Corn bio-fertilized by mycorrhizal and microbial consortium can improve yield, reduce mycotoxins, enhance biochemical parameters in chicken broilers and preserve the nutritive value after a long storage: a multifaceted study

Mycorrhized corn from field to broilers and pigs

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Abstract

Bio-fertilizers are prominent tools in a sustainable agriculture, but the products modified by such "botanic" way could be harmful, or useless, or useful. For this reason a multi-faceted experiment was sequenced to assess the feasibility of a bio-fertilization in the corn to poultry supply chain.

In two crop trials (Corn-I & II) corn was fertilized by Micosat F© the results showed that yield was improved by some 4-19% and increase modify resistance to fungal attacks. Furthermore secondary metabolites as well as fatty acid composition, NIRS properties and Electronic Nose profiles were modified. As regard to feed properties fortification by use of mycorrhizal corn can substantially reduce the oxidant power , respectively -47% in the corn grain flour and -19% in the feed thus conversely increasing the antioxidant properties. Notice that this property could be a marker of effective bio-fertilization. Moreover, very interesting result, the shelf life has been remarkably increased after a 21 months storage, preserving palatability; in fact the broilers, fed control diet, reduced the feed intake -26.7% with a corresponding -27.7 % final body weight at 33 d in the Poultry-II trial. Otherwise, in the Poultry-I trial productive performances, slaughter and meat color were not modified, but NIRS examination of ethanol preparates foreseeing differences in meat texture; otherwise the blood biochemical parameters revealed a concrete amelioration of the physiological functions and of the serum antioxidant capacity, improved +20% in fast and +14% in the slow fractions. In a Pig growth trial carried out with 40 animals but using the Corn-I product as fresh not storage, no effect was apparent on live or slaughter performances, while a NIRS test on ethanol muscle preparates enhanced an high discriminative 1-VR value (0.77), that can be connected to the above finding for meat in Poultry-I trial.

In conclusion the dreaded harmful and unproductive effect on the poultry and pig feeding has been excluded, even better the functional conditions and livability of poultry have been improved as well as in the short storage and even more in a long storage period.

Arbuscular Mycorrhiza (AM) and microbial fertilization may be a strategic key for the sustainability and usefulness of maize crop.

Key words: corn, mycorrhizal, yield, mycotoxins, antioxidant, poultry, shelf life, biochemical parameters, electronic nose, NIRS.

Highlights

- Bio-fertilizers as mycorrhizal and microbial factor improve corn yield, increase resistance to fungal attacks, and modify secondary metabolites.
- Fortification of feeds by mycorrhizal improve oxidant stability
- The shelf life of corn is preserved, increasing palatability.
- Blood biochemical traits are improved.

Introduction

Producing more food with fewer resources may seem too good to be true, but the world's farmers have trillions of potential partners that can help achieve that ambitious goal. Those partners are microbes (Rein and Green, 2012). A concise, but incomplete claim because Mychorrizal (fungi, not bacterial) must be strongly involved when *Connecting the green and brown worlds: allometric and stoichiometric predictability of above- and below-ground network* (Mulder et al., 2013).

The term mycorrhyza derives from the combination of two Greek words, *mycos* meaning "fungus" and *rhiza* meaning "root". It indicates a mutualistic relationship between fungi belonging to order *Glomales* and the roots of a large variety of terrestrial plants (Wang et al., 2006; Poshtvareh et al., 2011). Usually, the established association is a symbiosis based on the penetration of fungal hyphae within the roots of plant host cortical cells, to form a structure called Arbuscular Mycorrhiza (AM) (Ferrol et al., 2002). In sustainable agriculture, bio-fertilization by AM inoculation has been recognized as a favourable strategy to overcome plant nutritional problems (Alizadeh et al., 2011). In corn fertilized by a complex consortium Masoero and Giovannetti (2015) have found that the *in vivo* pH of the plant is acidified according a de-gradient from the root ($[H^+]$ + 214%) to the ear level in the stem (+ 85%). Important effects of AM on colonized plant are the increase of nutrients absorption (Willis et al., 2013), particularly phosphorus, with consequent stimulating effect on the plant growth, and enhancement of its resistance to biotic and abiotic stresses (Jackobsen, 1999; Chu et al., 2013). Qualitative modifications of the seeds will greatly affect in positive sense the primary (Berta et al, 2013) and the secondary (Ritieni et al, 2015) compounds, namely the antioxidants. Otherwise, corn is commonly colonized by filamentous mycotoxigenic fungi, which can cause plant spoilage and, contamination of grains (Susca et al., 2014). Moreover, if yield loss in field and reduced economic crop value are matters of concern for corn producers; for corn-based feeding industry, the presence of mycotoxins in kernels is a severe problem, due to the potential health hazard on livestock farming (Milani, 2013; Yang et al., 2014) and considering that corn is the main

ingredient of diets for pig and avian, as broiler chicken (Yegani et al., 2013). These claims are important reasons to study approaches aimed to adequately guarantee corn production and contextually mitigate mycotoxins contents. Regarding this last aspect, Ismail et al. (2013) reported that on tomato plants seedlings, the use of *Glomus irregular* reduced mycotoxins production of *Fusarium sambucinum,* confirming that AM can interact with soil biota and contrasts pathogens. An important point that needs further information concerns not only the achievement of best nutritional qualities but also their increased shelf life in medium-long storage. In the light of these considerations, a sequential set of researches was undertaken to study if and as corn fertilized by Mycorrhizal and microbial consortium can improve yield, reduce mycotoxins, enhance physiological parameters in broilers and preserve the nutritive value after a long storage. For this purpose corn crops was fertilized by commercial mycorrhizal and microbial consortium (MICOSAT ®) and then was used to feed chicken broilers after a short or after a long storage time. The objectives were: (1) to assess the effects on yield of the bio-fertilizers on corn crop irrigated or not and on its nutritional-antioxidant characteristics; (2) to testify a such antioxidant hypothetic differential when transferred in animal productivity and biochemical traits by using the corn as main ingredient in chicken broilers diet after a short or after a long storage delay.

Materials and Methods

Experimental plan. The protocol concerned two contemporary Corn production trials (Corn-I and Corn-II) which were pursued by three animal utilisation trials, respectively two sequential with poultry broilers (Poultry-I-fresh corn, and Poultry-II long stored corn) and one with heavy pigs. The Corn-I trial was carried out at the experimental farm of the Department of Agricultural, Forest and Food Sciences of University of Turin in the western Po plain, northern Italy (44°53’N, 7°41’E, altitude 232 m a.s.l.) on corn stands (PR32G44 named *Famoso*, FAO class 600, Pioneer Hi-Bred Italia, Gadesco-Pieve Delmona, Cremona, Italy) harvested as grain at physiological maturity (black layer). The soil was a sandy-loam textured alluvium soil with a pH measured in water of 7.6 with a

115 value of 33 of the Martonne's aridity index. The sand, silt, and clay contents of the soil were 470,
116 440, and 90 g/kg, respectively at 0–30 cm depth. Two factor were evaluated: i) inoculation with a
117 microbial consortium (M; Micosat F®, CCS Aosta Ltd., Quart, Aosta, Italy) composed of three AM
118 species (*Glomus caledonium* GM24, *Glomus intraradices* GG31, *Glomus coronatum* GU53, in
119 form of spores, hyphae and root fragments), and three Plant Growth Promoting Rhizobacteria
120 (PGPR) species (*Pseudomonas fluorescens* PA28, *Pseudomonas borealis* PA29, *Bacillus subtilis*
121 BA41) with a total concentration of 106 cells · g^{-1}; ii) rainfed, R *vs.* Irrigate, I), for a total of four
122 treatments in completely randomized blocks with four replications (4 blocks with plots of 13.5 m x
123 45 m): control (C) rainfed (C_RF), control irrigated (C_IR), inoculated without irrigation (M_RF)
124 and inoculated irrigated (M_IR). Micosat F® was distributed at seeding at a rate of 12 kg ha^{-1}. Corn
125 stands were planted at a theoretical planting density of 67,000 seeds/ha. Fertilizer was applied at a
126 rate of 40 kg ha^{-1} of P_2O_5 and 55 kg ha^{-1} of K_2O immediately before planting. An additional 160 kg
127 ha^{-1} of N was top-dressed as urea at the six leaf stage. Irrigation was provided by a sprinkler
128 irrigation system on two events at a rate of about 600 m^3 water ha^{-1}. The harvesting was carried out
129 on early October using a Wintersteiger plot combine harvester. The DM yield was determined by
130 harvesting the two central rows of each plot to a length of about 25 meters, for a total of 37.5 m^2 of
131 surface collection. For the feeding trial a total of 500 kg of dried grain were harvested for each
132 treatments. Grain was immediately dried at low temperature (50°C) to 10% moisture content, and
133 conserved indoor at 18°C protected for damaged by rats and insects.
134 The Corn-II trial-was carried out at the experimental farm of the CREA-SUI of Modena in the south
135 Po plain north Italy (44°34'N, 11°2'E, altitude 41 m a.s.l.) on with the cultivar NK FAMOSO (FM)
136 class FAO 500, 127 d (Syngenta Italia S.p.A, Milano, Italy) and the cultivar DK6666 (*DK*) class
137 FAO 600, 132 d (Dekalb, Monsanto Agricoltura Italia S.p.A., Milano, Italy). The pre-sowing
138 fertilization was performed in February distributing Potassium sulfate 50% (100 kg $ha^{-1}$of K_2O),
139 Triple superphosphate 46% (92 kg $ha^{-1}P_2O_5$) and Entec 46 to release fractionated (120 kg ha^{-1} of N).
140 Glyfosate at a dose of 3 l ha^{-1} was used to clean off the soil. The two main fields were split in two

subfields and seeded at a 6.6 plants m^{-2} investment. Inoculation by Micosat F® was performed at a rate of 13 kg ha^{-1}. Check of the yields were carried out in the four sub-fields in three homogeneous sup-plots, ranging from 5080 to 15240 m^2. Whole corn harvested from each of the 4 large plot was weighed directly on field to assess the yields and, after drying, carried out with drier movable capacity 18 t, it was stored in big bags of about 900 kg. The maize cv *Famoso* was transported at the experimental farm of the University of Torino after six months from harvesting then conserved indoor, as before described, for a supplementary fifteen months period, prior to start the feeding trial, thus the whole storage period raised to 21 months.

Corn analyses On harvesting 10 corn samples were drawn, then separated in sub-samples:

a) 250 g packed in bags Nallophan, sealed and stored at - 20 ° C until the sending unit operating in CRA-FLP, Casale Monferrato, AL, Italy, for subsequent NIR and electronic nose analyses;

b) in Corn-I experiment an aliquot of the grain was dried and analysed for Crude Protein (CP), Ether Extract (EE), Ash, Starch, Neutral Detergent Fiber (NDF) and Acid Detergent Fiber (ADF) according Borreani and Tabacco (2014) and for mycotoxins compounds: Aflatoxin B_1 (AFB1), deoxynivalenol (DON), Fumonisin B_1 (FB_1) Ochratoxin A (OTA), T-2 toxin (T-2), and Zearalenone (ZEA) analysed by ELISA test.;

c) in Corn-II experiment 250 g oven-dried at 60 ° C to constant weight under vacuum packed in bags until ground in a Cyclotec mill (Tecator, Herndon, VA, USA) to pass a 1 mm screen for the determination in duplicate of the dry matter (DM), CP, EE, A, phosphorus and NDF, ADF and Lignine according Van Soest et al.(1991); in the Famoso cultivar a fatty acid profile was determined as reported in Della Casa et al. (2010);

d) in Corn-II experiment, 500 g, dried in oven at 60 ° C to constant weight, and then vacuum packed and transferred to the laboratory of the CRA-MAC, Bergamo, Italy, for prediction of constituents via NIRS spectra equations, as described by Brenna and Berardo (2004) for these constituents: starch, lutein, zeaxantin and carotenoids.

167 The antioxidant activity of the Corn-I and of the diets of Poultry-I experiment was determined
168 according the DPPH method as reported by Prola et al. (2011) and expressed as Trolox Equivalent
169 100 g^{-1}.
170 The electronic Nose (EN, PEN2 model, Win Muster AirSense Analytics GmbH, Schwerim,
171 Germany) as described in Torri et al., 2013 and NIRS (Model LSP 350-2500P LabSpec Pro
172 portable spectrophotometer, ASD; Analytical Spectral Devices, Inc.; Boulder, CO, equipped to
173 collect spectra from 350 to 2500 nm) were used to qualitatively ascertain the discriminability of the
174 main factors: Micosat, Irrigation, Corn cultivar. Chemometrics was performed by WinISI II version
175 1.04 spectral analytical software (InfraSoft International LLC, State College, PA, USA), using
176 cross-validated modified partial least square equations in calibration of fixed effects; prediction
177 capacity was then evaluated with the 1−VR parameter (Maximum is 1), where VR is the ratio of
178 unexplained variance, and with the relative prediction deviation (RPD, useful if>2) that codifies the
179 reliability over new samples. Detailed procedures and test for differences are discussed in
180 Concollato et al. (2015).
181
182 *Poultry Trials* The feeding trials were performed at the experimental farm Department of
183 Agricultural, Forest and Food Sciences of Turin University. In Poultry-I trial 240 one day-old
184 female broiler chicks (Ross 308) were purchased from a commercial hatchery and randomly allotted
185 to 24 pens (10 chicks per pen). The feeding program consisted of a starter period until d 21 of age
186 and a finisher period until d 33 of age. The diets based on a concentrate (400 g kg^{-1} diet) whose
187 composition differed for the two periods, and corn (600 g kg^{-1} diet). The ingredient composition and
188 analysed chemical contents of the diets for the starter and finisher periods were given in Table 1. A
189 2×2 factorial experiment was designed to evaluate the effect of the type of corn used to complete
190 the concentrate in diets formulation: control not irrigated (C_RF), control irrigated (C_IR),
191 inoculated without irrigation (M_RF) and inoculated irrigated (M_IR). The pen was considered as
192 experimental replicate and each treatment had 6 replicates. Rearing temperature was maintained

within the thermo neutral zone. Feed and water were provided for *ad libitum* consumption. For each pen, body weight and feed intake were recorded on day 1, on day 21 and on day 33. Feed conversion ratio and body weight gain were calculated for the starter period, the finisher period and the entire period of trial.

In Poultry-II trial 120 one day-old female broiler chicks (Ross 308) were purchased from a commercial hatchery and randomly allotted to 12 pens (10 chicks per pen). The feeding program consisted of a unique period till d 33 of age (Table 1). At slaughtering and on the carcasses the same parameters were measured as in Poultry-I

Pig Trial

The trial was performed by using 40 crossed pigs Duroc x Large White Italian, with starting live weight of 52 kg, divided into two experimental groups Control (C) and Micosat Treated (M) each consisting of 10 castrated males and 10 entire females, in their turn subdivided in two boxes according to the live weight to avoid as much as possible the competition at the trough. Diets were formulated (Tab. 1) according to the live weight, with the highest possible content of corn (over 80%) in order to highlight any differences between the two treatments. The animals were individually weighed every 28 days and contextually were calculated the feed:gain ratio. The feed was wet fed with water: feed ratio of 2.5: 1 in two daily meals for 13 meals per week. The dayli ration was scheduled as follows: initial ration 1.68 kg / head / d, increase of 140 g / head / d. for 8 weeks, increase of 50 g / head / d for 5 weeks up to 3.05 kg/head/d, maximum dose of administration.

The animals were slaughtered at 165 kg of live weight. At slaughtering the hot carcass weight, the lean meat content of carcasses by Fat O Meter , the weight of the thighs and the pH 45' after slaughter of the muscles semimembranosus and biceps femoris were recorded. After 24 hours weight of the cooled thighs, the pH on the before mentioned muscles and the color of semimembranosus muscle with colorimeter Minolta CR-300 according to the CIELab system with

illuminant C.

Blood biochemical In Poultry-I trial samples of blood from 12 broilers per group were collected via wing vein puncture before slaughtering, by Veno Jet tube (Terumo, Leuven, Belgium), with a 19-Gauge pin according to Good Veterinary Practices. The blood was allowed to clot and was then centrifuged at 3000 rpm for 10 min at room temperature. The serum was then separated and stored at -70° C until analysis. The ILab Aries analyzer (Instrumentation Laboratory, Milan, Italy) was used to analyze serum levels of cholesterol, triglycerides, liver enzyme alanine aminotransferase (ALT), albumin, and glucose. Serum antioxidants capacity were evaluated by the anti-ROMs test (Diacron s.r.l., Grosseto, Italy). This method exploits the ability of antioxidants to reduce ferric iron to ferrous iron, giving rise to a red-purple coloration, due to the reaction with αα'-dipyridyl. Color intensity increases proportionally according to the quantity of iron reduced by the antioxidants present in the sample. This test enables discrimination between the concentration of the so-called "fast antioxidants", determined at the start by the instrument, i.e. those which are fast-acting, such as Vitamin C or Vitamin E, and the concentration of "slow antioxidants", subsequently determined by the instrument, such as thiol-SH groups, uric acid, polyphenols and anthocyanins. Results are expressed in μEq of reduced iron/liter using ascorbic acid as a standard according to Giongo et al. (2011).

Slaughter procedures and analyses At the end of the trials, two chicks per pen were randomly selected and individually weighed. Birds were slaughtered by stunning and exsanguinated. The carcasses were plucked, eviscerated and weighted without head, neck, feet, and abdominal fat. At 24 h after slaughter, breast and thigh muscles pH were measured with a Crison MicropH 2001 (Crison Instruments, Barcelona, Spain) using a combined electrode. At the same time, the colour of meat was measured on breast skin, breast muscle and thigh muscle using a portable Minolta CR-331C Colorimeter (Minolta Camera, Osaka, Japan). Colorimetric results were expressed in terms of

lightness (L*), redness (a*) and yellowness (b*) in the CIELAB colour space model (CIE, 1976). Samples of the breast muscle were taken in the Poultry-I, prepared in ethanol medium according (Masoero et al. 2007; Concollato et al. 2015) and then stored in cold dark until the aerated samples were examined by NIRS.

Statistical analyses Corn yields were analyzed by the Friedman's test for paired observations (StatBox software vs 6.5, Grimmer Logiciel, Paris). Several mono and bi-factorial ANOVA models using PROC GLM by SAS V. 9 software (SAS Institute, Cary, NC, USA) evaluated the effect of the factors of the realized data, within each corn trial, as well within the two subsequent poultry trials. Chemometrics of the NIRS vibrational spectroscopy and of the electronic nose traces was performed by exporting the digital data to the WinISI II vers.1.04 software (Infrasoft International, LLC) and performing qualitative calibration / cross-validation Partial Least Squares (PLS) processes on the dummy discriminative variables. A similar PLS bi-component model was applied to discriminate the Micosat vs. Conventional condition on the basis of the DPPH test values of the corn and of the derived diets.

Results and Discussion

Corn trials

In Corn-I the yield was on average 12.63 t ha^{-1} near 50% superior to Corn-II (8.47 t ha^{-1}, Tables 2 and 3). The different quantity of N (160 vs. 120 kg ha^{-1}) can explain the results, but the basal fertility must be a main contributing cause. The microbial fertilisation significantly improved the yield in both trials, with different amplitude according to the farm, the water supply, and the cultivar; in fact the raising was +4% in rainfed and +6% in irrigate conditions, pertinent to Corn-I. In Corn-II an average significant improvement of the Micosat raised +19%, but in the cultivar *Famoso* the increment was +8%, while in the cultivar *DK* a very high value (+29%) was recorded. Under a semi-field conditions Berta et al. (2013) have observed massive increases vs. the negative control, ranging from +34 to +53% for AM and/or *Pseudomonad*. In field surveys (Masoero and

Giovannetti, 2015) observed a significant (P< 0.02) growth effect for the Micosat on the stalk weight with an improvement of +14%, while a +12% increase in the ear was not significant (P< 0.20), and the total green mass yield (+12%) was nearly significant (P< 0.07). Celebi et al. (2010) confirmed that AM inoculation increased the corn silage yield, as green and dry matter, in a whole irrigation regimen but even under restricted water; they observed an increase in leaf and stem ratios, the green parts, but a decrease in ear ratios, that looks similar with the cited silage features but different from the positive results of the present work on mature grain corn. Other works recovered strong grain mass increment (Berta et al., 2013) or green mass increment at an early stage of growth, with *Famoso* cultivar too (Zoppellari et al., 2014).

Qualitative features on the bio-fertilized corn involved several aspects. Preliminary assessment of the biodiversity were provided by the NIRS and the EN instruments (Table 4). In Corn-I experiment the irrigation effects were absent in NIRS radiation and EN sensors, while mild pronouncements (1-VR= 0.38 and 0.42) emerged for Micosat factor; however when the relationships were studied separately, the irrigate condition appeared more favorable to an enhancement of the Micosat factor in the NIRS radiation (0.67). In Corn-II experiment the examination of the integer grain as well as of the dried flour ensured a perfect discrimination of the two cultivars (0.97 and 0.99, respectively) that appeared also already high according the EN analyses (0.83). The Micosat factor instead was overall lowered (0.20 and 0.42, respectively for NIRS and EN), but when considered separately according the cultivar, the discriminative values raised more in the *DK* cultivar (0.57 and 0.82) than in the *Famoso* (0.39 and 0.43). After milling the NIRS discriminative ability for the Micosat factor became considerable higher reaching levels of 0.73 and 0.85 for the two cultivars.

The announcement of biodiversity of Corn-I, both in spectroscopy and aromatic profiles, was then corroborated by a different composition in primary and secondary compounds (Table 2). Protein content and ether extract were increased in Micosat treated corn by some 2% and 4%, the report on protein agreed with Sabia et al (2015). But more important were the decrement in the mycotoxin

297 complex as Fumonsin (-72%) and DON (-44%), while in the ZEA toxin emerged an interactive
298 effect with the system of crops watering. Rainfed regimen was clearly unfavourable to DON
299 (+125%) and ZEA (+15%) somewhat reducing OTA (-28%). DPPH score measures the oxidant
300 power of the matrix, a reciprocal of the antioxidant values: Micosat reduced DPPH by -47% in the
301 Corn which was reduced to -19% when the corn meal was included in the complete poultry diets.
302 Important to observe that the feed preparates after milling, mixing and storing increased their
303 oxidant power of some +36% the Control and +67% the Micosat groups. On behalf to characterize
304 the Mycorrhizal corn a partial least square (PLS) relationships was developed to discriminate the
305 Micosat treated condition providing the DPPH values of the Corn-final Feed couples:
306 Conventional (score 1) vs. Micosat (score 2) = 6.799 - 1.197 DPPH_Corn -3.546 DPPH_Feed
307 (R^2=0.89). Cross validated reclassification was 100%. The Figure 1 illustrates the bi-plot of the
308 DPPH antioxidant values of the corn (X axis) and of the derived feeds (Y axis), and suggests how to
309 testify the two origin of the corn, namely Mychorrizal vs. Conventional.
310 Recently in durum wheat, treated by Micosat, Migliore et al (2015) recovered significant rise in
311 antioxidant compounds (FRAPS +64%; ABTS + 24%) as related with higher contents of
312 carotenoids (+43%) and Phenols (+231%).
313 In Corn-II unfortunately we had not applied DPPH oxidant measurements of grain; moreover the
314 Cultivar factor was very prominent as revealed from vibrational and aromatic profiles, but very
315 often it was interacting with the Micosat effects. In fact after the bio-fertilization treatment, protein
316 and starch appeared whether decreased and increased respectively, but ether extract level did not
317 change; marked increases were in charge of the neutral cell wall constituents of the grain (NDF
318 +13%) while lignin presence was reduced by some 32%. Within the secondary compounds
319 noticeable the reduction in Lutein (-2%) a compound present in a double quantity vs. the average
320 value of 13 mg kg^{-1} - ranging between 1.03 and 21.00 mg kg-1 dm - in the EUMLCC panel
321 (Berardo et al., 2009) and already noticeable, because this is the first time observation, the increase
322 in mono unsaturated Palmitoleic acid (+34%) and a decrease of the saturated Stearic acid (-3%).

This last fact may be hypothetically related to a superior protonic availability in the corn plant as a consequence of the AM network; Masoero and Giovannetti (2015) observed the pH in vivo decrease, in Maize as well as in vine (Masoero et al., 2015) and in other four species, except Kiwi (unpublished data).

Poultry trials

Broilers mortality was minimal, limited to one chicken in both trials. Feed intake, conversion rate and growth performance in Poultry-I trial were very similar in all four groups fed C_RF, C_IR, M_RF and M_IR diets from day 1 to day 33 (Table 4). Already, no statistically significant effects emerged in carcass traits or in carcass colour examination except two interactions for yellowness (P=0.010) and Hue variables (P= 0.026). In this study, the growing data of chickens are consistent with previous broiler feeding trials reported in literature (McNaughton et al., 2008; Yegani et al., 2013). The lack of significant effects in both starter and grower periods indicates that the performances of birds fed the different diets are comparable. In view of the results reported about corn grain quality and its mycotoxins content these outcomes were expected. Presumably, it is possible to assume that although Micosat had a significant a effect on both percentage of CP and EE, the magnitude of the range obtained for these results is little (8.38 to 8.67 for CP and 3.29 to 3.50 for EE), having no practical relevance in the formulation of a chicken diet. Similarly, also mycotoxins results of harvested grains were affected by experimental arrangement but they were largely under the limits fixed for products intended for animal feeding (European Commission, 2013), without any perceivable impact on growth performances of chickens. Both chicken carcass and meat quality were generally not influenced by imposed treatments with the exception of some colorimetric variables. Reasons for these significances are not clear. In *Longissimus thoracis* muscle of Piemontese cattle steers fed Mycorrhized corn (Tassone et al in prep) observed a significant increment in the ultimate muscle pH (+2%) correlated with color opacification, which affected the chroma (-9%), mainly reducing the yellow component (b: -13%); in these cattle,

already the water holding capacity, under compression, was significantly improved. In Poultry-I meat an interesting difference in muscle characteristics was foreseen by the NIR spectroscopy of the ethanol preparates. In fact the 1-VR discriminative values for the effects pertinent to Micosat and Irrigation factors were 0.56 and 0.46 respectively. These values were not only statistically significant, but also meaningful about differences in texture properties. In cattle veal Brugiapaglia et al. (2011) reported a strong correlation of the NIRS test with the prediction of texture (1-VR= 0.91) in a large panel experiment. Moreover Concollato et al. (2016) in salmon stunned by CO reported high 1-VR relationships of the spectra of ethanol samples with the maximum *rigor mortis* time (0.70) and with the tenderness sensory scores (0.54). In the present experiment unfortunately no texture analysis was carried out, but an effect probably positive of the mycorrhized corn on the texture traits, may be furtherly explored, even in other meat species.

Totally different was the growth model observed in Poultry-II trial (Table 5). The feed containing the mychorrized corn was consumed as normally attended, and realized in the Poultry-I trial, on the contrary the control corn was intaken with a -26% rate. So an enormous body weight difference , namely +26,7% for the mycorrhizal group distinguished the broilers along the growth. The explanation of this different palatability must be attributed to a very different shelf life which was maintained for 21 months in mycorrhized corn but which has disappeared in the conventional corn. The feed conversion index was very similar in both groups. As in the Poultry-I both relative composition of chicken carcass and meat quality traits were not influenced by the feed origin. Biochemical traits of poultry in Poultry-I (Table 6) opened a very different scenario beyond the indifferent zootechnical performances. All the biochemical parameters were significantly improved by the Micosat corn intake. In particular were modified in a favourable sense: fast antioxidants (+20%); slow antioxidants (+14%); uric acid, an antioxidant in poultry (+22%); ALT, alanine amino transferase (-8%); Cholesterol (-5%); Triglycerides (-4%); Albumin (+2%); Glucose (-1%).

Pig trial. In the Pig trial no effect was apparent on live or slaughter or color performances (Table 7) while the NIRS test on ethanol muscle preparates enhanced an elevated discriminative 1-VR value (0.77), which can be connected to the above finding for meat in Poultry-I trial.

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Conclusion

The corn trials have shown capabilities for the microbial fertilisation at a real scale, that could be a key for sustainability in front of the mineral support of the intensive maize crop. It must put in evidence that official cultivar-tester fear to contaminate the soil with AM and microorganisms, that will survive and could bias the test trials in the next years; furthermore, multiple interactions between the soil biota, the bio-fertilizer biota and the genetic capabilities of the cultivars can display sparse results. The application of Micosat F® can obstacle mycotoxin advances and can enhanced the levels of several secondary metabolites. Environmental condition and modality of AM inoculation could module both primary and secondary metabolites. The general trend verified in these works is a reduction of the oxidant power of the corn and of the derived feeds, which could be objectively testified, and that this antioxidant energy can be transferred in the animal health and welfare but even more in a shelf life dramatic improvement.

In conclusion the harmful and unproductive effect on the poultry feeding has been excluded, even better the functional conditions and livability have been improved as well as in the short storage and even more in long storage period. AM and microbial fertilization may be a strategic key for the sustainability and usefulness of maize crop.

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MIPAAF programma AMICO

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Table 1. Ingredients of the diets for the chicken broilers in the two Poultry and in the Pig trials and chemical composition .

	Poultry-I		**Poultry-II**	**Pig** [3]		
	Starter[1]	**Finisher**[2]	**Unique**[1]	**Live Weight kg**		
Items	**1-21 d**	**22-33 d**	**1-33d**	**40-80**	**80-120**	**120-165**
Ingredients (%)						
Corn meal	60	60	60	79.5	84	88.5
Soybean meal 50%	33.32	31.21	32.27	17	12.5	8
Vegetable fat	2.5	5	3.66	0	0	0
Dicalcium phosphate	1.3	1.24	1.27	1.12	1.14	1.15
Calcium carbonate	1.15	1.12	1.13	1.14	1.15	1.17
Sodium chloride	0.23	0.22	0.23	0.4	0.4	0.4
Sodium bicarbonate	0.13	0.15	0.14	0	0	0
DL-methionine	0.39	0.25	0.32	0.02	0.01	0
L-lysine	0.2	-	0.2	0.24	0.22	0.2
Threonine	0.08	0.11	0.08	0	0	0
L-Thriptophan	0	0	0	0.03	0.03	0.03
Vitamin and mineral premix[1]	0.5	-	0.5	0.55	0	0

Vitamin and mineral premix[2]	-	0.5	-		0.55	0.55
Choline chloride	0.1	0.1	0.1	0	0	0
3-phytase; (E-300; natuphos bio/G500)	0.1	0.1	0.1	0	0	0
Crude Protein (g kg^{-1} DM)	224	227	226	163	144	125
Ether Extract (g kg^{-1} DM)	49	49	49	33	34	33
Ash (g kg^{-1} DM)	65	68	66	44	42	41

[1]

[3] Provided vitamins and minerals per kilogram of feed (Istituto delle Vitamine S.p.A., Segrate, MI, Italy): vitamin A, 15,000 IU ; vitamin D3, 2,000 IU; vitamin E, 50 mg; vitamin K3, 2.5 mg; vitamin B1, 2 mg; vitamin B2, 5 mg; vitamin B6, 4 mg; vitamin B12, 0.03 mg; biotin, 0.15 mg; niacin, 25 mg; D-pantothenic acid, 15 mg; choline chloride, 350 mg; Mn, 25 mg as manganous oxide; Fe, 150 mg as ferrous sulfate; Cu, 15 mg as copper sulfate; Zn, 100 mg as zinc oxide; I, 1.5 mg as calcium iodide; and Se, 0.1 mg as sodium selenite.

Table 2. Results of the Corn-I trial (Torino) in the four groups: Control Rainfed (C_RF), Control Irrigate (C_IR), Micosat Rainfed (M_RF) and Micosat Irrigate (M_IR).

	Means of groups					***P >F***		
	Control Rainfed (C_RF)	**Control Irrigate (C_IR)**	**Micosat Rainfed (M_RF)**	**Micosat Irrigate (M_IR)**	**RMSE**	**Micosat**	**Irrigation**	**Interaction**
Grain yield (t DM ha^{-1})	12.1	12.5	12.6	13.3	1.42	**	*	
Moisture at harvest (g kg^{-1})	167	169	163	166	0.131			
Crude Protein (CP, g kg^{-1} DM)	83.8b	85.8ab	86.7a	86.5a	3.4	*		
Ether Extract (EE, g kg^{-1} DM)	32.9b	33.5b	34.2a	35.0a	17.0	**		
Ash (g kg^{-1} DM)	11.2	11.6	11.4	11.6	0.3			
Starch (g kg^{-1} DM)	689	710	708	703	38.6			
Neutral Detergent Fiber (aNDF, g kg^{-1} DM)	118	110	113	118	8.0			
Acid Detergent Fiber (ADF, g kg^{-1} DM)	28	27	28	29	1.7			
DPPH Corn (Trolox Equivalent 100 g^{-1} DM)	0.71c	1.05a	0.62d	0.90b	0.014	***	***	**
DPPH poultry diets (Trolox Equivalent 100 g^{-1} DM)	1.24b	1.41a	1.11c	1.11c	0.025	***	***	***
Aflatoxin (AFB_1, ppb DM)	<1.00	<1.00	<1.00	<1.00	-			
Zearalenone (ZEA, ppb DM)	35.5b	26.94c	36.12a	35.34b	3.35	***	***	**

Fumonisin (FB_1, ppm DM)	1.57a	1.32a	0.76b	0.92b	0.17	***	
Deoxynivalenol (DON, ppm DM)	0.17a	0.06c	0.10b	0.06c	0.02	*	***
Ochratoxin (OTA, ppb DM)	1.53b	2.65a	2.41a	2.86a	0.43		*
T-2 toxin (T-2, ppm DM)	< 0.025	< 0.025	< 0.025	< 0.025	-		

Means followed by the same letter are not significantly different based on Tukey's HSD test at P < 0.05; *P>F*: * <0.05; ** >0.01; *** < 0.001

Table 3. Results of the Corn-II trial (Modena) in the four groups: Control Famoso (C_FM), Control Dekalb (C_RK), Micosat Famoso (M_FM) and Micosat Dekalb (M_DK).

	Means of groups					***P >F***		
	Control *Famoso* (C_FM)	**Control *Dekalb* (C_DK)**	**Micosat *Famoso* (M_FM)**	**Micosat *Dekalb* (M_DK)**	**RMSE**	**Micosat**	**Cultivar**	**Interaction**
Moisture at harvest (g kg^{-1})	141b	224a	144b	205a	13		***	
Grain yield (t DM ha^{-1})	8.12b	6.77c	8.81a	8.77°	0.65	**		
Crude Protein (CP, g kg^{-1} DM)	84.8c	91.9a	85.6c	88.7b	1.8	**	***	***
Ether Extract (EE, g kg^{-1} DM	38.0a	38.3a	38.1a	38.1a	0.82			
Starch (g kg^{-1} DM)	700a	696c	701a	698b	1.2	*	***	
Crude fiber (CF, g kg^{-1}DM)	23.3a	20.7b	22.9a	21.0b	6		**	
NDF (g kg^{-1} DM)	126.2a	92.5b	126.0a	126.1a	1.7	***	***	***
ADF (g kg^{-1} DM)	29.0b	33.7a	34.2a	27.2b	4.6			***
ADL (g kg^{-1} DM)	12.1b	18.7a	10.5b	12.9b	4.6	**	**	
Phosphorus (g kg^{-1} DM)	1.43 b	1.53 a	1.43 b	1.50 a	0.03		***	
Carotenoids (g kg^{-1} DM)	24.0a	11.0c	22.9a	13.9b	0.95		***	*
Lutein (mg kg^{-1} DM)	24.5a	22.8c	24.0b	22.3d	3.78	**	***	

Zeaxantin (g kg^{-1} DM)	6.26d	9.18a	7.49c	8.12b	7.19		***	***
Fatty Acids %								
Palmitic 16:0	15.4a	14.9b	15.8a	15.2a	0.37		**	
Palmitoleic 16:1	0.14 ab	0.10b	0.17a	0.15a	0.04	*		
Stearic 18:0	2.49b	2.56a	2.45b	2.47b	0.04	**		
Oleic 18:1	30.0a	26.5b	30.2a	25.9b	0.63		***	
Linoleic 18:2	49.9b	54.0a	49.3c	54.4a	0.38		***	*
α-Linolenic 18:3	1.52a	1.43b	1.55a	1.46b	0.05		**	
Eicosenoic 20:1	0.27	0.26	0.24	0.26	0.03			
Sum of saturated FA	18.0a	17.5b	18.3a	17.7b	0.38		**	
Sum of unsaturated	81.9ab	82.4a	81.6b	82.2a	0.38		**	

RMSE: root mean square error;

Means followed by the same letter are not significantly different based on Tukey's HSD test at $P < 0.05$; *P>F*: * <0.05; ** >0.01; *** < 0.001

Table 4. NIRS and Electronic Nose results on the corn grain in the two Corn-I and Corn-II Trials,

			Codes		NIRS -			EN		
Experiment	**NIRS Instrument \ Corn preparate Laboratory**	**Samples**	**Factor**	**Contrast**	**No.**	**1-VR**	**RPD**	**No.**	**1-VR**	**RPD**
Corn-I Torino	ASD	All	*Micosat F*	C_1\M_2	45	0.38a	1.3	38	0.42a	1.3
	Whole grain	All	*Irrigation*	IR_1\RF_2	40	0.03b	1.0	45	0.02b	1.0
		Irrigate	*Micosat F*	C_1\M_2	37	0.67a	1.7	21	0.38	1.2
		Rainfed	*Micosat F*	C_1\M_2	43	0.13b	1.1	22	0.44	1.3
Corn-II Modena	ASD	All	*Cultivar*	FM_1\DK_2	262	0.97a	6.1	33	0.83a	2.4
	Whole grain	All	*Micosat F*	C_1\M_2	291	0.20b	1.1	35	0.42b	1.3
	Casale	Famoso (FM)	*Micosat F*	C_1\M_2	139	0.39b	1.3	22	0.43b	1.3
	Monferrato	DK6666 (DK)	*Micosat F*	C_1\M_2	142	0.57a	1.5	20	0.82a	2.4
	FOSS 6500	All	*Cultivar*	FM_1\DK_2	72	0.99a	12.8			
	Dry flour	All	*Micosat F*	C_1\M_2	75	0.69b	1.8			
	Bergamo	Famoso (FM)	*Micosat F*	C_1\M_2	38	0.73	2.0			
		DK6666 (DK)	*Micosat F*	C_1\M_2	37	0.85	2.0			

a > b on columns; P<0.05. 1-VR: 1-variance ratio in cross-validation mode; RPD = standard deviation / standard error in cross validation.

Table 5. Results of the Poultry-I trial in the four groups: Control Rainfed (C_RF), Control Irrigate (C_IR), Micosat Rainfed (M_RF) and Micosat Irrigate (M_IR).

			Means of the groups					***P>F / 1-VR***		
Stage	**Variables**		**C_RF**	**C_IR**	**M_RF**	**M_IR**	**RMSE**	**Micosat**	**Irrigation**	**Interaction**
Live	Final Body Weight (33 d)	g	1804.7	1630.1	1858.3	1792.6	39.4			
(No.=12 per group)	Feed Intake	g	2552.8	2309.9	2619.8	2532.5	53.6			
	Feed Conversion Ratio	$g\ g^{-1}$	1.45	1.46	1.44	1.45	0.01			
NIRS of ethanol preparate	Discrimination of main effects	1-VR						0.56	0.46	
Biochemical parameters	Fast antioxidants[1]	µEq of $Fe^{+++}l^{-1}$	191.4	198.6	232.5	233.7	0.92	***	***	***
(No.=10 per group)	Slow antioxidants[2]	µEq of $Fe^{+++}l^{-1}$	966.2	1008.7	1127.3	1128.2	1.21	***	***	***
	Uric Acid	$mg\ dl^{-1}$	3.80	4.11	4.74	4.93	0.13	***	***	
	ALT, alanine amino transferase.	$U\ l^{-1}$	33.82	32.10	30.72	29.61	0.93	***	***	
	Cholesterol	$mg\ dl^{-1}$	176.4	172.2	165.3	164.5	0.73	***	***	***
	Triglycerids	$mg\ dl^{-1}$	52.6	51.9	50.5	49.6	0.77	***	**	
	Albumin	$g\ dl^{-1}$	2.03	1.99	2.05	2.03	0.08			
	Glucose	$mg\ dl^{-1}$	221.7	221.5	220.0	220.1	0.93	***		

[1] First concentration of antioxidants (µEq of Fe^{+++}/l) determined by anti-ROMs test

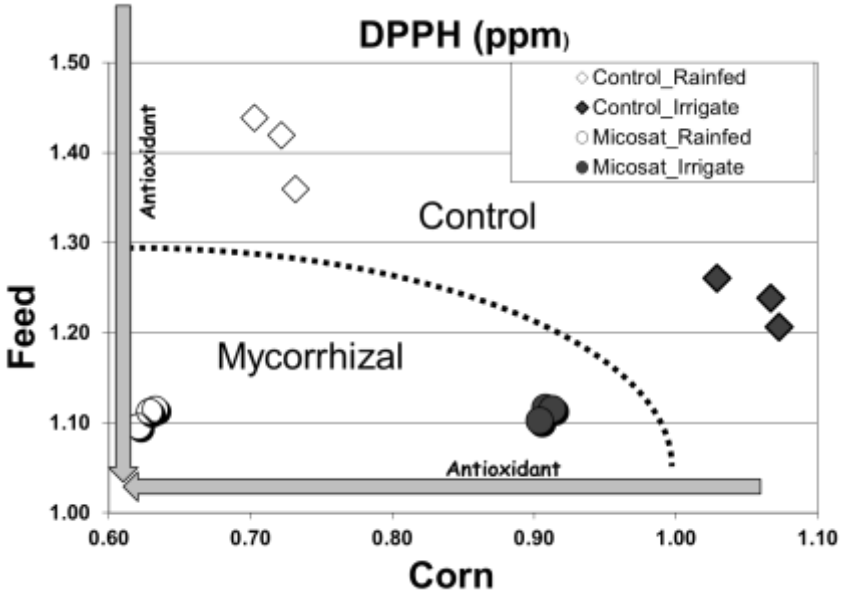
[2] Second concentration of antioxidants (µEq of Fe^{+++}/l) determined by anti-ROMs test

Table 6. Results of the Poultry-II trial in the two groups: Control *Famoso* (C_FM) vs. Micosat *Famoso* (M_FM) .

		Means of the groups			***P>F***
Variables		**C_FM**	**M_FM**	**RSME**	**Micosat**
Final Body Weight (33 d)	g	1354.1	1715.8	52.6	***
Feed Intake	g	2372.5	2981.5	105.2	***
Feed Conversion Ratio	$g\ g^{-1}$	1.81	1.78	0.04	

Table 7. Results of the live stage Pig trial in the two groups: Control *Famoso* (C_FM) vs. Micosat *Famoso* (M_FM) (N=40).

			Means of the groups		
	Variables		**C_FM**	**M_FM**	**RSME**
Live stage	Final Body Weight	kg	169	166.2	1.98
	Average Daily Gain	g d^{-1}	840	820	572
	Feed Conversion Ratio	g g^{-1}	3.18	3.26	0.091
NIRS of ethanol preparate	Discrimination of Micosat effect	1-VR		0.77	


DPPH (ppm)
Control_Rainfed
Control_Irrigate
Micosat_Rainfed
Micosat_Irrigate
Antioxidant
Control
Mycorrhizal
Antioxidant
Feed
Corn
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1.40
1.30
1.20
1.10
1.00
0.60
0.70
0.80
0.90
1.00
1.10

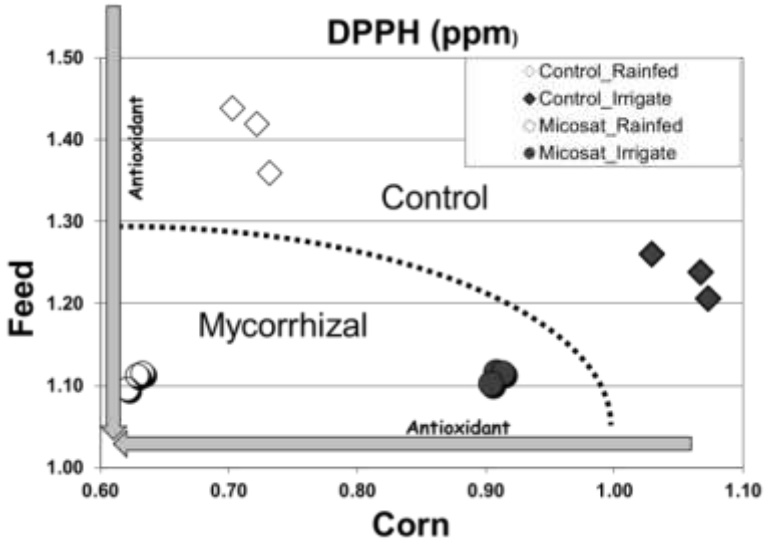

Figure 1. Bi-plot of the DPPH oxidant-antioxidant values of the corn and of the derived feeds.

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