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

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Abstract

A feeding strategy that maintains high content of functional molecules in buffalo milk has been verified by giving *Sorghum vulgare* as green fodder, but it is not available all year round. The aim of this study was to evaluate the inclusion of former food products (FFPs) containing 87% biscuit meal (nonstructural carbohydrate: 60.1%; starch 14.7; crude protein 10.6), in the diet of buffaloes in terms of: (a) fermentation characteristics through gas production technique; (b) milk yield (MY) and quality; (c) content of some biomolecules and total antioxidant activity. The experiment was performed involving 50 buffaloes divided into two groups: Green group and FFPs group (animals fed Total Mixed Ration with either green forage or FFPs respectively). Daily MY was recorded and milk qualitative analyses were determined monthly for 90 days. Furthermore, fermentation characteristics of the diets were studied in vitro. No significant differences were recorded in feed intake, BCS and MY and quality. Similar in vitro fermentation data of two diets were found, with slight differences in terms of gas production and degradability. During the incubation, kinetic parameters showed a faster fermentation process with the diet of the FFPs group in relation to Green group ($p < 0.05$). Green group had higher levels ($p < 0.01$) of γ-butyrobetaine, glycine betaine, L-carnitine and propionyl L-carnitine in milk, whereas no differences were observed for δ-valerobetaine and acetyl L-carnitine. Total antioxidant capacity and iron reduction antioxidant assay were higher ($p < 0.05$) in the plasma and milk of the Green group. The administration of a diet high in simple sugars, obtained with FFPs, seems to favour the ruminal biosynthesis of some metabolites in milk, such as δ-valerobetaine and acetyl-L-carnitine, similar to green forage administration. Overall, the use of biscuit meal can be an alternative to green fodder when it is

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not available to ensure environmental sustainability and optimize costs without compromising milk quality.

KEYWORDS
fermentation kinetics, green feed, health-promoting biomolecules, in vitro degradability, milk quality

1 | INTRODUCTION

In recent years, consumer attention has focused on functional foods of animal origin (Díaz et al., 2020; Vecchio et al., 2016), especially when produced with respect for the environment and animal welfare (Kalač, 2011). This can be achieved by improving farm management that takes into account the nutritional requirements of the animals. For this reason, innovative breeding and management techniques have been used in recent years to improve the nutritional quality of milk (Servillo, D'Onofrio, Giovane, et al., 2018). In a previous study on buffaloes, increased space allocation (10 vs. 15 m^2/head) was associated with higher levels of beneficial molecules (e.g., γ-butyrobetaine, L-carnitine and so on) in milk and dairy products, likely due to improved animal welfare (Salzano et al., 2019). Similarly, the addition of green forage (30% alfalfa) to the diet of dairy buffaloes has been reported to improve health-promoting biomolecules, antioxidant and antineoplastic properties of milk (Salzano et al., 2021). The addition of fresh forage improves rumen metabolism (Neglia et al., 2023) and ensures higher levels of carnitine precursors (betaines and their derivatives), which contribute to numerous metabolic processes and can prevent various diseases by stimulating sirtuin pathways (D'Onofrio et al., 2019; D'Onofrio, Cacciola, et al., 2020; D'Onofrio, Mele, et al., 2020; Zhao et al., 2018). However, as green forages are not available all year round, nutritional strategies need to be found to improve the content of these molecules through the intake of their precursors and by promoting rumen metabolism.

The use of alternative feedstuffs is an interesting option from many points of view (Vastolo, Calabrò, et al., 2022). Countries participating in the 2030 Agenda have committed to take measures for sustainable management and efficient use of natural resources, reduce food losses along supply chains and manage waste throughout its life cycle (Sustainable Development Goal 12), in order to minimize negative impacts on both human health and environment (FAO, 2021). According to the EU Catalogue of Feed Materials (Regulation [EU, 2017] No 2017/1017), former food products (FFPs) are feeds manufactured for human consumption but for many reasons (e.g., logistics, production, packaging and so on) are no longer intended for human consumption, although it does not pose any health risks when used as feed (Giromini et al., 2017). Providing soluble sugars, these industrial byproducts (e.g., broken biscuit, wrongly shaped bread and so on) can be used to increase the rumen fermentability of the diet (Vastolo, Matera, et al., 2022). By giving FFPs a secondary destination as feed for livestock nutrition, losses along the food chain can be reduced and at the same time nutrients can be recovered from unusable FFPs, together with a reduction in feed costs (Giromini et al., 2017).

The hypothesis tested was that FFPs could be used as an alternative to green feed to improve rumen metabolism and ensure higher levels of carnitine precursors. The inclusion of FFPs containing 87% biscuit meal in the diets of dairy buffaloes was evaluated on: (a) fermentation characteristics using the gas production technique; (b) milk yield (MY) and quality; (c) the content of betaines, L-carnitine and short-chain acylcarnitines; and (d) antioxidant activity in milk and plasma.

2 | MATERIALS AND METHODS

All in vitro procedures involving animals were approved by the Ethical Animal Care and Use Committee of the University of Napoli Federico II (Protocol Number 13729/2019). For the in vivo trial, standard veterinary practices were followed and institutional approval was obtained from the Ethical Animal Care and Use Committee of the University of Napoli Federico II (Protocol Number 0025532/2022).

2.1 | Animals and diets

The trial was carried out in a commercial buffalo farm located in the South of Italy over 90 days, during the summer period. Fifty pluriparous (4.6 ± 0.3 years old and 2.9 ± 0.2 parity) Italian Mediterranean buffaloes (676 ± 16 kg live weight, 111 ± 3.63 days in milk [DIM] and 11.7 ± 0.7 kg MY), kept in free stall housing conditions that allowed 15 m^2/head and 80 cm manger, were involved in the study. Animals were machine milked twice daily at 05:00 a.m. and 04:00 p.m. Buffaloes were randomly divided into two homogeneous groups, according to DIM, parity and MY. Two iso-energetic and iso-proteic diets were administered as total mixed ration (TMR) with the presence of *Sorghum vulgare* L. green forage (Green group; $n = 25$) or a commercial product (Top Star®; Stella Mangimi) containing 87% of biscuit meal (FFPs group; $n = 25$) respectively (Table 1). Nutritional characteristics of both the Top Star® and the two diets are showed in Tables 2 and 3 respectively. In particular, the two diets were characterized also by the same nonstructural carbohydrates (NSC) and ether extract (EE) content, together with similar fibre. The animals in either group underwent a 14-day adaptation period to the two diets before starting data collection.

TMRs were administered twice/day in each group and refusals were daily weighed and sampled, in order to calculate average feed

TABLE 1 TMR formula of the diets tested in vivo in the two groups.

Ingredient (kg)	Green diet	FFPs diet
Corn silage	10	16
Brewers' grain	15	15
Ryegrass hay	4.0	2.0
Fresh sorghum	16	–
Flaked corn	2.7	1.6
Top Star® feed	–	3.0
Soyabean meal	0.8	0.7
Hydrogenated fats	0.3	0.3
Vitamins	–	0.1
Salt 1:3	0.1	–
Calcium carbonate	0.2	0.1

Note: Green diet including fresh forage, FFPs diet including Former Food Products (Top Star®).

Abbreviations: FFPs, former food products; TMR, total mixed ration.

TABLE 2 Nutritional characteristics of the commercial product (Top Star®, Stella Mangimi) utilized during the trial.

Nutrients	Top Star®
Dry matter, %	89.8
Crude protein, % DM	10.6
EE, % DM	12.8
Crude fibre, % DM	3.60
Ash, % DM	2.63
Starch, % DM	14.7
NDF, % DM	13.7
ADF, % DM	7.50
NSC, % DM	60.1
MFU/kg DM	1.35

Note: 1 MFU = 1700 Kcal.

Abbreviations: ADF, acid detergent fibre; DM, dry matter; EE, ether extract; MFU, milk forage units; NDF, neutral detergent fibre; NSC, nonstructural carbohydrates.

intake. Feedstuffs and orts for each group were analysed once a week according to AOAC (2005), to calculate dry matter intake and diet composition. Individual feed intake and differences (Δ) between nutritive intake and relative requirements were estimated as previously reported (Campanile et al., 1998; Salzano et al., 2021):

$$\text{Dry matter (DM) intake} = 91\ g * \text{MW} + 0.27\ \text{kg} * \text{kg ECM}$$

Energy values (milk forage units = 1700 kcal) were calculated using equations provided by Institut National de la Recherche Agronomique (2010). Finally, the body condition score (BCS) of each buffalo was recorded weekly using a 1–9 scale (Wagner et al., 1988).

TABLE 3 Nutritional characteristics of the two diets.

Nutrients	Green diet	FFPs diet
Dry matter, %	16.7	16.8
Crude protein, % DM[3]	14.7	14.6
EE, % DM	6.56	7.38
Crude fibre, % DM	18.3	17.9
Ash, % DM	7.02	6.57
Starch, % DM	14.7	18.5
NDF, % DM	34.9	35.0
ADF, % DM	20.6	21.8
NSC, % DM	36.82	36.45
MFU/kg DM	0.95	0.95
Phosphorus, % DM	0.44	0.44
Calcium, % DM	0.85	0.87
Forage:concentrate ratio	62:38	53:47

Note: Green diet including fresh forage, FFPs diet including Former Food Products (Top Star®). 1 MFU = 1700 Kcal.

Abbreviations: ADF, acid detergent fibre; DM, dry matter; EE, ether extract; FFPs, former food products; MFU, milk forage units; NDF, neutral detergent fibre; NSC, nonstructural carbohydrates.

2.2 | In vitro fermentation characteristics

The in vitro fermentation characteristics of the two diets utilized in the in vivo experiment, were tested using the in vitro gas production technique as described by Vastolo et al. (2020). For this goal, the two diets (representatively collected in several points of the feeder and dried at 65°C for 24 h) were ground to pass 1.0 mm screen (SM 100, Retsch). For the in vitro trial, they were incubated under anaerobiosis condition with buffered rumen liquor, collected at slaughterhouse, according to EU legislation (EU Council, 2004), from four adult Italian Mediterranean Buffalo cows fed a TMR containing corn silage, oat hay and concentrate (crude protein 12.4 and neutral detergent fibre 43.5% on dry matter basis). After 120 h of incubation, the following parameters were recorded: pH using pH meter (Thermo Orion 720A +); organic matter degradability (dOM, %) after filtration throughout crucibles (Porosity #2) and burning in muffle at 550°C; cumulative gas production (OMCV, mL/g) recorded with a manual pressure transducer (Cole and Palmer Instrument) and related to incubate OM; volatile fatty acids (VFAs, mM/g) detected by gas chromatography (GC Focus AI 3000, Thermo Scientific) using an external standard solution composed of acetate, propionate, butyrate, iso-butyrate, valerate and iso-valerate (Musco et al., 2017). Kinetics parameters (A, mL/g: potential gas production related to incubated OM; B, h: time to produce half of A; R_{max} mL/h: maximum fermentation rate; T_{max}, h:

time to reach R_{max}) were obtained fitting the experimental data to a sigmoidal model (Groot et al., 1996).

2.3 | Milk and plasma sampling and analysis

Individual MY was recorded daily by the automated milking system. On monthly basis, milk sampling was carried out by collecting milk samples in the morning and afternoon: the samples of each animal were proportionally mixed according to MY at each milking. From each sample, two aliquots were collected: the first was immediately analysed for the determination of milk quality (fat, protein, casein, lactose, urea, β-hydroxy butyrate [BHB], fatty acids profile and chlorides) through mid-infrared spectroscopy (Milkoscan FT6000®, Foss Electric), whereas the second aliquot was stored at −80°C until analyses for biomolecules content. The pH metre with burette 24 1 S (Crison) has been used for the measurement of pH. Somatic cell count was obtained by Fossomatic FC® counter (Foss). Energy corrected milk (740 kcal) was also calculated through the following formula (Campanile et al., 2003), which considers fat and protein content of milk:

$$\text{ECM} = ([\{\text{fat}(\text{g} \cdot \text{kg}^{-1}) - 40 + \text{protein}(\text{g} \cdot \text{kg}^{-1}) - 31\} * 0.01155] + 1) * \text{milk yield}.$$

At the same time, individual serum and plasma from two groups, collected separately, were obtained by centrifugation at 1800*g* for 20 min and stored at −80°C until analysis.

2.4 | High-performance liquid chromatography electrospray ionization-tandem mass spectrometry (HPLC-ESI-MS/MS) analysis

The content of L-carnitine, acetil-L-carnitine, propionyl-L-carnitine, glycine betaine, γ-butyrobetaine and δ-valerobetaine was determined in duplicate pooled 3 kDa milk extracts as previously described (Servillo, D'Onofrio, Giovane, et al., 2018). Milk samples were boiled, cooled and then centrifuged at 3000*g* for 15 min at 4°C. The central aqueous phase was sampled, ultrafiltered using 3 kDa Amicon filters and centrifuged at 4°C for 30 min at 13,000*g*. Volumes of 10 µl of 3 kDa sample or standard solution were used for analysis by HPLC-ESI-MS/MS with Agilent 1100 LC-MSD SL quadrupolar ion trap mass spectrometer in positive multiple reaction monitoring mode as previously described (Servillo, D'Onofrio, Giovane, et al., 2018). Quantification of each substance was obtained by comparison of the peak area of its most intense MS2 fragment with the respective calibration curve built with standard solutions. The mass spectrometer was operated utilizing nitrogen as the nebulizing and drying gas. The instrumental conditions were as follows: nebulizer pressure, 30 psi; drying temperature, 350°C; drying gas 7 L/min. The ion charge control was applied with target set at 30,000 and maximum accumulation time at 20 mS. The linearity of the instrumental response was assessed by correlation coefficients (r^2) > 0.99 for all analytes. For each compound, the concentrations were determined comparing the relative calibration curve.

2.5 | Antioxidant activity

The total antioxidant capacity (TAC) was evaluated by using a colorimetric assay (ab65329, Abcam), following the manufacturer's instruction. In brief, milk and plasma samples were mixed with 100 µL of the Cu^{2+} working solution and then incubated for 90 min at room temperature in the dark. The reaction was detected by measuring the absorbance at 570 nm by using a microplate reader model 680 Bio-Rad (Bio-Rad). Absorbance was interpolated with the standard curve of Trolox and the TAC value expressed as nM of Trolox equivalents. The reducing ability of the samples [reduction of ferric iron ($Fe3^+$) to ferrous iron ($Fe2^+$)] was performed using the ferric reducing antioxidant power assay (FRAP) (MAK369, Sigma Aldrich). The reducing power was calculated by reacting 10 µL of sample with 190 µL of the reaction mixture and monitoring the increase in absorbance at 594 nm for 1 hour at 37°C and finally, determined using a ferrous iron standard curve and results are expressed as Fe^{2+} equivalents (nM).

2.6 | Meteorological information

Environmental temperature and relative humidity (ET and RH respectively) were recorded from June 2022 to August 2022 at a weather station located 1 km from the commercial farm.

The average ET was 27.1 ± 0.21°C, ranging from 22°C (10 June 2022) to 29°C (20 July 2022). The average RH was 50.3 ± 0.96, ranging from 28 (20 July 2022) to 68 (28 August 2022).

2.7 | Statistical analysis

Statistical analyses were performed by analysis of variance (ANOVA) for one-way (JMP®, Version 14 SW, SAS Institute, 1989–2019) to compare the in vitro results after 120 h of incubation (pH, dOM, OMCV, A, B, T_{max}, R_{max}, VFA) of the two diets. Qualitative (fat, protein, casein, lactose, urea, BHB, fatty acids profile and chlorides, functional biomolecules content) and quantitative (daily MY) milk parameters and antioxidant activity (TAC and FRAP) in blood and milk were analysed by repeated-measures ANOVA using the SPSS software package (IBM Corp., 2021) for Windows 10 (SPSS). A statistically significant difference was accepted at $p < 0.05$.

3 | RESULTS

3.1 | In vitro fermentation characteristics

The results of the in vitro fermentation are shown in Table 4 and Figure 1. The pH values measured after 120 h of incubation were

within the ranges suitable for the activity of cellulolytic bacteria. When comparing the two diets, only potential gas production was different between diets (A: 281 vs. 271 mL/g, $p < 0.05$ for diet FFPs and Green diet respectively). The kinetic parameters showed statistically significant differences (T_{max}: 0.17 vs. 3.03 h and R_{max}: 12.4 vs. 9.56 mL/h, $p < 0.05$, for the FFPs and Green diet respectively), indicating a more intense and faster process in the FFPs-containing diet in relation to the diet with green forage. However, the difference in the rate profile was only evident in the first 20 h of incubation and disappears in the following hours (Figure 1).

TABLE 4 In vitro fermentation characteristics of the diets tested *in vivo* in the two groups.

Diet	Green	FFPs	MSE
pH	6.60	6.57	0.003
dOM, %	70.3	72.2	10.7
OMCV, mL/g	240	235	9.76
A, mL/g	271[a]	281[b]	10.5
B, h	19.2	20.6	1.18
R_{max}, mL/h	9.56*	12.4*	1.36
T_{max}, h	3.03*	0.17*	0.05
VFA, mM/g	130	133	13
Acetic acid, % tVFA	68.4	63.1	2.17
Propionic acid, % tVFA	19.2	19.7	0.08
Butyric acid, % tVFA	9.57	12.2	0.07

Note: Green is the group fed with fresh forage, FFPs is the group fed with Former Food Products (Top Star®).

Abbreviations: A, potential gas production related to incubated OM; B, time to produce half of A; dOM, organic matter degradability; FFPs, former food products; MSE, mean square error; OMCV, cumulative gas production related to incubated OM; R_{max}, maximum fermentation rate; T_{max}, time to reach R_{max}; tVFA, total volatile fatty acids.

*Statistically significant differences, $p < 0.05$.

3.2 | Qualitative and quantitative milk production

Average daily DM intake was 16.5 ± 0.15 vs. 16.5 ± 0.02 in Green and FFP diet, respectively, without significant difference between groups. Similarly, no differences were present for BCS on average (7.25 ± 0.1 vs. 7.32 ± 0.2). Average daily MYs and quality were similar in both

TABLE 5 Milk production and quality.

Group	Green	FFPs
MY, kg/d	9.75 ± 0.07	9.86 ± 0.07
Fat, %	9.23 ± 0.30	9.46 ± 0.27
Protein, %	4.69 ± 0.07	4.55 ± 0.06
ECM, kg/d	15.8 ± 0.54	15.6 ± 0.62
Lactose, %	4.41 ± 0.06	4.45 ± 0.05
SCC, ×10^3 cells/ml	170 ± 40.0	206 ± 73.4
DSCC, %	41.9 ± 1.96	40.4 ± 2.30
Casein, %	3.86 ± 0.07	3.75 ± 0.05
Urea, %	31.4 ± 3.15	34.5 ± 2.73
BHB, %	0.20 ± 0.03	0.19 ± 0.02
pH	6.61 ± 0.01	6.61 ± 0.02
EC, mS/cm	605 ± 13.1	616 ± 13.9

Note: Values are expressed as mean ± SEM. Green is the group fed with fresh forage, FFPs is the group fed with Former Food Products (Top Star®).

Abbreviations: BHB, β-hydroxy butyrate; DSCC, differential somatic cell count; EC, electrical conductivity; ECM, energy corrected milk; FFPs, former food products; MY, milk yield; SCC, somatic cell count.

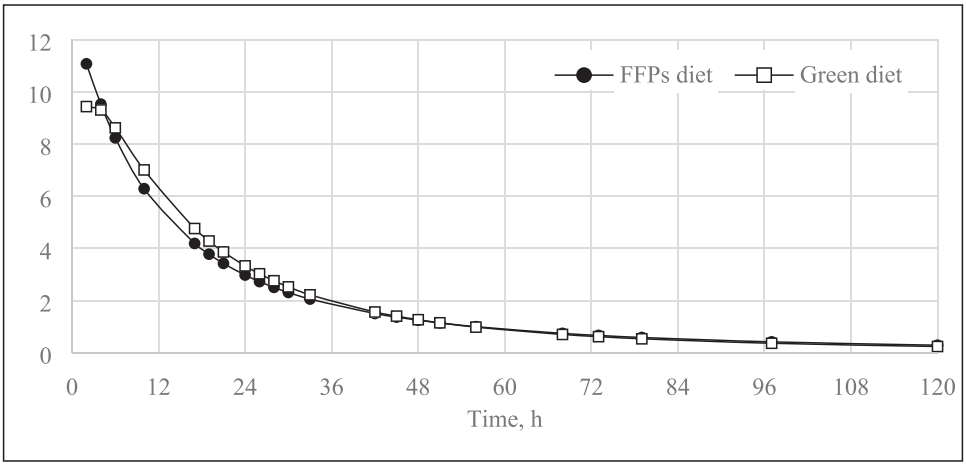

FIGURE 1 In vitro fermentation rate over time of the diets tested in vivo. Green diet include fresh forage; FFPs diet include Former Food Products (Top Star®).

TABLE 6 Milk fatty acid composition (% of total fatty acid).

Group	Green	FFPs
MCFA	3.63 ± 0.10	3.62 ± 0.09
SCFA	1.12 ± 0.05	1.11 ± 0.04
MUFA	2.85 ± 0.11	3.04 ± 0.10
LCFA	4.00 ± 0.16	4.25 ± 0.14
PUFA	0.23 ± 0.01	0.25 ± 0.01
SFA	6.29 ± 0.21	6.28 ± 0.19
UFA	2.99 ± 0.11	3.23 ± 0.10
TFA	0.24 ± 0.01*	0.29 ± 0.01*
Myristic acid	0.97 ± 0.04	0.94 ± 0.03
Palmitic acid	2.51 ± 0.08	2.57 ± 0.08
Stearic acid	0.92 ± 0.02**	0.98 ± 0.02**

Note: * and ** indicate statistically significant differences. Values are expressed as mean ± SEM (standard error of the mean). Green is the group fed with fresh forage, FFPs is the group fed with Former Food Products (Top Star®).

Abbreviations: LCFA, long-chain fatty acids; MCFA, medium-chain fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SCFA, short-chain fatty acids; SFA, saturated fatty acids; TFA, trans fatty acid; UFA, unsaturated fatty acids.

*$p < 0.01$; **$p < 0.05$.

TABLE 7 Functional biomolecules content in milk (mg/L).

Group	Green	FFPs
γ-Butyrobetaine	6.24 ± 0.10*	5.87 ± 0.10*
δ-Valerobetaine	17.4 ± 0.14	17.2 ± 0.21
Glycine betaine	15.5 ± 0.14*	15.0 ± 0.12*
L-Carnitine	39.6 ± 0.36*	37.3 ± 0.17*
Acetyl-L-carnitine	45.4 ± 0.59	43.8 ± 0.71
Propionyl-L-carnitine	25.0 ± 0.36*	22.3 ± 0.32*

Note: * indicate statistically significant differences. Values are expressed as mean ± SEM. Green is the group fed with fresh forage, FFPs is the group fed with Former Food Products (Top Star®).

Abbreviation: FFPs, former food products.

*$p < 0.01$.

groups (Table 5), except for trans fatty acid (TFA) ($p < 0.01$) and stearic acid ($P < 0.05$ in the Green group in relation to the FFP group (Table 6). Furthermore, milk parameters, indicating mammary gland health and animal metabolism, did not differ between the two groups (Table 5). The Green group had higher levels ($p < 0.01$) of γ-butyrobetaine, glycine betaine, L-carnitine and propionyl L-carnitine in relation to the FFPs group, whereas no differences were observed for δ-valerobetaine and acetyl L-carnitine (Table 7). The antioxidant capacity of milk was higher in the animals of the Green group in relation to the FFPs group, both when determined with TAC ($p < 0.05$) and with the FRAP assays ($p < 0.01$) (Table 8). Similarly, plasma also showed higher TAC ($p < 0.01$) and FRAP ($p < 0.05$) levels in the Green group in relation to the FFPs group (Table 8).

TABLE 8 TAC and FRAP in blood and milk.

	Green	FFPs
Milk		
TAC	276.51 ± 9.31*	252.45 ± 7.66*
FRAP	240.62 ± 6.00**	213.86 ± 4.89**
Plasma		
TAC	77.52 ± 1.43**	71.66 ± 1.74**
FRAP	58.53 ± 2.22*	51.10 ± 1.89*

Note: * and ** indicate statistically significant differences. Values are expressed as mean ± SEM. Green is the group fed with fresh forage, FFPs is the group fed with Former Food Products (Top Star®).

Abbreviations: FRAP, ferric reducing antioxidant power assay; TAC, total antioxidant capacity.

*$p < 0.05$; **$p < 0.01$.

4 | DISCUSSION

The aim of the trial was to increase the concentration of bioactive compounds in buffalo milk when the 'zero grazing' technique (e.g., grass harvesting and administration to housed animals as TMR; Velarde-Guillén et al., 2019) cannot be applied. We hypothesized that the administration of a commercial concentrate characterized by the presence of biscuit meal (Top Star®) may increase fermentability in the rumen similarly to green forage administration. Comparable fermentation kinetics were observed between the two diets in vitro, supporting the decision to use FFPs as a replacement for green forage: the high content of simple sugars available to the rumen microbial population in both diets allowed recording similar results. As reported in previous in vitro studies, the fermentation characteristics of the tested diets are consistent with their chemical composition in terms of protein, fibre and starch content (Musco et al., 2017; Vastolo, Matera, et al., 2022). Moreover, a similar degradability of the organic matter, combined with a relatively low production of gases and total VFAs, confirm that the chemical-nutritional characteristics of the two experimental diets were completely comparable and provide a useful amount of energy and nutrients for the production phase of the studied species. Indeed, no differences were observed between the two experimental buffalo groups in terms of MY and quality, as already reported with the use of alfalfa green forage (Salzano et al., 2021) and *S. vulgare* forage (Neglia et al., 2023). In addition, the fatty acid profile of the milk of the two groups did not differ in terms of saturated, monounsaturated and polyunsaturated fatty acids. On the contrary, a higher concentration of TFA and stearic acid was found in the FFPs in relation to the Green group. These differences could be due to the high proportion of partially hydrogenated vegetable oils found in various commercial products, such as spreads and baked goods. Some studies

have demonstrated the harmful effects of industrial TFAs on heart health (FAO, 2010), a systematic review and meta-analysis commissioned by World Health Organization reports that nonruminant TFAs are associated with the risk of coronary heart disease (de Souza et al., 2015). On the contrary, recent evidence shows that the biological activities of industrial TFAs differ from ruminant TFAs. In particular, rumenic, vaccenic and t-palmitoleic acids can be associated with beneficial effects on human health (de Souza et al., 2015). Relatively to stearic acid, neutral effects on serum cholesterol levels (Yu et al., 1995) and cardiovascular disease in humans have been demonstrated (Astrup et al., 2020).

The metabolomic profile of buffalo milk and dairy products revealed the presence of a wide range of natural bioactive compounds with recognized health benefits such as antimicrobial, antineoplastic, antidiabetic and antioxidant properties (Basilicata et al., 2018; Cacciola et al., 2022; Chanu et al., 2018; D'Onofrio, Cacciola, et al., 2020; D'Onofrio, Mele, et al., 2020; Huma et al., 2018; Tenore et al., 2015). Regarding the metabolic profile of milk, buffaloes fed green fodder showed higher levels of γ-butyrobetaine, glycine betaine, L-carnitine and propionyl-L-carnitine in milk, while no differences were found for δ-valerobetaine and acetyl-L-carnitine in relation to the FFPs group. Similar results were previously found (Neglia et al., 2023; Salzano et al., 2021), although the use of FFPs reduced the differences in the metabolomic profile of milk in relation to green forage. Interestingly, the increase in rumen fermentability achieved by these feeding strategies resulted in similar concentrations of δ-valerobetaine and acetyl-L-carnitine in relation to TMR diet alone. The δ-valerobetaine is a biomolecule synthesized in the rumen from Nε-trimethyl-lysine and is present in milk (Servillo, D'Onofrio, Neglia, et al., 2018). This betaine has several interesting biological properties, such as antioxidant, anti-inflammatory and anticancer properties (D'Onofrio et al., 2019; D'Onofrio, Cacciola, et al., 2020a; D'Onofrio, Mele, et al., 2020b; D'Onofrio et al., 2021), and has been reported to be able to suppress pro-inflammatory cytokines, involving modulation of SIRT1 and SIRT6 (D'Onofrio et al., 2019). Acetyl-L-carnitine shows important antioxidant and anti-inflammatory activities, particularly at the level of endothelial cells and platelets (Mohammadi et al., 2016). Acetyl-L-carnitine counteracts endothelial oxidation by downregulating NADPH oxidases and upregulating superoxide dismutase and glutathione levels (Mohammadi et al., 2016). It is likely that the levels of the latter two bioactive compounds found in this study depend on the amount of VFA and isoacids in the rumen (Bergman, 1990; Rychlik et al., 2002). Interestingly, the higher TAC and FRAP levels in milk and plasma in the Green group were associated with both higher L-carnitine content, a biomolecule with antioxidant and anti-inflammatory properties (Bene et al., 2018), and with a high vitamin and antioxidant content of green sorghum (Etuk et al., 2012). The higher levels of antioxidant activity may be due to reduced free radical production and increased antioxidant content and anti-free radical activity provided by L-carnitine. It is likely that L-carnitine interacts with the phospholipid forming arachidonic acid and protein kinase C to reduce lipid peroxidation and oxidative stress (Pignatelli et al., 2003; Saluk-Juszczak et al., 2010). These interesting results are also consistent with previous studies carried out in dairy buffalo (Salzano et al., 2021).

5 | CONCLUSIONS

Our results showed that the administration of FFP or green forage in the diet of lactating buffaloes does not cause any significant difference in terms of feed intake, BCS, MY and milk quality. Furthermore, similar results were also recorded in in vitro fermentation studies, with slight differences in gas production and degradability. The administration of a diet high in simple sugars seems to favour its fermentability and the metabolic pathways in the rumen useful for the synthesis of L-carnitine, short-chain acylcarnitines and betaines. This supports our hypothesis that the FFPs diet could be used as an alternative to green forages to improve the production of buffalo milk with a high nutritional and health profile. This approach opens new perspectives for the feeding strategy that can contribute to improve the metabolomic structure of buffalo milk and promote the health aspect of animal products.

AUTHOR CONTRIBUTIONS

Gianluca Neglia: Conceptualization, resources, data curation, writing—review and editing, investigation, supervision, funding acquisition. **Serena Calabrò**: Methodology, software, validation, writing—original draft preparation. **Alessio Cotticelli:** Software, formal analysis. **Angela Salzano**: Conceptualization, data curation. **Roberta Matera**: Software, validation, investigation writing—original draft preparation. **Alessandro Vastolo**: Methodology, validation, writing—original draft preparation. **Nunzia D'Onofrio**: Methodology, validation, data curation, writing—original draft preparation. **Andrea Giorgino**: Software, writing—review and editing. **Elisa Martino**: Methodology, formal analysis. **Maria L. Balestrieri**: Methodology, investigation, writing—review and editing, project administration. **Giuseppe Campanile**: Conceptualization, resources, supervision, writing—review and editing funding acquisition. All authors have read and agreed to the published version of the manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors declare no known conflict of interest.

DATA AVAILABILITY STATEMENT

The data sets used and/or analysed during the current study are available from the corresponding author on reasonable request.

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