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The potential of sorghum meal as a replacement of corn meal in the diet for lactating buffaloes: impacts on milk yield and nutrient digestibility

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ABSTRACT

Under the current climate change scenario and due to its lower irrigation water requirement, sorghum (*Sorghum bicolor*) meal has been proposed as a viable option in ruminant diet to replace corn meal. The aim of the present study was to evaluate animal performance and *in vivo* digestibility of lactating buffaloes fed a total mixed ration in which sorghum or corn meal was the primary energy source. Twenty multiparous buffaloes were equally divided into two balanced dietary groups fed the same basal diet supplemented with sorghum meal (Sorghum diet) or corn meal (Corn diet). After a 2-week adaptation period, feed intake and milk yield and components, including somatic cell count and coagulation characteristics, were recorded for 5 weeks. At the end of the experimental period, *in vivo* nutrient digestibility was assessed using acid insoluble ash as a marker of indigestibility. The use of sorghum meal did not negatively effect on dry matter intake, body weight, or body condition score. Milk yield did not differ between the diets, nor did the content of milk macro components and coagulation characteristics. However, milk urea concentration increased significantly ($p < 0.05$) in buffaloes fed the sorghum-based diet, indicating reduced dietary nitrogen utilisation. Additionally, these buffaloes exhibited a significant reduction in total tract starch digestibility (-2.8% points, $p < 0.05$) and a tendency towards lower crude protein digestibility ($p = 0.06$). We concluded that corn meal can be replaced by sorghum meal in the diet of lactating buffaloes without affecting their productive performance.

HIGHLIGHTS

- Dairy buffalo farms need to diversify energy feed sources due to supply risks.
- Sorghum meal is a cost-effective alternative to corn, requiring fewer production inputs.
- Replacing maize meal with sorghum meal does not compromise buffalo milk production or quality.

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Introduction

The growing demand for buffalo mozzarella cheese has led to a significant expansion of dairy buffalo (*Bubalus bubalis*) farming in Italy over the past decades (Uzun et al. 2020). This has resulted in a shift towards more intensive production methods (Sannino et al. 2018; 2024), which have been facilitated by genetic improvement of the animals (Cesarani et al. 2021). As a result, both milk production per cow and herd

size have increased, with Italian buffalo currently being the most productive in the world (Serrapica et al. 2022). However, this high milk yield necessitates supplementation of energy- and protein-rich concentrates to fulfil nutritional requirements. Current practices in most Italian buffalo farms involve a simplified feed production strategy. Farms typically grow forage crops that maximise dry matter yield per hectare, such as maize for summer silage and ryegrass for winter

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hay or haylage, while protein and energy concentrates are purchased from the market (Uzun et al. 2018; Serrapica et al. 2020a). However, this reliance on external feed sources, historically driven by low grain prices, exposes farmers to the volatility of the market price of concentrates and raises concerns about the long-term economic and environmental sustainability of buffalo farming. Moreover, depending on their geographical origin, feedstuff imported from outside the EU may act as carriers of transboundary animal diseases and pests. Additionally, harmful chemicals, including pesticides banned in the EU but permitted in some non-EU regions, can contaminate feed (Gasparini et al. 2024). Imported corn grain is currently the most common starch-based high-energy feed for buffaloes (Rossi et al. 2023). Thus, identifying alternative local grown low-input crops as a source of starch for buffalo feed has become a research priority.

Sorghum (*Sorghum bicolor* (L.) Moench) is an increasingly important cereal species in many regions of the world, especially as an alternative to corn (*Zea mays* L. subsp. *mays* L.), due to its adaptability to a wide range of environmental and agronomic conditions (Pino and Heinrichs 2017; Pontieri et al. 2022a). Compared to corn, sorghum has excellent drought tolerance, greater resistance to pests and diseases, and higher nitrogen use efficiency, resulting in lower water, fertiliser and agrochemical requirements (Colombini et al. 2012; Cattani et al. 2017). As a summer crop, sorghum ensures more stable yields in regions prone to biological adversities, high radiation and temperatures, insufficient and erratic rainfall, and lack of irrigation (Cattani et al. 2017). These characteristics offer potential in the Mediterranean area, where water is and will continue to be decreasing supply (Berti Suman et al. 2023; Ceci et al. 2023).

Although sorghum and corn grains have similar starch content, differences exist in the rate and extent of their ruminal starch degradation, which varies depending on differences in the type of endosperm and other intrinsic factors, such as the content of condensed tannins and other secondary compounds (i.e. saponins and phytates) able to modulate rumen bacterial activity (McCustion et al. 2019; Ronda et al. 2019). Tannins, in particular, are known to reduce rumen microbial activity over a broad range of ruminal pH (3.5–7) by inhibiting the release of extracellular bacterial enzymes or by forming strong complexes with macromolecules (e.g. proteins, starch, structural carbohydrates, sugars) and metal ions that protect the macromolecules from degradation by ruminal microorganisms. The extent of these effects is especially

pronounced when tannin concentrations exceed 5% of dry matter (Besharati et al. 2022). Several *in vitro* (Streeter et al. 1990; Herrera-Saldana et al. 1990a; Shipandeni et al. 2021) and *in vivo* studies (Herrera-Saldana et al. 1990b; Poore et al. 1993; Oliveira et al. 1995; Shipandeni et al. 2023) have confirmed that sorghum has a lower ruminal starch degradability compared to corn. The rate and extent of rumen fermentation of dietary carbohydrates (especially starch) are important parameters that determine the nutrient supply to the animal (Hall et al. 2010). As for other dairy ruminants, altering the ruminal degradability of starch can affect dairy buffalo performance by influencing intake, partitioning, and absorbed protein and, consequently, milk production and composition (Bovera et al. 2007; Bartocci and Terramocchia 2010). However, to the best of our knowledge, studies on the use of sorghum in the diet of lactating buffaloes are lacking. Thus, this study aimed to evaluate the potential of sorghum as a primary energy source for dairy buffaloes by assessing the impact of replacing corn meal with sorghum meal on milk production, feed intake, digestibility, and body condition.

Materials and methods

Sorghum and corn meals

Fontanelle 1000 hybrid sorghum seeds were supplied by Mr. Earl Roemer (Nu Life Market LLC, Scott City, KS USA). In 2022, an open field cultivation of sorghum was carried out in San Bartolomeo in Galdo (BN) located in the Fortore area of the Campania Region, Southern Italy (41°25'N, 15°01'E and 597 m a.s.l.).

At harvest, the sorghum seeds were stored in a dry environment at 16 °C. Starting one month later, they were finely ground using the Partisani Combi Junior MB250 stationary roller mill. The corn meal was prepared from high vitreous corn grains purchased on the local market, finely ground following the same procedure.

Animals, housing, and experimental design

All procedures involving handling and treatment of animals have been approved by the Institutional Ethics Committee of the University of Naples Federico II (protocol code PG/0025485) and in compliance with the EU requirements concerning the protection of animals used for scientific purposes (Directive 2010/63/EU) as implemented by the Italian legislation (DL n. 26, 4 March 2014). The trial took place from April to June 2023 at a dairy buffalo (*Bubalus bubalis*,

Mediterranean type) farm located in Campania, a region in southern Italy (41°02'N, 14°18'E; 33 m above sea level, 1153 mm annual precipitation, 15.7 °C average annual temperature). Twenty lactating buffaloes were enrolled in the study and randomly and equally assigned to two homogeneous groups ($n = 10$) receiving a total mixed ration (TMR) with corn as the primary energy source (Corn group) or corn replaced by sorghum meal (Sorghum group). The groups had similar (mean \pm standard deviation) parity (3.90 ± 1.44 and 3.88 ± 1.18 , respectively for Corn and Sorghum group), days in milk (105.8 ± 18.8 , range $77 \div 134$ days, and 107.2 ± 18.7 days, range $83 \div 145$ days), milk yield (9.60 ± 1.87 and 9.33 ± 1.26 kg/head/d), body weight (BW; 644.1 ± 27.1 and 638.4 ± 30.8 kg) and body condition score (BCS; 6.0 ± 0.22 and 6.2 ± 0.13). The composition and nutritional characteristics of the sorghum and corn meals and the experimental TMRs are shown in Tables 1 and 2, respectively.

Both groups were fed once a day (08:00 h) with a preliminary TMR containing meadow hay, soybean meal and a mixture of minerals and vitamins. This TMR was placed on the cement floor in front of each feed bunk, mixed with a pitchfork with an equal amount (4.3 kg/head as fed) of either corn or sorghum meal, depending on the treatment, and then offered at approximately 110% of the expected intake. The TMRs were re-applied on a three-times-daily basis (at 12:00, 16:00, and 18:00 h). The diets were developed in accordance with the recommendations proposed by Bartocci et al. (2002) and Campanile et al. (1998) for an expected daily dry matter

Table 1. Chemical composition (% of dry matter, unless noted) and *in vitro* rumen digestibility of corn and sorghum meal used in the formulation of the experimental diets (mean \pm SD, $n = 5$ samples).

Item	Corn meal	Sorghum meal
Chemical composition		
DM, % of fresh matter	88.80 \pm 0.37	91.20 \pm 0.43
Ash	1.52 \pm 0.51	1.66 \pm 0.66
CP	9.55 \pm 0.29	12.93 \pm 0.35
Ether extract	3.18 \pm 0.17	2.66 \pm 0.14
NDF	13.78 \pm 0.72	10.25 \pm 0.85
ADF	3.14 \pm 0.81	3.95 \pm 0.67
ADL	0.68 \pm 0.25	0.99 \pm 0.33
NFC	79.98 \pm 0.36	77.47 \pm 0.41
Starch	71.98 \pm 0.26	70.18 \pm 0.18
Soluble protein, % CP	21.98 \pm 0.32	13.92 \pm 0.42
NPN, % CP	5.13 \pm 0.27	3.56 \pm 0.25
NE _L , MJ/kg DM	8.49 \pm 0.40	8.19 \pm 0.40
<i>In vitro</i> rumen digestibility		
IVDMD, % DM	87.28 \pm 0.70	84.83 \pm 0.73
IVNDFD, % NDF	73.50 \pm 0.62	62.46 \pm 0.53

DM: dry matter; CP: crude protein; NDF: neutral detergent fibre; ADF: acid detergent fibre; ADL: acid detergent lignin; NFC: non-fibrous carbohydrate; SP: soluble protein; NPN: non-protein nitrogen; NE_L: net energy of lactation; IVDMD: *in vitro* DM rumen digestibility; IVNDFD: *in vitro* NDF rumen digestibility; SD: standard deviation.

intake (DMI) of 16.0 kg/head. The cows were housed in two free-stall barns, each with similar access to feed bunks and water, and were milked twice a day (05:00 and 15:30 h) in a 2 \times 3 auto-tandem milking parlour. The experimental period consisted of 2 weeks of adaptation to the diets, followed by 5 weeks for data recording and sample collection. At the time of group formation and at the conclusion of the trial, immediately following the morning milking and for three consecutive days, the cows were weighted and scored for BCS by two independent evaluators who were unaware of the treatments, using a 9-point scale adapted for buffalo cows (Campanile et al. 2006).

Table 2. Ingredients, chemical composition (% of dry matter, unless noted), *in vitro* rumen digestibility, and particle size distribution of the experimental diets with corn or sorghum as the main starch source (mean \pm SD, $n = 5$ samples).

Item	Experimental diets	
	Corn	Sorghum
Dietary ingredients, kg as fed		
Mixed hay	11.00	11.00
Corn meal	4.30	–
Sorghum meal	–	4.30
Soybean meal	3.00	3.00
Vitamin and mineral premix ^a	0.40	0.40
Water	12.60	12.60
Chemical composition		
DM, % of fresh matter	52.85 \pm 1.08	53.07 \pm 1.17
Ash	9.96 \pm 0.70	9.98 \pm 0.70
CP	15.86 \pm 0.38	16.64 \pm 0.33
Ether extract	1.26 \pm 0.29	1.14 \pm 0.41
NDF	42.70 \pm 1.21	41.48 \pm 1.13
ADF	27.55 \pm 0.88	27.71 \pm 0.93
ADL	4.16 \pm 0.85	4.22 \pm 0.78
NFC	30.30 \pm 1.16	28.63 \pm 1.27
Starch	18.13 \pm 0.22	17.78 \pm 0.33
Soluble Protein, % CP	24.47 \pm 0.95	22.89 \pm 0.88
NPN, % CP	16.07 \pm 0.95	15.26 \pm 0.65
NE _L , MJ/kg DM	5.85 \pm 0.60	5.80 \pm 0.60
<i>In vitro</i> rumen digestibility		
IVDMD, % DM	69.48 \pm 1.93	66.75 \pm 2.57
IVNDFD, % NDF	26.98 \pm 4.62	22.14 \pm 5.31
Particle size distribution ^b		
>19.0 mm	8.12 \pm 0.38	8.19 \pm 0.65
19.0–8.0 mm	3.72 \pm 0.83	3.17 \pm 0.74
<8.0 mm	4.73 \pm 0.87	5.20 \pm 1.05
pef	0.72 \pm 0.05	0.69 \pm 0.07
peNDF	29.92 \pm 1.91	29.26 \pm 2.81

DM: dry matter; CP: crude protein; NDF: neutral detergent fibre; ADF: acid detergent fibre; ADL: acid detergent lignin; NFC: non-fibrous carbohydrate; SP: soluble protein; NPN: non-protein nitrogen; NE_L: net energy of lactation; IVDMD: *in vitro* DM rumen digestibility; IVNDFD: *in vitro* NDF rumen digestibility; pef: physical effectiveness factor; peNDF: physically effective NDF; SD: standard deviation.

^aPremix containing (per kg, based on the manufacturer' declared content): 4,000,000 IU of vitamin A; 100,000 IU of vitamin D3; 1500 mg of vitamin E; 1400 mg of vitamin B6; 1400 mg of vitamin C; 1100 mg of vitamin B1; 500 mg of vitamin B2; 5000 mg of choline chloride; 1000 mg of biotin; 800 mg of pantothenic acid; 700 mg of niacinamide; 180 g calcium; 38 g of phosphorus; 70 g of sodium; 15 g of magnesium; 1000 mg of S as copper-II-sulphate; 1600 mg of Mn as manganese-II-oxide; 5400 mg of Zn as zinc sulphate, monohydrate; 80 mg of I as calcium iodate, anhydrous; 10 mg of Se as sodium selenite.

^bParticle size determined by a Penn State Particle Separator, which has a 19-mm screen (long), an 8-mm screen (medium), pan (fine, <8 mm).

Experimental measures, sampling procedure, and analysis

Feeds and diets

Ration intake was measured weekly on a group basis by subtracting the morning feed-bunk residues from the amount of TMR offered the previous day. At the same time, samples ($n = 5$) of TMR and refusals were also collected on a group basis in accordance with the methodology outlined by Robinson and Mayers (2010). A subsample of the feed and refusals was immediately used to determine the particle size distribution using a Penn State Particle Separator sieve (PSPS; model C24682N, Nasco, Fort Atkinson, WI) in accordance with the procedure described by Lammers et al. (1996), while the remaining subsample was sent to the laboratory. The sieved fractions, along with the TMR and refusal samples, were dried in a forced-air oven at 65°C and ground by a hammer mill (Brabender rotary mill; Brabender GmbH & Co., Duisburg, Germany) to pass through a 1-mm screen, pending further analysis. All samples were separately analysed for each sampling time.

Ash, crude protein (CP), and crude fat by ether extract (EE) were determined following AOAC International (2002) methods 942.05, 976.05, and 954.02, respectively. Neutral detergent fibre (NDF) and acid detergent fibre (ADF), including residual ash, were sequentially determined using the method of Van Soest et al. (1991), adapted for the Ankom 200 Fibre Analyser apparatus (Ankom Technology Corporation, Fairport, NY, USA). Sodium sulphite was added to the solution for NDF determination, and both corn meal and sorghum meal were pre-treated with thermostable α -amylase (activity 17,400 Liquefon units/mL, Ankom Technology). Acid detergent lignin (ADL) was determined according to Robertson and Van Soest (1981). Starch content was assessed by the Ewers' polarimetric method (ISO 2000). Non-protein nitrogen (NPN) and soluble protein (SP) were determined according to the procedure of Licitra et al. (1996). The net energy for lactation (NE_L) value was estimated according to Nozière et al. (2018). The NFC content was calculated as 100 minus the sum of %NDF, %EE, %CP, and %ash (NRC (National Research Council) 2001). The *in vitro* digestibility of DM (IVDMD) and NDF (IVNDFD) of corn and sorghum meal as well as of the two TMRs were determined in a Daisy II system (Ankom, Tech. Co., Fairport, NY, USA) as previously detailed by Serrapica et al. (2019). All analyses were performed at least in triplicate.

The physically effective NDF (peNDF) was calculated by multiplying the NDF content by the physical

efficiency factor (pef), which is the sum of the percentages of DM retained on the two sieves (19 and 8 mm) (Lammers et al. 1996). The feed sorting index (SI) for each fraction was calculated as the percentage of actual intake relative to predicted intake, which is the product of the DMI and the percentage of the DM fraction offered in the diet. Values of SI that are equal to, exceed, or fall below 100 indicate no sorting, sorting for a specific particle size, and sorting against a specific particle class, respectively (Leonardi and Armentano 2003).

Milk production

Milk yield was recorded daily in the milking parlour and sampled twice a week using in-line milk samplers (Ambic Equipment Ltd, Witney, UK), alternating between morning and evening milking. The samples were immediately refrigerated (4°C) and analysed in triplicate (ISO (International Organization for Standardization) 2013) the day after collection for fat, protein, lactose, milk urea nitrogen (MUN) and somatic cells counts (SCC) by infra-red spectrophotometry (CombiFoss™ FT+, Foss, Hillerød, Denmark). Additional sets of milk samples were taken on weeks 2, 3 and 4 to determine milk coagulability and on week 2 and 4 for fatty acids (FA) composition of milk fat. Rennet coagulation time (RCT, min), curdling time (K_{20} , min) and curd firmness (A_{30} , mm) were measured at the technical time of 30 min using Hansen standard rennet solution (200 μ l/10 ml of milk) and a mechanical lactodynamograph (Formagraph instrument, Foss Electric).

For fatty acid composition, an aliquot of milk (about 30 ml) was centrifuged at $17,800 \times g$ for 30 min at 8°C, the fat cake was removed and stored at -20°C until analysis. The extraction of fat (Röse-Gottlieb method (FIL-IDF 1996), the *trans* esterification of triglycerides into the fatty acid methyl esters, and the gas chromatography analysis was performed as extensively described in Romano et al. (2010). Briefly, a Perkin-Elmer Auto System XL GC apparatus (Waltham, MA, USA), equipped with 100 m fused silica capillary column (model SP 2380, Supelco Inc., Bellefonte, PA, USA), a flame ionisation detector, and a programmed temperature vaporiser, was used. The identification and quantification of separated peaks were performed using the Supelco 37 Component FAME MIX, a CLA isomer mixture (Nu-Chek Prep., Inc. Elysian, MN, USA), as external standards, and GC retention data from the literature. Values <0.1 were not quantified. Standard buffalo milk (fat and protein corrected milk, FPCM, with 8.3% fat and 4.73% protein)

and mozzarella cheese yield were calculated based on milk composition as detailed by Serrapica et al. (2020a, 2020b).

Digestibility trial

At week 5 of the experimental period, *in vivo* total tract digestibility was assessed using acid insoluble ash (AIA) as an intrinsic marker (Sales and Janssens 2003) and following the procedure described by Masucci et al. (2011). For a four-day period, faecal samples were collected from the rectum of each animal at 09:00, 13:00, and 17:00 h and immediately refrigerated (4 °C). The two TMR were also sampled, and the DMI was determined on a pen basis by calculating the difference between the offered and refused TMR. All samples were dried at 65 °C for DM determination and ground through a 1-mm sieve. The faecal samples were pooled per animal and then analysed for ash, CP, NDF, and AIA using the 2 N hydrochloric acid procedure of Van Keulen and Young (1977). The daily samples of the two TMR were combined by diet and subjected to the same analytical assessments as the faecal samples.

Statistical analysis

Prior to statistical analysis, normality of the data distribution and homogeneity of variance were tested using the Shapiro-Wilk and Levene's tests, respectively. Since SCC were not normally distributed, the values were \log_{10} -transformed. Data were analysed by SAS, version 8.1 (SAS Institute Inc., Cary, NC). Milk yield ($n=350$ /group) and composition ($n=100$ /group, except clotting ability, $n=30$ /group) underwent two-way ANOVA per repeated measures (Proc MIX) with the dietary treatment as a non-repeated factor, sampling time and diet \times sampling time as repeated factors and cow as a random effect. Data on DMI, feed sorting, total tract digestibility ($n=5$ /group), BW, BCS, ($n=5$ /group) and milk FA composition ($n=20$ /group) were analysed by one-way ANOVA (Proc GLM) to determine the fixed effects of the dietary treatment. The pen was used as the experimental unit for DMI and feed sorting ($n=5$ measures), while the cow was the experimental unit for BW, BCS, milk FA composition, and total tract digestibility. Statistical significance was declared at $p < 0.05$ and tendencies discussed at $p < 0.10$. Post hoc pairwise comparisons were performed using Student's *t*-test correction. Data are reported as least square means \pm standard error (SEM) of the means.

Results

Diets, feed intake, body weight and body condition score

The composition and nutritional characteristics, together with the particle size distribution, of the experimental diets are given in Table 2. Compared to the control diet, the Sorghum diet had a comparable energy content but a slightly lower starch content (0.35% points). In addition, the CP content was 0.8% points higher, whereas the SP and NPN contents were 1.58% and 0.81% points lower, respectively. Finally, the Sorghum diet had lower values for both IVDMD and IVNDFD by 4 and 3% points respectively. Replacing maize with sorghum meal did not significantly impact the physical characteristics of the diets, as the proportions of particle size classes remained largely unchanged. Table 3 shows the BW, BCS, DMI and feed sorting of experimental groups. Both BW and BCS of lactating buffaloes remained unaffected by diet at the end of the study. Similarly, no differences in DMI and no selective intake of long, medium and fine particles were observed (Table 3).

Milk yield and composition

The effects of dietary treatments on milk yield and composition are presented in Figure 1 and Table 4. Diet by time interactions were not significant for any parameter, while, apart from SCC, the effect of time

Table 3. Body weight and BCS (LSM, $n=10$) and dry matter intake and sorting index (LSM, $n=5$) of lactating buffaloes fed the experimental diets with corn or sorghum as the main starch source.

Item	Dietary treatment		SEM	<i>p</i> value
	Corn	Sorghum		
Initial BW, kg	648.89	642.02	9.28	0.61
Final BW, kg	656.53	647.12	8.31	0.12
Initial BCS	6.02	6.12	0.06	0.23
Final BCS	7.02	6.95	0.10	0.18
DMI, kg/d	15.49	15.09	0.17	0.13
Actual intake ^a , kg/d				
Long	7.47	7.32	0.16	0.51
Medium	3.51	2.89	0.27	0.14
Fine	4.51	4.88	0.31	0.43
Sorting index ^b				
Long	98.47	98.15	0.68	0.74
Medium	100.28	100.79	0.63	0.58
Fine	102.48	102.66	0.88	0.88

BCS: body condition score; BW: Body weight; DMI: dry matter intake; LSM: least square means; SEM: standard error of mean.

^aThe actual intake of each fraction was calculated as the difference between the amount of each fraction in the offered feed and that in the refused feed (on DM basis).

^bThe sorting index (SI) was calculated as $100 \times (n \text{ actual DMI} / n \text{ predicted DMI})$, where n = particle fraction by a Penn State Particle Separator. Sorting values equal to 100% indicate no sorting, >100% indicate a preferential consumption (sorting for), and <100% indicate a selective refusal (sorting against).

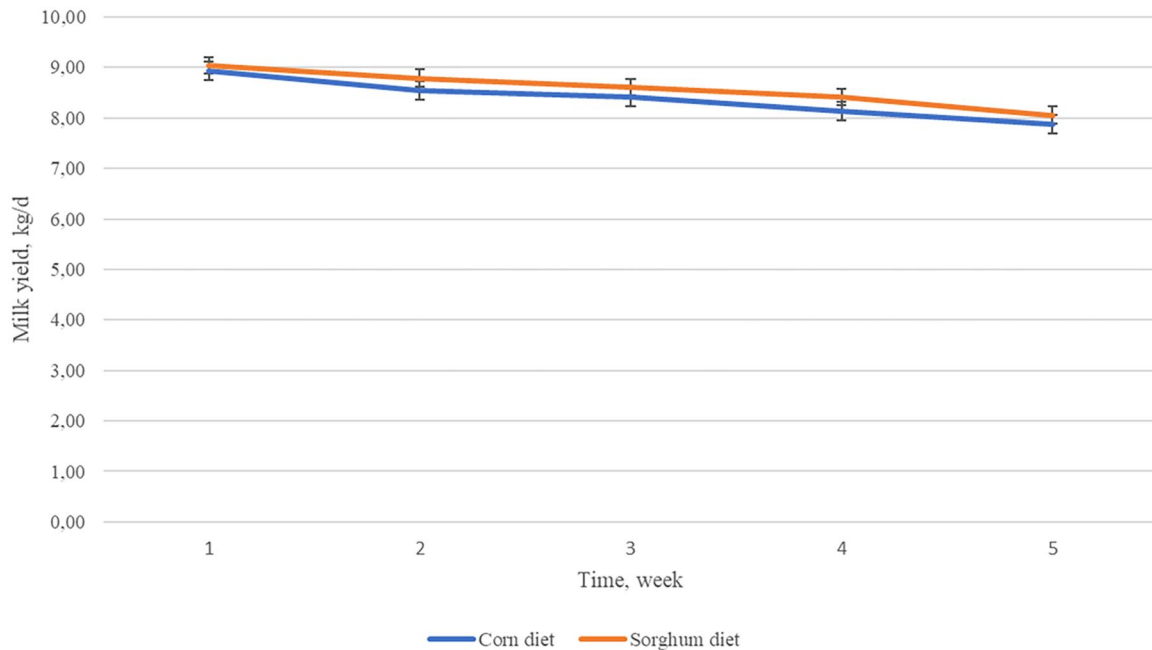


Figure 1. Milk yield over time (kg/d ± SEM) of lactating buffaloes fed the experimental diets with corn or sorghum as the main starch source. Daily data were averaged at weekly intervals.

Table 4. Milk yield (LSM, $n = 350$), milk quality and estimated mozzarella cheese yield (LSM, $n = 100$) and clotting properties (LSM, $n = 30$), of lactating buffaloes fed the experimental diets with corn or sorghum as the main starch source.

Item	Dietary treatment			<i>p</i> value		
	Corn	Sorghum	SEM	Diet	Time	Interaction
Milk yield, kg/d	8.54	8.60	0.64	0.92	<.0001	0.87
FPCM ^a , kg/d	8.76	8.92	0.40	0.71	<.0001	0.54
Fat, %	8.84	9.06	0.34	0.54	<.0001	0.63
Protein, %	4.74	4.62	0.14	0.67	0.04	0.40
Lactose, %	4.60	4.54	0.08	0.17	<.0001	0.32
MUN, mg/dL	21.17	22.71	0.21	0.001	<.0001	0.18
SCC, log ₁₀ cells/mL	5.15	5.12	0.04	0.52	0.12	0.66
Clotting properties						
RTC, min	17.27	17.32	0.13	0.68	<.0001	0.49
K ₂₀ , min	3.42	3.43	0.09	0.93	<.0010	0.17
A ₃₀ , min	39.15	39.26	0.14	0.46	<.001	0.99
Mozzarella cheese yield ^b , kg/d	26.61	26.65	0.88	0.96	<.0001	0.47

MUN: milk urea nitrogen; SCC: somatic cells count; RCT: rennet coagulation time; K₂₀: curd-firming time; A₃₀: curd firmness; LSM: least square means; SEM: standard error of mean.

^aFat and protein-corrected milk at 8.3 and 4.73% of fat and protein, respectively. Calculated as $\{[(g \text{ of fat}/L - 83) + (g \text{ of protein}/L - 47.3)] \times 0.00687\} + 1 \times \text{milk yield (kg/d)}$.

^bCalculated as $\text{milk yield (kg)} \times [3.5 \times (\text{milk protein, \%}) + 1.23 \times (\text{milk fat, \%})] - 0.88$.

was consistently significant ($p < 0.05$), reflecting normal changes in milk production as lactation progressed. The isoenergetic substitution of corn for sorghum meal had no effect on the actual and FPCM milk yield, as well as on the content of milk macro-components (i.e. fat, protein and lactose) and on the SCC. Nevertheless, the MUN value was higher ($p < 0.05$) for cows fed the sorghum-based diet (Table 4). No adverse effects were observed between groups on estimated mozzarella cheese yield, rennet coagulation time (RTC), curd-firming time (K₂₀) and curd firmness (A₃₀) (Table 4).

As summarised in Table 5, milk FA composition was largely unaffected by the dietary treatment, except for C17:0 significantly higher ($p < 0.05$) for Corn group.

In vivo digestibility

Table 6 shows the apparent total tract digestibility as influenced by dietary treatments. Compared to the Corn diet, the buffalo fed Sorghum diet exhibited a significant reduction in the starch digestibility coefficient ($p < 0.05$) and a tendentially lower CP digestibility ($p = 0.06$). While confirming the same trend, the

Table 5. Fatty acids composition (% of total FA) of milk (LSM, $n = 20$) produced by lactating buffaloes fed the experimental diets with corn or sorghum as the main starch source.

Fatty acid	Dietary treatment		SEM	p value
	Corn	Sorghum		
C4:0	3.50	3.39	0.05	0.17
C6:0	2.09	2.07	0.08	0.89
C8:0	1.00	0.95	0.03	0.28
C10:0	2.01	2.10	0.09	0.47
C12:0	2.30	2.98	0.08	0.96
C14:0	13.0	13.04	0.13	0.85
C14:1	0.96	0.99	0.02	0.33
C15:0	1.16	1.01	0.04	0.07
C16:0	35.53	35.72	0.22	0.55
C16:1	2.37	2.30	0.03	0.22
C17:0	0.48	0.45	0.01	0.04
C17:1	0.20	0.18	0.01	0.15
C18:0	10.99	11.19	0.17	0.67
C18:1c9	18.88	18.81	0.20	0.82
C18:1t9	0.43	0.33	0.04	0.08
C18:2c9,12	1.50	1.52	0.05	0.85
C18:2t9,12	0.2419	0.2123	0.01	0.10
C18:3	0.22	0.24	0.01	0.37
Mix CLA	0.39	0.37	0.01	0.39
Others	2.04	2.22	0.20	0.53
SFA	72.76	72.82	0.24	0.87
MUFA	22.84	22.62	0.21	0.48
PUFA	2.35	2.33	0.05	0.80

CLA: conjugated linoleic acids (C18:2t9,c11 + C18:2c9,t11); SFA: saturated fatty acids; MUFA: monosaturated fatty acids; PUFA: polyunsaturated fatty acids; LSM: least square means; SEM: standard error of mean.

differences between the digestibility coefficients did not reach the significance threshold for DM, OM and NDF.

Discussion

The inclusion of sorghum in the cropping systems of Mediterranean dairy farms has gained renewed interest due to the increasing need to minimise the environmental and economic footprint associated with feed production (Negri et al. 2024; Vastolo et al. 2024). This issue is particularly acute for dairy buffalo farms in southern Italy, where the scarcity of arable land has favoured the development of intensive forage cropping systems, largely based on maize silage production (Bragaglio et al. 2022; Zicarelli et al. 2023; Balivo et al. 2025). It is therefore not surprising that most of the literature dealing with the use of sorghum as an alternative to corn-based feeds in dairy buffalo diets has focused on its use potential as silage (Barile et al. 2007; Ann Huws et al. 2012; Sarubbi et al. 2014; Tudisco et al. 2021) neglecting its role as an energy-rich concentrate.

The chemical composition of the locally grown sorghum was within the expected range, with higher CP content and lower starch and solubility of CP compared to corn meal (NRC (National Research Council) 2001; Nozière et al. 2018; Gholizadeh et al. 2021;

Table 6. Total tract apparent digestibility coefficients (LSM, $n = 10$) of buffaloes fed the experimental diets with corn or sorghum as the main starch source.

Item	Dietary treatment		SEM	p value
	Corn	Sorghum		
DM	62.29	60.96	1.07	0.41
OM	64.94	62.25	1.12	0.12
CP	59.79	56.34	1.04	0.06
NDF	43.09	39.97	1.93	0.28
Starch	82.86	80.78	0.36	0.02

DM: dry matter; OM: organic matter; CP: crude protein; NDF: neutral detergent fibre; LSM: Least Square Means; SEM: standard error of mean.

Pontieri et al. 2022b). Similarly, the lower *in vitro* degradability of DM and NDF compared to corn was expected due to the higher proportion of proline-rich storage proteins and the more complex carbohydrate-protein matrix in sorghum. These characteristics lead to increased higher endosperm vitreousness, which makes sorghum protein matrices less diffusible and restricts accessibility for rumen microbes, thereby delaying rumen degradability (McCustion et al. 2019; Ronda et al. 2019). Additionally, the varying proportion of SP and NPN may also affect the characteristics of protein matrices (Gholizadeh et al. 2021). Finally, the presence of polyphenols and phytates in sorghum can inhibit bacterial amylase activity (Ronda et al. 2019; Gholizadeh et al. 2021). Apart from a slight difference in CP content, the Corn and Sorghum TMRs were largely equivalent and capable of meeting the nutritional requirements for mid-lactation buffaloes (Bartocci et al. 2006). Furthermore, the close proportion of TMR particles retained by the PSPS sieves suggests that both cereal meals have a similar ability to bind coarse feed particles when water is added to the mixer wagon. The particle size distributions were found to be consistent with the recommendations of Kononoff et al. (2003) and with those observed by Heinrichs et al. (2019) in a large on-farm survey of dairy buffalo farms in southern Italy, indicating the experimental TMRs representative of practical buffalo diets.

Extensive research has explored the impact of cereal grains (primarily corn and barley) on dairy cow production (Ferraretto et al. 2013; Gómez et al. 2016). However, data on sorghum grain is scarce in dairy cows (Buonaiuto et al. 2021; Shipandeni et al. 2023) and, to the best of our knowledge, lacking in lactating buffaloes. The present study demonstrated that the substitution of corn meal with sorghum did not affect DMI, thereby confirming the findings of previous research on lactating cows (Buonaiuto et al. 2021; Shipandeni et al. 2023). Furthermore, the DMI values observed were consistent with those reported by

Serrapica et al. (2022) for mid-lactating buffaloes fed hay-based diets. It is well documented that dietary NDF is a key factor influencing feed intake in ruminants through rumen fill (Mertens 1997), but the similar NDF content of the two experimental diets (ranging from 41.2% to 42.7%) is unlikely to have a negative effect on intake. Furthermore, these NDF levels might even promote chewing, salivation, and maintain a high rumen pH, potentially masking some of the effects caused by differences in rumen degradability (Mertens 1997). The absence of sorting against long particles, which represent the physically effective component of the ration, may provide indirect support for this hypothesis. In any case, the sorting activity observed aligns with the typical sorting behaviour reported for lactating buffaloes under practical conditions (Esposito et al. 2023; Masucci et al. 2024). Overall, the lack of effect on DMI and SI suggests that sorghum inclusion likely does not negatively impact TMR palatability.

Consistent with findings in dairy cows (Buonaiuto et al. 2021; Shipandeni et al. 2023), substituting corn with sorghum did not influence milk yield, and fat and protein content and, consequently, the composition-related cheesemaking parameters like FPCM yield, mozzarella cheese yield milk clotting characteristics. Since buffalo milk is almost exclusively used for cheesemaking (Sannino et al. 2018), these results are particularly significant.

The higher CP content in the Sorghum diet did not affect milk yield and composition, which is in line with Naveed-Ul-Haque et al. (2018) who reported that buffalo milk production remained unchanged when dietary CP content was modified while maintaining a constant energy intake. Furthermore, the absence of productive responses observed may be indicative of the fact that both diets met the animals' protein and energy requirements since, unlike dairy cows, buffaloes tend to prioritise maintenance over milk production when dietary deficiencies occur (Serrapica et al. 2020a). As further confirmation, cows BW and BCS at the beginning and end of the trial did not vary between groups and were within the expected range for lactating buffaloes of similar parity and days in milk (Bartocci et al. 2006; Negretti et al. 2008; Masucci et al. 2024).

Similar to findings in dairy cows (Buonaiuto et al. 2021), substituting corn for sorghum had no significant effect on milk FA composition. Modifying milk FA profile typically relies on pasture, use of fresh forage, or oil-rich diets (Esposito et al. 2014; Uzun et al. 2018; Salzano et al. 2021). Since our study did not include

any of these feeding strategies, the effect of the diet on FA composition was not observed. Overall, the FA profile of the buffaloes in both groups was comparable to that reported for buffalo fed similar diets in previous research (Serrapica et al. 2020a, 2020b, 2022).

Milk from cows fed sorghum showed increased MUN probably due to a less efficient ruminal nitrogen utilisation compared to the Corn diet. The slightly higher CP content of the diet, combined with an imbalance between the rate and extent of protein and carbohydrate degradation in the rumen, may have led to ammonia accumulation (Hall and Huntington, 2008) and consequently increased MUN in the Sorghum group. The higher concentration of ammonia in the rumen fluid observed by Shipandeni et al. (2023) in cows fed finely ground sorghum supports this hypothesis. Interestingly, the extent of MUN increase recorded in our study was less pronounced than that observed when sorghum meal was fed to dairy cows as substitute to corn (Buonaiuto et al. 2021; Shipandeni et al. 2023), probably due to more efficient rumen nitrogen recycling in buffalo compared to cow (Campanile et al. 2010; Vastolo et al. 2022). However, the close link between MUN and urinary nitrogen excretion raises the possibility that sorghum meal may increase nitrogen excretion (Spanghero and Kowalski 2021). Unfortunately, the lack of established methods for estimating urinary nitrogen excretion in dairy buffaloes hinders confirmation of this (Neglia et al. 2014; Patra et al. 2020). In addition, the tendentially lower CP digestibility of the Sorghum diet may indirectly indicate a lower efficiency of the Sorghum diet group in utilising dietary nitrogen. These findings are consistent with previous research reporting reduced total tract digestibility in cows fed sorghum grain-based diets (Mitzner et al. 1994; Oliveira et al. 1995; Theurer et al. 1999; Shipandeni et al. 2023). This suggests the sorghum-based diet in our study may have allowed more dietary starch and CP to escape rumen digestion, increasing the amount of undegraded nutrients reaching the small intestine. Furthermore, the digestibility outcomes are consistent with studies on Mediterranean buffaloes fed diets with similar nutritional profiles (Campanile et al. 2008; Serrapica et al. 2020b; Guerra et al. 2024). However, although no effect on lower nutrient absorption was observed during the 5-week observation period, the possibility of a long-term negative impact on milk production, body condition, and the incidence of metabolic disorders should not be excluded. Additionally, potential confounders, including season, microclimate conditions,

and genetic variability within the buffalo population, should also be considered.

Conclusion

The study investigated the feasibility of home-grown sorghum meal as a substitute for corn meal as a starch source in a practical hay-based diet fed to mid-lactating buffaloes. Within the constraints of the five-week observation period, the results indicate that sorghum meal can be a viable substitute for corn meal, maintaining milk production and cheese-yield quality parameters. However, a reduction of DMI, along with lower starch digestibility and potential inefficiency in nitrogen utilisation, as evidenced by the increase in MUN, were observed with the sorghum-based diet. These aspects warrant further investigation in long-term trials as they could potentially impact feed efficiency along with fertility and nitrogen excretion. Future research should explore combining sorghum meal with other grains in balanced concentrate mixtures to promote efficient rumen digestion in buffaloes.

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Ethical approval

The experimental design of the current study was evaluated and approved by the Institutional Ethics Committee of the University of Naples Federico II (protocol code PG/0025485), in compliance with EU requirements concerning the protection of animals used for scientific purposes (Directive 2010/63/EU), as implemented by Italian legislation (DL n. 26, 4 March 2014). Furthermore, the experimental procedures adhered to the ARRIVE guidelines.

Disclosure statement

The authors declare no conflict of interest. Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture. USDA is an equal opportunity provider and employer.

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Data availability statement

The data of this study are available from the corresponding author upon reasonable request.

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