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Connecting the use of Biofertilizers on Maize silage or Meadows with Progress in Milk Quality and Economy

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Abstract

A systematic use of biofertilizers can improve both the quality of a farming system and the parameters of milk. Some issues related to biofertilization experiments on six farms in the Po Valley (NW Italy) involved in the production of milk from dairy cattle fed maize silage or grazed on hay produced from permanent meadows are reported in this paper. Biofertilized maize was found to lower the live stem pH by about 2.3%, and NIR spectroscopy foreshadowing major changes in the composition. Overall, the plant silage was improved in quantity (+10%) but also in quality, as shown by the delayed maturity stage of the leaves (crop maturity index -4%), the lower indigestible NDF content (-7%), and the higher digestible carbohydrates and protein in the whole plants. Such favorable feeding conditions, together with the improved palatability of the feed ration, boosted the nutrient values of the protein (+4.6%) and fat contents (+5.7%) in the milk. Moreover, the functional properties of the milk were ameliorated, as testified by the higher levels of vitamin A (+27%) and vitamin E (+25%) and the reduced levels of saturated fatty acids (-6%), especially myristic (-18%) and stearic (-32%) acids, while the unsaturated acids increased by 15%. As far as economy aspects are concerned, the biofertilization of maize for silage has led to consistent rewards pertaining to the marginal price of the milk, which in turn has led to a value chain increase of about 9%, because of the fields cultivation, but mainly of the cow transformation in milk quality issues. On another farm, intensive maize was substituted with permanent biofertilized meadows, over a greening path, and a + 17% value chain increase was obtained that already derived mainly from the best price for milk quality parameters. Such an evolutionary leap toward a new vision of sustainable agriculture for the environment and for animals, in which a better quality of products, animal welfare and company budget are combined with soil biofertilization, can be considered a bonanza.

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Introduction

Biofertilizers, whose use is considered an acclaimed strategy for multifunctional soil management, as regard the biological and physical features, and an increasing plant resistance to pathogens and to biotic and abiotic stress, represent a promising tool that may provide a response to the new challenges of modern agriculture [1]. In general, the quantitative aspect is considered the most, after encouraging favorable evidence has emerged concerning legumes (+19%), vegetables (+17%), cereals (+15%) and roots (+10%) [2]. At the same time, advantages have been found more for the use of P (+6.7 kg yield kg P^{-1}) than of N (+1.8 kg yield kg N^{-1}). From the operational point of view, the challenge is now to verify whether the law of minimum is applicable or whether the second law of diminishing return, which relates a dose to the response, can be verified and modeled in a production plan considering the interactions with each autochthonous microflora. Johnson et al [3] applied the Law of Minimum to the mycorrhizal factor and showed that mutualism is favored in P-limited systems, while commensalism or parasitism is favored in N-restricted systems. The second challenge for biofertilizers concerns the possibility of the sequestration and bioavailability of N for plants: the most apparent manifestation of plant luxuriating, after a successful biofertilization, is the intense green color, which phenotypically corresponds to a "nitration ". Thirkell et al. [4] in a microcosm experiment, showed that allowing hyphae access to an organic material improved the total N and P contents, with a dramatic +66% of mass increase.

However, the impacts of a biofertilizer on the qualitative improvements of the yield have been studied less, and those studies that have been conducted have mainly focused on the first chain of the raw feeds [5-8] or foods [9-11] but in the latter case, the studies have only rarely been protracted to the animal supply chain for poultry and pigs [12], meat cattle [13] or the milk chain [14-16]. The source of forage for dairy cows and the energy supply affect both the quality of the milk [17] and problematic methane emissions to a great extent [18, 19]. Many studies have been conducted on fatty acids profiles. It has been recognized that an increase in unsaturated FAs leads to a reduction of the ratio between saturated fatty acids (SFA) and PUFAs, thus improving the nutritional quality of milk [20] as well as its antioxidant properties [21].

The present study reports some issues pertaining to medium-term biofertilization experiments, focused on milk quality, which were carried out on intensive dairy cattle farms in Italy. Considering the high value of the lands in Italy and the need to maximize production, the dominant food system is based on the cultivation of maize (*Zea mays*) for silage, mash and corn. In order to face the problems of reorienting agricultural production in the EU toward a green deal, biofertilization could be used as a tool to support chemical restrictions in fields, reduce mycotoxins [12] and improve animal health.

The practice of permanent hay meadows, which had been abandoned by conventional agriculturists for about half a century, is now considered, by a prescient minority, a winning high quality choice, with benefits and substantial help from the use of a controlled biofertilizer management. In the present study, we examine the impact of such a reorientation, that is, from maize to meadows, on the quality of milk and on a company budget.

Experimental Procedure

An asynchronous model was utilized to compare the production of milk – from a quantity and quality perspective - under normal conventional feeding (C) and under an improved regimen (B). The improvement that was tested was due to the use of a biofertilizer consortium applied to the main crops considered in order to satisfy the feeding necessities of a herd, pertaining to maintenance, reproduction and production, that is, a maize crop and a meadow. Four farms were enrolled in the maize experiment over a period of two years, while one farm was followed over three years for the meadow study.

Material and Methods

Maize Experiments

In the first year, four farms were involved in producing a trench silo of biofertilized whole-plant maize, in a quantity considered sufficient to feed milking *Holstein Italian* cows over a period of almost two months in the second year. Four farms in the Po valley, in the Piedmont Region (Carmagnola), and a farm in Soncino in the Cremona Region were involved in the ProLacte Project. MICOSAT F wp® seed for tanning cereals was used for biofertilization purposes at a dose of 1 kg/ ha. This is a somewhat complex consortium in which 100 g contains symbiotic fungi (32 g of sorghum root inoculum, spores and hypha of the *Glomus coronatus* GO01 and GU53 species, *Glomus caledonium* GM24, *Glomus intraradices* GB67 and GG32, *Glomus mosseae* GP11 and GC11, and *Glomus viscosum* GC41), bacteria (Bacillus subtilis BR48), saprophytic fungi (*Beauveria* spp. BB48, *Trichoderma harzianum* TH01, *Trichoderma atroviride* TA28) and inert media ama 100.

Details of the methods used for cropping and monitoring the growth and yield were published in a study by Masoero and Giovannetti [22], which focussed on the variations of the in-vivo stem pH at a cereus status. Each plant was cut at 2/3 of the internode below the ear, and a measurement was made of the in vivo stem pH at the up position (pH_up). A second cut was then made at ½ of the 2nd internode, and the stem pH was measured at the bottom position (pH_bot). The pH measurements were conducted using a BORMAC "XSpH 70" pH meter, provided with a combined plastic-glass electrode Hamilton PEEK Double-Pore F, with 35×6 mm dimensions (LxØ), and two decimals. All the on-plant measurements were conducted at the center of the cut sections. One leaf was retained from each plant and the thus obtained 1278 leaves were then examined in a laboratory, by means of vibrational spectroscopy, using a portable LabSpec 4 Standard-Res Lab UV-Vis-NIR Analyzer fiber optic diode array spectrophotometer (ASD, Analytical Spectral Device Inc., Boulder, CO) to scan the lower pages of the leaves over a 350–1025 nm range (676 absorbance points).

The spectra were imported in a format that was compatible with the WinISI II v1.04 software (FOSS NIRSystem / Tecator, Infrasoft International, LLC) for chemometric processing, by means of a Partial Least Squares (PLS) method, to calibrate the binary condition C *vs.* B, as the 1,2 dummies. No leaf quality prediction model was available at that time for the ASD instrument. Therefore, in order to consider the modifications of the biofertilizer on the leaves and to extend and validate the features of the present study, we included a collection of the NIR spectra of 1627 leaves obtained from a series of later carried out biofertilization published experiments [23].

A low cost NIR device was used for this purpose; the SCiO™ [23] and the spectra were downloaded, imported in WinISI and the quality traits were predicted using models validated in a previous sorghum study [25] and further improved in a vine study [26].

Analyses were carried out on chopped green whole maize plants, by means of a NIRSystem 5000, which is currently used by the Regional Breeder Association.

Meadow Experiment

In the first year, one farm changed its crop organisation from intensive maize feeding and dairy production to meadow feeding and hay -haylage production, using the aforementioned biofertilizer over the entire crop area. The meadows included *Trifolium pratensis, Medicago sativa*, *Dactilys glomerata*, *Lolium multiflorum*, *Festulolium* and *Festuca pratense*. No comparative analyses were carried out on the grasses or hay.

In the transition period, the ration of the milking was gradually enlarged for the forage-to-concentrate ratio.

Milk yield and quality

Several kinds of measurements involved the controls on milk. Individual dairy surveys were conducted on the milking cow farms by means of a functional control conducted by the Italian Herd Breeder Association. A total of 14833 yield records (kg) were collected and several paired standard analyses (fat%, protein%, somatic cell count 000, linear score, dressing% (fat% * 0.9979 + protein% * 4.19 -5.24) and cheese yield (kg*dressing%) were carried out in the two C and B maize silage feeding phases. Over the whole 2013-14 years, about 90% of the controls were conducted during the conventional feeding period and the remaining 10% during the biofertilized feeding period.

Bulk milk samples (No. 98) were regularly examined to establish their quality and cheesemaking properties using a MilkoScan FT120 [27] for routine analyses of the fat, protein and lactose and for the registered interferograms (n=1154 digits), and a Fossamatic 90 for the somatic cell count, which was transformed into a Linear Score. Special laboratory analyses were conducted concerning the functional milk properties, namely the fatty acid profile [28, 29] and the vitamin A and vitamin E contents [30].

Results

Maize yield and quality

A significant rise in yield was obtained as a result of the biofertilizer treatment, more so for the stem system (+15%) than for the ears (+2%), and an increased S to H ratio (+10%) was observed (Table 1).

The pH of the stem was lowered significantly by the biofertilizer, more so at the bottom measuring point (-2.9%) than at the top point (-0,8%), although the up-bot difference was more pronounced (-144%) (Table 2).

As far as the putative leaf composition is concerned, as fingerprinted in the 1278 NIR spectra, the difference between the two theses was extremely high, with an R^2 of 0.82 (Figure 1).

Where considering the 1627 extra leaves (Table 3), the foliar pH varied in parallel as the stem pH varied, thus confirming a 3% lower value in the biofertilized maize.

As far as the foliar composition, predicted by means of SCiO™ NIRS, is concerned (Table 3), the general feature that was observed is a delay in maturity after the treatment, as represented synthetically by the -4% in the crop maturity index. The more juvenile status was testified by more protein % (+6.4), ash (+7), hemicellulose (+13), cellulose (+3) and, conversely, by less lignin (ADL, -5) indigestible NDF (-3) and ADF (-2). The whole-plant composition reflected the consequences of the preponderant part of the stem vs. the ears, and especially in the biofertilized plants, which exceeded the average stem/ear ratio by 10%. The biomass was obviously increased much more in the stem system (+15) than in the ears (+2). Thus, the whole plant reflected the leaf composition as being mixed with the stem and ears parts, and the result was a relative reduction in the fibers, compensated by a rise in protein (+3), but mainly in total sugars (+11). In short, a more palatable and nutritive silage food was obtained.

All items unitless are % dry matter; differences in ln(B/C)% are shown in parentheses; * $P<0.05$

Milk yield and Quality on the Maize Farms

In the context of the ProLacte project, the results pertaining to the quantity of milk produced by individual cows, carried out by the AIA on 13171 cases of feeding with conventional silomais (C) and 1662 of biofertilized feeding (B), indicated a slightly negative production trend (-2.7%) after a comparative analysis. This is mainly due to the voluntary reduction of the feeding level verified on farm #2 and to sanitary reasons on farm #3 (Table 4).

On the other hand, the results on the composition of the milk are interesting. Overall, the fat content increased by +5.5%, while the protein content increased by +3.3%. This has led to an increase in the dairy dressing percentage of 5.3%, and +2.3% in cheesemaking. In parallel, the results of the bulk-milk analyses (Table 5) pointed out even higher increases in fat (+5.7), protein (+4.6) and in the dressing percentage (+6.9).

A clear characterization of the differences is presented in Figure 2, where the regression of the milk protein on the daily yield in the biofertilized (B) and in the control (C) periods highlights two nearly parallel trends, but a dramatic upper value of 3.3% (in relative log dimension). It should be noted that the B rhombus point in this figure is the actual situation on a farm that has applied a systematic biofertilization for ten years, while the C rhombus point represents the breed average from 682 conventional herds.

Functional Properties of the milk on the maize farms

Functional results of considerable dietary interest were obtained concerning the vitamins and the composition of the fatty acids. In fact, significant increases were observed for the Vitamin E (+25%) and Vitamin A (+27%) contents on 3 out of the 4 farms (Table 6), with a more favorable response for the lower levels of biofertilization in the conventional period (Figure 3). The two functional properties are correlated (r =0.81).

The amount of saturated fatty acids decreased significantly (-6%): myristic acid (-18); stearic acid -

Table 1. Yields of the conventional (C) and biofertilized (B) waxy maize plots before silage for the stem and ears, and the total green mass.

Farms	C\B	Plant # m^{-2}	Stems kg m^{-2}	Ears kg m^{-2}	S/H	Total mass kg m^{-2}
1	C	6.5	14.0	7.4	1.9	21.4
1	B	7.5	15.1	7.7	2.0	22.8
2	C	5.9	15.3	15.3	1.0	30.5
2	B	7.7	18.5	12.5	1.5	31.0
3	C	6.3	10.4	9.3	1.1	19.6
3	B	7.4	12.4	11.0	1.1	23.4
4	C	8.1	23.2	12.3	1.9	35.4
4	B	8.5	25.9	12.7	2.0	38.5
5	C	6.8	17.4	9.9	1.8	27.3
5	B	7.5	20.6	11.3	1.8	31.8
Mean	C		16.0	10.8	1.5	26.8
Mean	B		18.5 (15) *	11.0 (2)*	1.7 (10)*	29.5 (10)*

ln(B/C)% is shown in parentheses; * P<0.05

Table 2. In vivo stem pH for the top (pH_up) and bottom (pH_bot) heights of the conventional (C) and biofertilized (B) maize.

No. 760	C- conventional	B-biofertilized
pH_up	4.83	4.79 (-0.8)
pH_bot	4.90	4.76 (-2.9)*
Diff. (pH up - pH_bot)	-0.07	0.03 (-144)*

ln(B/C)% is shown in parentheses; * P<0.05

Table 3. Composition and properties of the conventional (C) and biofertilized (B) maize leaves and the whole plants at the waxy stage.

	Leaves - No.1627		Whole plants - No. 8	
Items	C	B	C	B
Foliar pH, unit	5.14	4.97 (-3)*		
Stem pH bot, unit			4.90	4.76 (-2.9)*
Crop maturity index, n	2.45	2.35 (-4)*		
Lignin, ADL	8.11	7.68 (-5)*		
Ether extract	1.31	1.27 (-2)*	2.67	2.65 (-1)
ADF	42.81	42.13 (-2)*		
Crude fiber	28.01	27.83 (-1)		
Indigestible NDF	25.43	24.56 (-3)*	10.31	9.55 (-7)
N-free extract	46.02	46.99(2)*		
Gross energy, MJ/kg DM	17.43	17.42 (0)		
In vitro tot. digestibility, IVTD %	71.58	71.64 (0)		
NDF	46.20	46.53 (1)*	39.88	38.21 (-4)
NDF digestibility, %	44.93	46.75 (4)*	74.22	75.06 (1)
Hemicellulose	7.91	8.92 (13)*		
Digestible NDF	21.24	21.67 (2)*	29.57	28.66 (-3)
Cellulose	27.64	28.44 (3)*		
Crude protein	8.83	9.40 (6.4)*	6.86	7.05 (3)
Dry matter, %	27.05	28.09 (4)*	35.58	35.61 (0)
Ash	6.43	6.89 (7)*	4.03	3.73 (-7)
Non -structural carbohydrate, NSC			46.56	48.33 (4)
Starch			36.95	37.91 (3)
Total Sugars			5.57	6.05 (11)

All items unitless are % dry matter; differences in ln(B/C)% are shown in parentheses; * $P<0.05$

Table 4. Dairy survey of individual cows in the two considered periods with conventional (C) and biofertilized (B) maize silages (Herd 1-4) or meadows (Herd 5) (No. 14833).

Herd	1	1	2	2	3	3	4	4	5	5	1-5
Conventional \Biofertilized	C	B	C	B	C	B	C	B	C	B	
No	2397	187	2416	387	5970	387	1205	353	1183	348	
kg milk	27.26	28.52	24.36	21.01	32.64	29.93	26.88	28.04	31.42	31.26	
Fat %	3.78	4.15	3.91	4.44	3.95	3.89	3.73	3.89	3.73	3.80	
Protein %	3.34	3.53	3.54	3.76	3.41	3.38	3.44	3.58	3.46	3.51	
Somatic Cells 000	334	296	309	267	483	660	395	274	358	517	
Linear Score	3.10	3.03	3.04	2.99	3.11	3.29	4.33	4.07	4.10	4.11	
Dressing %	12.52	13.67	13.50	14.94	13.00	12.78	12.88	13.64	12.97	13.25	
Cheese kg	3.41	3.90	3.29	3.14	4.24	3.83	3.46	3.83	3.98	4.04	
	P	lnB/C	P	lnB/C	P	lnB/C	P	lnB/C	P	lnB/C	lnB/C
kg milk	0.01	4.6%	<.0001	-13.8%	<.0001	-8.3%	0.0217	4.3%	0.304	-0.5%	-2.7%
Fat %	<.0001	9.7%	<.0001	13.5%	0.13	-1.7%	0.0014	4.2%	0.033	1.7%	5.5%
Protein %	<.0001	5.6%	<.0001	6.2%	0.04	-1.1%	<.0001	4.2%	0.001	1.5%	3.3%
Somatic Cells	0.48	-11.3%	0.37	-13.9%	0.01	36.5%	0.0775	-30.6%	0.484	44.4%	5.0%
Linear Score	0.14	-2.1%	0.09	-1.6%	<.0001	5.8%	0.3846	-5.9%	0.422	0.3%	-0.7%
Dressing %		9.2%		10.7%		-1.7%		5.9%	0.001	2.2%	5.3%
Cheese kg		14.2%		-4.5%		-9.9%		10.5%	0.458	1.4%	2.3%

Table 5. Bulk-milk analyses from four herds in the two periods with conventional (C) and biofertilized (B) maize silages.

No. 98		C	B
Fat	%	3.45	3.65 (5.7)
Protein	%	3.32	3.47 (4.6)
Casein	%	2.58	2.59
Dressing %	%	12.10	12.94 (6.9)
Lactose	%	4.50	4.61
Somatic Cells	000	296.31	299.79
Linear Score	Log	3.44	3.45
Bacterial charge	000	106.32	107.50
Cryostatic point	°C	-0.5284	-0.5268

Significantly different percentages of ln(B/C)% are shown in parentheses.

Table 6. The Vit-A and Vit-E contents in the milk from the conventional (C) and bio-fertilized (B) feeding periods in four herds.

N= 97		Farms				All Farms
		1	2	3	4	
Vit.A µg/100g	C	30.9	32.8	34.3	54.0	38.0
	M	42.4	40.6	52.5	56.8	48.1
	Ln(B/C)%	37%	24%	53%	5%	27%
	Prob	0.0023	0.0238	<.0001	0.6009	<.0001
Vit.E IU	C	177	188	195	298	215
	M	261	242	255	312	268
	Ln(B/C)%	48%	28%	31%	5%	25%
	Prob	<.0001	0.0008	<.0001	0.57	<.0001

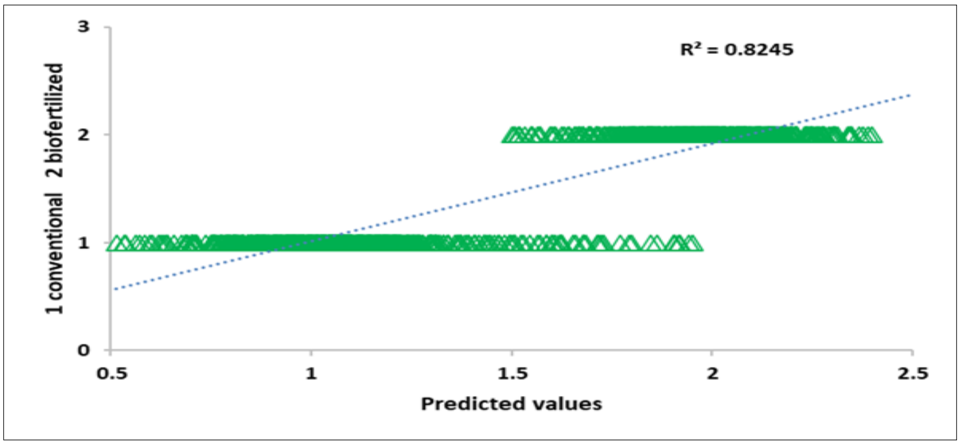

Figure 1. Foliar NIR Spectroscopy of the conventional (C, 1) and biofertilized (B, 2) maize leaves (No. 1278).

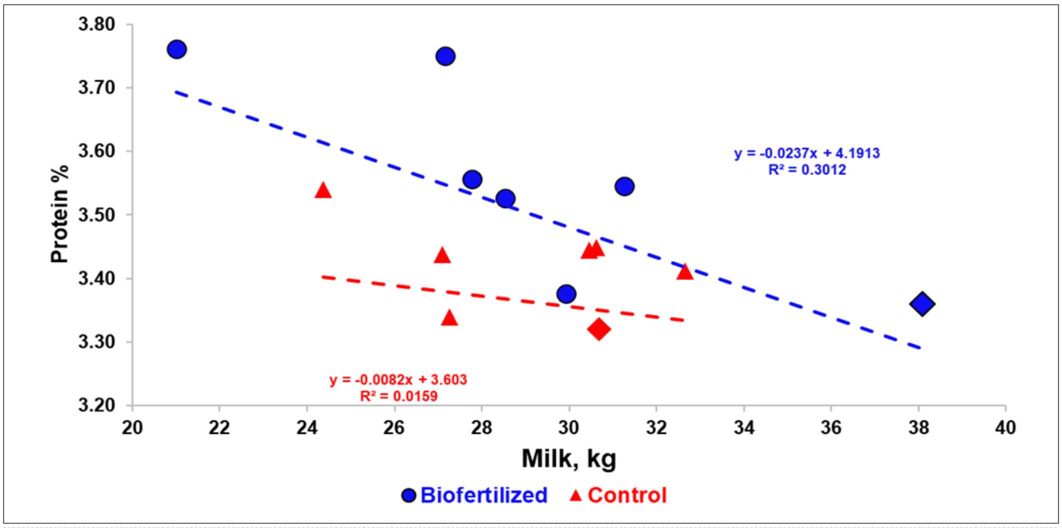

Figure 2. Regression of the milk protein% on the daily yield in the biofertilized (B) and in the control (C) periods, all farms grouped.

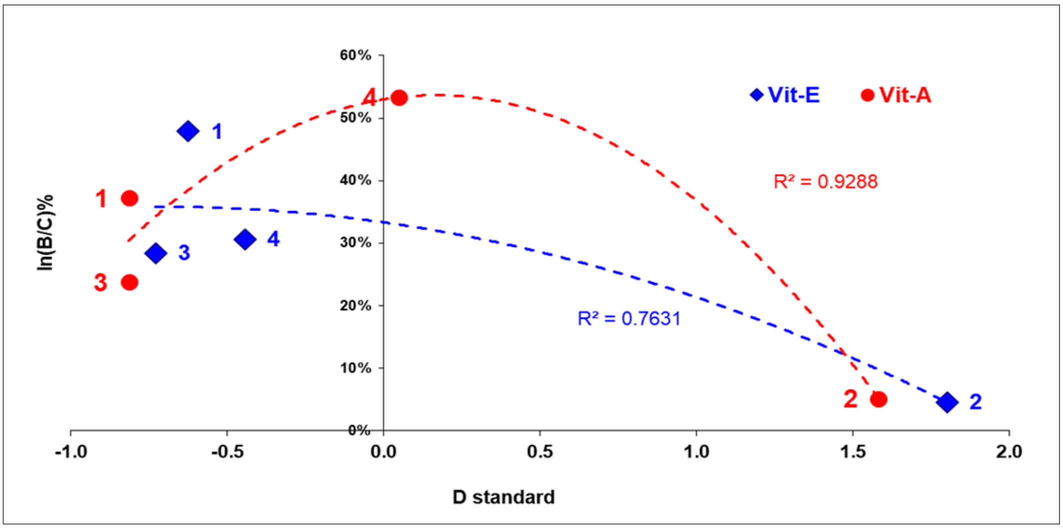

Figure 3. Percentage increase in the vitamin E and vitamin A contents on the four farms in relation to the original standard deviation of the conventional (C) and biofertilized (B) maize.

32%, with a balanced increase in unsaturated acids (+15%) and a decrease in the saturated / unsaturated ratio (-25%) (Table 7).

Milk yield and quality on the meadow farm

The transition from conventional maize to biofertilized meadow was accompanied by a reduction in the individual milk yield of about 8% (Table 8), in part due to a reduction from a triple milking to the normal double one. Importantly, the living conditions of the cows were dramatically improved, as testified by the calving order, which passed from 1.72 to 2.02 (+18%), together with a reduced need for medical treatments. The reduction in energy in the ration was compensated for by a good roughage, and this was reflected in a superior quality of the milk, not of the fat content, but of the protein content (+7% and +9% respectively for T and B) which influenced the dressing% to a great extent (+8% and +10%), while the cheese yield was penalized by the minor yield.

Economic balance

The value chain examined in the unique farm and pertaining to the biofertilized meadow (B) stage was compared with results obtained for the conventional stage (Table 9). A reduction in the operative cost was relevant, as a result of the reduction in chemicals (fertilizers, herbicides, pesticides) and in mechanical processing in the field operations. Moreover, a major advantage was observed for the dairy system in the stable, as a result of the lower feeding costs, but mainly because of the superior quality of the milk parameters, which compensated for the lower quantity, thus resulting in a positive balance, that is, of about 17% of the yearly milk income.

The value chain pertaining to the biofertilized maize was compared with the results verified in the conventional stage for the average of the four farms (Table 10). A certain advantage was observed for the field operations after the reduction in phosphatic fertilizers, as well as an improved yield, for constant mechanical processing costs. However, as before for the meadows study, a major advantage was observed for the stable and dairy system, as a result of the superior quality of the milk parameters, which compensated for the lower quantity, thus resulting in a positive balance, that is, of about 9% of the yearly milk income.

Discussion

Maize

The pivotal role of the foliar pH as a marker of mycorrhization - as previously published [22] has been revealed in different crops [31] and confirmed in further experiments on olive trees in Apulia [32], but mainly in maize experiments [23], where the foliar pH in a set of 13 pairwise inoculation comparisons on average acidified (-3.7%), while the yield responses ranged from +25.2% to -9.2%. However, the relationship was not confirmed in one experiment with biofertilized tomato [33], but the examined samples were not in a good state of conservation. This multifaceted parameter had already been revealed as being important for the predictability of grape yield variations, almost important as the whole foliar NIR spectra [34]. In short, we confirm here a general relevance since the foliar pH has been shown to be a parameter that is inversely correlated with the temperature and with a direct UV-AB exposition in *Lepidium sativum* [35], but is also directly correlated with the soil moisture [36] and with the UV in the 24th solar cycle for grapevine leaves [37].

According to Sabia et al [10], the stalks of plants biofertilized with Micosat F showed significantly lower levels of crude fiber, NDF, ADF and ash (but the P% was raised) than the control plants, according to the presented data, although a greater yield increase (+18%) was observed. Pinar et al. [15], using an AM spore -based biofertilizer, observed a +20% increase and + 39% in a synchronous experiment with sorghum silage, but observed no difference in maize silage composition, while a reduction in NDF (-5%) and an increase in the protein content (+21%) were observed in sorghum, as in the present case.

It seems absurd that corn, the main bioenergy crop throughout the world, is not affected by the results in biofertilization studies. Literature comparisons, limited to the early stages in pot experiments [38, 39] were mostly focused on physiological agronomic traits and showed evidence of a higher leaf-supply of N, P, Mg and Ca, but not of K.

A different consideration derives from an expansion of the category of biofertilizers to the "biofertilizers" issued from biogas. Such products cannot be defined exactly as bio-inoculants since they are

Table 7. Percentage content of fatty acids in the conventional (C) and biofertilized (B) milk from the four herds.

	Fatty acid	C-Conventional	B- Biofertilized
C8_0	Caprylic	0.53	0.52
C10_1	cis-9C10:1	0.04	0.04
C10_0	Capric	0.96	0.97
C12_0	Lauric	1.95	1.99
C12_1	cis9C12:1	0.14	0.15
C14_0	Miristic	10.40	8.52 (-18)
C15_0	pentadecanoic	0.08	0.07
C14_0_m12	12MTD	0.16	0.16
C15_0_m14		0.04	0.04
C16_0	Palmitic	47.50	48.34
C16_2	Palmitolitic	0.73	0.74
C17_0	Margaric	0.19	0.19
C17_1	9-eptadecenoic	0.09	0.09
C18_0	Stearic	9.97	6.74 (-32)
C18_1cis9	Oleic	33.12	33.85
C18_1_11	Vaccenic	0.50	0.49
C18_1_m14	14-metil-esadecanoic	0.13	0.14
C18_1_m15	15-metil-esadecanoic	0.13	0.13
C18_2	Linoleic	0.10	0.11
C19_1		0.04	0.04
C20_1		0.07	0.07
C20_3_n3	Ecosatrienoic	0.53	0.55
Total	Saturated	71.78	67.54 (-6)
Total	Unsaturated	28.22	32.46 (15)
	Ratio S / U	3.11	2.35 (-25)

Significantly different percentages in ln(B/C)% are shown in parentheses.

Table 8. Dairy records from the individual cows after substituting maize silage with biofertilized meadows. Comparisons of the three stages: C- conventional before changing, T- transition, B- biofertilized.

No. 4578	C-Conventional before changing	T-Transition	B- Biofertilized
N° Records	2800	702	1076
Calving order	1.716	1.823 (6)	2.019 (18)
Daily Milk Yield	30.5	27.1 (-11)	27.9 (-8)
Total Milk Yield, kg	7075	6938	6520 (-8)
Fat %	3.80	3.80	3.83
Protein %	3.45	3.70 (7)	3.75 (9)
Dressing %	13.0	14.1 (8)	14.3 (10)
Cheese, kg	3.83	3.66 (-4)	3.74 (-3)

Significantly different percentages in ln(T/C)% and in ln(B/C)% are shown in parentheses.

Table 9. Value chain pertaining to the biofertilized meadows period (B) *vs.* conventional maize period (C)

	Items	B-C Per farm, k€ Y^{-1}	B-C Per cow, € Y^{-1}
Fields	Chemicals: B-meadows *vs.* C- maize cost	-6	
	Labor: B-meadows *vs.* C- maize cost	-1.5	
	Difference in total field cost	-7.5	-84
Herd & dairy	External feeds	-10	
	Reduced production	-18	
	Bonus from milk quality	57.5	
	Differences in dairy incomes	29.5	331
Total		37	416 (+17%)

Table 10. Value chain for the biofertilized maize (B) *vs.* conventional maize (C) for fields and stable and dairy value chain.

		C- Conventional	B- Biofertilized	B-C	B-C
	Items			Per maize, € ha^{-1}	Per cow, € Y^{-1}
Fields	Biofertilizer cost			150	
	Silage mass value			400	
	Net maize value			250	23
Stable and dairy	Milk, kg d^{-1}	27.62	28.52 (3.2)*		
	Fat%	3.45	3.65 (5.8)*		
	Protein%	3.32	3.47 (4.5)*		
	Final price, .00€ kg^{-1}	39.43	41.53 (5.3)		
	Gross income, € cow^{-1} d^{-1}	10.9	11.8 (8.7)		
	Gross income, € cow^{-1} Y^{-1}	3321.6	3612.5		291
Total	Total chain value, € cow^{-1} Y^{-1}				314 (+9%)

Percentages in ln(B/C)% are shown in parentheses; * P<0.05

derived from anaerobic fermentation, while the useful microflora in the soil, which represent the core of a biofertilizer, are aerobic [40]. These products are simply amendments, that is, products used at exponential doses compared to real inoculant biofertilizers, which provide the soil with a mainly organic-N supply. Studies conducted on juvenile plants at the Embrapa Dairy Cattle research unit [41], witnessed a significant increase in the quantity of fresh and dry matter in plants receiving consistent ferti-biofertirrigation through sprinkling, at around 800-1600 mc ha^{-1}, in comparison with the controls, but without any variations in the dry matter, NDF, ADF, CP or N levels.

Corn silage, despite being scarce in protein, is the most relevant feed for intensive ruminant production. Other important pawns on the cultural chessboard are needed in the sustainable farm, especially in cropping systems. In this strategy, chemical and chicken manure fertilizers, biofertilizers based on N-fixing bacteria and phosphate-solubilizing bacteria have shown profitability in intercropping corn-soybean systems [42] and have ranked high in NDF and ADF contents but less in protein and mass yield, while corn-soybean intercrops could slightly increase forage yields and quality, and produce 2% more total protein yields, but also overall produce 39% more than zero-fertilized crops. However, the chemical-organic treatments elevated the zero-fertilizer by about 118%. This is exactly the overly critical point of a biofertilizer advance in real fields: a dose \ response should be adequate for medium – high levels of production, and the results must be confirmed over years and not months. The litterbag-NIRS [33, 34] is a recommended indirect simple method apt to monitor the evolution of the soil biota, especially after the use of biofertilizers.

Milk

In stable practice, the difference between a conventional feeding and a biofertilized one resides in several aspects that are derived from a better palatability and lower level of mycotoxins in the ration, as shown in the Amico project in which the maize grain was measured directly, as well as after poultry feeding experiments [12]. The only references in literature for dairy cows are two experiments conducted by the CREA group. According to Chiariotti et al [14], an improvement in the milk protein content (+6% relative) and in the overall animal condition was found, as confirmed by a greater feed intake (+6%) and by an accentuated weight gain during lactation (+118%). A microscopic investigation of the rumen microflora revealed an excess of protozoa (+15%) in a wide variety of shapes and multicolored microbial species, because of the feeding with corn grains treated with Microbial fertilizer microbial biota Micosat F. In the second replicate experiment [16], the AA speculated that a treatment, even under sub-optimal conditions, could affect some peculiar features of the maize and ration, favouring appetite and digestibility. In fact, the group fed biofertilized maize showed a significantly higher and smoother feed intake over the first period of the trial, thus suggesting that the cows were less subject to the physiological disorders caused by a lack of energy during the first part of lactation. The average daily milk yield was higher in the cows in the mycorrhized group (+5.4%), although there were no notable qualitative differences in the bulk milk, except for a favorable reduction of 16% in amyloid A, a marker used to indicate mastitis, and an unfavorable 1% reduction in lactose. Moreover, the cheesemaking features were significantly improved, with -12% in the coagulation time, a sign of a positive interference on the casein features.

The energy balance of dairy cows must be analytically measured from input-output measures and changes in the body reserves, and as this requires the measurement of all the energetic inputs (feed intake) and outputs (milk, fetus, growth), but this it is not feasible under the current commercial conditions. An indirect option that has been put forward for measuring the energy balance is to consider the changes in the milk composition, for example, the changes in the fat and protein contents of the milk. According to the proposal of Friggens et al [43], a tentative PLS relationship:

Energy Balance (Mj d^{-1}) = 82.4 + 5* Fat% - 80.5 (Fat/Protein), has provided an average comparison of ln(B/C) negative on farms 1 (-17%) and 2 (-26%), null on farm 3 and positive on farm 4 (+15%), but +55% on the farm with meadows, where the real livable conditions were improved, as pointed out by the lower elimination rate of the cows that raised the order of the calving.

Milk producers are concerned about the raw commercial features, but consumers may have different opinions, and some are willing to pay for top quality products. Different Spectroscopy and Electronic Nose Techniques can be used to objectively characterize milk features [44] build blueprints of different milk systems [45] and track feeding regimes and geographic origins [46]. Vibrational spectroscopy can be used to distinguish mountain milk from pasture milk or from the milk of cows fed maize on farms on the plain [47]. A quick down-up approach, which can be used to organize the variability of the commercial, nutritional, and aromatic properties of cow milk and grade them into multifaceted feature quality classes, which would be useful for producers, transformers, and consumers, was described [48].

In that survey, which involved a total of 106 dairy farms in Puglia, the farms were classified as belonging to four characteristic dairy Types: 3-SCH (Silage Concentrate-High, n=25); 4-HCH (Hay Concentrate-High, n=33); 5-HCL (Hay Concentrate- Low, n=32); 6-PCZ (Pasture Concentrate Zero, with Podolic cows, n=16).

The comparisons in Figure 4 highlight that the saturated FAs decreased in parallel to a lower intensity of the feeding-cow system, and, conversely, the unsaturated FAs grew. Vitamin A appeared slightly different, for the systems in the two experiments.

According to Bernardini et al. [49], with reference to *Holstein* dairy cows fed two isoenergetic diets, based on either grass hay or maize silage, the milk from animals fed the green diet contained lower concentrations of saturated FAs and higher levels of polyunsaturated FAs.

Confirmation of the high correlation of the two functional properties (r =0.81) observed in the present work can be found in the work of Strusińska et al [50], who observed that the inclusion of pasture swards in the feed rations raised the contents of liposoluble vitamin A, vitamin E and β-carotene in the milk. A higher concentrate share in the diet – corresponding to raise in yield - also determined a raise in both vitamins but not in the β-carotene.

As the health of a dairy cow depends on the quality of the feed, how much of this health will be transferred to the biologically active components (particularly vitamins and trace elements) that are fundamental for promoting the neonatal and adult health of humans? Previous experiments with mycorrhized corn grain [12] showed that antioxidant qualities were conferred to the animals and transformed into products. In the case of milk, it appears that a better fatty acid profile, less saturated acids, and a greater antioxidant power is conferred to milk for greater vitamin A and E contents. Therefore, the presence of a greater amount of vitamin E in the raw milk analyzed in this study could also have a positive effect on cows and therefore could avoid the onset of infertility phenomena or problems related to disorders of the musculoskeletal system.

However, it is the protein in milk that appears to be a kind of cornerstone of the construction that starts from the mycorrhizal symbiosis, runs through energized metabolic pathways, highlighted by a lower foliar pH in the stalk, and finally determines a better nitrogen efficiency of the entire soil-root-plant-cow system. The experimental evidence obtained from the quantitative analysis of vitamin A and vitamin E in the raw milk of cows fed mycorrhized corn silage could be related to a greater availability of nitrogen present in the mycorrhized cultivar, compared to the untreated cultivar. The results of this study have in fact confirmed many findings in the literature. Some studies have shown how the availability of nitrogen determines a variation in the photosynthetic pigment content, for example, in the chlorophyll a, chlorophyll b, lutein, β-carotene, neoxanthin, xanthine violet, zeaxanthin and xanthine anther contents. A study by Lipppert et al [51], conducted on *Phragmites autralis* (common reed), highlighted that the photosynthetic pigment content depends on the position of the leaves along the stem. It also stated that the leaves containing a greater quantity of nitrogen also have a greater pigment content; the synthesis of chlorophylls, proteins and amino acids in fact depends on the availability of nitrogen.

Kopsell et al. [52] showed that increasing the total nitrogen concentration, causes a linear increase in carotenoids in the dry plant. Furthermore, the molecular form of nitrogen can alter the accumulation of pigments; an increase in carotenoids was observed in cabbage cultivars where the NO_3-N titer was increased in the "nutrient solution".

Another study [53], conducted on the pea plant (*Petroselinum crispum*), showed that a higher concentration of nitrogen in the nutrient solution leads to a significant increase in the carotenoid content, in particular lutein, zeaxanthin and β-carotene, and to an increase in the biomass of the plant.

A further study [54] conducted on AM symbiosis in *Zea mays,* revealed that an accumulation of isoprenoids takes place in the cortical cells of the plant root, in particular of "micorradicin" and of cyclohexenone derivatives. These two groups of compounds can be synthesized through two routes: a direct route, in which the IPP coming from the MEP route is directly converted into these two compounds, and an indirect route, in which the IPP is initially converted into carotenoids and subsequently, through their oxidative degradation, into micorradicin and cyclohexenone derivatives. Furthermore, it has been seen that the mycorrhized roots of *Zea mays* contain a greater quantity of carotenoids than the control , especially of violaxanthin and neoxanthin esters. Furthermore, by treating a mycorrhizal *Zea mays* plant with a herbicide (norflurazon), which inhibits the enzyme phytoene desaturase (PDS) present in the biosynthesis of carotenoids, an accumulation of phytoene was found, which was not present in the non-mycorrhizal corn roots. This indicates that the biosynthesis of phytoene is more active in mycorrhized roots than in non-mycorrhized roots. In fact, it was found that there is a greater transcription of the gene of the enzyme phytoene desaturase in mycorrhizal roots, which was not found in the non-mycorrhized roots. It should be pointed out that the pH showed a basipetal trend, as can be seen in Table 2, a sign of energetic mycorrhizal reactions, even from the roots.

The better protein yield of cows fed with biofertilized forages can be an indicator of a lower dispersion of methane into the environment by lactating cows fed biofertilized maize. Methane is formed from a bacterial biotransformation of hydrogen, as generated by rumen protozoa [55]. The defaunation of the rumen from the protozoa is a way of trying to reduce the emissions of ruminants. Various products (bentonite, tannins, yeasts) have been tested in vitro, but the results cannot be measured in practice, except indirectly from the acid profile of the milk [19]. In an in-vivo experiment on cows in individual head chambers [56], an Italian product, supplemented at 8 g head d^{-1} Enteric, showed a significant reduction in methane emissions of about 20%, while the protein % of the milk increased parallelly by around 5%.

As a corollary, we here report the experience of two colleagues [14, 16] who, on examining the rumen fluid of cows fed mycorrhized and non-mycorrhized corn under a microscope, discovered a multiform and multicolored collection of microflora in the former, that is, a colored photograph vs. a black and white one.

Conclusion

In this work, it has been shown that a subtle *fil rouge* connects the brown world, vitalized by a biofertilizer consortium with mycorrhizae, to the growth and composition of plants up to the transformation into a milk protein, of a nobility that is even superior to the standard gold of the egg. In fact, Schaafsma [57] found 121 *vs.* 118 Protein Digestibility-Corrected Amino Acid Scores (PDCAAS), for milk and eggs, respectively, that is, an unequivocal assertion that milk proteins are superior to plant proteins in cereal-based diets (83 PDCAAS for heated soybean meal).

Biofertilizers based on arbuscular mycorrhizae have so far been noted for their P solubilizing performances in the soil. But in this longer way, it is the nitrogen that excels in efficiency, from the brown of the soil to the white of the milk. Moreover, the functional properties of a milk labeled as biofertilized or a "symbiotic" dimension, equipped with a particular set of antioxidant vitamins, that is, A and E, and fewer saturated fatty acids, allows a real evolutionary leap to be made toward a new vision of sustainable agriculture for the environment and for animals, by combining a better quality of products, animal welfare and at the same time improving the company budget about 9-17%.

In conclusion, the experimental observations summarized in this work constitute the analytical core of an interdisciplinary study related to the repercussions of the use of Micosat F in *Zea mays* crops on the dairy sector. However, further investigations need to be carried out on the still unknown aspects in the bromatology and agronomic fields.

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