Effects of Seed Dressing with Microbial Inoculum on Nutrient Composition and Biological Yield of Silage Corn

Ruihuan DU[1,2], Yanhua YAN[1,2], Lei WANG[1,2], Liang ZHANG[1,2], Shuai WANG[1,2], Shuo YANG[1,2,3], Yajing WANG[1,2,3], Huihui CAO[1,2,3]*

1. Tangshan Food and Drug Comprehensive Testing Center, Tangshan 063000, China; 2. Hebei Agricultural Products Quality and Safety Testing Innovation Center, Tangshan 063000, China; 3. Tangshan Institute of Industrial Technology for Functional Agricultural Products, Tangshan 063000, China

Abstract [**Objectives**] This study was conducted to understand the effects of microbial inocula on the biological yield and nutritional components of corn. [**Methods**] Silage corn varieties suitable for planting in Hebei Province were selected, and set with an experimental group and a control check group each. Meanwhile, at the late stage of milk ripening of silage corn growth cycle, the nutritional components and indexes of silage corn were determined and analyzed by a near-infrared analyzer and the NIRS technique. Meanwhile, the biological yield of silage corn was determined at the maturation stage of its growth period, aiming at comprehensively evaluating the regulation effects of the microbial inoculum tested on silage corn from the aspects of basic nutritional components, mineral element contents, energy and related indexes of corn, combined with agronomic characters and biological yield. [**Results**] The microbial inoculum improved the biological yield and nutritional indexes of silage corn, and had a positive regulation effect on the growth of silage corn. [**Conclusions**] The results of this study provide a theoretical basis for popularizing the planting methods and cultivation methods of silage corn with high yield and high quality.

Key words Silage corn; Microbial inoculum; Near-infrared technology; Energy index; Nutritional quality analysis

DOI:10.19759/j.cnki.2164-4993.2023.05.012

With the continuous development of animal husbandry, the demand for silage is increasing day by day. Due to its short production cycle, low cost and high economic benefits, silage corn feed has become an important feed resource in animal husbandry[1]. From the perspective of animal nutrition, silage corn feed is rich in nutrients such as dry matter, crude fat and cellulose, which can not only help herbivorous livestock to improve their palatability, but also facilitate their digestion and absorption, thus greatly improving the production performance of animal husbandry[2-3]. Silage corn feed can not only provide sufficient feed for herbivorous livestock, but also solve the problems of resource waste and environmental pollution caused by corn stalk burning, which conforms to the scientific concept of "green development" of China[4-5].

The structure of rhizosphere microbial community can reflect soil fertility and plant health, and it is a response to the comprehensive factors of plant growth environment[6]. In recent years, the research on rhizosphere microbial community has been widely carried out in many crops[7-8], and some research results show that some microorganisms in rhizosphere can not only positively regulate crop biomass, but also enhance crop disease resistance, so we call them beneficial microorganisms[9]. As a kind of biocontrol bacterium, *Bacillus subtilis* has a good effect of preventing diseases and promoting root growth. The environment-friendly microbial inocula made of *B. subtilis* can not only promote seed germination and improve germination rate and emergence rate, but also improve plant biological yield and nutritional quality. In this study, the seed dressing of silage corn was carried out by a *B. subtilis* microbial preparation before sowing, and the positive regulation of the microbial preparation on nutritional quality and yield of silage corn was verified by measuring the agronomic characters, biological yields and nutritional components of experimental groups and control check groups. The results of this study provide a theoretical basis for popularizing the planting methods and cultivation methods of silage corn with high yield and high quality.

Materials and Methods

Experimental materials

(1) Microbial inoculum: It was a biocontrol microbial inoculum made of *B. subtilis* JTFM 1001[10] (strain preservation number: CCTCC No: 2016145) with antibacterial and detoxifying effects, and the effective viable count was equal to or greater than 500 million/ml.

(2) Seed dressing method: For the seed dressing group, the concentration of microbial inoculum used for seed dressing was 75 ml/kg. Through field experiments, it was found that the sowing density of corn is generally 66 000-69 000 seeds/hm^2, and it is generally 30.0-37.5 kg/hm^2 according to different seed sizes. According to the sowing density of 30 kg of corn seeds per hectare, the amount of microbial inoculum needed was 2 250 ml/hm^2. The bacterial suspension was directly sprayed on the surface of seeds, which were stirred evenly, dried in the shade and sown. The application rate was increased or decreased according to different

Received: July 5, 2023 Accepted: September 7, 2023

Supported by Fund Project for Transformation of Agricultural Scientific and Technological Achievements in Hebei Province in 2022; Agricultural Products Quality and Safety Technology Innovation Center of Hebei Province; Tangshan Talent Project (A202110026); Tangshan Science and Technology Planning Project (23150210A); High-level Talent Fund of Hebei Province (C20231087).

Ruihuan DU (1985-), female, P. R. China, senior engineer, master, devoted to research about analysis of nutritional components in crops.

* Corresponding author.

conditions of crop seed size/seeding rate/seed coat thickness. The control group (CK) was not treated with microbial inoculum.

(3) Silage corn varieties: Dongdan 1331 (Liaoning Dongya Seed Industry Co., Ltd.), Woyu 3.

Determination indexes and methods

Determination of agronomic traits and biological yield In this study, "Dongdan 1331" and "Woyu 3" were used as research materials, and the seeds were dressed with *B. subtilis* before sowing. At the maturation stage of the growth cycle, following biological characters of silage corn of different varieties in different treatment conditions were measured: plant height, stem diameter, ear height, number of leaves, number of yellow leaves, single plant weight (kg/plant), total weight (kg/4 rows ×2.5 m), and weight per square meter (kg). The values of three biological replicates were averaged for each biological character.

Determination of nutritional components In the late stage of milk ripening of silage corn growth cycle, whole-plant silage corn was harvested while leaving 15 cm stubbles. A FOSS-2500 multifunctional near-infrared analyzer was used to determine the nutritional components in whole-plant silage corn of the two treatments of the two varieties, and three biological replicates were set for each variety. The NIRS technique was applied to analyze the indexes of forage nutrition components. In specific, 100 g of each sample to be tested was crushed with a cyclone mill to obtain powder, which was sieved through a 1 mm sieve, and spectral scanning was performed with the FOSS5000 near-infrared analyzer (FOSS Company, Denmark) under following working parameters: wavelength range of 1 100 – 2 500 nm, scanning times of 32 times, and spectral interval of 2 nm. Based on the imported near-infrared detection model of alfalfa from National Forage Testing Center of the United States, the nutritional components such as dry matter (DM), crude protein (CP), water-soluble carbohydrate (WSC), crude fat (EE), starch and lignin, mineral elements such as ash, calcium (Ca), phosphorus (P), potassium (K) and magnesium (Mg), and energy indexes such as net energy for production (NEL), net energy for gain (NEG), milk yield per ton of feed and non-fibrous carbohydrate (NFC), were obtained.

Results and Analysis

Measurement and analysis of biological characters of silage corn with different treatments

Following biological characters of silage corn under different treatment conditions were measured by hands: plant height, stem diameter, ear height, number of leaves, number of yellow leaves, fresh weight of single plant (kg/plant), total weight (kg/4 rows ×2.5 m) and weight per square meter (kg), and the values of three biological replicates were averaged for each biological character. The results are shown in Table 1.

Table 1 Measurement results of biological characters of silage corn samples

Variety	Plant height//m	Stem diameter//cm	Ear height//m	Number of leaves	Number of yellow leaves	Fresh weight of single plant//kg/plant	Total weight ($6\ m^2$, 4 rows × 2.5 m)//kg	Weight per square meter//kg
Dongdan 1331 (seed dressing)	2.61	2.60	1.05	15	2	1.15	38.39	6.73
Dongdan 1331	2.60	2.40	0.89	14	2	1.11	36.20	6.03
Woyu3 (seed dressing)	2.92	2.63	1.06	15	2	1.38	46.08	8.01
Woyu 3	2.91	2.40	1.02	15	2	1.32	43.33	7.22

The results in Table 1 showed that, compared with the control check (CK), the plant height, stem diameter and ear height of silage corn treated with the microbial inoculum were higher than those of the control group, and the fresh weight per plant, total weight of 6 m^2 and weight per square meter of silage corn in the experimental group were significantly higher than those in the CK. It indicated that seed dressing with the *B. subtilis* inoculum significantly improved the biological yield of silage corn.

Analysis of nutritional components of silage corn under different treatment conditions

At the later stage of milk ripening, the whole-plant silage corn was harvested while leaving stubbles with a height of 15 cm, and the nutritional components of the experimental group and the CK were determined by a FOSS-2500 multifunctional near-infrared analyzer. The measurement results are shown in Table 2 and Table 3.

The results showed that seed dressing with *B. subtilis* significantly improved the nutritional quality of silage corn, and promoted the contents of crude protein, carbohydrates, ash, Ca, K and Mg in the late stage of milk ripening. Among them, the crude protein content and carbohydrate content of silage corn treated by seed dressing with *B. subtilis* were as high as 8.80% DM and 18.3% DM, respectively. Generally speaking, the silage corn with starch content greater than 28%, crude protein content greater than 7%, acid detergent fiber content less than 22% and neutral detergent fiber less than 45% is of high quality[3-4], and the nutritional indexes of silage corn in the experimental group were significantly better than those in the CK.

Analysis on the differences in energy indexes of silage corn under different treatment conditions

As shown in Table 4, for each of Dongdan 1331 and Woyu 3, the net energy for production (NEL), net energy for gain (NEG), milk yield per ton of feed, non-fibrous carbohydrate (NFC) and other values in the experimental group were significantly higher than those in the CK. It indicated that *B. subtilis* significantly improved the net energy for production, net energy for gain, milk yield per ton of feed and non-fibrous carbohydrate of corn while increasing the biological yield of corn.

Table 2 Analysis on nutritional components of silage corn with different treatments

Variety	Moisture	DM//%					
		Dry matter	Crude protein	Water-soluble carbohydrates (WSC)	Crude fat	Starch	Lignin
Dongdan 1331 (seed dressing)	71.3	28.70	8.80	18.30	2.56	22.70	3.15
Dongdan 1331 (CK)	70.5	29.50	8.35	14.10	2.88	26.50	3.32
Woyu3 (seed dressing)	73.0	28.75	8.47	13.50	2.98	27.90	6.99
Woyu 3 (CK)	72.1	27.90	8.40	13.20	2.73	23.75	3.66

Table 3 Analysis on mineral components of silage corn with different treatments

Variety	DM//%				
	Ash	Ca	P	K	Mg
Dongdan 1331 (seed dressing)	5.39	0.23	0.23	1.29	0.15
Dongdan 1331 (CK)	5.12	0.20	0.22	1.24	0.13
Woyu3 (seed dressing)	5.17	0.19	0.24	1.38	0.14
Woyu 3 (CK)	4.82	0.17	0.243	1.26	0.12

Table 4 Analysis on energy indexes of different silage corn varieties

Index	NEL//MJ/kg	NEG//MJ/kg	Milk yield per ton of feed//kg/t	NFC//%/DM
Dongdan 1331 (seed dressing)	1..595	1.135	1 513.5	44.9
Dongdan 1331 (CK)	1.590	1.120	1 389.0	44.2
Woyu 3 (seed dressing)	1.595	1.130	1 539.0	44.9
Woyu 3 (CK)	1.555	1.075	1 433.5	41.8

Conclusions and Discussion

Agronomic characters and biological yield are important indexes to evaluate the quality of silage corn[3-4]. Studies have shown that the plant height, ear height, stem diameter and the number of green leaves of silage corn are all factors that affect the biological yield[3]. The higher the plant height and ear height of silage corn, the higher the biological yield; the thicker the stem[1], the stronger the lodging resistance of the plants[2]; and leaves serve as the main structure of organic matter accumulation in maize plants, and the number of leaves is also positively correlated with the biological yield.

Fertilization is a major measure to increase crop yield in agriculture. Excessive fertilization will lead to a series of ecological and environmental problems, such as soil degradation, water pollution, low utilization rate of crop nutrients, and increased crop diseases and pests. Long-term application of chemical fertilizers significantly changes the bacterial community structure and reduced the bacterial diversity. *B. subtilis* can not only improve the stress resistance of crops such as disease resistance, drought resistance and cold resistance, but also increase soil nutrients and improve soil structure and the utilization rate of chemical fertilizers. It also has certain functions of nitrogen fixation, phosphorus dissolution and potassium dissolution, thus promoting the decomposition of organic matter in soil into humus, accelerating crop growth and increasing yield and income. The results of this study provide a theoretical basis for popularizing the planting methods and cultivation methods of silage corn with high yield and high quality.

References

[1] WU X, XU HL, CHEN W, *et al.* Comprehensive evaluation and development trend of silage maize varieties approved by the state[J]. Journal of Agriculture, 2019, 9(9): 5-10. (in Chinese).

[2] WANG R. Significance and planting techniques of silage corn planting [J]. Technical Advisor for Animal Husbandry, 2020(1): 33-34. (in Chinese).

[3] COOK RJ, ROVIRA AD. The role of bacteria in the biological control of *Gaeumannomyces gram* in is by suppressive soils [J]. Soil Biology&Biochemistry, 1976(8): 267-273.

[4] WANG J, YANG ML, WANG ZH, *et al.* Varieties selection and evaluation of food and fodder dual-purpose silage maize[J]. Journal of Agriculture, 2019, 9(8): 10-13. (in Chinese).

[5] PIERSON EA, WELLER DM. Use of mixture of fluorescent pseudomonas to suppress take-all and improve the growth of wheat[J]. Phytopathology, 1994(84): 940-947.

[6] WENG QY, HUANG XJ, XU HL, *et al.* Effects of corn/soybean intercropping model on yield, quality, soil nutrition and rhizosphere microorganisms of silage corn[J]. Acta Agriculturae Nucleatae Sinica, 2021, 35 (21): 0462-0470. (in Chinese).

[7] CHEN HS, QIN CX, PENG C, *et al.* Effects of sugarcane intercropping peanut on microbial population and enzyme activity in rhizosphere soil[J]. Jiangsu Agricultural Sciences, 2019, 47(3): 223-226. (in Chinese).

[8] DU YQ, WANG MJ, SUO RZ, *et al.* Soil characteristics of intercropping forage hybrid soybean with silage maize[J]. Grassland and Prataculture, 2022, 34(3): 25-32. (in Chinese).

[9] BULGARELLI D, GARRIDO-OTER R, MÜENCH PC, *et al.* Structure and function of the bacterial root microbiota in wild and domesticated barley[J]. Cell Host & Microbe, 2015, 17(3): 392-403.

[10] YAO YP, ZHENG BQ, LI AJ, *et al.* *Bacillus subtilis* JTFM1001 and its application in prevention and control of aflatoxin pollution in maize[P]. China, ZL201610282974.2 20160503. (in Chinese).

[11] GUO Y, WANG N. Benefit analysis of silage corn planting, feeding and harvesting[J]. Agricultural Engineering, 2021, 11(3): 140-143. (in Chinese).

[12] ZHANG SL, HE ZH, WANG ZH, *et al.* Preliminary evaluation of 23 silage maize combinations containing African germplasms[J]. Mod Agric Sci Technol, 2015(22): 48-53. (in Chinese).

(Continued on page 58)

Table 4 Recovery and precision of 14 kinds of β-receptor agonists (n = 6)

Compound	Amount of added standard 5.0 μg/kg		Amount of added standard 10.0 μg/kg		Amount of added standard 100.0 μg/kg	
	Recovery//%	*RSD*//%	Recovery//%	*RSD*//%	Recovery//%	*RSD*//%
Cimaterol	83.1	6.4	88.2	5.1	93.4	4.4
Terbutaline	82.8	7.2	89.3	6.4	94.7	5.2
Salbutamol	85.7	5.8	92.7	4.7	103.8	3.1
Fenoterol	85.6	6.8	90.1	5.3	96.1	4.7
Ractopamine	83.4	7.3	87.6	6.0	95.2	4.2
Clorprenaline	86.2	6.9	91.0	4.1	102.7	3.8
Clenbuterole	83.2	7.5	86.9	5.8	94.8	5.0
Tulobuterol	84.8	6.1	92.7	5.1	105.5	2.8
Penbuterol	87.3	6.8	93.4	4.6	106.4	3.7
Zilpaterol	88.2	7.0	94.1	6.2	108.9	4.7
Cimbuterol	86.3	7.2	90.7	5.9	102.3	3.4
Brombuterol	85.8	6.8	89.3	6.1	97.8	4.1
Clenproperol	86.9	6.9	91.1	5.2	99.8	4.8
Bambuterol	87.9	5.9	92.5	4.7	104.2	3.8

An HPLC-MS/MS method for the determination of 14 β-receptor agonists in mutton was established by optimizing the conditions of chromatography and mass spectrometry. When the mass concentration was in the range of 0.05 – 0.5 μg/ml, the linear relationship of β-receptor agonists was good, with correlation coefficients (r) ≥0.999 2. The detection limits of the method were in the range of 0.04 – 0.87 μg/kg, and the quantitative limits were in the range of 0.35 – 1.86 μg/kg. The average recovery values were in the range of 82.8% – 108.9%, with RSDs (n = 6) in the range of 1.9% – 6.7%. The method is simple, sensitive, reproducible, accurate, and can be used for simultaneous determination of the 14 kinds of β-receptor agonist residues in mutton.

References

[1] CHENG QP, HAN F, WANG Y, *et al.* Research progress on β-agonists in food-borne animal tissues[J]. Journal of Food Safety and Quality, 2019, 10(2): 385 – 393. (in Chinese).

[2] ZHANG RY, LIANG MJ, ZHAO L, *et al.* Determination of β-agonists in food by solid phase extraction-high performance liquid chromatography-tandem mass spectrometry[J]. Chinese Journal of Food Hygiene, 2017, 29(2): 167 – 171. (in Chinese).

[3] CABAN M, STEPNOWSKI P, KWIATKOWSKI M, *et al.* Determination of β-blockers and β-agonists using gas chromatography and gas chromatography-mass spectrometry: A comparative study of the derivatization step[J]. J Chromatogr A, 2011, 1218(44): 8110 – 8122.

[4] SUO DC, WEI SL, XIAO ZM, *et al.* Simultaneous determination of 18 β-agonists in blood products for feeds by liquid chromatography tandem mass spectrometry[J]. Scientia Agricultura Sinica, 2019, 52(24): 4613 – 4623. (in Chinese).

[5] YANG M, YIN Y, HU XJ, *et al.* Determination of 14 β-agonists in infant formulas using QuEChERS-high performance liquid chromatography-tandem mass spectrometry[J]. Science and Technology of Food Industry, 2019, 40(11): 273 – 277.

[6] GAO Z, ZHANG Y, ZHANG YN, *et al.* Progress in the detection of residual β2-receptor agonists in animal tissues[J]. Chin J Sports Med, 2014, 33(4): 370 – 378.

[7] CHEN HL, XIE WP, WANG CL. Determination of β2-agonists in pig liver by liquid chromatography-tandem mass spectmetry conbined with acid hydrolysis for extraction[J]. Physical Testing and Chemical Analysis (Part B: Chemical Analysis), 2018, 54(12): 31 – 35. (in Chinese).

[8] WANG GN, WU NP, HE X, *et al.* Magnetic graphene dispersive solid phase extraction-ultra performance liquid chromatography-tandem mass spectrometry for determination of β-agonists in urine[J]. Journal of Chromatography B, 2017(1067): 18 – 24.

[9] YE P, YUE ZF, XIAO CG, *et al.* Determination of 28 β2-agonists in pork by liquid chromatography-tandem mass spectrometry[J]. Journal of Food Safety and Quality, 2013, 4(3): 682 – 688. (in Chinese).

[10] LI DN, YAN F, WU JP, *et al.* Simultaneous determination of seven β2-agonists in livestock manure by on-line cleanup liquid chromatography-tandem mass spectrometry[J]. Chinese Journal of Analytical Chemistry, 2014, 42(12): 1797 – 1803. (in Chinese).

Editor: Yingzhi GUANG Proofreader: Xinxiu ZHU

(Continued from page 54)

[13] NA RS, XIANG KF, LIANG QW, *et al.* Comparison of production performance and feed quality of eight silage maize varieties[J]. Feed Research, 2022, 45(13): 105 – 110. (in Chinese).

[14] YU XL, SONG Q, MA SM, *et al.* Study on agronomic characters and grass yields of 13 silage corn varieties in Longdong area[J]. Gansu Animal Husbandry and Veterinary, 2022, 52(4): 31 – 34. (in Chinese).

[15] WU XM, FANG ZH, CHI HW, *et al.* Comparison of 30 maize (*Zea mays*) varieties for food and feed in the Yanmenguan area[J]. Acta Prataculturae Sinica, 2022, 31(1): 205 – 216. (in Chinese).

[16] LIU Z, SHAO HF, WEN W, *et al.* Evaluation of agronomic traits and nutritional quality of 34 silage maize varieties in southern Ningxia[J]. Feed Research, 2021, 44(11): 98 – 104. (in Chinese).

[17] KE M, ZHU H, LIANG WW, *et al.* Grey correlation analysis of main agronomic characters and its yield and quality traits in *Sorghum sudanense*[J]. Pratacultural Science, 2016, 33(5): 949 – 955. (in Chinese).

Editor: Yingzhi GUANG Proofreader: Xinxiu ZHU