Arch Lebensmittelhyg 71,
45–49 (2020)
DOI 10.2376/0003-925X-71-45

ISSN 0003-925X

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Characteristics of the milk of Italian Holstein dairy cows fed a diet including mycorrhized maize grain

Merkmale der Milch von italienischen Holstein-Milchkühen, die mit Mykorrhiza-Mais gefüttert wurden

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Summary

The objective of this study was to evaluate the characteristics of the milk obtained from dairy cows fed a diet including mycorrhized maize during early lactation. MICOSAT F® containing symbiotic fungi, rhizosphere bacteria, saprophytic fungi and yeast was applied at maize sowing. A feeding trial was carried out with two groups of Italian Holstein dairy cows during early lactation. One group was fed a diet containing mycorrhized maize, and the other group was fed a diet containing nonmycorrhized maize.

The group fed mycorrhized maize (M) showed a significantly higher and smoother feed intake over the first period of the trial compared with the group fed nonmycorrhized maize. This trend suggests that the mycorrhized group was less subject to the physiological disorders caused by lack of energy during the first part of lactation.

The average daily milk yield was higher in modulus in the mycorrhized group (+1.6 kg) than in the nonmycorrhized group. The lactose content was higher in the M milk than in the nonmycorrhized milk. No significant difference was found in the somatic cell count (5.26 $\log_{10}$/ml vs 5.32 $\log_{10}$/ml). In addition to the somatic cell count, milk amyloid A was also used as a marker of mastitis. The value of milk amyloid A was higher in the milk of the group fed nonmycorrhized maize (NM) than in the group fed mycorrhized maize.

The use of mycorrhized maize led to an improvement in some milk quality traits, particularly the coagulation properties. The effect of mycorrhized maize on the milk quality and properties reflects the positive effect on the animals' overall condition, confirmed by a higher dry matter intake. We speculate that treatment could affect some intrinsic characteristics of the maize and ration, such as palatability and degradability.

Keywords: milk quality, coagulation properties, milk amyloid A

Journal of Food Safety and Food Quality 72, Heft 2 (2020), Seiten 29–58

Introduction

The interest in the production of quality and sustainable food and feed is strongly increasing throughout the world and drives research towards the development of solutions for agri-food systems in order to obtain better and more environmentally friendly products. Mycorrhizae could represent a feasible option. Indeed, the roots of approximately 80% of the known plant species can live in symbiosis with arbuscular mycorrhizal (AM) fungi (Smith and Read, 2008). The advantages derived from AM colonization include enhanced resistance to foliar-feeding insects and soil pathogens, improved resistance to drought (Celebi et al., 2010; Zoppellari et al., 2014), tolerance to salinity and heavy metals, maintenance of soil aggregate stability and improved plant nutrition (Gosling et al., 2006). These aspects make the use of AM fungi very appealing to improve plant nutrition, and several studies have emphasized the positive effect of AM crop inoculation (Candido et al., 2013; Douds et al, 2007; Sharma et al., 2007). Moreover, according to Siddiqui and Pichtel (2008), the use of inocula could exert a positive effect on seed characteristics and mycotoxin control, stimulating plant resistance. Some results regarding the important effect of AM on mycotoxin production suggested that the biocontrol effect was due to the stimulation of plant defences by inducing the expression of defence-related genes (Ismail et al. 2013).

To the best of our knowledge, there are few studies on the administration of mycorrhized feed in animal production. Among the available literature, two papers that used mycorrhized plants as feed, Meo Zilio et al. (2014) and Chiariotti et al. (2015), found higher protein content (+0,13%; $P < 0.05$) in the milk from cows fed diets including mycorrhized maize grain. However, those results were relevant to cows in an advanced lactation phase. As feed intake is a critical limiting factor during early lactation of high-producing cows, the improvement of feed intake during that phase could be of major interest for producers. Moreover, metabolic and reproductive disorders in dairy cows during early lactation have been attributed to a negative energy status, resulting from a genetic potential for high milk production accompanied by a delay in feed intake in the peripartum period (Veerkamp et al., 2003). The use of mycorrhized crops for animal feed could lead to increased yield and quality and improved overall condition.

The objective of this study was to evaluate the characteristics of the milk obtained from dairy cows fed a diet including mycorrhized maize during early lactation.

Material and methods

Maize production

MICOSAT F®, a product containing symbiotic fungi, rhizosphere bacteria, saprophytic fungi and yeast, patented by an Italian company, was used to inoculate maize plants for grain production. The treatment was performed at sowing, according to manufacturer's instructions. Maize produced under the same conditions, but without inoculation, was included in the control diet.

Animals

The experiment was carried out over 4 months in 16 lactating Italian Holstein dairy cows. The animals were equally divided into two groups, the control (NM) and experimental (M) group and exhibited similar milk yield (kg 28.35 ± 6.80), days in milk (46 ± 7), parity (2.4 ± 1.4), and liveweight (572 ± 113 kg). Liveweight was recorded at the beginning of the trial, at the end of the trial and every 15 days. Milk yield was measured at the onset of the trial and every 15 days. All animals were visited weekly by the veterinarian of the experimental farm to evaluate the overall condition of each individual animal and the occurrence of potential clinical manifestations of diseases.

Diets

Two diets (forage to concentrate ratio 55:45 % on a dry matter (DM) basis) were used in the trial, and they differed only in the type of maize used (10 % as fed). TheM diet contained 5 kg of mycorrhized maize grain, and the NM diet contained 5 kg of non-mycorrhized grain on a wet basis. The cows were fed using a *unifeed* technique (30.06 kg/day DM). The diets were isoenergetic (0.94 milk forage units (FU)/kg DM), had the same amount of protein (16 % crude protein (CP) on a DM basis) and contained second-cut alfalfa hay, triticale/sorghum silage and, depending on the group, two different maize grains. The cows of each experimental group received 4.4 kg on a DM basis of maize grain (16.44 % of diet DM), and the relative percentage of the constituents of both the diets as fed was as follows: hay 13 %, silage 50 %, and concentrate 37 %.

Sample analysis

The following analyses were performed monthly on the feeds and diets: DM, CP, crude fibre (CF), ether extract (EE), ash (AOAC 1995), neutral and acid detergent fibre (NDF and ADF), and lignin (ADL) (Goering and Van Soest 1970). Non-structural carbohydrates (NSC) were calculated (Van Soest et al. 1991). The net energy, expressed as milk FU/kg DM, was determined by the chemical composition and the digestibility of organic matter (INRA 1988). The residuals were collected and weighed at least twice per week throughout the trial. Starch was quantified according to the AOAC 996.11 and AACC 76-13.01 methods.

The aflatoxin B1 (AFB1), fumonisins (FBs), deoxinivalenol (DON) and zearalenone (ZEA) contents were analysed in the maize grain samples. The mycotoxin concentration levels were determined using an enzyme-linked immunosorbent assay (ELISA). The Ridascreen® R-Biopharm kit tests were performed using the Chemwell Automatic Awareness Engineer (inc.). The grain samples were milled with a Retsch – ZM 200 mill using a 0.5 mm sieve. Mycotoxin extraction and tests were performed according to the manufacturer's instructions (Berardo et al., 2011).

The antioxidant activity of the maize grain samples was evaluated by the DPPH (1,1-diphenyl-2-picryl-hydrazyl radical) method (Yang and Zhai, 2010).

All milk samples, representative of the two daily milkings (12-h interval) and collected every 15 days, were analysed every two weeks. The fat, protein and lactose contents were measured by an infrared methodology (Milkoscan FT 600, Foss) according to the reference method ISO 9622/IDF 141:2013. The urea content was measured by the reference method (ISO 14637:2004/IDF 195:2004). The coagulation property analysis (r = rennet clotting time, K20 = curd firming time, A30 = curd firmness at 30 min, A2r = curd firmness at 2r) was conducted by means of Formagraph (Maspres), as reported by Zannoni and Annibaldi (1981). The total somatic cell count (SCC) was determined by the fluoro-opto-electronic flow cell method (Somacount 150, Bentley Instruments) (ISO 13366-2:2006/

TABLE 1: *Maize characteristics. Crude protein, ash, crude fibre, ADF, ADL, NDF, ether extract % DM, Dry matter % as fed, DPPH mg TE/kg as fed.*

Maize	Dry matter	Crude protein	Ash	Crude Fibre	ADF	ADL	NDF	Ether extract	Starch	DPPH
M	89.69	8.91	1.26	2.69	3.97	1.61	15.81	3.66	69.64	294
NM	90.36	9.24	1.26	3.08	4.45	1.57	14.56	3.98	71.11	293

IDF 148-2:2006). In addition to somatic cells, milk amyloid A (MAA) was measured as a mastitis biomarker by an ELISA test produced by the Bentley company.

TABLE 2: *The content of common mycotoxins in M and NM maize grain (μg/kg) and their limits (μg/kg) in feed materials with a moisture content of 12% established by the EU Regulation 574/2011, EU Recommendation 2006/576.*

Maize	Aflatoxin B1	Fumonisins	Deoxinivalenol	Zearalenon
M	0.03	450	2.90	0.00
NM	0.02	510	38.84	0.00
Risk threshold value for livestock	20	60,000	8,000	2,000

Statistical analyses

All data were analysed using SAS GLM procedures (SAS 2012) and a mono-factorial model, with diet as a fixed effect, as follows:

$$Yij = \mu + \alpha i + \varepsilon ij;$$

where μ = general mean, αi = treatment (i = 1, 2), and εij = error of the model.

A P-value greater than 0.05 was not considered significant.

The group feed intake means, expressed as dry matter, were compared by a Student's t-test for independent data in SAS with Satterthwaite adjustment for unequal variances. A statistical significance threshold (type I error) of 0.05 was used.

Results and discussion

The maize characteristics are shown in Table 1. Preliminary analyses regarding the content of the main mycotoxins in mycorrhized and nonmycorrhized maize grain were performed (Table 2). The aflatoxin B1 (AFB1), fumonisins (FBs), deoxynivalenol (DON) and zearalenone (ZEA) contents were far below the established limits (Table 2, EU Regulation 574/2011, EU Recommendation 2006/576) in all the samples. Probably due to the unfavourable weather conditions for the development of mycotoxins in 2017 and, consequently, to the low detected concentration in general, it was not possible to determine a direct effect of the treatment on maize grain, rendering meaningless any significant difference that could have been present. In addition, the mycotoxin content of the milk was not analysed. In an analogous study on mycorrhized maize grain (Tripaldi et al., 2017), the mycotoxin content was close to the limit of detection in all samples, and no conclusion was drawn regarding this aspect. Total antioxidant activity, as measured by DPPH activity, in maize grain was similar in the two types of maize grain, 294 and 295 mg TE/kg, respectively, on an as fed basis. Maize is a good source of carotenoid compounds that have antioxidant activity (Žilić et al., 2012), and our data indicate that there is no effect of mychorrizal treatment. Following the results of the maize grain, we did not investigate the possible effects on the antioxidant activity of the milk.

In Table 3, we report the chemical composition and fibre fraction of the administered ration.

The cows did not show any deflection from normality regarding general condition and liveweight, confirming a consistent plane of nutrition, ration design and delivery. Health condition, regarding in particular nutritionally related problems, was regularly monitored, and no sign of significant metabolic diseases, such as laminitis, bloat or other non-infectious hoof and rumen diseases, appeared among animals during the experiment. The feed intake at peak lactation is reported in Figure 1. Compared with the NM group, the M group showed a higher feed intake (DMI) for the first period (P< 0.05), from the trial beginning to the peak of lactation that corresponds to the physiological negative energy balance phase. According to the curve, there is a more regular trend of feed intake in the M group when compared to the NM group. This trend led us to suppose that the M group was less subject to the physiological

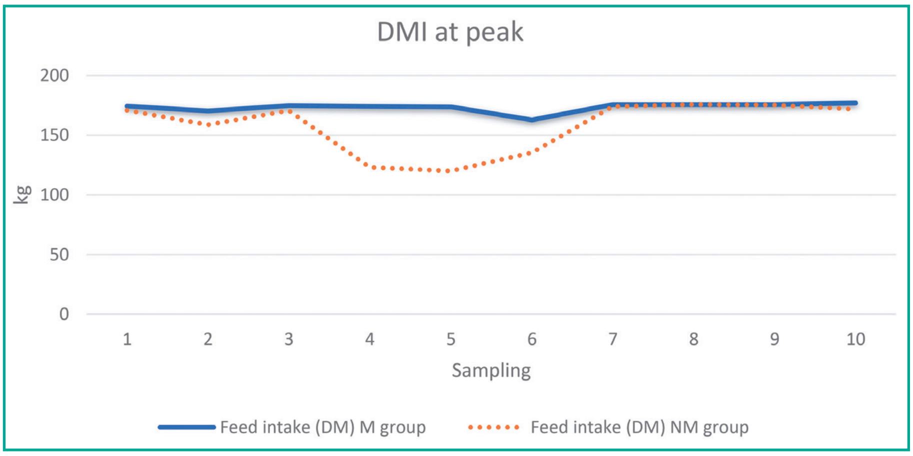

FIGURE 1: *Feed intake over the first month of the experiment.*

TABLE 3: *Chemical composition of utilized ration (%).*

	D.M.	Crude protein	Crude fibre	Ether extract	Starch	Ash	NDF	ADF	ADL
As fed	59.7	9.87	11.23	1.97	16.86	3.84	24.3	13.66	2.39
D.M.	–	16.54	18.81	3.3	28.24	6.43	40.7	22.87	4.01

disorders represented by a lack of energy in the first part of lactation. It is worth mentioning that, in theory, peak lactation occurs at approximately 60 days from calving. Italian Holsteins reach peak lactation at approximately 80 days from the beginning of lactation, on average (Fantini, 2012). The negative energy balance (NEB) is a major limiting factor for milk production and animal health. We speculate that treatment could affect some intrinsic characteristics of maize (Berta et al., 2014) and ration (i.e., palatability, degradability, etc.). However, those topics are not the object of the present study and will be addressed in a further paper on animal feeding and nutrition.

Table 4 reports the overall means of milk yield and sample analyses for the observed period.

The average daily milk yield was higher in modulus in the M group (+1.6 kg), but the difference was not significant.

The somatic cells analysed according to the routine method were, on average, were recorded to be 5.26 log_{10}/ml/ml in the M group milk and 5.32 log_{10}/ml in the NM group milk, and no significant differences were found. In contrast, the MAA-ELISA test values of the M group were significantly lower than those of the NM group (2.15 vs 2.57 log_{10} µg/ml). The MAA is an acute phase protein produced by bovine mammary epithelial cells (McDonald et al., 2001). The MAA provides relevant information about udder health (Eckersall et al., 2001) and is a reliable biomarker for both subclinical and clinical mastitis (Jaeger et al. 2017). The results of the MAA test showed that animals fed mycorrhizal maize grain had lower susceptibility to mammary gland infections than the animals fed the nonmycorrhizal maize grain. Moreover, comparing the results of the MAA test to the SCC results, the response of this acute phase protein, which is supposed to be more sensitive for assessing the animal‘s response to the presence of mastitogenic bacteria, may be confirmed.

No meaningful differences emerged regarding the fat and protein content of the two groups, while the lactose content was significantly higher in the M group compared with the NM group. Because milk lactose is derived almost entirely from plasma glucose, its concentration in milk could be used as a possible indicator of energy balance (Buckley et al., 2003). The higher lactose content is likely due to the higher dry matter intake observed in the M group (25.8 vs 24.3 kg head/d). This result confirms the results from a previous study, in which +6% of dry matter intake was observed in the mycorrhized maize diet group (Meo Zilio et al., 2014).

Even though a more favourable trend of all milk coagulation parameters was observed in the M group, the only significant difference was found in milk coagulation time, which was shorter in the M group than in the NM group (23.56 vs 26.80 min). According to many authors, a shorter coagulation time and a greater curd firmness are positively correlated with an increase in yield and quality of cheese (Bittante et al., 2012). Again, higher dry matter ingestion may have positively influenced the coagulation properties of the milk from the M group, as previously observed in dairy cows (Vertes et al., 1989; Macheboeuf et al., 1993).

Conclusions

The use of mycorrhized maize as feed led to an improvement in some milk quality characteristics, particularly coagulation properties. The effect of mycorrhized maize on the milk quality and properties reflects its positive effect on animals' overall condition, confirmed by a higher DMI and probably by a lower susceptibility of the mammary glands to bacterial infection and inflammation. Further in-depth studies are expected to confirm our results and to clarify the actual role of mycorrhization on the mycotoxins in feed and milk.

TABLE 4: *Yield, health and physio-chemical characteristics and coagulation properties of milk from the mychorrized (M) and the non-mychorrized (NM) group.*

	M group	NM group	SEM	P Value
Milk Yield (kg/d)	30.67	29.11	0.606	0.1993
SCC (log_{10}/ml)	5.26	5.32	0.058	0.2802
MAA (log_{10} µg/ml)	2.15b	2.57a	0.148	0.0494
Fat (%)	3.63	3.68	0.063	0.6769
Protein (%)	3.08	3.11	0.020	0.5781
Lactose (%)	4.90a	4.85b	0.013	0.0353
Urea (mg/dl)	38.07	35.91	0.735	0.1441
pH	6.73	6.75	0.004	0.0825
r (min)	23.56b	26.80a	0.731	0.0261
K20 (min)	6.78	7.37	0.357	0.4042
A30 (mm)	18.51	15.46	1.051	0.1476
A2r (mm)	25.83	24.88	1.260	0.7093

a, b: P < 0,05; SEM: Standard error of mean

Acknowledgements

This study was funded by the EU Rural Development Programmes of Regione Lombardia within the project "Use of non-toxic strains of Aspergillus flavus and mycorrhized microbial consortia to reduce the mycotoxin content of maize".

The authors are grateful to the Amedeo Ardigò farm for providing maize grain for the feeding trial.

Conflict of interest

Authors declare that they have no conflict of interest.

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