J. Dairy Sci. 107:9426–9441
https://doi.org/10.3168/jds.2024-24902

Replacing maize silage with hydroponic barley forage in lactating water buffalo diet: Impact on milk yield and composition, water and energy footprint, and economics

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ABSTRACT

This study investigated the feasibility of integrating hydroponic barley forage (HBF) production into dairy ruminant production, focusing on its effect on milk yield and components, energy and water footprints, and economic implications. Maize silage (MSil) was used as a benchmark for comparison. The research was conducted on a water buffalo dairy farm equipped with a fully automated hydroponic system producing approximately 6,000 kg/d of HBF as fed (up 1,000 kg/d on DM basis). Thirty-three lactating water buffaloes were assigned to 3 dietary treatments based on the level of MSil or HBF in the diet: D0 (100% MSil), D50 (50% MSil and 50% HBF), and D100 (100% HBF). The feeding trial lasted 5 wk, plus a 2-wk adaptation period during which each cow underwent a weighing, BCS scoring, recording of milk yield and components, including SCC and coagulation characteristics. Based on the data obtained from the in vivo study, the water and energy footprints for the production of MSil and HBF and buffalo milk, as well as income over feed cost, were evaluated. Complete replacement of MSil with HBF resulted in a slight increase in milk yield without significant impact on milk components. The resource footprint analysis showed potential benefits associated with HBF in terms of water consumption. However, the energy footprint assessment showed that the energy ratio of HBF was less than 1 (0.88) compared with 11.89 for MSil. This affected the energy efficiency of milk yield in the 3 diets, with the D50 diet showing poorer performance due to similar milk yield compared with D0, but higher energy costs due to the inclusion of HBF. The production cost of HBF was about 4 times higher than that of farm-produced MSil, making feed costs for milk yield more expensive. Nevertheless, HBF can potentially improve income over feed costs if it increases milk yield enough to offset its higher production costs. Overall, the results suggest that the current practice of using HBF to replace high-quality feedstuffs as concentrates is likely to result in energy and economic losses.
Key words: hydroponic barley forage, maize silage, milk yield and components, income over-feed cost, energy and water footprint

INTRODUCTION

Conventional methods of forage production, in addition to requiring large areas of land, are subject to unpredictable weather conditions and high variability in yield and quality of production from season to season (Hanna et al., 2018; Rognli et al., 2021). Hydroponics is an alternative vertical growing method in which plants are grown without soil under controlled conditions of temperature, water availability, and light timing (Velazquez-Gonzalez et al., 2022). Hydroponics as a method of forage production in intensive dairy systems has gained attention in recent years (Ceci et al., 2023; Zang et al., 2024). Numerous hydroponic systems have been developed for forage production, varying in size and level of automation, but all are based on the germination and indoor growth of fast-growing seeds, primarily cereals, especially barley (Ahamed et al., 2023). These systems can produce fresh, high-quality forages consistently throughout the year, typically within a short timeframe of 6 to 10 d and are not constrained by weather or soil conditions (Ahamed et al., 2023). Thus, hydroponic forage (**HF**) production has the potential to not only address the main challenges associated with traditional forage crops, but also enhance overall milk yield on farms by providing year-round forage of high nutritional quality (Ma et al., 2023; Pastorelli et al., 2023). Furthermore, because HF production has been reported to reduce the needs for irrigation water (Ghasemi-Mobtaker et al., 2022; Elmulthum et al., 2023), it could

Received March 12, 2024.
Accepted May 13, 2024.
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The list of standard abbreviations for JDS is available at adsa.org/jds-abbreviations-24. Nonstandard abbreviations are available in the Notes.

potentially address the challenges associated with forage production in livestock farms facing constraints such as limited arable land and water for irrigation (Newell et al., 2021). However, to the best of our knowledge, there is still a lack of consolidated data assessing both the potential improvement effect of HF on milk yield under intensive farming conditions, given the contrasting reports in the literature (Núñez-Torres and Guerrero-López, 2021; Pastorelli et al., 2023), and the energy and water use efficiency of HF in terms of DM production (Afzalinia and Karimi, 2020; Afzalinia et al., 2020).

Due to its high DM yield per hectare, maize for silage remains an important forage crop in many intensive ruminant feeding systems, despite its significant requirements for water, fertile land, and fertilizer (Borreani et al., 2018; Gallo et al., 2022; Rossi et al., 2023). However, the rising cost of production inputs and the uncertainty of water supply, both directly from rainfall and from irrigation sinks, are reducing the production efficiency of maize forage (Gallo et al., 2014; Bonfante et al., 2019). This emerging challenge is of increasing concern in areas vulnerable to ongoing climate change, where significant increases in summer temperatures and reductions in irrigation water are expected (Casolani et al., 2020). Such conditions are evident in the buffalo (*Bubalus bubalis* Mediterranean type) mozzarella Protected Designation of Origin (**PDO**) area situated in the coastal plains of central and southern Italy, characterized by a maritime Mediterranean climate (Uzun et al., 2018). Efforts are therefore underway to change the current practice of producing forages for intensive dairy cow and water buffalo farming (Tabacco et al., 2018; Serrapica et al., 2022). In this context, hydroponics has been proposed as an alternative production method for warm-season forage crops. The existing studies on the use of HF in dairy ruminant diets have yielded conflicting results and have predominantly examined these products as substitutes for concentrates, administered at relatively low levels (Núñez-Torres and Guerrero-López, 2021; Pastorelli et al., 2023). This contradicts one of the current product claims, which emphasizes the production of high-quality forage (Chand et al., 2022). Another issue that has emerged from the literature is that the DM yield of HF may be lower than that of seed (Fazaeli et al., 2012; Soder et al., 2018). Therefore, it is crucial to assess the overall efficiency of HF in terms of both milk yield and the associated economic and energetic costs.

Motivated by the insufficient scientific evidence regarding the potential benefits of hydroponically produced forages on milk yield and components under intensive farming conditions and the lack of comprehensive data on its technical efficiency, this study aims to examine the feasibility of integrating HF production into dairy farming. To this end, the nutritional benefits of hydroponically produced forage for lactating buffaloes were evaluated, while also analyzing its efficiency in terms of energy and water use and assessing its economic return. Maize silage (**MSil**) was selected as the benchmark for comparison.

MATERIALS AND METHODS

All procedures described in this experiment involving handling and treatment of animals have been approved by the Institutional Ethics Committee on Animal Use of the University of Naples Federico II (protocol code PG/0025485) and in compliance with the European requirements concerning the protection of animals used for scientific purposes (Dir. 2010/63/UE) as implemented by the Italian legislation (DL n. 26, 4 March 2014). Animal procedures are reported according to the Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines 2.0 (Percie du Sert et al., 2020).

Hydroponic Barley Forage Production

The study was conducted from February to April 2022 on a water buffalo farm situated in Campania, a region in southern Italy (41°16′N 14°27′E; 120 m above sea level, 987 mm annual rainfall, 18.7°C average annual temperature). The farm is equipped with a fully automated, continuous HF production system (EA-38*2, Eleusis International Sau, Spain) capable of producing up to 1,000 kg/d on DM basis (6,000 kg as fed) of hydroponic barley forage (**HBF**). Within this chamber, 14 polypropylene conveyor belts (38 × 1.5 m) are arranged in 2 racks of 7 belts, spaced about 50 cm apart. The daily production unit consists of 2 belts, one per rack, placed at the same level. Environmental conditions are regulated by a single electronic unit in an adjacent chamber, controlling a LED lighting system (24 W, 3000 Kelvin, PF 0.50), an air-conditioning system (relative humidity 70% and 15–18°C), and an irrigation system with chlorine-enriched water (hypochlorite 0.2 mg/L) to prevent mold formation. During the production cycle, the growing forage is illuminated and watered at set intervals (see Supplemental Table S1, see Notes). Approximately 1,000 kg of not-soaked barley seed (usually 6-row varieties) is added to a production unit daily, allowing it to grow for 7 d. The resulting HBF, consisting of young leaves and roots, and ungerminated or germinated residual seeds, is then dumped into a mixer wagon for inclusion in the TMR.

Experimental Design, Animals, and Diets

In December 2021, the performance and health status of the lactating buffaloes of the herd were evaluated.

Thirty-three lactating buffaloes were enrolled in the study based on the recorded database for milk yield, visual examination for health status and BCS. The cows were blocked by DIM (57.1 ± 22.1 d [± SD]), milk yield (12.36 ± 3.42 kg/head per day), parity (2.54 ± 1.88), and BW (635.5 ± 30.41 kg/head) and randomly assigned to 1 of 3 homogeneous groups of 11 animals each according to the following dietary treatments: D0 (control, TMR based on MSil), D50 (50% of MSil replaced by HBF), and D100 (all MSil replaced by HBF). The composition and nutritional characteristics of MSil, HBF, and TMR are detailed in Tables 1 and 2. The diets were formulated to meet nutrient requirements for an expected daily DMI of 16 kg (Campanile et al., 1998; Bartocci et al., 2002). The buffaloes were housed in 3 adjacent freestall barns with similar access to feed bunk and water. An electronic water flow meter equipped with an internal memory (DigiFlow 8100T; Savant Electronics Inc., Taichung City, Taiwan) was installed on the water pipe that supplying the drinkers in each barn and used to estimate drinking water intake (**DWI**). Following the farm routine, the buffaloes were milked twice a day (at 0500 and 1530 h) in a 2 × 6 autotandem milking parlor, while the rations were provided once a day in the morning (at 0800 h) and were pushed up to the buffaloes several times throughout the day. The experimental period comprised a 2-wk adaptation to the diets, during which milk yield was monitored daily, followed by 5 wk of data recording and sample collection.

Table 1. Chemical composition (% DM, unless noted), in vitro DM and NDF digestibility (IVDMD and IVNDFD), and mold score of maize silage and hydroponic barley forage used in the formulation of the experimental diets (mean ± SD of 5 samples)

Item	Forage	
	Maize silage	Hydroponic barley forage
Chemical composition		
DM, % of fresh matter	30.5 ± 0.63	15.4 ± 0.96
Ash	6.0 ± 0.26	3.7 ± 0.30
CP	9.3 ± 0.40	14.0 ± 0.63
Ether extract	2.5 ± 0.37	3.3 ± 0.26
NDF	43.4 ± 1.05	35.0 ± 0.66
ADF	22.3 ± 1.6	20.2 ± 0.5
ADL	2.5 ± 0.34	1.1 ± 0.21
NFC	38.9 ± 1.4	43.9 ± 0.8
Starch	29.4 ± 0.67	11.0 ± 0.43
Water-soluble carbohydrates	1.4 ± 0.39	25.4 ± 1.39
SP, % CP	3.1 ± 0.47	8.4 ± 0.37
NPN, % CP	0.58 ± 0.19	6.46 ± 0.29
NE_L, MJ/kg DM	6.60 ± 0.17	7.35 ± 0.11
In vitro digestibility		
IVDMD, % DM	76.5 ± 1.6	81.3 ± 1.1
IVNDFD, % NDF	29.4 ± 0.9	46.5 ± 0.6
Mold score[1]	—	0.4 ± 0.55

[1]According to the 5-point scale of Soder et al. (2018).

Experimental Measures, Sampling Procedure, and Analyses

Animals and Diets. At the beginning and end of the trial, immediately after morning milking and for 3 consecutive days, the cows were weighed and scored for body condition using a 9-point scale (1 = emaciated to 9 = obese) adapted to buffaloes (Campanile et al., 2006) by 2 independent treatment-blind evaluators. The average of 3 measurements were used for statistical analysis. Feed intake was measured weekly on a group basis by subtracting the morning feed-bunk residues from the amount of TMR offered the previous day and dividing the estimated intake by the number of animals. Two samples of HBF, MSil, TMR and refusals were collected, one for immediate determination of particle size distribution and the other for chemical analyses. Particle size distribution (Lammers et al., 1996) of the samples was determined using a 3-tiered (2 sieves with a pore size of 19 and 8 mm and one pan) Penn State Particle Separator (Nasco, Fort Atkinson, WI). The DM and NDF content of particles collected in the 2 sieves and in the pan were determined separately for each sampling time (n = 5). The physical effectiveness factor was calculated as the sum of the percentages of DM retained on the 2 sieves and the product between physical effectiveness factor and NDF content provided the physically effective NDF (Lammers et al., 1996). Differences between the particle size distribution of TMR and refusals allowed the calculation the sorting index of particles, which represents the percentage between actual particle intake and the particle distribution in the TMR. Values >100 indicate selective consumption and <100 selective refusal (Leonardi and Armentano, 2003). Samples of HBF and MSil were collected weekly, just before discharge into the mixing wagon, for chemical analysis; HBF was also scored for mold incidence using the 5-point scale of Soder et al. (2018).

In laboratory, the TMR and feeds samples (n = 5) were dried at 65°C to determine DM content, ground to pass through a 1-mm screen (Brabender rotary mill; Brabender GmbH & Co., Duisburg, Germany), composited by diet and analyzed for ash (method 942.05), CP (method 976.05), ether extract (method 954.02) according to AOAC International (2002); NDF and ADF according to Van Soest et al. (1991); and ADL according to Robertson and Van Soest (1981) adapted for Ankom 2000 fiber analyzer using Ankom F57 filter bags (Ankom Technology Corp., Fairport, NY). For the determination of NDF, sodium sulfite was added to the solution and the MSil, HBF and concentrate samples were treated with thermostable α-amylase (activity 17.400 units/mL, Ankom Technology). Nonprotein nitrogen and soluble protein (**SP**) were determined according to the procedure of Licitra et al. (1996). The Ewers polarimetric method

Table 2. Ingredients (as fed and on a DM basis in parentheses), chemical composition (% of DM, unless noted), in vitro DM and NDF digestibility (IVDMD and IVNDFD), and particle size distribution of the experimental diets fed to lactating buffaloes (mean ± SD)

Item	Experimental diet[1]		
	D0	D50	D100
Dietary ingredients, kg as fed			
Maize silage	16.0 (4.88)	9.0 (2.75)	—
Hydroponic barley forage	—	16.0 (2.46)	25.0 (3.85)
Alfalfa hay	4.0 (3.73)	4.0 (3.73)	4.0 (3.73)
Alfalfa wrapped silage	4.0 (1.91)	4.0 (1.91)	4.0 (1.91)
Mixed hay	1.0 (0.94)	1.0 (0.94)	2.0 (1.88)
Maize meal	3.8 (3.40)	3.8 (3.40)	3.8 (3.40)
Concentrate mix[2]	2.0 (1.77)	2.0 (1.77)	2.0 (1.77)
Chemical composition			
DM, % of fresh matter	54.0 ± 1.18	42.6 ± 1.09	40.5 ± 1.55
Ash	7.2 ± 0.47	6.9 ± 0.28	6.9 ± 0.44
CP	14.6 ± 0.63	15.1 ± 0.46	15.5 ± 0.66
Ether extract	3.0 ± 0.32	3.1 ± 0.24	3.0 ± 0.38
NDF	39.7 ± 1.03	38.5 ± 0.97	39.2 ± 0.90
ADF	24.1 ± 0.83	23.7 ± 0.87	24.7 ± 0.98
ADL	3.7 ± 0.33	3.5 ± 0.54	3.5 ± 0.30
Starch	23.5 ± 0.39	21.0 ± 0.86	17.5 ± 0.88
Water-soluble carbohydrates	2.7 ± 0.23	6.2 ± 0.35	8.3 ± 0.38
NFC	35.6 ± 1.37	36.4 ± 1.15	35.4 ± 1.04
SP, % CP	30.3 ± 0.39	34.1 ± 0.48	35.9 ± 0.83
NPN, % CP	20.0 ± 0.49	24.6 ± 0.44	27.6 ± 0.48
NE_L, MJ/kg DM	6.37	6.42	6.42
In vitro digestibility			
IVDMD, % DM	76.6 ± 0.99	78.7 ± 0.50	79.7 ± 0.46
IVNDFD, % NDF	38.9 ± 0.45	44.7 ± 0.63	50.14 ± 1.94
Particle size distribution[3]			
>19.0 mm	56.7 ± 1.50	64.9 ± 0.45	67.4 ± 0.77
19.0 to 8.0 mm	7.0 ± 0.51	7.5 ± 0.36	6.8 ± 0.33
<8.0 mm	36.3 ± 1.21	27.6 ± 0.69	25.8 ± 2.07
pef[4]	0.64 ± 0.16	0.72 ± 0.17	0.70 ± 0.18
peNDF[5]	24.4 ± 0.92	27.9 ± 0.84	30.3 ± 1.05
Intake			
DWI,[6] L/d	51.1 ± 1.83	45.0 ± 1.92	42.9 ± 1.50
DMI, kg/d	14.6 ± 0.81	14.4 ± 1.06	14.9 ± 0.95
Actual intake,[7] kg/d			
Long	8.2 ± 0.19	9.3 ± 1.20	11.6 ± 0.88
Medium	1.1 ± 0.27	1.1 ± 0.16	1.2 ± 0.10
Fine	5.71 ± 0.41	4.23 ± 1.13	4.45 ± 0.10
Sorting index[8]			
Long	96.7 ± 0.45	98.3 ± 1.38	99.8 ± 0.13
Medium	103.9 ± 2.81	102.8 ± 1.92	100.8 ± 0.52
Fine	104.2 ± 0.31	103.0 ± 2.70	100.2 ± 0.19

[1]D0 = maize silage-based diet; D50 = 50% replacement of maize silage with hydroponic barley forage; D100 = 100% replacement of maize silage with hydroponic barley forage.

[2]Commercial concentrate based on whole flaked soybean, maize meal, sodium bicarbonate, magnesium oxide, wheat middling, calcium carbonate, sodium chloride, *Saccharomyces cerevisiae* culture products, sugar cane molasses, and vitamin and mineral supplements.

[3]Particle size determined by a Penn State Particle Separator with a 19-mm screen (long), an 8-mm screen (medium), and pan (fine, <8 mm).

[4]pef = physical effectiveness factor.

[5]peNDF = physically effective NDF.

[6]DWI = drinking water intake.

[7]The 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).

[8]The sorting index was calculated as 100 × (n actual DMI/n 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).

(ISO, 2000) was used to assess the starch content. Water-soluble carbohydrates were assayed using the Dubois method as described by Covino et al. (2020). The energy concentration expressed as NE_L was estimated according to Nozière et al. (2018), while NFC were calculated as 100 − (%NDF + %ether extract + %CP + %ash). The in vitro digestibility of DM (**IVDMD**) and NDF (**IVNDFD**) of HBF, MSil, and TMR was determined in a Daisy II system (Ankom, Tech. Co., Fairport, NY) using the procedure of Robinson et al. (1999) as previously detailed (Serrapica et al., 2019). Briefly, 3 filter bags/sample (Ankom F57; Ankom Technology Corp., Fairport, NY) were filled with 250 mg of milled sample and incubated for 48 h in 3 digestion jars at 39°C in presence of buffered rumen fluid collected postmortem at a local abattoir (RO.C.A. S.R.L., Mercato San Severino, SA, Italy) from 4 buffaloes selected at a local farm before slaughtering. The buffer solution was prepared by combining 2 solutions (Robinson et al.,1999). Ruminal fluid was collected within 3 min after death, and coarsely filtered to remove larger particles and immediately transferred to preheated (39°C), 2-L airtight glass bottles filled with carbon dioxide. In the laboratory, the ruminal fluids were mixed and further filtered through 2 layers of cheesecloth under continuous CO_2 flushing and transferred to the jars. The time from rumen fluid collection to incubation was approximately 50 min.

Milk Yield and Components. Milk yield was recorded daily at milking time. Individual milk samples were collected twice a week using in-line milk samplers (Ambic Equipment Ltd., Witney, UK), alternating morning and evening milking. Only one sample was collected in the last week of the experiment due to a problem with the milking plant's automatic sampler. The samples (n = 297) were immediately refrigerated (+4°C) and analyzed for fat, protein, and lactose by infrared spectrophotometry (CombiFoss TM7; Foss, Hillerød, Denmark) on the day after collection. No preservatives were added to the milk samples. Three additional sets of milk samples (n = 99) were collected on d 14, 21, and 28 to assess clotting properties of milk. Milk coagulability was determined by measuring the rennet coagulation time (min), curd-firming time (min), and curd firmness (mm) at a technical time of 30 min using Hansen rennet standard solution (200 μL/10 mL of milk) and a mechanical lactodynamograph (Formagraph instrument; Foss Electric, Hillerød, Denmark). Fat- and protein-corrected milk (**FPCM**), with 8.3% of fat and 4.73% of protein, was calculated from the milk composition as previously described (Serrapica et al., 2020). Mozzarella cheese yield was estimated using the equation of Altiero et al. (1989).

Water and Energy Footprint. The water and energy footprint for the production of both MSil and HBF, as well as buffalo milk, were evaluated from a life-cycle perspective. To avoid bias in comparing HBF and MSil yields, factors such as spatial differences (soilless vs. arable units), temporal variations (weekly vs. seasonal harvest cycles), and the preservation phase were taken into account. Then the assessment was conducted at the farm gate and covered one calendar year (January 1–December 31). The foreground system was confined to milk production focusing on gate-to-gate process flows associated with forage production, feed management, cow feeding, and milk yield, and 1 kg of DM and 1 kg of FPCM were used as functional units. The inputs associated with replacement heifers, dry cows, and manure storage and application were excluded from the analysis. A cradle-to-gate life-cycle inventory was developed to assess the input-output flows of raw materials, energy, and water within the defined system boundary. Based on the data collected, various indices were calculated to assess the effect of HBF production on energy and water efficiency. The full list of indices and the equations used for calculation are provided in Supplemental Table S2 (see Notes).

Energy Data Acquisition. Primary data for the foreground system, including information on rations (ingredients and nutritional values) and animal-related data (feed and water intake, milk yield), were gathered from the in vivo trial. Additional primary data spanning 3 yr (2019–2021) were derived from farm records, and information on forage production was sourced directly from the farmer. Secondary data (e.g., the type and quantity of raw materials used to build feed storage and milking facilities, and hydroponic chamber components) and energy conversion coefficients were sourced from literature, with a preference for production contexts similar to the analyzed one, where available (Pagani et al., 2016). Data relating to the background system, such as the energy and water used to build the factories producing fertilizers, chemicals, or machinery, were not included in the analysis (Krauß et al., 2015; Pagani et al., 2016). Similarly, the energy used to transport equipment and materials to the farm was omitted as it was not possible to track the full logistics of production inputs. For the energy analysis, direct inputs included electricity, diesel fuel, lubricants, and human labor for on-farm feed production, while the energy embedded in purchased feeds, buildings, stationary equipment, and machinery were counted as indirect inputs (Pagani et al., 2016; Oğuz and Yener, 2019). Daily electricity consumption by the HBF system was derived from electricity recorded over 3 yr using a 3-phase digital meter (OR-WE-520; ORNO Group, Gliwice, Poland) installed upstream from the growth chamber. Specific fuel consumption for crop management and cow feeding was provided by the farmer and lubricant consumption was estimated on the basis of fuel consumption as suggested by Uzal (2013). Embodied energy in seeds, chemicals, and fertilizers was allocated on a mass basis (Supplemental Table S3, see Notes) using the specific energy inputs reported in Supplemental Tables S4 and S5 (see Notes). Embodied energy in structures and stationary equipment was estimated using a "bottom-up approach," considering specific building materials reported in the construction layout and their expected lifespan (Koesling et al., 2015). The inventoried building materials and the average embedded energy per year of the expected life cycle are summarized in Supplemental Table S6 (see Notes). The energy embodied in machinery was calculated following Bechini and Castoldi (2009) and detailed in Supplemental Table S4. Energy embodied in feeds used for concentrate formulation was derived from Pagani et al. (2016).

Water Data Acquisition. Water consumption for FPCM production was calculated according to Prochnow et al. (2012) as the sum of water used for feed production and water consumed by the animals through drinking, the latter derived from data obtained during the feeding trial. The water used to produce pesticides, machinery and building materials and direct water consumption of cleaning water were excluded as negligible (De Boer et al., 2013; Doering et al., 2013).

The average daily water consumption (m^3/d) for HBF production was calculated using data over 1 yr from a continuous flow water meter (MI02002103; NieRuf GmbH, Besigheim, Germany) installed on the growth chamber's inlet pipe. Water inputs for the other forage crops produced on farm were modeled at field scale (m^3/ha). This modeling considered the sum of actual crop evapotranspiration (**ETc**, mm/d) from precipitation (m^3/ha) and irrigation water (m^3/ha) distributed over the growing season, utilizing the empirical formula of Hargreaves (1994) for estimating reference crop evapotranspiration. Daily weather data from a local meteorological station (41°10′N, 14°13′E, 7.7 km from the farm) were used, averaged over a 10-yr period (2012 to 2021) to capture interannual temperature and precipitation variations. Specific dynamic values of crop coefficients for southern Italy were used to calculate ETc for each stage of plant development (Lazzara and Rana, 2010). Water from precipitation was determined from daily effective precipitation (mm/d) during growing cycles, estimated using the equations of Brouwer and Heibloem (1986) for soils with a slope of less than 4% to 5%. The irrigation water was calculated as the difference between daily cumulative ETc and effective precipitation (FAO, 2019). Virtual water corresponding to off-farm feed resources used for the concentrate formulation was obtained from Ibidhi and Salem (2020).

Forage Costs and Income Over Feed Cost. The data collected for water and energy footprint were also utilized to estimate the production cost of HBF and MSil as well as to assess their respective effects on milk production cost. The milk income over feed cost (**IOFC**; €/d per cow) was estimated within a partial budget model. It involved calculating the difference between milk revenue and feeding costs, utilizing average farm-gate values recorded during the accounting years 2019 to 2021 (Ferreira and Teets, 2020). Because there is no quality-related premium price applied in the water buffalo milk market, the income from milk was calculated by multiplying the actual milk yield per cow by the average selling price of buffalo milk (€2.0/kg). Daily feed costs per cow were calculated on a DM basis, considering the cost of each ingredient (€/kg DM) and its quantity in the experimental rations. Concentrates were assigned a purchase price of €0.35/kg. The barley seed price was set at €0.25/kg, as it was certified for high germinability. Given the absence of a market and farm-gate price for HBF and recognizing that HBF production involves changes in both operating and durable inputs, variable and fixed costs were calculated for all home-grown forages used in the experimental rations (i.e., HBF, MSil, alfalfa hay, alfalfa wrapped silage, and mixed hay). The production costs (€/t DM) of conventional forages were computed per hectare of crop and then converted into a cost per unit of stored feed, while the cost of HBF was calculated on an annual basis by dividing the total cost by the total DM production. For variable costs, labor, energy (fuel and electricity), and materials used during the cultivation cycle and forage storage were inventoried using physical input flows traced in the energy analysis. Labor costs were calculated using an hourly wage of €7.30 (Serrapica et al., 2021), while data sourced from the farmer were used for other costs. Annual repair and maintenance costs for the hydroponic chamber were estimated at 5% of the initial cost, whereas those for machinery and equipment were determined based on ASAE Standards (2000) standards and allocated to each feed source according to the hours worked. Repair and maintenance costs were considered negligible for forage storage facilities (Rotz et al., 2022).

As for the fixed costs, the opportunity cost of owning land was set at €1,000/ha per year, divided equally if 2 crops were produced on the land (Bellingeri et al., 2019). For depreciable assets, the annual cost was calculated based on the initial cost, capital recovery factor, salvage value, and accounting life of each specific asset, as outlined in Supplemental Table S7 (see Notes). The capital recovery factor was calculated using an interest rate of 5% (Serrapica et al., 2021), and the annual insurance rate was set at 0.5% of the initial cost (Rotz et al., 2022).

Statistical Analysis

The data were analyzed using SAS, version 8.1 (SAS Institute Inc., Cary, NC). Milk yield and composition underwent 2-way repeated measures ANOVA (Proc MIX), with diet (D0, D50, D100), sampling time, and their interaction included as model factors, and cow considered as a random effect. A significant time by diet interaction was observed for milk yield. Therefore, the data collected daily were averaged to obtain biweekly data at the same time as milk quality sampling. Statistical contrasts between the diets were performed at each time point. For data on initial and final BW and BSC, a one-way ANOVA (Proc GLM) was employed to determine the fixed effects of diet. Data were tested for normality of residuals and homogeneity of variance using the Shapiro-Wilk and Levene tests, respectively. The Tukey test was then used to compare treatment means. Results are presented as LSM, and significance was declared at $P < 0.05$, with tendencies discussed at $P < 0.10$.

RESULTS

Feeds and Diets

Table 1 presents the chemical and nutritional characteristics of HBF and MSil. The occurrence of mold in HBF was minimal, reaching only level 1 (mold on 10% of the tested area) in samples collected in wk 2 and 3. Compared with MSil, HBF exhibited less than half the DM along with increased variability. Additionally, it contained approximately a third of the starch and reduced amounts of NDF and lignin. However, HBF had numerically higher levels of NFC, soluble sugars, and CP, primarily consisting of NPN and SP. These characteristics resulted in a numerically higher IVDMD (+ 6%) and IVNDFD (+ 58%) for HBF than for MSil.

Table 2 shows the ingredients, as fed and on a DM basis, and the chemical composition of the 3 experimental diets along with data on DMI, DWI, and feed sorting. Because of the different DM content of MSil and HBF, the 16 kg of MSil in the D0 control diet was replaced 50% by 16 kg of HBF in the D50 diet, and 100% by 25 kg of HBF plus 1 kg of mixed hay in the D100 diet. Adjustments were made on a DM basis. Despite these modifications, the diets had an almost similar energy and CP concentration. The main differences among the TMR reflect the different composition of MSil and HBF. Notably, the D100 diet contained less starch and more soluble carbohydrates and protein compared with D0, whereas D50 exhibited intermediate values. In terms of particle size distribution, the increase in HBF content from D0 to D100 resulted in higher proportions of particles >19.0 mm and physically effective NDF, accompanied by a decrease in particles <8.0 mm.

Similar values of DMI were observed, but the actual intake of long particles was 3 kg/d lower in D0 group resulting in a sorting against long particle lower than 100. Drinking water intake was on average 8 L/d lower in D100 group.

Table 3. Body weight and BCS of lactating buffalo fed experimental diet containing maize silage and hydroponic barley forage (LSM)

Item	Experimental diet[1] D0	D 50	D 100	SEM	*P*-value
Initial BW, kg	633.6	631.6	641.2	8.95	0.73
Initial BCS	7.2	7.1	6.9	0.35	0.77
Final BW, kg	639.9	637.6	648.2	9.89	0.72
Final BCS	7.4	7.1	7.0	0.27	0.55

[1]D0 = maize silage-based diet; D50 = 50% replacement of maize silage with hydroponic barley forage; D100 = 100% replacement of maize silage with hydroponic barley forage.

Intake and Milk Yield and Composition

Table 3 shows the BW and BCS of the 3 experimental groups of lactating buffaloes. No differences were observed between groups for BCS and BW at baseline or at the end of the study.

The milk yield and composition of the experimental groups is presented in Table 4. As an interaction between diet and time was detected ($P < 0.0002$), the milk yield of the 3 groups over time is shown in Figure 1. Milk yield decreased in all experimental groups (time effect $P < 0.0001$). However, from 2 wk after the baseline of the experiment, the D100 group showed a less pronounced decline than the D0 group, resulting in a significantly higher milk yield (Table 4). Conversely, the milk yield of D50 group did not differ compared with the other groups.

With regard to the milk components (Table 4), there was a tendency for the protein percentage to increase in the D100 group ($P = 0.063$), whereas the milk fat and lactose content were not affected by the diet. Furthermore, SCC, rennet coagulation time, curd coagulation time, and curd firmness did not change between the groups. Consequently, there were no significant differences in FPCM, milk fat yield, and estimated mozzarella yield. However, milk protein yield was significantly higher in the D100 group due to the combined effects of increased milk yield and the tendency for a higher milk protein percentage. As observed for milk yield, FPCM and mozzarella yield also decreased with lactation progression (time effect $P < 0.0001$), although this reduction was less pronounced due to the concomitant increase in milk fat ($P < 0.0001$; data not shown). A significant time effect was also observed for protein ($P = 0.0390$) and lactose ($P = 0.0064$) percentages, reflecting their irregular trend during the observation period. Specifically, there was a significantly lower lactose and higher protein values at d 28 compared with the data at d 4 and d 8 (data not shown). Nevertheless, the variation of protein across time did not result in variation in clotting properties.

Table 4. Milk production, clotting properties, and estimated mozzarella cheese yield of lactating buffalo fed experimental diet containing maize silage and hydroponic barley forage (LSM)

	Experimental diet[1]				*P*-value		
Item	D0	D 50	D 100	SEM	Diet	Time	Interaction
Milk yield, kg/d	11.00[b]	11.41[ab]	11.83[a]	0.23	0.0616	<0.0001	0.0002
FPCM,[2] kg/d	11.36[a]	11.68[a]	12.08[a]	0.23	0.1008	<0.0001	0.0539
Fat, %	9.14	9.01	8.80	0.13	0.2031	<0.0001	0.6568
Fat, kg/d	1.00	1.02	0.99	0.03	0.8638	0.0180	0.8179
Protein, %	4.37	4.39	4.53	0.005	0.0630	0.0390	0.7290
Protein, kg/d	0.48[b]	0.50[b]	0.53[a]	0.01	0.0016	<.0001	0.1718
Lactose, %	4.88	4.86	4.90	0.004	0.8443	0.0064	0.2982
SCC, $\log_{10}$ cells/mL	4.98	5.02	4.97	0.017	0.1112	0.1896	0.1382
Clotting properties[3]							
RCT, min	22.99	22.99	23.16	0.77	0.9847	0.8868	0.8477
K_{20}, min	5.05	4.89	4.69	0.22	0.5111	0.4239	0.8953
A_{30}, min	34.80	31.25	31.88	2.82	0.6391	0.6964	0.9346
Mozzarella cheese yield,[4] kg/d	2.82	2.91	3.0	0.07	0.1712	<0.0001	0.7362

[a,b]Mean values in the same row with different superscripts are significantly different at $P < 0.05$.

[1]D0 = maize silage-based diet; D50 = 50% replacement of maize silage with hydroponic barley forage; D100 = 100% replacement of maize silage with hydroponic barley forage.

[2]FPCM = fat- and protein-corrected milk at 8.3% and 4.73% of fat and protein, respectively. Calculated as ({[(g of fat/L − 83) + (g of protein/L − 47.3)] × 0.00687} + 1) × milk yield (kg/d).

[3]RCT = rennet coagulation time, K_{20} = curd-firming time, A_{30} = curd firmness.

[4]Calculated as milk yield (kg) × [3.5 × (milk protein, %) + 1.23 × (milk fat, %)] − 0.88.

Energy and Water Footprint and Economic Costs for MSil and HBF Production

To compare the water and energy footprints of MSil and HBF forages, inputs, outputs, and efficiency indices were calculated for production of 1,000 kg DM, as summarized in Table 5. Due to the higher energy concentration of HBF compared with MSil (refer to Supplemental Table S3 and S5), producing 1 t of HBF slightly increases energy output by 3.2% (19,617.29 vs. 20,254.22 MJ for MSil and HBF, respectively). However, the energy expenditure per tonne of DM was over 16 times higher for

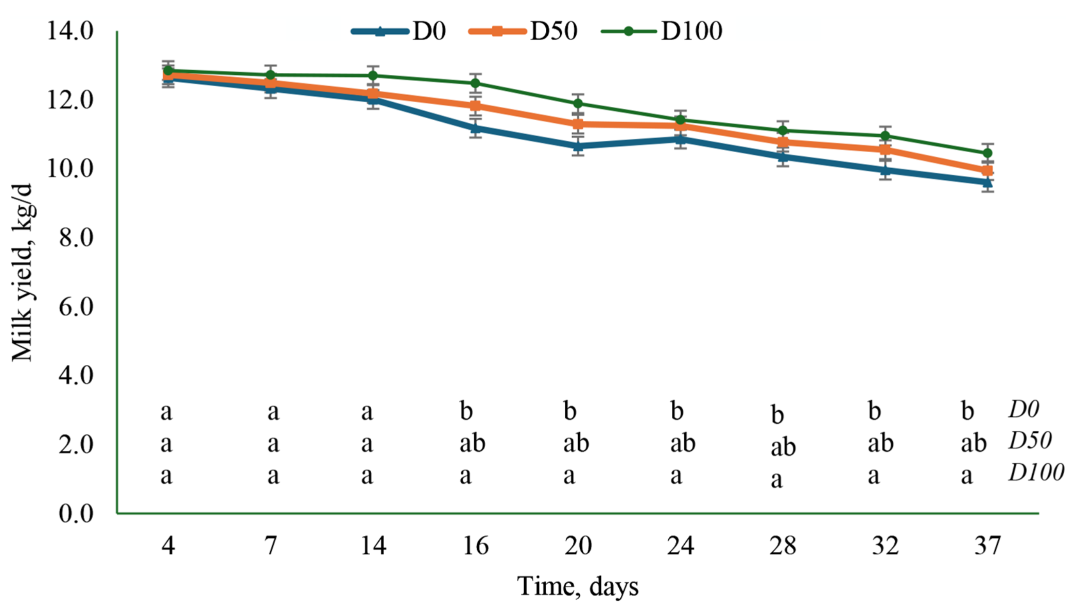

Figure 1. Milk yield over time (k/d ± SEM) of the 3 experimental groups. Daily data were averaged at bi-weekly intervals. D0 = maize silage-based diet; D50 = 50% replacement of maize silage with hydroponic barley forage; D100 = 100% replacement of maize silage with hydroponic barley forage. Different letters (a, b) in the same column are significantly different at $P < 0.05$.

Table 5. Energy and water balance to produce 1 t DM of maize silage and hydroponic barley forage; the percentage of each input category (direct and indirect) is shown in parentheses

	Forage	
Item	Maize silage	Hydroponic barley forage
Energy input, MJ		
Direct	955.96 (58.0)	2,221.05 (9.62)
Electricity	577.93 (35.1)	2,218.54 (9.61)
Diesel fuel	357.67 (21.7)	—
Others[1]	20.36 (1.24)	2.51 (0.01)
Indirect	692.31 (42.0)	20,866.64 (90.39)
Fertilizers	505.60 (30.67)	—
Seeds	92.75 (5.63)	18,585.91 (80.51)
Embedded energy[2]	72.55 (4.40)	2,277.60 (9.87)
Others[3]	21.40 (1.30)	3.14 (0.01)
Total, MJ	1,649.28 (100)	23,085.18 (100)
Energy output, MJ	19,617.29	20,254.22
Water Input[4]		
Wprec	13.78 (4.90)	—
Wirr	267.55 (95.10)	41.12 (100)
Total, m^3	281.33 (100)	41.12 (100)
Efficiency indices[5]		
ER	11.89	0.88
SE, MJ/kg DM	1.65	23.09
EP, kg DM/MJ	0.61	0.04
SW, m^3/kg DM	0.28	0.04
WP, kg DM/m^3	3.55	24.32

[1]Includes direct energy inputs from lubricants and human labor.

[2]Includes indirect energy inputs in form of energy embodied in buildings (i.e., silage storage facility, hydroponic chamber, barley seed silos) and agricultural field machinery.

[3]Includes indirect energy inputs from chemicals (i.e., pesticides, herbicides, sodium hypochlorite, and so on) and plastic sheeting used to seal the silo.

[4]Includes precipitation (Wprec) and irrigation water (Wirr).

[5]Energy and water use efficiency indices. ER = energy ratio [Energy output (MJ)/Energy input (MJ)]; SE = specific energy [Energy input (MJ)/DM output (kg)]; EP = energy productivity [DM output (kg)/Energy input (MJ)]; SW = specific water [Water input (MJ)/DM output (kg)]; WP = water productivity [DM output (kg)/Water input (m^3)].

Table 6. Cost of production of maize silage and hydroponic barley forage (€/t DM); the percentage of each input category (variable and fixed) is shown in parentheses

	Forage	
Item	Maize silage	Hydroponic barley forage
Variable costs	79.6 (62.5)	406.7 (75.9)
Fertilizers	18.7 (14.7)	—
Fuel	18.0 (14.2)	—
Seeds	11.1 (8.7)	316.1 (59.0)
Labor	6.3 (5.0)	9.4 (1.8)
Chemicals	6.9 (5.5)	1.1 (0.2)
Electricity	4.0 (3.1)	80.1 (14.9)
Others[1]	14.4 (11.3)	—
Fixed costs	48.7 (37.5)	129.4 (24.1)
Depreciation[2]	1.9 (1.5)	93.9 (17.5)
Insurance[3]	5.5 (4.3)	31.9 (6.0)
Repair and maintenance	1.5 (1.2)	3.5 (0.7)
Land ownership	38.9 (30.6)	—
Total cost[4]	127.4 (100)	536.0 (100)

[1]Includes cost of lubricants and silage making products (inoculants, plastic sheeting, and so on).

[2]Includes machinery and facility depreciation cost.

[3]Includes machinery and facility insurance cost.

[4]Calculated as the sum of variable and fixed costs.

HBF (1,649.28 vs. 23,085 MJ). Notably, although for MSil the energy inputs are rather equally allocated to direct (58%) and indirect inputs (42%), the primary energy expenditure for HBF is attributed to the energy embedded in the barley seeds (80%). All the energy efficiency indices were worse for HBF. The energy ratio value for HBF is less than 1 (0.88) compared with 11.9 for MSil, the amount of DM produced per MJ input is only 0.04 kg for HBF in respect to 0.6 kg for MSil, and the specific energy requirement per 1,000 kg DM for HBF (23.09) is 16 times higher than that for MSil (1.65). Further differences in energy expenditure between HBF and MSil are sensitive to the agricultural techniques employed and the intensity of fixed equipment used.

In terms of water requirements, producing 1 t DM of HBF necessitates ~41 m^3 of water, while demands about 281 m^3 for MSil. Consequently, the water efficiency indices of HBF were superior to those of MSil, with HBF being approximately 7 times more efficient. This is evident in the water consumption per kilogram of DM, with HBF requiring 0.04 m^3 compared with 0.28 m^3 for MSil, and in the DM yield per cubic meter of water, which stands at 3.55 for HBF and 24.32 for MSil.

The estimated economic cost for production of MSil and HBF are shown in Table 6. The production cost (€/t DM) of HBF (536.0) was about 4 times higher than that of MSil (127.4), with the main cost driver being the cost of barley seed (59%). Consequently, variable costs accounted for 75.9% of the total cost for HBF, whereas for MSil, fixed (37.5%) and variable (62.5%) costs were more evenly distributed, consistent with previous observations on energy balance. Electricity (80.1 €/ t DM, 15% of total cost) and machinery and facility depreciation (93.9 €/t DM, 17.5% of total cost) were the other more relevant costs for HBF production.

Energy and Water Footprint and Economic Costs for Milk Production

Table 7 provides a summary of the daily energy and water requirements per cow assessed for each dietary treatment investigated in the in vivo study. Due to the increased energy demands associated with HBF production, the energy requirement increases steadily from the control, maize-based diet (D0, 107.32 MJ) to D50 (+53 MJ) and further to D100 (+82 MJ). Thus, the D100 diet exhibits a 40% decrease in energy productivity (kg FPCM/MJ) compared with D0, corresponding to a 64% higher milk energy footprint (MJ/kg FPCM). Mirroring the data on forage production, an opposite trend was ob-

Table 7. Energy (MJ/cow) and water (m^3/cow) expenditure for milk production and efficiency indices by dietary treatments

	Experimental diet[1]		
Input	D0	D50	D100
Energy inputs			
Energy from feed[2]	45.08	98.51	126.83
Maize silage	7.89	4.44	—
Hydroponic barley forage	—	56.88	88.88
Other feeds	37.19	37.19	37.95
Other inputs[3]	62.25	62.29	62.43
Total	107.33	160.80	189.26
Water input			
Water from feed[4]	9.22	8.73	8.17
Maize silage	1.35	0.76	—
Hydroponic barley forage	—	0.10	0.16
Other feeds	7.87	7.87	8.01
Other inputs[5]	0.05	0.04	0.04
Total	9.27	8.77	8.21
Efficiency indices[6]			
MEP, kg FPCM/MJ	0.10	0.07	0.06
MEF, MJ/kg FPCM	9.72	14.09	15.96
MWP, kg FPCM/m^3	1.19	1.30	1.44
MWF, m^3/kg FPCM	0.84	0.77	0.69

[1]D0 = maize silage-based diet; D50 = 50% replacement of maize silage with hydroponic barley forage; D100 = 100% replacement of maize silage with hydroponic barley forage.

[2]Calculated as the energy equivalent of each dietary ingredient (MJ/kg DM) multiplied by the proportion of the ingredient in the diet (kg DM ingredient/kg DM diet).

[3]Includes direct and indirect energy for feeding (human labor, fuel, and lubricants for mixing and delivering TMR, and embedded energy in the mixer wagon) and milking cows (electricity and the embedded energy in milking parlor).

[4]Calculated as the water equivalent of each dietary ingredient (m^3/kg DM) multiplied by the proportion of the ingredient in the diet (kg DM ingredient/kg DM diet).

[5]Includes drinking water consumed by cows.

[6]Energy and water efficiency indices. MEP = milk energy productivity [milk yield (kg/FPCM per cow)/Energy inputs for milk production (MJ/cow)]; MEF = milk energy footprint [Energy inputs for milk production (MJ/cow)/milk yield (kg FPCM/cow)]; MWP = milk water productivity [Milk yield (kg/FPCM per cow)/Water inputs for milk production (m^3/cow)]; MWF = milk water footprint [water input for milk production (m^3/cow)/milk yield (kg FPCM/cow)].

served for water use efficiency for milk yield. This is evidenced by the higher milk water productivity and lower milk water footprint per unit of FPCM for the D100 diet compared with D50 (+11% and −10% for milk water productivity and milk water footprint, respectively) and D0 diets (+21% and −17%, respectively).

Table 8 reports the feeding costs per cow along with the IOFC estimated for the 3 experimental diets. Based on the average data of milk yield and HBF production costs, the D100 diet shows the numerically highest values for both income (€23.66) and feed costs (€5.55), with the barley seed cost accounting for about 60% of the total cost (see Supplemental Tables S5 and S7). However, despite the increase in feed cost (€1.49 per cow), which largely offsets the additional milk revenue of D100 (€1.66 per cow), the overall result is a net benefit of €0.17 per cow in the IOFC of D100 compared with the D0 diet. Conversely, diet D50 demonstrates the poorest IOFC due to a combination of 4% lower milk yield (compared with D100) and 25% higher feed costs resulting from the inclusion of HBF (compared with D0).

Table 8. Daily milk income, feed cost and income over feed cost (€/cow) by dietary treatments

	Experimental diets[1]		
Item	D0	D50	D100
Milk income	22.0	22.82	23.66
Feed cost			
Purchased feeds	1.86	1.86	1.86
Home-grown forage	2.19	3.21	3.69
Maize silage	0.70	0.40	—
Hydroponic barley forage	—	1.32	2.06
Other forage sources[2]	1.49	1.49	1.63
Total[3]	4.06	5.07	5.55
IOFC[4]	17.95	17.75	18.11
Additional revenue[5]		0.82	1.66
Additional cost[5]		1.0	1.49
Net benefit[6]		−0.19	0.17

[1]D0 = maize silage-based diet; D50 = 50% replacement of maize silage with hydroponic barley forage; D100 = 100% replacement of maize silage with hydroponic barley forage.

[2]Includes feed cost for alfalfa hay, alfalfa wrapped silage, and mixed hay.

[3]Calculated as the sum of purchased and home-grown forage costs.

[4]Calculated as difference between milk income and feed cost.

[5]Calculated as the difference from D0.

[6]Calculated as the sum of additional revenue and additional cost.

DISCUSSION

To the best of our knowledge, this study marks the first exploration of the effects of high levels of HF in the diet of lactating animals in total substitution of a forage source (Núñez-Torres and Guerrero-López, 2021; Pastorelli et al., 2023). Our approach also allowed the effect of HBF on animal performance to be assessed as a function of dose, minimizing the confounding effects of diet characteristics and dietary HF levels inherent in cross-study comparisons. In contrast, the assessed water and energy footprints, feed costs, and income versus feed costs are inevitably diet and farm specific. This was unavoidable as it was not possible to extend our dataset due to the very small number of hydroponic plants currently in operation, which also vary widely in size, level of automation and ruminant species reared. Although the data were obtained from a specific on-farm study and some of the assumptions are specific to the buffalo farming and market context, these analyses have nevertheless facilitated the identification of general trends that allow us to draw some general conclusions.

Feed and Animal Performance

The composition of HBF is consistent with the existing literature (Núñez-Torres and Guerrero-López, 2021; Pastorelli et al., 2023). The higher IVDMD and IVNDFD along with the higher solubility of proteins and carbohydrates, and the lower starch content in HBF compared with MSil, can easily be attributed to the production process. Indeed, HBF contains no rigid stems for mechanical support, consisting solely of young leaves and roots, along with ungerminated or germinated seed remains. In addition, the remarkably short growth cycle prevents the development of cell wall carbohydrates, especially lignin (Kim et al., 2024). Finally, in HBF, the elevated levels of SP, NPN, and water-soluble carbohydrates, coupled with low starch content, are a result of the germination process. During germination, enzymes produced in the aleurone layer and scutellum initiate the breakdown of starch and storage proteins, releasing them into the barley grain's endosperm (Shaik et al., 2014). The fluctuating DM content of HBF is consistent with the literature, particularly the findings of Zang et al. (2024), who also observed how it could jeopardize the stability of the TMR composition from day to day. The low cell wall polysaccharide content of HBF may contribute to its higher palatability, possibly leading to the small but significant increase of consumption of long size particles in the D100 group compared with D0. Notably, our previous work with lactating cows fed HBF at levels comparable to the D50 diet did not reveal any differences in feed sorting (Ceci et al., 2023), as well as for DMI and BCS. In contrast, Zang et al. (2024) reported sorting against HF along with higher BCS for cows fed HBF.

The mean daily DWI observed in our study falls within the range reported for lactating buffaloes by Neglia et al. (2014) for similar levels of DMI and milk yield. As reviewed by Golher et al. (2021), as water intake from feed decreases, DWI typically increases, as high-moisture diets generally fulfill a substantial portion of the water requirement. Therefore, it is not surprising that cows fed the MSil-based diet presented a DWI 8 L/d higher compared with D100 group. The lack of negative effects on DM intake and milk yield in the D100 group indirectly suggests that the lower DWI was compensated by a higher intake of feed water from the HBF.

The typical gradual decline in milk yield as lactation progressed (Catillo et al., 2002; Macciotta et al., 2006) was less pronounced in the D100 group, resulting in a higher level of production compared with D0. A key factor influencing milk yield and component is the availability of nutrients in the udder (Rulquin and Pisulewski, 2006), which in turn is influenced by the intake of DM and digestible nutrients (Tagari et al., 2008). Consequently, the sustained milk production observed in the D100 group may be attributed to various factors, either individually or in combination, able to offset the natural reduction in milk yield as lactation progresses. Differences in in vitro digestibility may account for the higher milk yield of buffaloes fed the highest level of HBF, allowing for increased energy availability. Moreover, scientific reports concur that HBF contains a range of essential vitamins and minerals, bioactive enzymes, and soluble nitrogen, which may contribute to improved overall nutrition for the animals and subsequently increased milk production (Delis-Hechavarría et al., 2021; Kim et al., 2024). Finally, it can be hypothesized that the hydrolysis of starch and insoluble protein reserves into soluble forms could significantly enhance the efficiency of nitrogen and degradable sugar utilization in the rumen (Hafla et al., 2014; Ren et al., 2022). Although the tendency to increase milk protein in the D100 diet may provide indirect support for this hypothesis; unfortunately, direct measurement of nitrogen excretion was not possible due to the absence of established procedures and equations for measuring nitrogen excretion in lactating water buffaloes (Neglia et al., 2014; Patra et al., 2020). Our findings on milk yield, which showed no difference for D50 and increased milk production at D100 compared with D0, mirror the conflicting results found in the literature (Pastorelli et al., 2023) and suggest that HBF has the potential to increase milk yield when incorporated at high levels (i.e., 3.85 kg on a DM basis) as a replacement for feeds with lower nutritional value.

With respect to milk components, the lack of a significant effect of HBF on milk macro-components at any inclusion level is consistent with numerous reports (Reddy et al., 1988; Naik et al., 2014; Nugroho et al., 2015; Kaouche-Adjlanea et al., 2016; Soder et al., 2018; Salo, 2019; Fazaeli et al., 2021; Zang et al., 2024). Although sporadic studies have suggested an improvement in milk protein in lactating animal fed HBF at low levels of CP in the diet (Bari et al., 2020; Barwant and Barwant, 2020; Kumar Naik et al., 2020), our results suggest that the increase in nutrient supply to the udder, which promotes higher milk yield, has occurred without a concomitant improvement in the supply of nutrients required for the production of milk constituents. It's worth noting that as buffalo milk is not directly consumed by humans, the lack of significant differences in both FPCM and estimated mozzarella yield effectively negates any positive effect of HBF on milk yield. The lack of effect of HBF on SCC and milk clotting characteristics is also noteworthy. As moldy forage can potentially increase SCC even in the absence of obvious signs of disease (Gallo et al., 2015; Cogan et al., 2017), SCC may serve as an indirect indicator of the hygienic quality of HF, confirming the results of the visual analysis reported previously. In addition, the maintenance of favorable coagulation characteristics is crucial for buffalo milk, which is mainly used in the production of mozzarella (Serrapica et al., 2020). In a previous study where we tested a lactating cow diet containing 10 kg of HBF, we observed a tendency for coagulability to deteriorate (Ceci et al., 2023). However, in light of the present results, it is evident that this deterioration was not necessarily directly related to HBF itself.

Energy and Water Footprint

As noted above, by using 1 t of DM as the functional unit for comparing production efficiency of MSil and HBF eliminates discrepancies resulting from their different production timelines and input sources (land vs. factory). The primary finding regarding energy utilization efficiency is the notably high energy footprint of HBF, which is closely linked to the unique characteristics of the production system. As shown by the data in Table 5, and consistent with the observations of Ghasemi-Mobtaker et al. (2022) and Zang et al. (2024), the brief growth period of HBF not only fails to fully exploit the plant's growth potential but also yields insignificant energy output increment, because the photosynthetic process doesn't generate adequate energy sugars to offset the losses incurred during the germination process and the initial stages of seedling growth (Fazaeli et al., 2012). These results are consistent with the observations of Soder et al. (2018) on the low efficiency of HF production in terms of DM yield. Consequently, with about 1.1 MJ of HBF (DM basis) produced for 1 MJ of barley seed, it's evident that the primary energy input of HBF is attributed to the energy embedded in the seed. In addition, the energy ratio less than 1 (0.88) we estimated implicate that the production of HBF result in energy costs that exceed the energy produced. It follows that using HBF as a substitute for concentrated feed, as is commonly done and proposed, may likely result in overall energy inefficiency. Finally, although extending the production cycle to improve the energy yield of HBF may be of interest and has been proposed (Zang et al., 2024) and studied up to 25 d of length (Elmulthum et al., 2023), it is important to recognize that such an approach would likely result in an overall reduction in annual HF yield, potentially reducing the efficiency of the system compared with conventional forages.

The survey by Ghasemi-Mobtaker et al. (2022) on the energy efficiency of various HF systems reported that, on average, 44% of the total expenditure is attributed to energy embedded in seeds, a figure notably lower than our own findings (80.5%). However, Ghasemi-Mobtaker et al. (2022) opted to conduct their analysis using biomass yield as the functional unit instead of DM yield. This approach could introduce bias into the results in terms of actual energy cost and is not relevant from a livestock feeding perspective (Tabacco et al., 2018). Moreover, the magnitude of the energy expenditure of HBF and its origin in the energy embedded in the seed suggest that it cannot be significantly reduced by any type of energy-saving equipment (e.g., use of solar energy, reduction of energy embedded in the plant and equipment).

For water footprint, in consistency with the indication of others (Afzalinia et al., 2020; Ghasemi-Mobtaker et al., 2022; Elmulthum et al., 2023) our analysis confirms that HBF production can reduce water consumption compared with MSil production. It is noteworthy that this water saving is achieved without any water-recycling equipment and is therefore sensitive to further improvement if water-recycling systems are implemented. In a scenario with minimal meteorological inputs, this result is even more relevant because, despite the total reliance of HBF on irrigation, approximately 95% of the water used for maize production also comes from irrigation. Moreover, according to Altobelli et al. (2018) in the PDO Buffalo Mozzarella area, only 2% of the water used for maize irrigation is green water.

Cost Analysis and Remarkable Trends

Although the economic aspects of HBF viability are particularly sensitive to the specifics of the case under study, some generalizable observation can be done. The cost differential between HBF and MSil highlights the significant economic impact of HBF production and also suggests that it is unlikely to compete with highly productive, nutrient-rich forage crops. As seed costs represent a significant portion of total costs, it follows that not only are production costs difficult to reduce, but they are also particularly sensitive to fluctuations in the price of barley seed on the international market, as for example has been seen in recent years following the conflict in Ukraine (Chepeliev et al., 2023; Liadze et al., 2023). The higher cost of HBF production compared with MSil is mirrored in the feeding cost for milk production and IOFC estimated for the 3 experimental diets. In particular, the negative economic results for D50 compared with both the MSil-based control diet (D0) and the HBF-based diet (D100) clearly indicate that the higher production costs associated with HBF can only be justified if it results in higher milk yield. Otherwise, it results in an economically unsustainable system characterized by losses. It should be emphasized that the cost analysis was conducted in the context of the dairy buffalo market, where milk prices are generally much higher than those for cow's milk. Consequently, any extension of these findings to the context of cow milk production demands careful evaluation and should be approached with caution. In contrast, although we have calculated the cost of MSil produced on the farm, if purchased, it is undoubtedly more expensive than our estimate. It follows that the feasibility of producing HF is also strongly influenced by the availability of arable land for maize for silage production. Finally, the HF plant we examined is fully automated, which means a high incidence of maintenance and electricity costs, which can be reduced in plants with lower levels of automation for higher labor costs. Thus, the production costs we observed for these inputs may vary depending on the level of automation of the plant.

Beyond the cost analysis, but perhaps equally important, a serious limitation of HF compared with traditional forage crops is the lack of land for manure disposal and nutrient recovery. Maize, like other gramineous crops, has the inherent ability to reduce the nutrients load on farmland and effectively recycle them. In the case of landless forage production, the critical issue is how to effectively recycle livestock excreta without further exacerbating the environmental impacts of livestock production. Although not considered in this study, this issue needs to be evaluated and cannot be ignored in the decision to adopt this type of forage production, especially in agricultural contexts characterized by heavy livestock pressure and limited land for crops for human consumption that could otherwise support nutrient recycling from manure.

CONCLUSIONS

This study investigated the feasibility of HBF production as an alternative to MSil, used as a forage benchmark, and provided insights into the potential challenges and benefits of integrating HF production into dairy farming. Our findings suggest that substituting conventionally produced MSil with HBF can either maintain or slightly enhance milk yield in lactating buffaloes without significantly affecting milk quality parameters. Moreover, HBF production demonstrates a reduced Wirr requirement compared with maize, indicating potential water-conservation benefits. However, the energy footprint assessment reveals an overall energy inefficiency associated with HBF production. Additionally, the production cost of HBF is approximately 4 times higher than that of on-farm-produced MSil. Our findings clearly indicate that the current application of HBF as a concentrate replacement may not be justified due to its energy inefficiency. Nonetheless, exploring HBF as a replacement for lower quality forage compared with MSil could yield more favorable results, as HBF has the potential to improve IOFC if it increases milk yield enough to offset its higher production cost. Therefore, further analysis is needed to assess the potential cost-effectiveness of HBF compared with lower-quality and less-productive forages or forages produced off farm.

NOTES

The research was funded by Programma per il Finanziamento della Ricerca di Ateneo UniNA, Call 2020, Line B, Project FORIDRO (Use of hydroponic forages in dairy ruminant farming; Project code: UNINA_CdA_75_2021_FRA_LINEA_B; Università degli Studi di Napoli Federico II, Napoli, Italy). The authors thank the Le Cerase farm (Castel di Sasso, Caserta, Italy) for their hospitality. Thanks are also due to Antonio Ambrosino (Università degli Studi di Napoli Federico II) for his helpful contribution during the sample collection and analysis. Supplemental material for this article is available at https://doi.org/10.17632/5vdmsrjr5c.1. All procedures described in this experiment involving handling and treatment of animals have been approved by the Institutional Ethics Committee on Animal Use of the University of Naples Federico II (protocol code PG/0025485) and in compliance with the European requirements concerning the protection of animals used for scientific purposes (Dir. 2010/63/UE) as implemented by the Italian legislation (DL n. 26, 4 March 2014). The authors declare no conflict of interest.

Nonstandard abbreviations used: A_{30} = curd firmness; DWI = drinking water intake; EP = energy productivity; ER = energy ratio; ETc = crop evapotranspiration; FPCM = fat- and protein-corrected milk; HBF = hydroponic barley forage; HF = hydroponic forage; IOFC = income over feed cost; IVDMD = in vitro digestibility of DM; IVNDFD = in vitro digestibility of NDF; K_{20} = curd-firming time; MEF = milk energy footprint; MEP = milk energy productivity; MSil = maize silage; MWF = milk water footprint; MWP = milk water productivity; PDO = Protected Designation of Origin; pef = physical effectiveness factor; peNDF = physically effective NDF; RCT = rennet coagulation time; SE = specific energy; SP = soluble protein; SW = specific water; Wirr = irrigation water; WP = water productivity; Wprec = precipitation water.

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