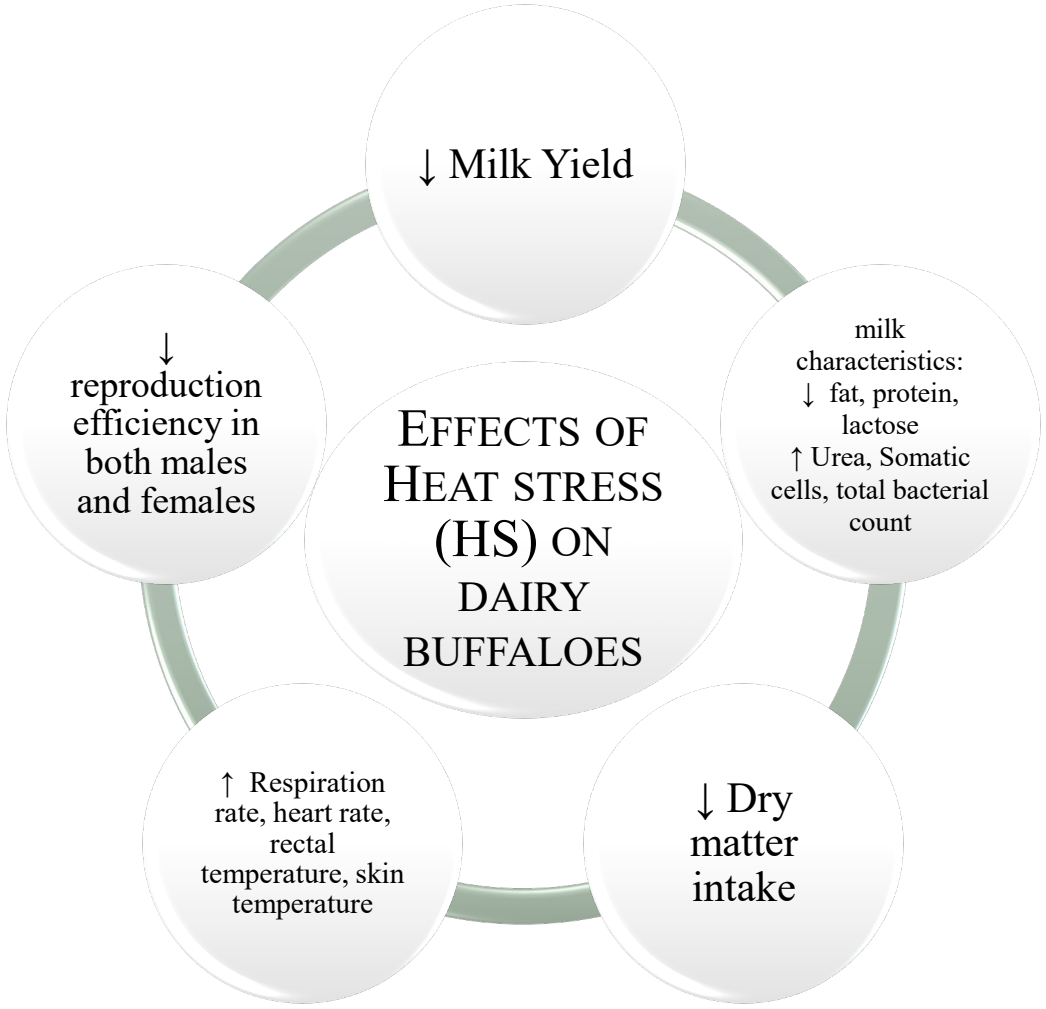
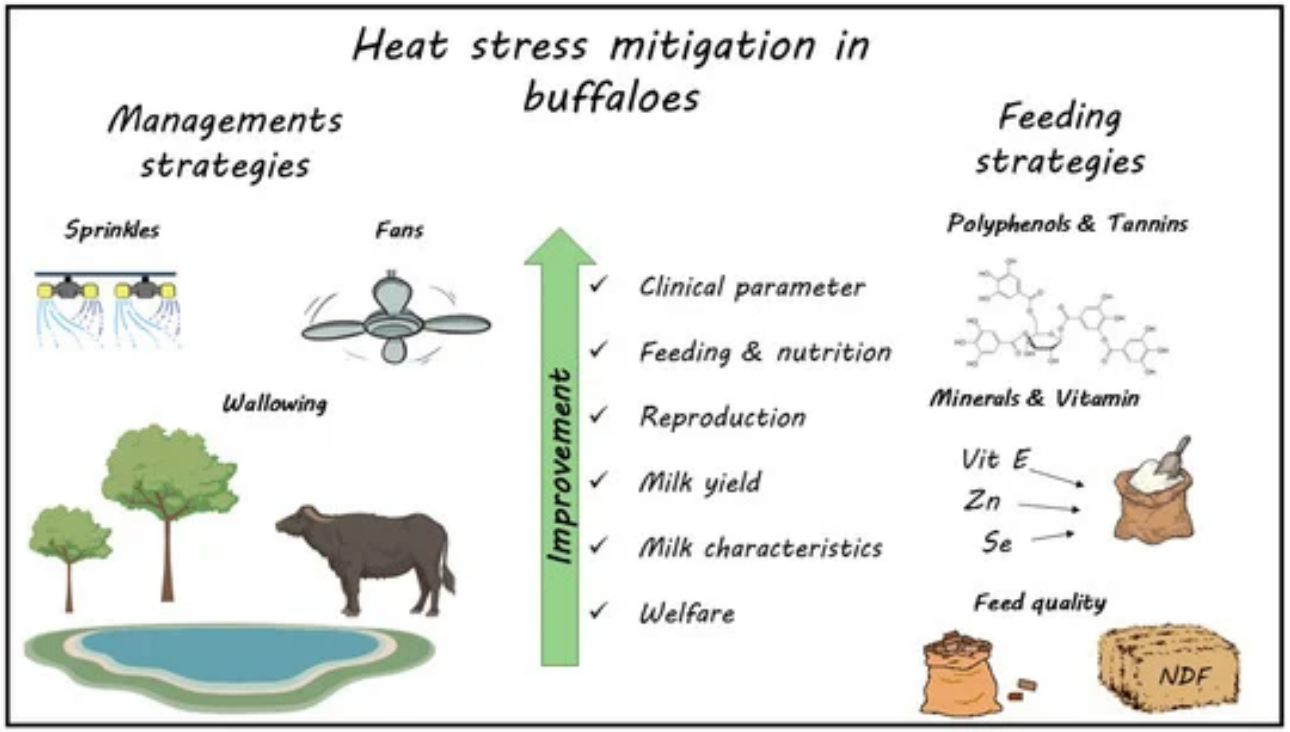
# INTRODUCTION

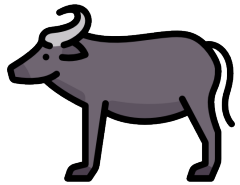


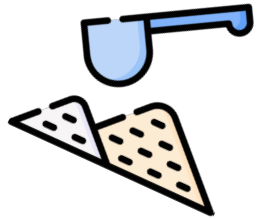




(Petrocchi Jasinski et al., 2023)

Heat stress can be simply defined as a condition that occurs when an animal cannot dissipate an enought quantity of heat (Bernabucci et al., 2014)

# AIM OF THE STUDY:





INVESTIGATE THE EFFECT OF A TWO DIFFERENT DOSES OF DIET SUPPLEMENT IN DAIRY BUFFALO BREEDING DURING HOT SEASON,

ON

MILK YIELD AND QUALITY

APPARENT DIGESTIBILITY

ANIMAL BEHAVIOURS

# MATERIALS & METHODS

**WHERE:** CREA-ZA Tor Mancina farm, Rome

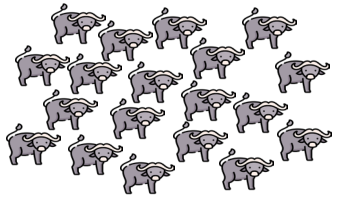
**WHEN:**

From June to September 2023
Total 93 days of experiment

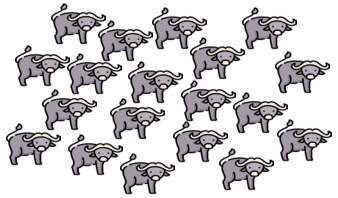
**NUTRITIONAL SUPPLEMENT:**


**Rumen Function Optimiser (RuFO)**

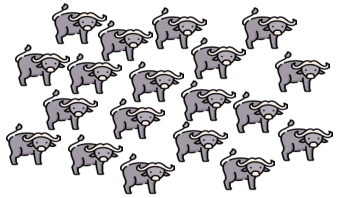
calcium carbonate, maerl, wheat bran, inulin (chicory), yeasts (brewers' yeast), Mojave yucca, garlic dried, cayenne pepper



**Control (C)**
Total mixed ration



**T1**
Total mixed ration
+ 100g/head/day
of Rufo



**T2**
Total mixed ration
+ 200 g/head/day
of Rufo

| Average±SD | C | T1 | T2 |
|---|---|---|---|
| **Buffaloes (n)** | 19 | 19 | 19 |
| **Days in milk (DIM)** | 82.89±34.95 | 76.84±41.05 | 78.79±34.41 |
| **Daily milk yield (L)** | 9.17±1.87 | 9.09±2.62 | 9.13±2.04 |
| **Parity (n)** | 3.21±1.40 | 3.05±1.28 | 3.11±1.41 |

All 3 groups were kept in the same conditions,
the only difference was in the supplementation.

## SAMPLING:

Every two weeks: **individual milk** and **total mixed ration samples** were collected
At the end: **individual faeces sampling**
Countinuously: **milk production**

| | Total mixed ration | Faeces |
|---|---|---|
| **Ash** | AOAC (1990) | AOAC (1990) |
| **Ash insoluble in hydrochloric acid (AIA)** | ISO 5985 (1978) | ISO 5985 (1978) |
| **Crude protein (CP)** | AOAC (2005) | AOAC (2005) |
| **Ether extract (EE)** | AOAC (2000) | AOAC (2000) |
| **Crude fiber (CF)** | AOAC (2005) | |
| **Starch** | AOAC (2005) | AOAC 2005 |
| **Neutral detergent fiber (aNDF), acid detergent fiber (ADF), and acid detergent lignin (ADL)** | Van Soest et al. (1991) | Van Soest et al. (1991) |
| **Physical characteristics** | Penn State Particle Separator (19, 8, 4 and < 4 mm) | |
| **Homogeneity index (HI) and sorting index (SI)** | PoliSPEC$^{NIR}$ (ITPhotonics, Breganze, VI, Italy) | |
| **physically effective NDF (peNDF)** | Mertens (1997) | |
| **Apparent digestibility** | Fredin et al. (2014) (AIA as internal markers) | |

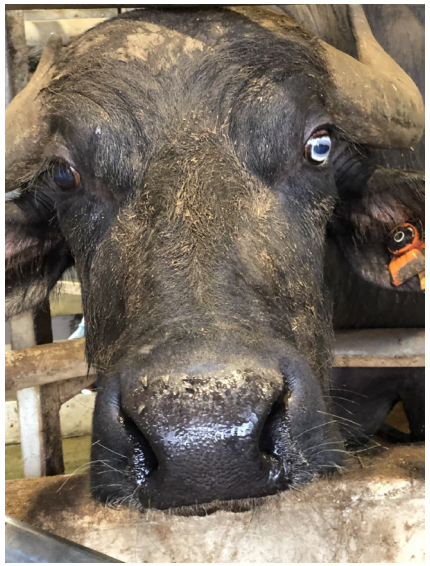
| Milk | |
|---|---|
| Milk was stored in 50 ml plastic tubes containing Bronopol® (2-Bromo-2-Nitropropane-1,3-Diol) at 4°C and analyses whitin 24-48h | |
| **Milk production** | Milking system |
| **Fat, protein, lactose, casein, solid not fat, urea, and freezing point** | FT-IR spectrophotometry - MilkoScanTM 7 RM (Foss Analytics, Hillerød, Denmark) |
| **Titratable acidity and pH** | Potentiometric method |
| **Somatic cell counts (SCC)** | Fluoro-optoelectronics method - FossomaticTM FC (Foss Analytics, Hillerød, Denmark). |
| **Somatic cell score (SCS)** | Wiggans and Shook, (1987) |
| **Energy corrected milk (ECM)** | Campanile et al. (1998) |
| **RCT, k20, a30** | Formagraph instrument (Mape System, Firenze, Italy) according to Zannoni and Annibaldi (1981) |

RCT: rennet coagulation time (min)
k20: curd firming time (min)
a30: compactness of curd (mm)

## SAMPLING:

**Animal behavior** and **environmental condition** continuosly recorded

| Other data | |
|---|---|
| **Animal behavior** | Ear tag. (CowManager, Harmelen, Netherlands) |
| **Environmental condition** | Datalogger (Mini Data Logger 174-H, TESTO, Italy) |

Behaviour registered:

- Eating time
- Ruminating time
- Total movement
- Not active time

Data obtained were used to calculate temperature-humidity index (THI) according to the following formula (Vitali et al., 2009):

$$\mathbf{THI} = (1.8 \times AT + 32) - (0.55 - 0.55 \times RH) \times [(1.8 \times AT + 32) - 58]$$

AT= ambient temperature; RH= relative humidity

## STATISTICAL ANALYSIS:

Statistical analysis was performed using Statistica 10 (Statsoft Inc, Tulsa, Oklahoma, USA).
The approach adopted was a **nested** analysis of variance (**ANOVA)** in which "**animal ID**" (**random factor**) was nested into "**group**" factor. The other factor was "**sampling**".
To test significance difference, Tukey test as post-hoc was performed.

Results are expressed as Lsmeans±SE, and significance was declared at $p < 0.05$.

$$Y_{ijk} = \mu + G_i + A_{j(i)} + S_k + \varepsilon_{ijk}$$

$Y_{ijk}$ is the dependent variable observed for the $j$j-th animal in the $i$i-th group at the $k$k-th sampling time.
$\mu$ is the overall mean.
$G_i$ is the effect of the $i$i-th group ($i$=$C$,$T1$,$T2$).
$A_{j(i)}$ is the effect of the $j$j-$^{th}$ animal nested within the $i$i-th group (random factor).
$S_k$ is the effect of the $k$k-$^{th}$ sampling time ($k$=1,2,3,…,7).
$\varepsilon_{ijk}$ is the random error associated with the observation $Y_{ijk}$.

# RESULTS

Table 1. Physical-chemical composition of the total mixed ration among 3 groups.

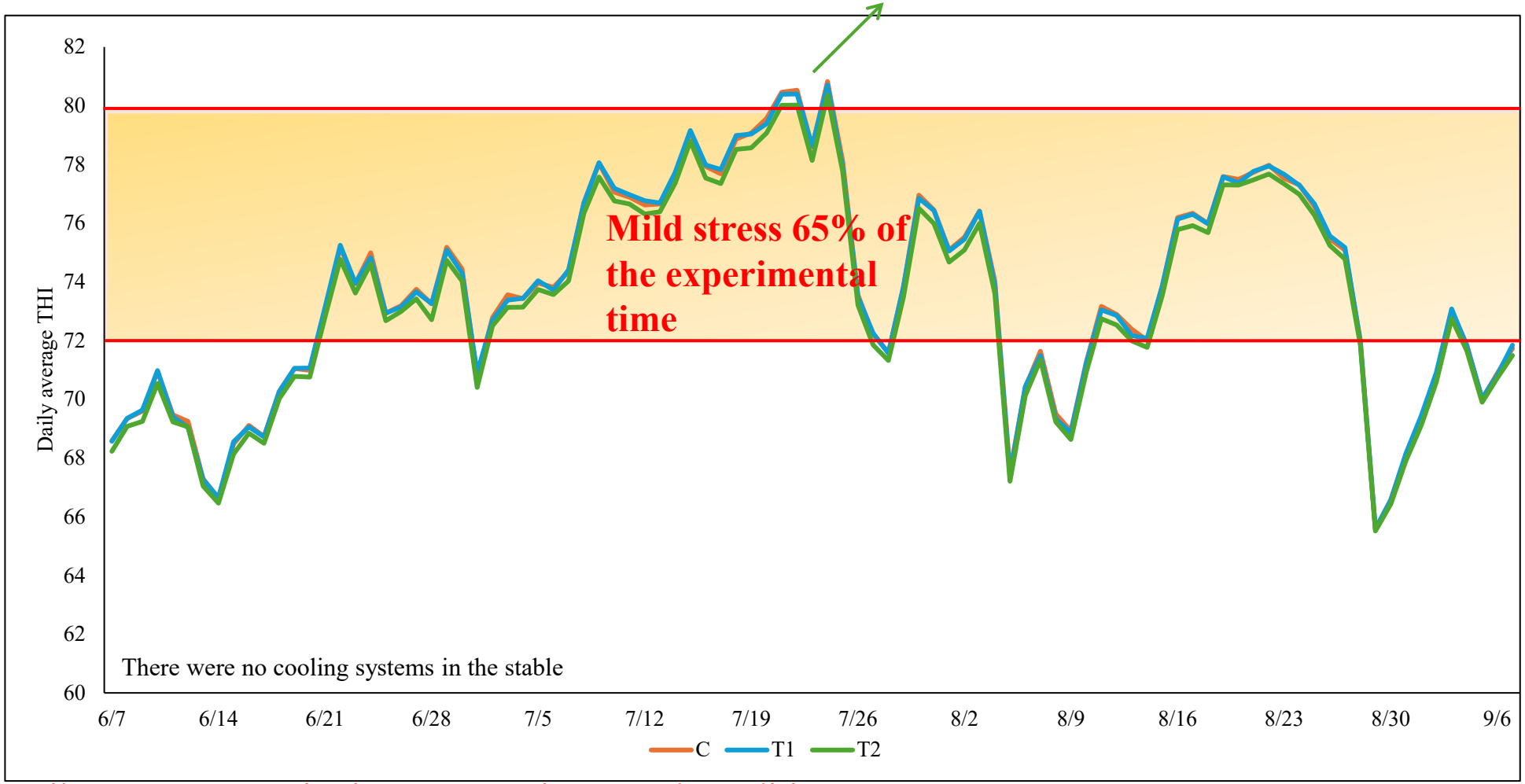
| | From start to July 11 (mean±SE) | | | | From July 12 to end (mean±SE) | | | |
|---|---|---|---|---|---|---|---|---|
| | C | T1 | T2 | p-value | C | T1 | T2 | p-value |
| **HI** | 67.33±1.93 | 63.13±4.72 | 72.07±5.72 | ns | 70.24±5.61 | 68.16±2.47 | 66.20±1.69 | ns |
| **SI** | 66.83±17.57 | 76.77±4.32 | 64.67±10.95 | ns | 47.42±7.87 | 57.64±6.78 | 46.64±7.26 | ns |
| **DM (%)** | 62.71±1.29 | 62.48±1.16 | 62.79±1.46 | ns | 62.22±1.06 | 63.29±0.94 | 62.35±0.93 | ns |
| **Ash (%)** | 9.66±0.64 | 9.41±0.65 | 9.71±0.67 | ns | 9.24±0.16 | 9.46±0,15 | 9.74±0.17 | ns |
| **Crude protein (%)** | 13.56±0.51 | 13.50±0.40 | 13.69±0.42 | ns | 14.86±0.37 | 14.95±0.37 | 14.99±0.31 | ns |
| **Ether extract (%)** | 1.72±0.16 | 1.66±0.13 | 1.63±0.09 | ns | 1.91±0.11 | 1.82±0.08 | 1.85±0.10 | ns |
| **Starch (%)** | 19.54±0.90 | 18.81±0.39 | 20.06±0.32 | ns | 17.90±0.42 | 18.41±0.62 | 17.53±0.57 | ns |
| **Crude fiber (%)** | 20.79±0.68 | 20.79±1.07 | 19.94±1.01 | ns | 22.78±0.50 | 22.23±0.41 | 22.17±0.43 | ns |
| **aNDF (%)** | 43.81±1.50 | 46.05±2.66 | 44.50±1.40 | ns | 45.63±0.52 | 45.37±0.51 | 44.64±0.94 | ns |
| **ADF (%)** | 31.13±0.99 | 31.15±1.97 | 30.70±1.25 | ns | 33.85±1.39 | 33.90±0.93 | 32.64±0.99 | ns |
| **ADL (%)** | 5.23±0.80 | 5.38±1.07 | 5.25±0.68 | ns | 6.00±0.23 | 6.03±0.16 | 5.86±0.23 | ns |
| **S1 (%)** | 15.46±1.09 | 14.76±0.53 | 16.75±1.24 | ns | 15.95±1.28 | 15.27±1.20 | 15.56±1.22 | ns |
| **S2 (%)** | 33.59±2.32 | 34.23±2.10 | 34.13±2.62 | ns | 31.44±1.22 | 31.22±0.90 | 30.91±0.93 | ns |
| **S3 (%)** | 21.98±1.13 | 21.75±1.20 | 21.49±1.28 | ns | 21.12±0.56 | 21.16±0.49 | 21.04±0.43 | ns |
| **Bottom (%)** | 28.98±1.74 | 29.25±2.00 | 27.64±1.50 | ns | 31.50±0.92 | 32.35±0.90 | 32.49±0.89 | ns |
| **peNDF (%)** | 31.07±0.43 | 32.50±1.72 | 32.17±1.09 | ns | 32.40±0.72 | 31.59±0.63 | 30.71±0.70 | ns |

HI=homogeneity index; SI=sorting index; DM=dry matter; NDF=neutral detergent fiber; ADF=acid detergent fiber; ADL=acid detergent lignin; S1-S2-S3-bottom=particles retained in the 19-8-4mm and bottom sieve; peNDF=physically effective NDF

The ration was similar to the 3 groups

# Environmental condition

They were subjected to moderate stress (average THI > 80) for 3 days (21,22 and 24 July 2023).

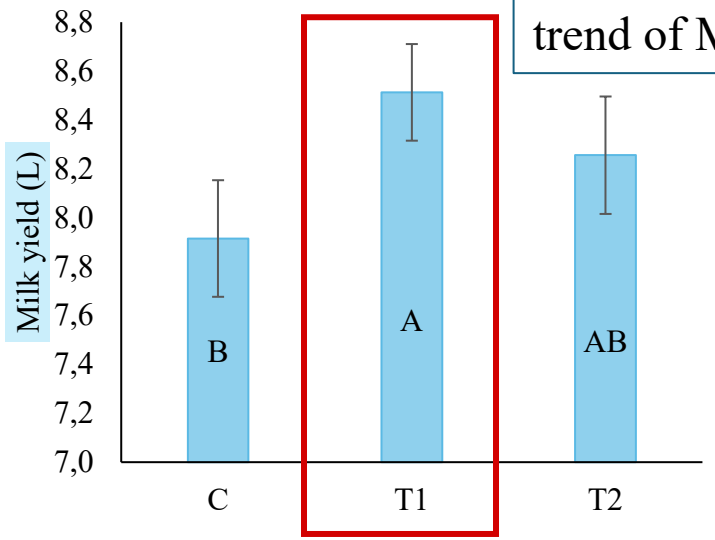



Literature data: difficulty in establishing a THI threshold for heat stress in dairy buffaloes; however Chaudhary et al. (2015) reported that for buffalo, a value of THI <72 is considered optimal, **THI between 72 and 79 is considered mild stress**, THI from 80 to 89 is considered moderate stress.

All 3 groups were in the some enviromental condition

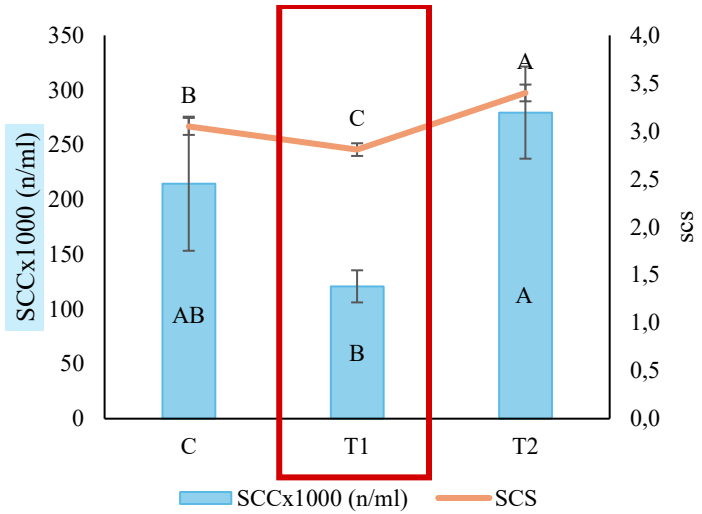
# RuFO effects on: Milk yield and characteristics

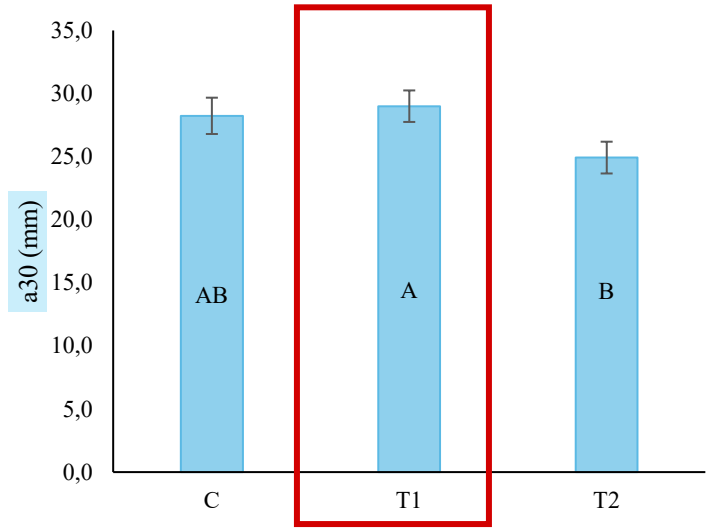
Parameters NOT influenced by treatment:
fat, protein, lactose, solid not fat and casein (%), freezing point (°C), urea (mg/dL), pH, titratable acidity (°SH), k20 (min)

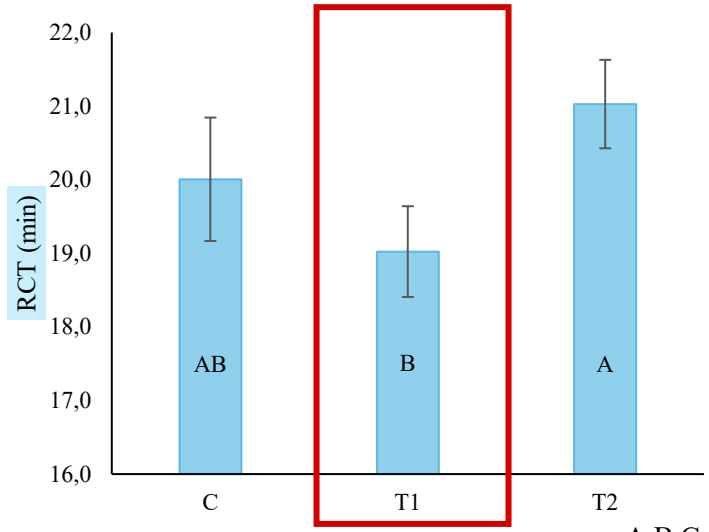



ECM follow the same trend of MY

- **Degirmencioglu et al., (2013)** and **Anjum et al., (2018)**: the administration of *Saccharomyces cerevisiae* to lactating buffaloes, *increase milk production* and *decrease SCS*.
- **An et al., (2023)**: supplementation of *Capsicum oleoresin*, *milk production increase* compared to control.
- **In all three studies**: the main milk components as fat, protein and lactose were not affected by treatment.



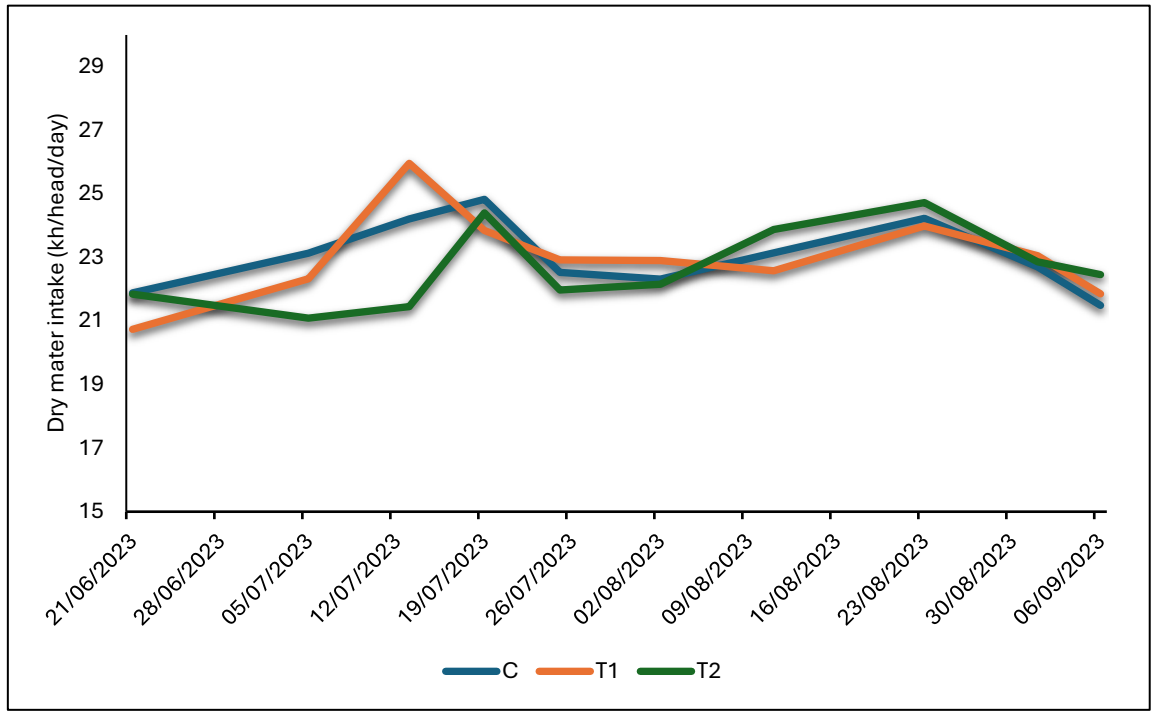




SCC=somatic cell count; SCS=somatic cell score; a30=curd firmness; RCT=rennet coagulation time; k20: curd firming time

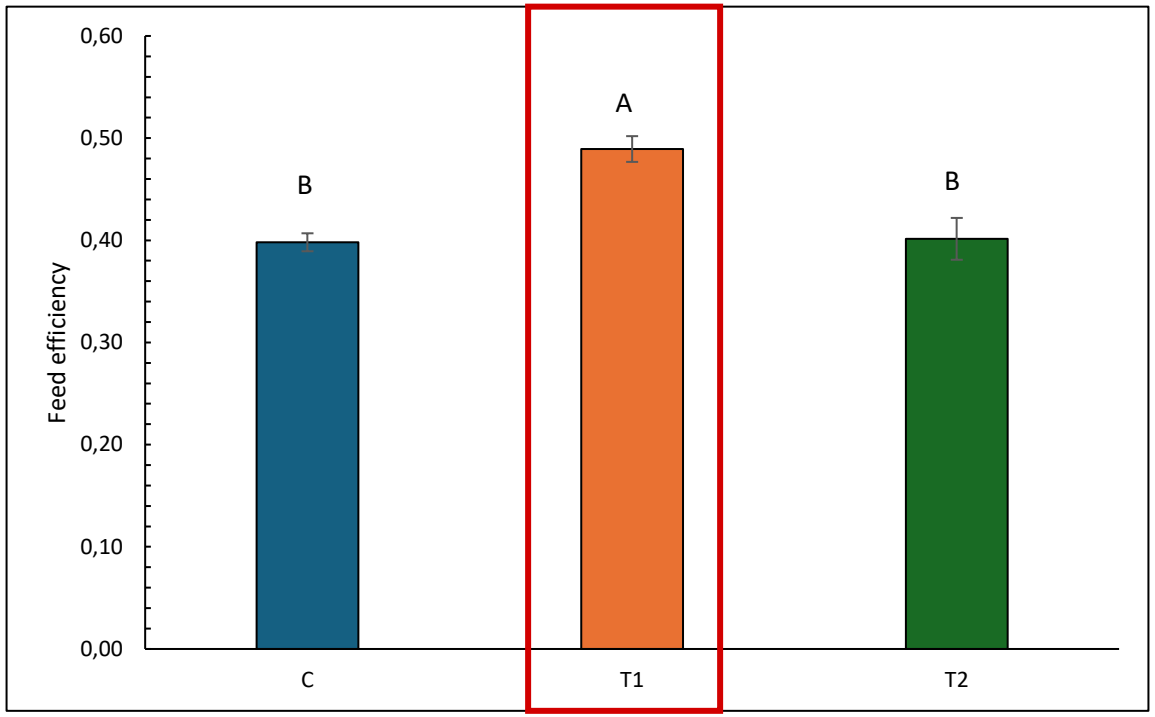
A,B,C $p < 0.001$

# RuFO effects on: Dry matter intake and feed efficency

Dry matter intake (DMI) (kg/head)



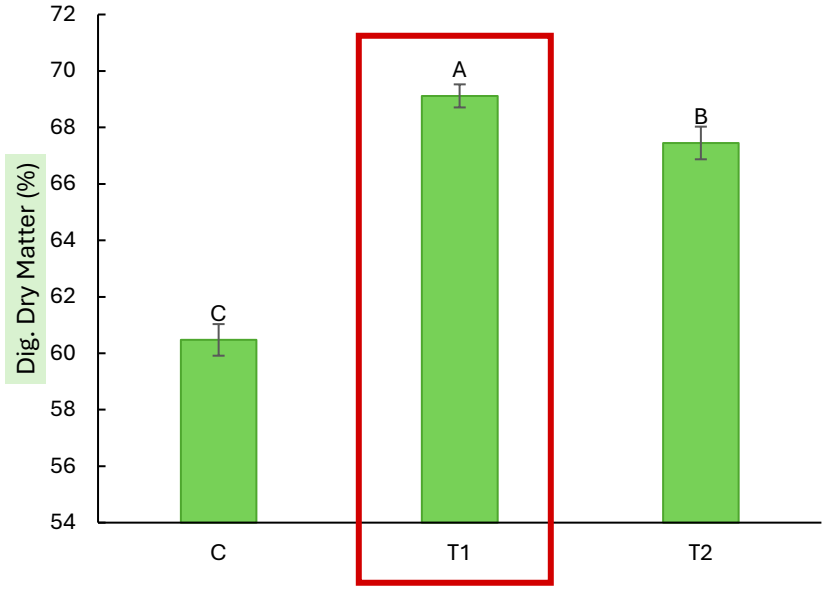

Feed efficency (Energy corrected milk/DMI)

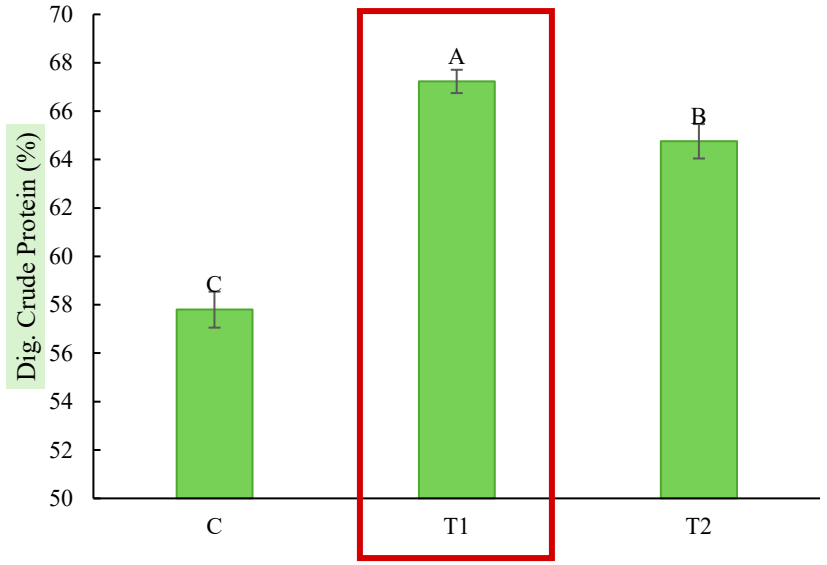


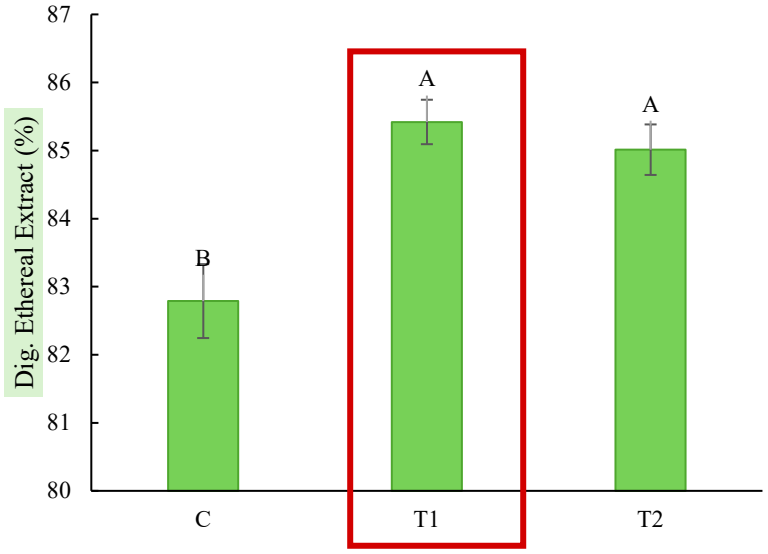

DMI was higher compared other studies (Salzano et al., 2021; Evangelista et al., 2022); feed efficiency was low compared to our previous study on dairy buffaloes (Evangelista et al., 2022).

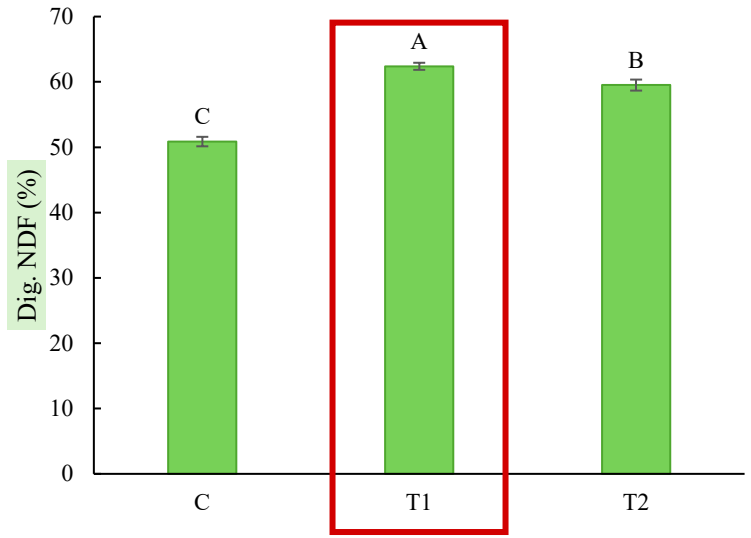
A,B,C $p < 0.001$

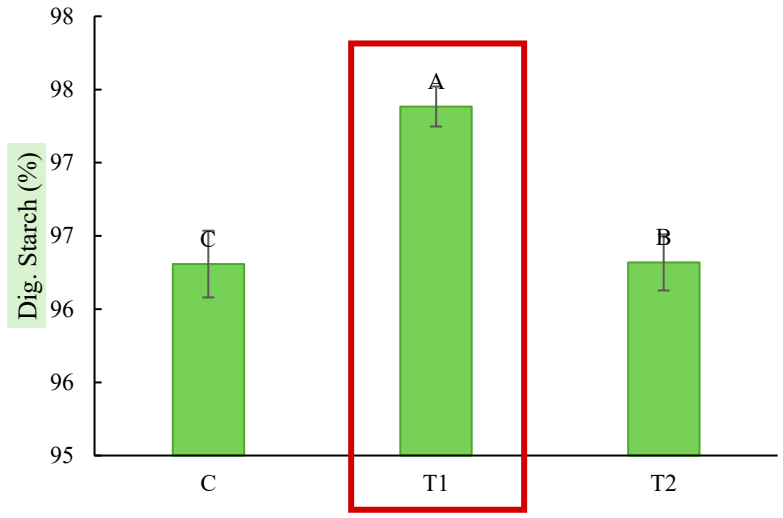
# RuFO effects on: Apparent digestibility of Dry matter, crude protein, ethereal extract, NDF and starch



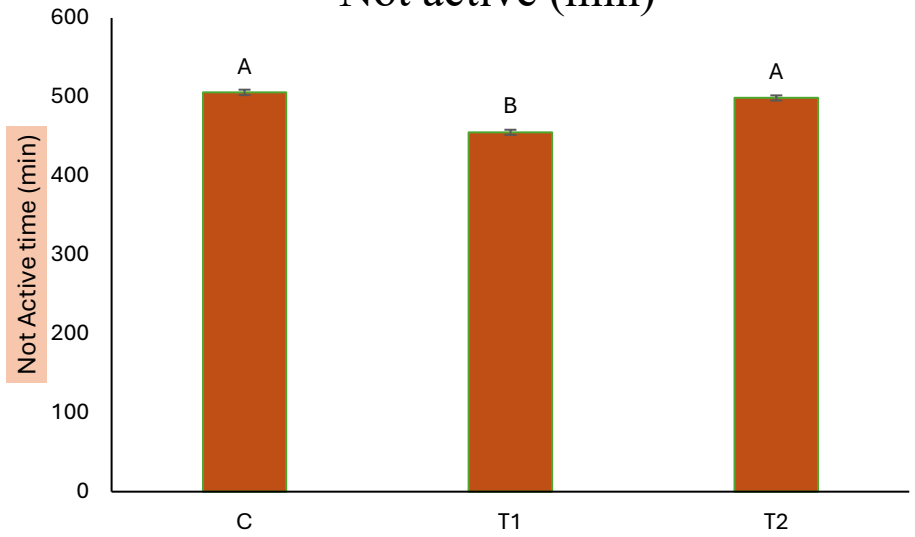

The apparent digestibility are consistent with values reported by Bovera et al. (2007) and Guerra et al. (2024).

A,B,C $p < 0.001$

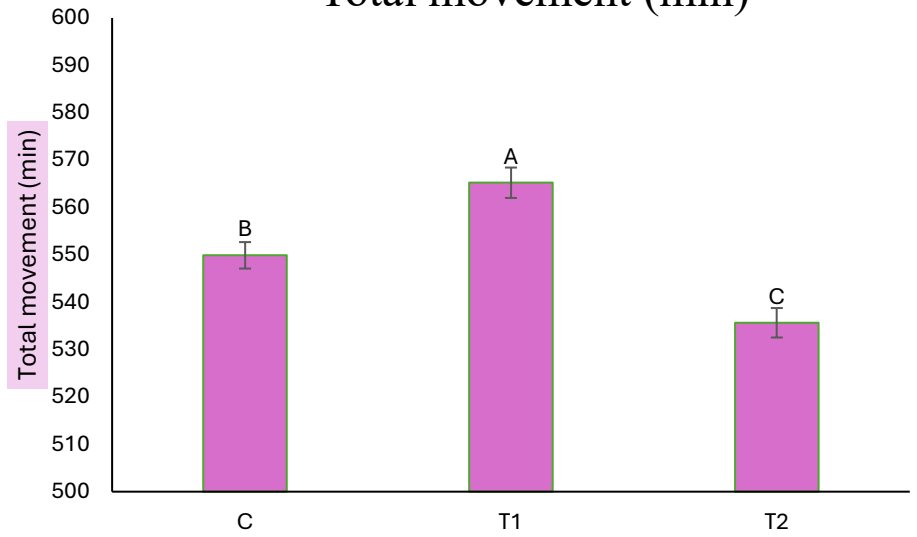
A,B,C p <0.001

# RuFO effects on: Animal Behaviours

## Not active (min)

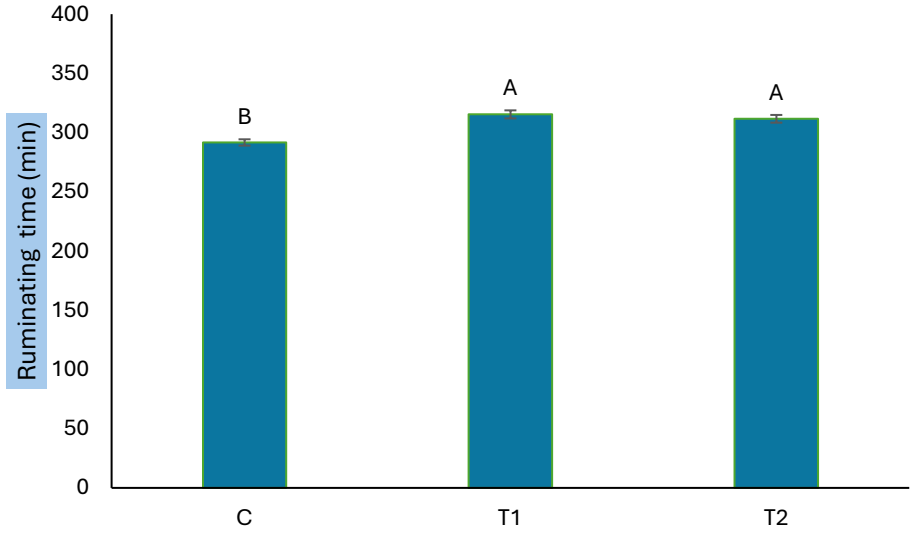



## Total movement (min)

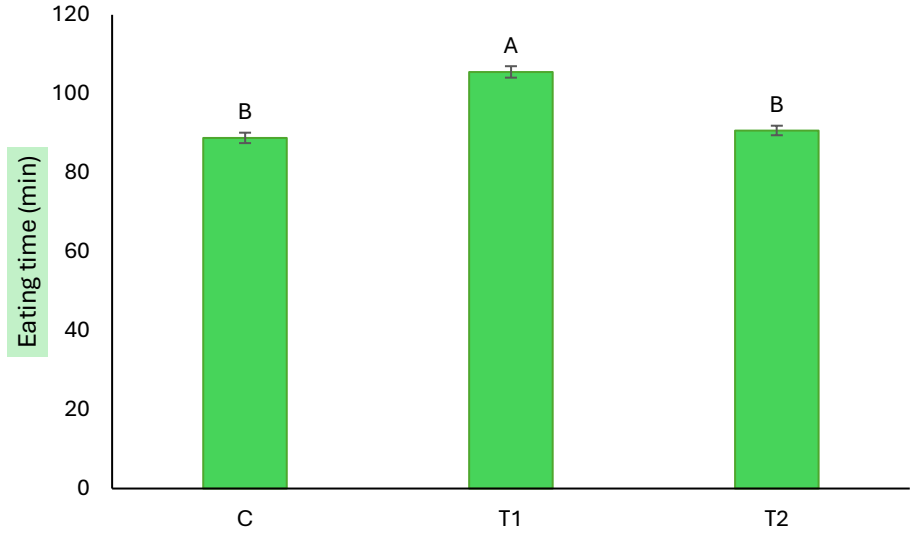



T1 seems less stressed than the others.

## Ruminating (min)




## Eating (min)




T1 group uses the ration better, in fact it has better production and digestibility.

Results agree with Meo Zilio et al. (2020) in a study conducted in the same farm some years earlier

# CONCLUSIONS

Administration of RuFO to TMR in dairy buffaloes in hot season allowed to:

- IMPROVE MILK YIELD
- DECREASE SOMATIC CELLS
- IMPROVE OF RHEOLOGICAL PARAMETERS (RCT AND A30)
- IMPROVE NUTRIENT DIGESTIBILITY
- IMPROVE FEED EFFICENCY

100 g/head/day

# THANKS FOR YOUR ATTENTION

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Acknowledges:

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# Supplemental material:

## Total mixed basal ration composition

| | From start to July 11 (kg/head/d) | From July 12 to end (kg/head/d) |
|---|---|---|
| **Mixed hay** | 8 | 8 |
| **Corn flour** | 3 | 4.50 |
| **Triticale silage** | 20 | 20 |
| **Barley grain** | 3.50 | 4 |
| **Sorghum grain** | 1 | - |
| **Soy flour** | 3.50 | 3.50 |
| **Commercial additives:*** | | |
| **a)** | 0.65 | 0.65 |
| **b)** | 0.30 | 0 |
| **c)** | 0.65 | 0.65 |
| **Total** | 40.60 | 41.30 |

## Rufo © (AHV, Piacenza, Italy):

calcium carbonate, maerl, wheat bran, inulin (chicory), yeasts (brewers' yeast), Mojave yucca, garlic dried, cayenne pepper

*Composition:
a) calcium carbonate, common wheat tritium, sodium chloride, dicalcium phosphate, sodium bicarbonate, magnesium oxide, Vitamin A, D3, E, B1, B2, B6, B12, niacin, choline chloride, manganous sulphate monohydrate, iron sulphate monohydrate, copper sulphate pentahydrate, zinc sulphate monohydrate, potassium iodide, sodium selenite, D-L methionine.
b) Calcium salts of palm oil fatty acids, sugar, zinc
c) Sodium bicarbonate, calcium carbonate, sodium chloride, calcium phosphate, common wheat tritium, zinc oxide, urea.

# Supplemental material: TMR

| | From start to July 11 (mean±SE) | From July 12 to end (mean±SE) | p-value |
|---|---|---|---|
| H.I. | 67.51±2.56 | 68.20±2.01 | ns |
| S.I. | $69.42^{A}$±6.38 | $50.57^{B}$±4.13 | <0.05 |
| DM (%) | 62.66±0.66 | 62.62±0.55 | ns |
| Ash (%) | 9.59±0.33 | 9.48±0.10 | ns |
| Crude protein (%) | $13.58^{B}$±0.22 | $14.93^{A}$±0.19 | <0.01 |
| Ether extract (%) | 1.67±0.07 | 1.86±0.05 | ns |
| Starch (%) | $19.47^{A}$±0.35 | $17.95^{B}$±0.31 | <0.01 |
| Crude fiber (%) | $20.51^{B}$±0.49 | $22.39^{A}$±0.25 | <0.01 |
| NDF (%) | 44.79±1.02 | 45.21±0.39 | ns |
| ADF (%) | $30.99^{B}$±0.74 | $33.46^{A}$±0.63 | <0.05 |
| ADL (%) | $5.29^{B}$±0.43 | $5.96^{A}$±0.12 | <0.05 |
| S1 (%) | 15.66±0.58 | 15.59±0.68 | ns |
| S2 (%) | $33.98^{A}$±1.18 | $31.19^{B}$±0.57 | <0.05 |
| S3 (%) | 21.74±0.61 | 21.10±0.27 | ns |
| Bottom (%) | $28.62^{B}$±0.91 | $32.11^{A}$±0.51 | <0.01 |
| peNDF (%) | 31.91±0.64 | 31.57±0.40 | ns |

HI=homogeneity index; SI=sorting index; DM=dry matter; NDF=neutral detergent fiber; ADF=acid detergent fiber; ADL=acid detergent lignin; S1-S2-S3-bottom=particles retained in the 19-8-4 and bottom sieved; peNDF=physically effective NDF

# Supplemental material: Milk results

| | C | T1 | T2 | p-value |
|---|---|---|---|---|
| Milk yield (L) | $7.92^{B}$±0.24 | $8.51^{A}$±0.20 | $8.26^{AB}$±0.24 | <0.001 |
| Fat (%) | 7.58±0.13 | 7.42±0.12 | 7.55±0.13 | ns |
| Protein (%) | 4.63±0.03 | 4.68±0.03 | 4.66±0.03 | ns |
| Lactose (%) | 4.47±0.03 | 4.47±0.03 | 4.47±0.03 | ns |
| SNF (%) | 9.86±0.05 | 9.92±0.05 | 9.96±0.05 | ns |
| ECM (kg/head/day) | $10.86^{b}$±0.55 | $12.16^{a}$±0.53 | $11.72^{ab}$±0.62 | <0.05 |
| SCC×1000 (n/mL) | $214.52^{AB}$±61.22 | $120.81^{B}$±14.66 | $279.43^{A}$±42.05 | <0.01 |
| SCS | $3.05^{B}$±0.09 | $2.81^{C}$±0.07 | $3.39^{A}$±0.09 | <0.001 |
| Freezing point (°C) | -0.512±0.002 | -0.509±0.002 | -0.515±0.002 | ns |
| Urea (mg/dL) | 48.24±0.77 | 46.78±0.67 | 47.28±0.71 | ns |
| Casein | 3.69±0.04 | 3.74±0.04 | 3.76±0.04 | ns |
| pH | 6.62±0.01 | 6.63±0.01 | 6.64±0.01 | ns |
| Titratable acidity (°SH/100mL) | 7.45±0.11 | 7.38±0.09 | 7.45±0.11 | ns |
| RCT (min) | $20.01^{AB}$±0.84 | $19.02^{B}$±0.62 | $21.03^{A}$±0.60 | <0.01 |
| k20 (min) | 4.90±0.34 | 4.88±0.28 | 5.55±0.32 | ns |
| a30 (mm) | $28.23^{AB}$±1.44 | $28.99^{A}$±1.25 | $24.93^{B}$±1.26 | <0.01 |
| a60 (mm) | 39.41±0.99 | 37.83±0.89 | 39.82±0.84 | ns |

SCC=somatic cell count; SCS=somatic cell score; RCT=rennet coagulation time; k20: curd firming time; a30=curd firmness

# Results

## Behavior

| | C | T1 | T2 | p-value |
|---|---|---|---|---|
| **Not active time (min)** | $505.68^{A} \pm 3.46$ | $455.13^{B} \pm 3.27$ | $498.67^{A} \pm 3.23$ | <0.01 |
| **Total movement (min)** | $549.89^{B} \pm 2.79$ | $565.21^{A} \pm 3.21$ | $535.65^{C} \pm 3.11$ | <0.01 |
| **Eating time (min)** | $88.82^{B} \pm 1.32$ | $105.53^{A} \pm 1.45$ | $90.70^{B} \pm 1.21$ | <0.01 |
| **Ruminating time (min)** | $291.67^{B} \pm 2.67$ | $315.39^{A} \pm 3.39$ | $311.65^{A} \pm 3.26$ | <0.01 |

Similar results to those reported by (Meo Zilio et al., 2020) in a study conducted in the same farm some years earlier