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Inoculation of Corn Seedlings with *Piriformospora indica* Influences Grain Biomass Yield, Forage Quality, Rumen Degradation Kinetics and Fourier Transformed Infrared Spectroscopy Molecular Structures

Research Article

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ABSTRACT

Piriformospora indica (*P. indica*) as a root growth promoting endophyte may improve corn grain production and forage quality in ruminant under semi-arid climate condition. The aim of current study was to evaluate the influence of corn seedling- *P. indica* endophyte inoculation on grain and forage biomass yield, nutritive value and Fourier transformed infrared spectroscopy (FTIR) of molecular structures. Corn seedlings were inoculated with *P. indica* and were grown under natural condition and were compared with control group (12 experimental boxes per each treatment). Half of the experimental boxes from each treatment selected to make corn silage and the other half remained for grain biomass yield. Inoculation with *P. indica* increased grain biomass yield (P=0.05) but had no impact on chemical composition except for total carbohydrate content which tended to be greater for *P. indica*-inoculated group (P<0.10). Leaf to stem ratio decreased (P=0.04) and ether extract increased in silages from treated corn compared with control group (P=0.04). *In situ* ruminal dry matter and organic matter degradability at 24 h of ruminal incubation were greater in *P. indica* corn grain from inoculated samples compared with control grain samples (P=0.02). Silage from *P. indica* group had greater *in vitro* rate of gas production (P<0.05) and tended to have greater volume of gas produced until 24 h of incubation (P=0.06). The FTIR ratio between amid I to amid II and amid II to lignin area tended to decrease in silages from *P. indica* group compared with control group samples (P=0.09). The principle component analysis differentiated the FTIR area related to carbohydrate between experimental groups. In conclusion, under semi-arid climate condition inoculation of corn seedlings with *P .indica* may improve the feeding value of corn seed and silage in ruminants.

KEY WORDS corn grain, corn silage, FTIR, nutritive value, symbiosis.

INTRODUCTION

Corn grain and corn silage are widely used in ruminants feeding due to their high carbohydrate content, good palatability and great energy value (NRC, 2001). Low digestibility of forage is mainly reflected in low voluntary intake and low energy supply that could limit milk and meat production of dairy cattle (Jung and Allen, 1995). However in the arid and semi-arid areas, it is a challenge to produce high quality grain and forage crops due to the abiotic stresses which restrict growing potential of crops (Waller *et al.* 2005). Increased plant productivity and crop improvement might therefore, rely on high chemical input and which is made at the expense of a huge negative impact on the environment (Chapin Iii *et al.* 2000; Parmesan and Yohe, 2003). An alternative to the use of chemicals is the application of

genetically-modified crops, although these also exhibit their own drawbacks and controversies (Mannion and Morse, 2013). For this reason, abiotic-stress tolerance can be evoked in crops by the exploitation of worldwide abundant endophytic arbuscular mycorrhiza fungi, which live in reciprocally beneficial relationships with 80% of land plants (Newman and Reddell, 1987).

Piriformospora indica (*P. indica*), a root endophyte fungus belonging to the Sebacinales in the Basidiomycota shows the increase in nutrient uptake and utilization in plant. The *P. indica* has a unique property and can be easily cultured axenically on a variety of medium (Varma *et al.* 1999). *P. indica* has a wide host range, including members of the *Brassicaceae*, which has been found to be involved in promoting the growth of various plants, including cereal crops such as rice, wheat, barley and corn, as well as many Dicotyledoneae (Verma *et al.* 1998; Peškan-Berghöfer *et al.* 2004; Ghabooli *et al.* 2013).

In the study of Kumar *et al.* (2009), Zhang *et al.* (2018), Wu *et al.* (2018) and Hosseini *et al.*(2018) corn plant colonized with *P. indica* increased the corn resistant to biotic and abiotic stresses and simultaneously also the biomass grain yield. None of those studies investigated the effect of corn seed inoculation with *P. indica* on its feeding value in ruminants. To our knowledge, only one research has been done on the influence of *P. indica* on the quantitative and qualitative characteristics of plants for use in the livestock feeding. This research showed that hay of alfalfa seedlings inoculated and co-inoculated with root growth promoting microorganisms had improved nutritional value compared with hay from non-treated alfalfa seedlings, and co-inoculation was the most effective, however, changes were relatively minor (Jafari *et al.* 2018). Molecular structures of feed cannot be determined using traditional wet chemical analysis, while Fourier transformed infrared spectroscopy (FTIR) can be used to reveal these structures including primary protein molecular structures (e.g. amide I, amide II), secondary protein structures (e.g. α-helix and β-sheet), lipid and carbohydrate molecular structures (Yu, 2005).

The hypothesis of current study was that *P. indica*-inoculated corn plant may have improved corn grain and corn silage feeding value in ruminant. Therefore, the aims of this study were to use chemical composition analysis, *in situ* ruminal degradation, *in vitro* gas production and FTIR to evaluate the effect of corn seedlings inoculation with *P. indica* on corn grain and silage nutritive value in ruminants.

MATERIALS AND METHODS

Culture of root endophyte fungus, *P. indica*

P. indica was cultured and maintained on CM (complex medium) (Pham *et al.* 2008) at 24 ˚C for 4 weeks.

This medium contains mineral salts, microelements, and also complex components such as peptone, yeast extract and casein hydrolysate (Pham *et al.* 2008). Former researches revealed that CM is one of the best mediums for *P. indica* culture. For solid medium, 15 g l^{-1} agar was used. The spore suspension was prepared (Ghabooli *et al.* 2013).

Co-culture of *P. indica* with corn seedling

For plant inoculation, corn seeds of the cultivar "640" were surface-sterilized with 70% ethanol (v/v) for 30 s followed by 6% sodium hypochlorite (NaCl) for 10-15 min. Then, they were rinsed in water, and were germinated for 2-3 days at 18/04/2014. Corn seedlings were inoculated by immersing in the spore suspension solution (adjusted to 5×10^5 spores per mL) with gentle shaking for 1 h. The other group of seedlings (control) was dipped in sterile water only. Inoculated and non-inoculated corn seedlings were later transferred into boxes under natural weather condition at Malayer University experimental farm (34.3020˚ N, 48.8146˚ E), filled with normal soil at date of 04/05/2014, two weeks after inoculation. The experiment was arranged in a completely randomized design with two fungus treatments (inoculated and non-inoculated) in twelve biological replications (n=12 per each treatment).

Microscopic analysis of colonization

Root samples from *P. indica*-inoculated and control plants were collected 14 days after plant inoculation. Root were softened by 10% KOH solution for 15 min, acidified with 1 M HCl for 10 min, and stained with 0.02% Trypan blue overnight, and then were distained with 50% lacto-phenol for 1 h prior to microscopic observation under a light microscope (Ghabooli *et al.* 2013).

Corn silage preparation

Among twelve experimental boxes, six boxes selected randomly for silage making and six boxes reminded to harvest corn as grain. Whole plants from six boxes from each of treatment were harvested when kernel milk was around 50% at date of 26/08/2014, and weighed to measure the total biomass yield. Harvested plants chopped to 1-cm length with a portable chopper (Nasr Company, Isfahan). Adequate samples used to measure dry matter (DM) content of fresh forages at 50 ˚C for 48 h. The rest of wet chopped forage from the experimental boxes was immediately compressed into a polyvinyl mini silo (15 cm diameter by 50 cm long cylinder) that was stopper at both ends and contained a valve to allow fermentation gas release (Sheaffer *et al.* 2006; Hashemzadeh-Cigari *et al.* 2014). The artificial silos were stored indoors at room temperature. The mini-silos were opened at date of 12/11/2014 and the enough samples was taken to measure pH according to and

the rest was dried at 50 ˚C for 48 for other measurements (Hashemzadeh-Cigari *et al*. 2014).

Corn grain collection

Plant from reminded six boxes allowed yielding corn grain. At date of 21/09/2014, corn grain was harvested and weighed and was oven dried at 50 ˚C for 48 h. Weight after drying was recorded to obtain grain biomass yield.

Chemical composition analysis

Standard procedures described by the Association of Official Analytical Chemists (AOAC, 1990) were used to determine DM (method 930.15), ash (AOAC method 942.05), crude protein (CP; AOAC method 984.13) and ether extract (EE; AOAC method 954.02). Neutral detergent fiber (NDF), assayed with heat stable alpha-amylase, and acid detergent fiber (ADF) were determined according to the methods for analysis of dietary fiber, neutral detergent fiber, and non-starch polysaccharides in relation to animal nutrition (Van Soest *et al.* 1991) with the ANKOM A200 Filter Bag Technique (Ankom Technology, Fairport, NY, USA). Acid detergent lignin (ADL) was determined by soaking the ADF filter bag residue in 72% sulphuric acid for 3 h followed by washes with water (AOAC method 973.18). All chemical analyses were performed in duplicate and repeated if required. Hemicellulose (NDF–ADF), cellulose (ADF–ADL), non-fiber carbohydrates, NFC= 100 - (NDF+CP+EE+ash) and total carbohydrates CHO= 100 - (CP+EE+ash) were calculated according to NRC (2001).

FTIR scanning of samples

The IR absorbance band of samples was determined using a FTIR spectroscopy (Bruker Tensor 27, Bruker Optics Inc., Billerica, MA, USA) coupled with a universal attenuated total reflectance accessory at the Isfahan University of Technology. The samples were finely ground and pressed uniformly against the diamond surface using a spring-loaded anvil, and the mid-IR spectra recorded from a resolution of 4000 to 600/cm at 2/cm. Typical spectral from corn grain and silages samples in the current study and absorbance bands of interest are shown in Figures 1 and 2 respectively. Each sample was scanned 2 times. Regions of IR spectra identified related to proteins (Amid I + Amid II; 1483 to 1708 cm^{-1}), total carbohydrate (1186 to 852 cm^{-1}), structural carbohydrate (1482.9 to 1188.9 cm^{-1}) and lipids (2770 to 3000 cm^{-1}) (Yari *et al.* 2013). Amide I and amide II bands are two major bands of the protein infrared spectrum. The amide I band (between 1600 and 1700 cm-1) is mainly associated with the C=O stretching vibration (70-85%) and is directly related to the backbone conformation. Amide II results from the N-H bending vibration (40-60%) and from the C-N stretching vibration (18-40%; Yu, 2005).

Functional spectral bands associated with carbohydrate and lipid molecular structures were assigned according to published studies (Refat *et al.* 2017; Yari *et al.* 2017) and identified after OriginPro (2019) noise elimination. Functional groups related to molecular structures of total carbohydrates (peak area baseline ca. 852 to 1186 cm-1), structural carbohydrates mainly associated with hemi and cellulosic compounds (peak area baseline ca. 1188 to 1482 cm-1), and lignin (peak area and height, baseline ca. 1498-1526 cm-1) were analyzed (Refat *et al*. 2017; Yari *et al*. 2017).

Functional groups related to lipids detected from ca 2770 to 3000 cm-1 and are related to vibration absorption of CH (asymmetric stretching of CH_2at ca. 2923 cm-1and CH_3at ca. 2967 cm-1; CH_3 symmetric and CH_2 symmetric groups were at ca. 2894 cm-1 and ca. 2874 cm-1 respectively) (Yari *et al*. 2017).

These features were identified after using second derivative and Fourier self-deconvolution functions of OriginPro (2019) (Figure 2 A and B) (Yari *et al*. 2017). Lipid unsaturation group (CH attached to C=C) peak area is detected between ca. 2996-3020 cm-1 with the center of the peak located at ca. 3000 cm-1 (Yari *et al*. 2017).

***In situ* ruminal degradability**

In situ ruminal incubations were performed as described by (Ørskov and McDonald, 1979) using 2 ruminal fistulated Mehraban ram lambs (body weight=35.5±2.5 kg) fed 1 kg DM/day (in g/kg DM; total mixed ration with 120 g chopped alfalfa hay, 540 g chopped wheat straw, 70 g wheat bran, 80 g ground barley, 170 g beet pulp, 10 g mineral-vitamin supplement, 5 g NaCl and 5 g bentonite) twice daily in equal portions. Feeding and animal husbandry of the lambs were according to procedures by the Iranian Council on Animal Care (1995) guidelines. Corn grain and corn silages samples were pooled in to two samples. Approximately 2 g of each sample was placed in Dacron bags (5 cm×10 cm and pore size of 40 micron). For corn silages, the bags number for incubation interval of 0, 3, 6, 12, 24, 48 and 72 h were 2, 2, 2, 2, 4, 6 and 8. For corn grain samples, ruminal incubation intervals were 0, 24 and 48 h. for both of grain and forage samples, bags for time 0 were manually washed under tap water. For other times, immediately after retrieval from the rumen, bags were manually washed and oven-dried at 50 ˚C for 48 h. The residues in the bags were analyzed for DM and ash as mentioned previously.

Rumen degradation parameters of DM and organic matter (OM) were estimated according to the Orskov and McDonald (1979), $Y= a + b (1–e^{-ct})$, modified first order kinetics model. Parameters were calculated using the nonlinear (NLIN) procedure of SAS (2015) and iterative least-squares regression (Gauss−Newton method).

Parameters were Y, the amount of degradable DM and OM at time t of incubation, washable degradation component (a), slowly degradable component (b) and the constant rate of degradation (kd). Effective rumen degradation (ed) was calculated using rate of passage (kp) of 5%. The potential of degradation (pd) was calculated as summation of a and b parameters.

In vitro gas production

A semi- automated system was used for *in vitro* gas production incubations as described by (Theodorou *et al.* 1994), using buffered rumen fluid prepared according to (Menke and Steinglass, 1988) for all treatments including grain and silage samples. Rumen fluid was collected before the morning feeding from 2 ruminal fistulated ram lambs (same animals as previously mentioned). After collection, ruminal contents were strained through 4 layers of cheese cloth, to eliminate large feed particles, and then transported to the laboratory in a pre-warmed thermal flasks.

Three bottles (125 mL) used as laboratory replicates for each sample that were filled with 0.2 g of feed, 10 mL of rumen liquid and 20 mL of buffer (Theodorou *et al.* 1994) and sealed and incubated for 72 h at 37.5 ˚C. Three bottles with buffered rumen medium, without sample, were incubated to correct for gas release from the inoculum. Head-space gas accumulation were measured using a pressure transducer (Razi Instruments, Mashhad, Iran) and head-space gas volume (*Gp*) was predicted by Boyle's Gas Law from pressure measurements as:

$$GP = (Vh/Pa) \times Pt$$

Where:
Vh: head-space volume (95 mL).
Pa: atmospheric pressure (101298.77 Pa; Meteorological Office, Hamadan, Iran).
Pt: represents pressure transducer reading (Theodorou *et al.* 1994).

Head-space pressure readings were taken at 0, 3, 6, 12, 24, 48 and 72 after the start of incubation. All incubations were repeated into 2 runs. The rate and extent of gas production were determined for each vial by fitting gas production data over time to the nonlinear equation:

$$Y = b\,[1 - e^{-ct}]$$

Where:
Y: volume of gas produced at time t.
b: asymptotic gas production.
c: fractional rate of gas production (Ørskov and McDonald, 1979).

Parameters *b* and *c* were calculated using the NLIN (non-linear) procedure of SAS using iterative least-squares regression (Guass-Newton method; SAS, 2015).

Statistical analysis

Data of forage yield, chemical composition, *in situ* DM and OM degradability, *in vitro* gas production kinetics were analyzed using PROC MIXED of SAS 9.4 (SAS, 2015) with the following statistical models:

$$Y_{ij} = \mu + T_i + B_j + e_{ij}$$

Where:
Y_{ij}: observation of the dependent variable ij.
μ: fixed effect of population mean for the variable.
T_i: fixed effect of treatments (i=2; control and P. indica).
B_j: random effect of pooled block (j=2).
e_{ij}: random error associated with the observation *ij*.

The Fisher's protected least significant difference test was used for multiple treatment comparisons using the LSMEAN statement of SAS (2015). For the different statistical tests, significance was declared at $P \leq 0.05$ and trends were considered at $P \leq 0.10$.

The FTIR spectra per corn grain and silage samples were subjected to multivariate and univariate analysis of variation (MANOVA and ANOVA, respectively). The MANOVA analysis was performed using cluster analysis principle component analysis (PCA) by OriginPro (2019). For the ANOVA, firstly the FTIR peak height and area for each functional group was measured by OriginPro (2019) and then by above statistical model the effect of treatment was considered (Yari *et al.* 2013; Refat *et al.* 2017).

RESULTS AND DISCUSSION

Corn seed samples had similar DM content at time of harvest while samples inoculated with *P. indica* had higher biomass yield (P=0.05). Samples had similar chemical composition except for total carbohydrate content which tended to be higher for control group (Table 1).

In corn forages, DM content of samples and biomass yield at harvest time was similar between treatments (Table 1). Leaf to stem ratio decreased in forage from treated corn (P=0.04) compared with control corn forage. Samples had similar chemical composition except for ether extract which was higher in treated samples with *P. indica* (P=0.04).

In forage samples, *in situ* ruminal degradability of DM and OM including washable fraction, slowly degradable fraction, fractional rate of degradation, potential of degradation and effective degradability from both experimental groups were similar (Table 2).

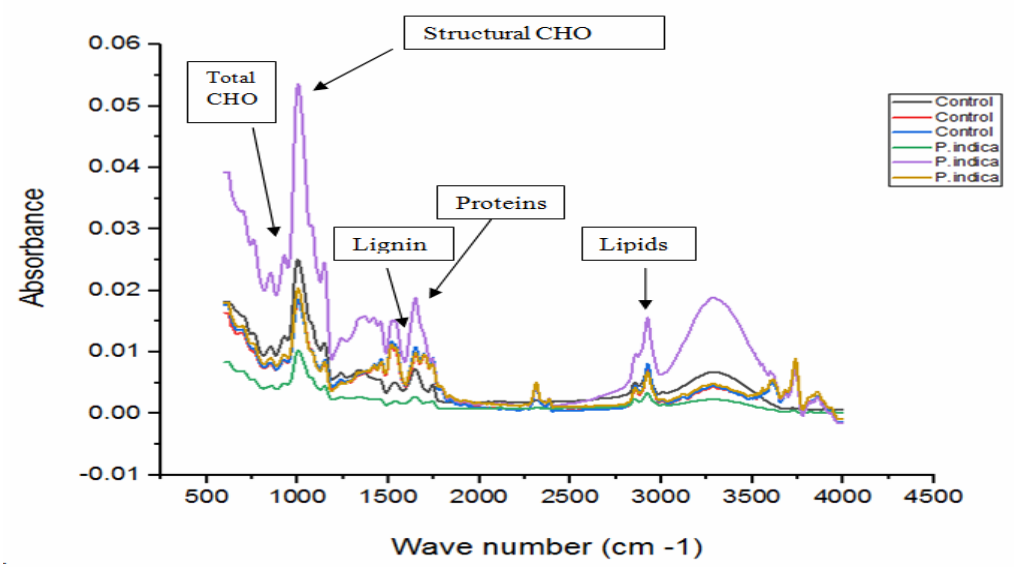

Figure 1 Full spectrum FTIR from ca 4000 to 600/cm of corn silages from control group and treated corn seedlings with *Priformospora indica*
Regions of IR spectra identified related to proteins (Amid I+Amid II; 1483 to 1708 cm^{-1}), total carbohydrate (1186 to 852 cm^{-1}), structural carbohydrate (1482.9 to 1188.9 cm^{-1}), lipids (2770 to 3000 cm^{-1}) and lignin (1498-1526 cm^{-1})

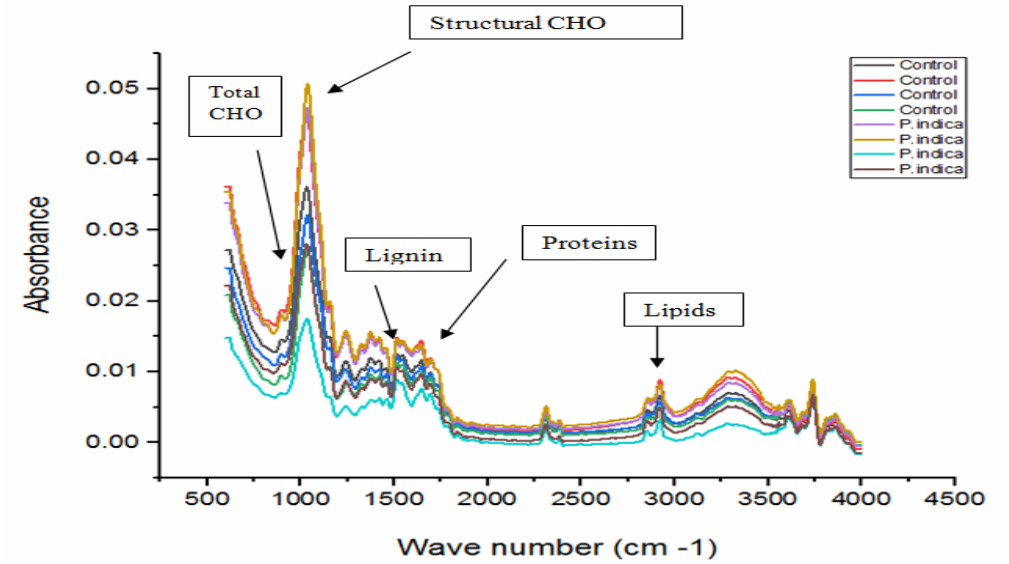

Figure 2 Full spectrum FTIR from ca 4000 to 600/cm of corn silages from control group and treated corn seedlings with *Priformospora indica*
Regions of IR spectra identified related to proteins (Amid I+Amid II; 1483 to 1708 cm^{-1}), total carbohydrate (1186 to 852 cm^{-1}), structural carbohydrate (1482.9 to 1188.9 cm^{-1}), lipids (2770 to 3000 cm^{-1}) and lignin (1498-1526 cm^{-1})

Table 1 Effect of inoculation of corn seedlings with *Priformospora indica* on botanical traits and chemical composition of grain and forage harvested as silage

Items[1]	Silage				Grain			
	Control	*P. indica*	SEM	P-values	Control	*P. indica*	SEM[3]	P-value
Botanical traits[1]								
DM (%)	33.6	31.4	1.02	0.16	74.4	75.1	4.24	0.89
Biomass (g DM)	262.4	234.8	16.44	0.26	34.3	138.5	22.93	0.05
Leaf:stem	0.80	0.67	0.039	0.04	-	-	-	-
Chemical composition (% of DM)								
OM	93.5	93.5	1.26	1.0	98.0	97.5	0.500	0.37
EE	1.8	2.4	0.41	.04	2.8	3.8	0.86	0.14
CP	7.3	7.7	0.42	0.55	8.2	8.1	0.17	0.70
NDF	61.5	59.6	3.12	0.37	-	-	-	-
ADF	33.3	31.0	0.93	0.16	3.8	3.8	0.43	1.00
ADL	6.5	6.0	1.74	0.61	1.00	1.00	0.001	0.99
Ash	6.5	6.5	1.26	1.0	2.0	2.50	0.50	0.37
pH	5.09	5.03	0.051	0.47	-	-	-	-
CHO	84.5	83.5	1.44	0.65	87.1	85.6	0.66	0.09
NFC	23.0	23.8	3.42	0.72	-	-	-	-

[1] Botanical traits were measured on fresh forages.
DM: dry matter; OM: organic matter; EE: ether extract; CP: crude protein; NDF: neutral detergent fiber; ADF: acid detergent fiber; ADL: acid detergent lignin; CHO: total carbohydrate calculated according the NRC (2001) as: CHO= 100 - (CP+EE+Ash); NFC: non-fiber carbohydrate calculated according the NRC (2001) as: NFC= 100 - (NDF+CP+Ash+EE).
SEM: standard error of the means.

Table 2 *In situ* ruminal degradability of dry matter and organic matter of corn silages from inoculated and non-inoculated corn seedlings with *Priformospora indica*

Items	Control	*P. indica*	SEM	P-value
***In situ* ruminal degradation of dry matter (% DM)**				
a	17.3	18.0	1.64	0.76
b	62.9	62.2	2.79	0.80
c (/h)	0.027	0.026	0.004	0.87
Pd	80.2	80.1	3.73	0.97
ed	40.8	40.33	1.40	0.75
***In situ* ruminal degradation of organic matter (% DM)**				
a	14.2	15.1	1.84	0.65
b	70.4	66.3	3.20	0.27
c (/h)	0.024	0.025	0.004	0.69
Pd	84.6	81.4	4.79	0.54
ed	38.2	38.6	0.79	0.62

a: washable fraction; *b*: slowly degradable fraction; *c*: rate of ruminal degradation; pd: potential of ruminal degradation and ed: effective ruminal degradation.

In corn seed inoculated with *P. indica*, *in situ* ruminal DM and OM degradability at 24 h of ruminal incubation were higher compared with control corn seed (Table 3; P=0.02).

In vitro rate of gas production and volume of gas produced until 24 h of incubation were higher for silage from treated corn compared with silage from control corn (P=0.02 and P=0.06 respectively). *In vitro* gas production kinetics were similar between corn seed samples (Table 4).

The FTIR height and area for functional groups of nutrient from control and treated corn with *P. indica* were similar (Tables 5 and 6, respectively) and for corn silages in Tables 7 and 8.

The ratio between amid I to amid II and amid II to lignin area tended to decrease in corn silage from treated corn with *P. indica* compared with control group (P=0.09; Table 7).

Other FTIR height and area and ratios related to functional group in corn and treated corn with *P. indica* were similar (Tables 7 and 8). The PCA did not discriminate between corn seed samples from control group and *P. indica* group at the FTIR area related to proteins and lipids but PCA discriminated the FTIR area related to carbohydrate between control group and *P. indica* group samples (Figure 3; a, b and c). The FTIR area related to proteins, lipids, carbohydrates and structural carbohydrates was not discriminated by PCA between corn silages samples from both experimental groups (Figure 4; a, b, c and d).

Grain

The nutrient contents of corn grain samples and corn silage samples including chemical composition, *in situ* ruminal degradability of DM and OM, *in vitro* gas production was consistent with those reported by NRC (2001).

Table 3 *In situ* ruminal degradation at different time of incubations of dry matter and organic matter of corn from inoculated and non-inoculated corn seedlings with *Priformospora indica*

Items	Control	*P. indica*	SEM	P-value
Dry matter (%)				
0	7.3	7.5	2.77	0.95
24 h	54.9	66.7	3.83	0.02
48 h	79.6	84.3	3.93	0.28
Organic matter (% DM)				
0	7.3	7.0	2.91	0.92
24 h	54.4	66.0	4.00	0.02
48 h	79.7	83.9	5.67	0.32

SEM: standard error of the means.

Table 4 *In vitro* gas production fermentation kinetics of silage and grain from inoculated and non-inoculated corn seedlings with *Priformospora indica*

Gas production measurements	Silage				Grain			
	Control	*P. indica*	SEM	P-value	Control	*P. indica*	SEM	P-value
b	251.4	259.7	15.66	0.61	333.0	331.4	3.03	0.75
c	0.02	0.03	0.002	0.02	0.031	0.032	0.0009	0.59
Gas 24 (mL/0.2 g DM)	23.6	27.4	1.27	0.06	202.2	200.3	7.08	0.85

b: potential of gas production; *c*: rate of gas production and Gas 24: cumulative gas production measurement after 24 h of incubation.
SEM: standard error of the means.

Table 5 Effect of inoculation of corn seedlings with *Priformospora indica* on the ANOVA of the FTIR area of functional groups of corn grain samples

Items	Control	*P. indica*	SEM	P-value
Amid II	0.1192	0.1030	0.02831	0.70
Amide I	0.2507	0.3287	0.1321	0.70
Cellulosic	0.1852	0.2861	0.1118	0.55
Protein	0.3699	0.4317	0.1563	0.79
CHO	0.2889	0.4689	0.1735	0.50
Starch	1.6551	2.0810	0.6503	0.66
Lipid	0.4796	0.5513	0.1886	0.80
CH_{2s}	0.1250	0.1315	0.04039	0.91
CH_{2a}	0.3545	0.4198	0.1482	0.77
FTIR area ratios				
AmidI_starch	0.1600	0.1340	0.03323	0.61
AmidII_starch	1.3928	0.8875	0.2613	0.24
Lipid_CHO	1.8139	1.2438	0.3306	0.28
Lipid_starch	0.3080	0.2543	0.05092	0.49
Pro_lipid	0.7749	0.7234	0.09068	0.70
Amide i_ii	2.1301	2.7629	0.6253	0.51

CHO: total carbohydrates (1186 to 852 cm^{-1}), total proteins (Amid I+Amid II; 1483 to 1708 cm^{-1}), structural carbohydrate (1482.9 to 1188.9 cm^{-1}), total lipid (2770 to 3000 cm^{-1}), and lignin (ca. 1498-1526 cm^{-1}); CH_{2s}: symmetric stretching of CH_2 groups was *ca*. 2874 cm^{-1}; CH_{2a}: asymmetric stretching of CH_2 at *ca*. 2923 cm^{-1}; AmidI_starch: ratio between amidI to starch; AmidII_starch: ratio between amid II and starch; Lipid_CHO: ratio between lipid and total carbohydrates; Lipid_starch: ratio between lipid and starch; Pro_lipid: ratio between protein and lipid and Amide i_ii: ratio between amid I and amid II.
SEM: standard error of the means.

In grain samples, chemical composition were similar but total carbohydrate was grater in samples from *P. indica*-treated corn. This chemical composition was calculated based on NRC (2001) by summation equations.

This finding was in accordance with PCA from total carbohydrates of FTIR area which was differentiated between control group corn and treated corn with *P. indica*. This may indicate that *P. indica* increased the magnitude of carbohydrate synthesis. This is also consistent with the increased biomass yield in grain production which was higher for *P. indica* group. Because most of the corn grain is consisted of carbohydrate (Khan *et al.* 2011; Macome *et al.* 2017), therefore it seems that the *P. indica* increased the synthesis of carbohydrates in grain which resulted to greater grain production.

In situ ruminal degradation amount of DM and OM was greater for *P. indica* group compared with control group. In corn varieties, magnitude of ruminal degradation is mostly determined by the starch quantity and quality (Khan *et al.* 2011; Macome *et al.* 2017).

Table 6 Effect of inoculation of corn seedlings with *Priformospora indica* on the ANOVA of the FTIR height of functional groups of corn grain samples

Items	Control	*P. indica*	SEM	P-value
Amid II	0.003963	0.003693	0.001382	0.89
Amide I	0.005023	0.005570	0.001991	0.85
Amide i_ii	1.4518	1.5676	0.2795	0.78
CHO	0.005320	0.008517	0.003128	0.50
Starch	0.01587	0.02314	0.007958	0.55
Cellulosic	0.005113	0.007553	0.003032	0.59
Ch_{2s}	0.003103	0.003250	0.000993	0.92
Ch_{2a}	0.005987	0.006427	0.002048	0.88
FTIR height ratios				
Starch_amidii	5.7516	8.2040	2.9143	0.58
Starch_amidi	3.4980	4.9773	1.1763	0.42
Cell_amidii	1.9571	2.4598	0.9790	0.73
Cell_amidi	1.1560	1.4838	0.3882	0.58
Starch_cell	3.1839	3.3461	0.2344	0.65
Chs_amidii	0.9211	1.2215	0.3599	0.58
Chs_amidi	0.6249	0.7469	0.1500	0.59
Cha_amidi	1.1981	1.4308	0.2588	0.55
Cha_amidii	1.7493	2.3347	0.6381	0.55
Starch_chs	5.4940	6.7557	1.0886	0.45
Starch_cha	2.8958	3.4761	0.6090	0.53
Cell_chs	1.8063	2.0685	0.4937	0.72
Cell_cha	0.9551	1.0607	0.2674	0.79

CHO: total carbohydrates (1186 to 852 cm^{-1}), total proteins (Amid I+Amid II; 1483 to 1708 cm^{-1}), structural carbohydrate (1482.9 to 1188.9 cm^{-1}); CH_{2s}: symmetric stretching of CH_2 groups was *ca*. 2874 cm^{-1}; CH_{2a}: asymmetric stretching of CH_2 at *ca*. 2923 cm^{-1}; Starch_amidi: ratio between amid I to starch; Starch_amidii: ratio between starch and amid II; Cell_amidi: ratio between cellulosic compounds and amid I; Cell_amidii: ratio between cellulosic compounds and amid II; Amid I_amid II: ratio between amid I and amid II; Starch_cell: ratio between starch and cellulosic compounds; Chs_amidii: ratio between CH_{2s} and amid ii; Chs_amidi: ratio between CH_{2s} and amid i; Cha_amidii: ratio between CH_{2a} and amid ii; Cha_amidi: ratio between CH_{2a} and amid i; Starch_chs: ratio between starch and CH_{2s}; Starch_cha: ratio between starch and CH_{2a}; Cell_chs: ratio between cellulosic compounds and CH_{2s} and Cell_cha: ratio between cellulosic compounds and CH_{2a}.

Current results indicated that the endophytic fungus *P. indica* affected the FTIR molecular structures of carbohydrate which resulted in higher total carbohydrate content and ruminal *in situ* degradation content. However, these changes did not affect fermentation profiles of samples in gas production medium. This might be due to the differences between *in situ* and *in vitro* methods for feed evaluation (Krizsan *et al.* 2012).

The silage samples from *P. indica* group had higher EE content. According to Jafari *et al.* (2018) co-inoculation of *Sinorhizobium meliloti* and *P. indica* increased the EE content compared with *P. indica*- inoculated alfalfa hay alone. The higher EE content may be due to higher absorption of soil nutrient by corn roots as a result of symbiosis with these endophytic microorganisms. The *P. indica* has been reported to stimulate the phosphorous and water absorption (Ghaffari *et al.* 2016), and the synthesis of ATP, cell walls and phospholipids in plants were previously found to increase with increasing phosphorous absorption (Kristek *et al.* 2005).

Inoculation or co-inoculation of alfalfa seedlings had no effect on forage yield compared with un-inoculated alfalfa seedlings in the study of (Jafari *et al.* 2018) while inoculation of barley seedlings with *P. indica* was found to increase biomass yield (Ghabooli *et al.* 2013; Ghabooli, 2014).

These authors indicated that *P. indica* changed some biochemical pathways in the plant which promoted higher biomass yield. The lack of response in forage yield in the current study may be due to different corn variety or growth conditions. Many studies have reported that *P. indica* improved the biomass yield of plants grown under environmental stress condition (Waller *et al.* 2005; Ghabooli *et al.* 2013; Murphy *et al.* 2014). Under normal growing conditions, the effect of plant inoculations with *P. indica* on forage biomass yield may be negligible or negative depending on plant species (Augé, 2001; Nadeem *et al.* 2014).

Forage

Forages from *P. indica* group had smaller leaf/stem ratio. Hosseini *et al.* (2018) reported that *P. indica* increased the leaf area and root volume. In corn forage the stem is consisted of mainly of structural carbohydrates such as cellulose, hemicellulose and lignin (Jung and Allen, 1995). However, the NDF, ADF and ADL components which measure the cell wall composition were similar between forage samples. Also, the FTIR functional groups related to cell wall components were similar between forage samples. It has been found that lignin (ADL) is essentially indigestible by most ruminant animals and believed to play a major role in the magnitude of digestion of plant by rumen microbes (Jung and Allen, 1995).

Table 7 Effect of inoculation of corn seedlings with *Priformospora indica* on the ANOVA of the FTIR area of functional groups of forage harvested as silage

Items	Control	*P. indica*	SEM	p- values
Amid II	0.3064	0.2859	0.01509	0.37
Amide I	0.1409	0.1717	0.02054	0.33
Amide i_ii	0.4550	0.5977	0.04936	0.09
TCHO	3.6276	3.6365	0.6116	0.99
Lignin	0.02776	0.02563	0.001211	0.25
SCHO	0.7178	0.7528	0.1686	0.88
CH_{2s}	0.09776	0.09593	0.01461	0.93
CH_{2a}	0.2898	0.2764	0.03923	0.81
Amidii_lig	11.0535	11.1293	0.2309	0.82
Amidi_lig	5.0260	6.6619	0.5649	0.08
Amidii_cha	1.0948	1.0832	0.1076	0.94
Amidi_cha	0.4829	0.6495	0.06582	0.12
Amidii_chs	3.2828	3.1320	0.3424	0.76
Amidi_chs	1.4404	1.8803	0.1955	0.16
Amidii_scho	0.4645	0.4471	0.07751	0.87
Amidi_scho	0.2015	0.2619	0.03561	0.27
Amidii_tcho	0.08728	0.08754	0.0004	0.98
Amidi_tcho	0.03851	0.05222	0.007250	0.22
Scho_tcho	0.1936	0.2023	0.01613	0.71
Lignin_tcho	0.007907	0.007939	0.001183	0.98
Lignin_scho	0.04197	0.04073	0.007445	0.90
Lignin_chs	0.2971	0.2833	0.03350	0.78
Lignin_cha	0.09912	0.09790	0.01065	0.93
Scho_chs	7.2058	7.5442	0.7659	0.76
Scho_cha	2.4224	2.6131	0.2740	0.64
Tcho_chs	37.4753	36.9623	2.0851	0.86
Tcho_cha	12.5444	12.7905	0.6476	0.79

CHO: total carbohydrates (1186 to 852 cm^{-1}), total proteins (Amid I+Amid II; 1483 to 1708 cm^{-1}), structural carbohydrate (1482.9 to 1188.9 cm^{-1}); CH_{2s}: symmetric stretching of CH_2 groups was *ca*. 2874 cm^{-1}; CH_{2a}: asymmetric stretching of CH_2 at *ca*. 2923 cm^{-1}; Starch_amidi: ratio between amid I to starch; Starch_amidii: ratio between starch and amid II; Cell_amidi: ratio between cellulosic compounds and amid I; Cell_amidii: ratio between cellulosic compounds and amid II; Amid I_amid II: ratio between amid I and amid II; Starch_cell: ratio between starch and cellulosic compounds; Chs_amidii: ratio between CH_{2s} and amid ii; Chs_amidi: ratio between CH_{2s} and amid i; Cha_amidii: ratio between CH_{2a} and amid ii; Cha_amidi: ratio between CH_{2a} and amid i; Starch_chs: ratio between starch and CH_{2s}; Starch_cha: ratio between starch and CH_{2a}; Cell chs: ratio between cellulosic compounds and CH_{2s} and Cell cha: ratio between cellulosic compounds and CH_{2a}.

Table 8 Effect of inoculation of corn seedlings with *Priformospora indica* on the ANOVA of the FTIR height of functional groups of forage harvested as silage

Items	**Control**	***P. indica***	**SEM**	**P-value**
Amid II	0.004715	0.004465	0.000164	0.32
Amide I	0.002485	0.002635	0.000224	0.65
TCHO	0.02636	0.02679	0.004472	0.94
Lignin	0.001578	0.001460	0.000071	0.28
SCHO	0.004088	0.003445	0.000699	0.53
CH_{2s}	0.002478	0.002450	0.000327	0.95
CH_{2a}	0.004368	0.004303	0.000520	0.93
Amide I_II	0.5239	0.5900	0.03506	0.23
Amidii_lig	2.9936	3.0646	0.05360	0.38
Amidi_lig	1.5654	1.8092	0.1034	0.14
Amidii_cha	1.1237	1.0622	0.09895	0.67
Amidi_cha	0.5721	0.6275	0.03803	0.34
Amidii_chs	1.9823	1.8988	0.2010	0.77
Amidi_chs	1.0093	1.1220	0.09130	0.41
Amidii_scho	1.2146	1.4327	0.1832	0.43
Amidi_scho	0.6181	0.8439	0.09419	0.14
Amidii_tcho	0.1837	0.1903	0.02943	0.87
Amidi_tcho	0.09408	0.1125	0.01712	0.47
Scho_tcho	0.1534	0.1409	0.02092	0.68
Lignin_tcho	0.06134	0.06166	0.008961	0.98
Lignin_scho	0.4044	0.4696	0.06132	0.48
Llignin_chs	0.6617	0.6181	0.06223	0.63
Lignin_cha	0.3751	0.3461	0.03103	0.53
Scho_chs	1.6439	1.4108	0.1562	0.33
Scho_cha	0.9321	0.7945	0.08993	0.32
Tcho_chs	10.7337	10.5883	0.8015	0.90
Tcho_cha	6.0852	6.0220	0.5544	0.93

CHO: total carbohydrates (1186 to 852 cm^{-1}), total proteins (Amid I+Amid II; 1483 to 1708 cm^{-1}), structural carbohydrate (1482.9 to 1188.9 cm^{-1}); CH_{2s}: symmetric stretching of CH_2 groups was *ca*. 2874 cm^{-1}; CH_{2a}: asymmetric stretching of CH_2 at *ca*. 2923 cm^{-1}; Starch_amidi: ratio between amid I to starch; Starch_amidii: ratio between starch and amid II; Cell_amidi: ratio between cellulosic compounds and amid I; Cell_amidii: ratio between cellulosic compounds and amid II; Amid I_amid II: ratio between amid I and amid II; Starch_cell: ratio between starch and cellulosic compounds; Chs_amidii: ratio between CH_{2s} and amid ii; Chs_amidi: ratio between CH_{2s} and amid i; Cha_amidii: ratio between CH_{2a} and amid ii; Cha_amidi: ratio between CH_{2a} and amid i; Starch_chs: ratio between starch and CH_{2s}; Starch_cha: ratio between starch and CH_{2a}; Cell_chs: ratio between cellulosic compounds and CH_{2s} and Cell_cha: ratio between cellulosic compounds and CH_{2a}.

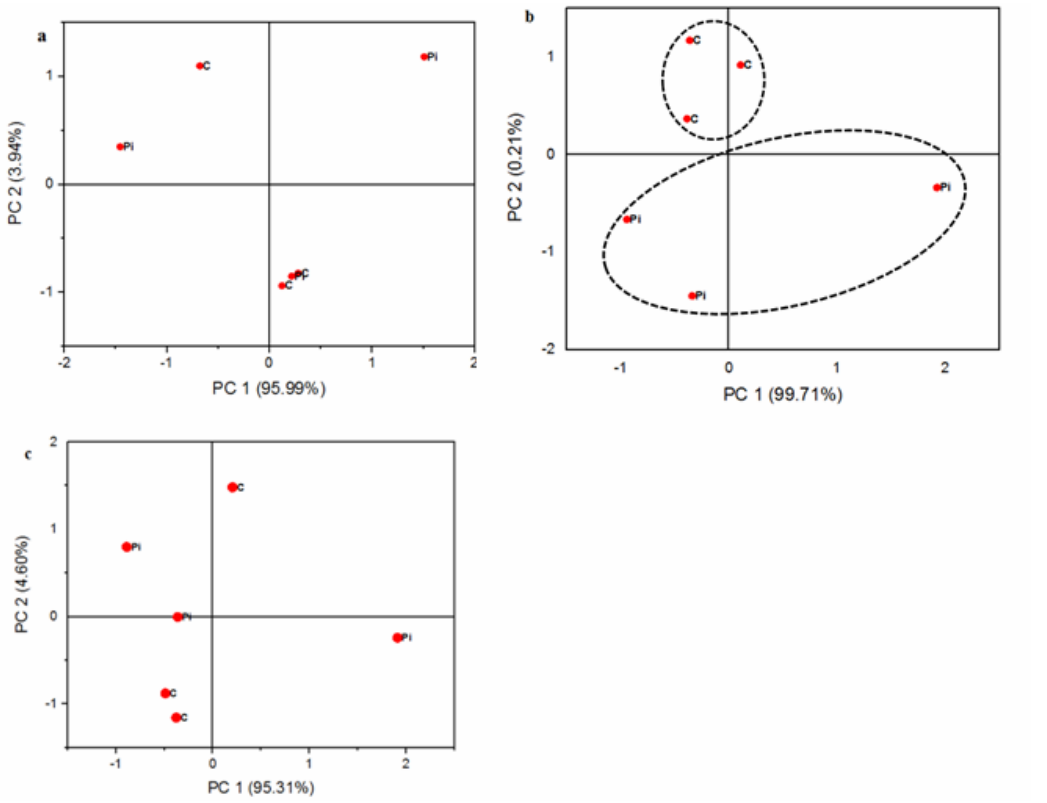

Figure 3 Multivariate PCA spectral analyses for grains from control group (code C) and treated corn seedlings with *Priformospora indica* (code Pi)
a:total proteins (Amid I+Amid II; 1483 to 1708/cm); b: total carbohydrates (1186 to 852/cm) and c: total lipid (2770 to 3000/cm)

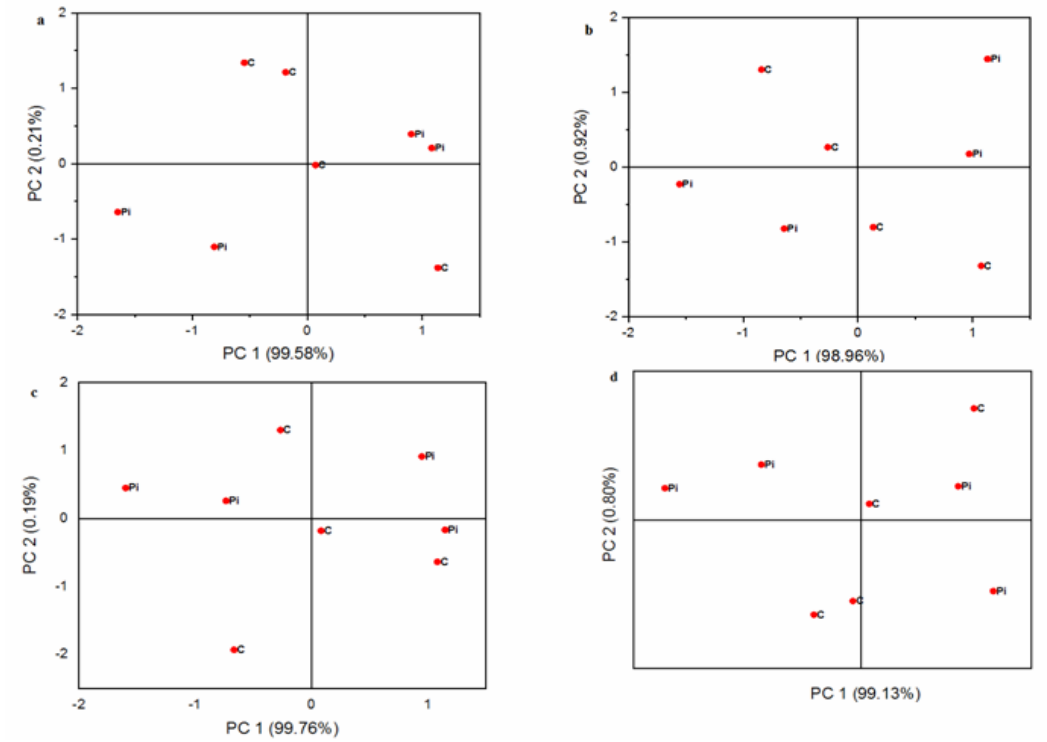

Figure 4 Multivariate PCA spectral analyses for silages of control group (code C) and treated corn seedlings with *Priformospora indica* (code Pi)
a: total proteins (Amid I+Amid II; 1483 to 1708 /cm); b: total carbohydrates (1186 to 852/cm); C: structural carbohydrate (1482.9 to 1188.9/cm) and d: total lipid (2770 to 3000/cm)

This was reflected in *in situ* ruminal DM and OM degradability in which the parameters for kinetics were similar between both silage samples.

However, in silages from *P. indica* group, rate of gas production was greater than silage from control group. The ADF and NDF component may be similar between two forages while their constitutions such as the cellulose, hemicellulose and properties related to them might be different (Van Soest *et al*. 1991; Krizsan *et al*. 2012). The nutritional value of corn silage largely depends on the content and degradability of the starch (Macome *et al*. 2017). The starch content, as well as the virtuousness of corn kernels, increases with maturity, and the fractional rate of ruminal starch degradation of corn decreases with maturity (Philippeau and Michalet-Doreau, 1997). It may be that the content and degradability of starch of corn kernel changed by *P. indica* in which influenced the fractional rate of gas production from corn silage. However, the results from gas production and *in situ* ruminal degradability did not show the same behavior in the case of rate of degradation and fermentation which may be due to the *in vitro* or *in situ* parameters which affect the obtained results (Macome *et al*. 2017).

In the study of Hosseini *et al*. (2018), in comparison with non-inoculated maize, inoculated maize showed lower catalase and ascorbate peroxidase activities which means that they experienced less oxidative stress induced by stressful conditions.

CONCLUSION

Inoculation of corn seeds with *P. indica* increased the biomass yield, mainly changed the FTIR carbohydrates structures and increased the *in situ* ruminal degradable component. Corn silages from *P. indica*-inoculated corn seeds had higher ether extract content and higher *in vitro* fermentation rate. This promises to create a new platform for exploitation of this endophytic fungus in improving the feeding value of corn seeds and silages under semi-arid climate condition.

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