

Effects of Microbial Fertilizer on Forage Growth and Soil Chemical Properties

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The purpose of this study is to explore the effect of microbial fertilizer on pasture improvement. The natural grassland of *Leymus chinensis* grassland was selected as a test plot in a base, and each kind of plot was treated with different kinds of microbial fertilizer. Through the analysis of plant growth status, community biological characteristics and soil physical and chemical properties, the research of this paper is expanded and discussed in depth, which provides new methods and technical support for grassland improvement. The results showed that the community density of *Leymus chinensis* grassland was increased by 64.90% under the treatment of molasses. The output of *Leymus chinensis* was increased by 5.41g/ml, and the biomass of *Leymus chinensis* was increased by 296.47%. Molasses fermentation is a kind of microbial fertilizer that can improve pasture production. In addition, under humic acid treatment, the yield and community yield of *Leymus chinensis* was decreased by 2.03g/m² and 19.61g/m², respectively. Humic acid can reduce grassland productivity, and it is not suitable for grassland protection and improvement. At the same time, alginic acid is not conducive to the growth of *Leymus chinensis*. After comprehensive consideration, alginic acid on the improvement of grass is not a good scheme. At last, three kinds of fertilizer mixed fertilizer in *Leymus chinensis* grassland community yield was increased by 6.01g/ml, and the yield of *Leymus chinensis* was increased by 27.54g/m². Based on above findings, we conclude that the mixed fertilizers can increase grassland productivity.

1. Introduction

As an important component of terrestrial ecosystem, grassland is widely distributed all over the world. Its total area accounts for about one-fifth of the land (Al-Juhaimi, et al., 2014). The grassland ecosystem provides human beings with net grade material production. It has multiple ecological protection functions, including carbon accumulation and carbon sink, water conservation, climate regulation, improved soil, soil and water conservation and windbreak and sand fixation and other products and services (Bashan, et al., 2014). As the largest land resource in our country, grassland has many functions, such as ecology, society and economy. It is not only the production base for the development of animal husbandry, but also a powerful barrier to protect the ecological environment and maintain the ecological balance (Bhoya, et al., 2014). In addition, it is an important guarantee for the development of the minority economy and the maintenance of social development (Hentati, et al., 2015). The improvement of grassland can restore soil fertility and grassland productivity, and restore grassland to its original ecological environment, so as to protect the natural environment (Lazcano, et al., 2013). It can also increase forage production and promote the development of animal husbandry (Möller, 2015).

2. Basic introduction of microbial fertilizer

2.1 Introduction of microbial fertilizers

Microbial fertilizers are also called bio-fertilizers, inoculants and fungi. It is a microbial organism with a specific fertilizer effect (Odlare, et al., 2014). As an important component of soil, microorganisms have the functions of decomposition, nutrition, transmission and regulation of plant growth and so on (Pan, et al., 2016). They have important practical significance for soil physical and chemical properties and biological function and element

cycle (Ramezani, et al., 2015). The active ingredients of microbial fertilizer are mainly microorganisms and other active substances. The essence of microbial fertilizer is to increase the species and quantity of microorganisms in the soil and reconstruct the biological community of the system, so as to improve the soil state (Schoebitz, et al., 2014). Studies have shown that the application of microbial fertilizer can significantly increase the species and number of microorganisms in the main soil, thereby enhancing soil physical fertility and biological fertility (Sarmadi, et al., 2016). At the same time, microbial fertilizer can promote the absorption of soil nutrients by plants, strengthen their resistance to diseases and insect pests, protect plant growth, and improve crop yield and quality. Microbial fertilizers can also repair soil ecology without harming the environment. At present, there are many reports about microbial fertilizers regulating soil ecosystems.

2.2 Types of microbial fertilizers

There are three kinds of microbial fertilizers: compound microbial fertilizer, microbial inoculum and biological organic fertilizer (Tiecher, et al., 2014). According to its biological species, it also contains bacteria, fungi, actinomycetes and algae and so on. There are many kinds of microbial fertilizers, and their mechanisms are different. So, according to the different uses of microbial fertilizer, scientists have developed rhizobia fertilizer, silicate fertilizer, fertilizer, phosphorus fertilizer, nitrogen fixing bacteria *Bacillus* microbial preparation, plant growth regulator of microbial fertilizer. In practice, different microbial fertilizers can be used in combination to increase the yield of crops. In the fertilizer, we can add some auxiliary elements. In this way, it can guarantee the sufficiency of the nutrient content of crops and realize the purpose of high yield and high quality.

3. Effects of microbial manure on herbage growth and soil chemical properties

3.1 Experimental design

In this experiment, grassland of *Leymus chinensis* grassland and *Stipa grandis* grassland were selected as the experimental plots for fertilization and drilling operations. Two grassland types were selected as experimental plots. Test materials include humic acid, alginic acid and molasses fermentation, and mixed fertilizers of three fertilizers. The interaction with drilling measures, and set a control, a total of 10 processing, each treatment set 3 repetitions. Each sample plot is divided into 30 small ones. The area of each cell is 3mX5m, and a 1m isolation band is provided between the cells. Each treatment is arranged in random blocks.

3.2 Experimental treatment

The trial was conducted from June 2014 to August 2015. In the early days of the grasslands, we used a conical iron fork with a diameter of 1 cm and a length of 17 cm to pierce vertically. The density of the punch is $18 \times 18 = 324 / \text{m}^2$. It is fertilized every June. The amount of fertilizer applied shall be in accordance with the standards of fertilizer use. Then, on rainy days, it is applied to the earth's surface and soaked by rain to the soil. Sampling and analysis were carried out in 7 and August. According to local time in the grass, every year at the beginning of September, we cut the grass. The standard of fertilizations is as shown in Table 1.

Table 1: Experimental treatment

Processing number	Fertilizer type	Fertilizer (kg, ml / cell)
T1	No	0
T2	Molasses fermented	90ml
T3	Humic acid	1.125kg
T4	Alginic acid	1.125kg
T5	Humic acid, molasses hair and alginic acid	0.375kg, 30ml, 0.375kg

3.3 Test method for sampling and sample determination

Plant samples: From 2014 to 2015, the peak season of plant growth season was selected from 1 to 8 months in each study area, and the natural height of each plant was investigated. The biomass of the plant community was measured by the harvest method. The aboveground biomass of the plant community was measured by the harvest method.

Underground root sample: From 2014 to 2015, the peak season of plant growth season was selected from 1 to 8 months in each study area. In the quadrat of vegetation, the root drill with a diameter of 7cm was used for sampling. The sampling depth was 30 cm, with a total of 0-10, 10-20, and 20-30 cm³. Each plot is repeated three times, mixing as a sample, the sample is stratified into 100 objective gauze bags in the back to the lab, washing the sample to measure the drift.

Soil sample: In the sample of the vegetation, a 5-cm diameter diamond is used for sampling. The sampling depth is 30cm. It is divided into 0-10, 10-20, 20-30cm³ layer. Each sample is repeated three times. After

mixing evenly, it is taken as a sample. Partial 0-10 cm soil samples were used to determine enzyme activity. The other samples were dried in the dark to 1 mm and 0.25 mm sieves, respectively, for further determination of nutrients.

Soil bulk weight: In August 2015, the ring knife method was used to determine soil bulk density. The depth is 30cm. It is divided into 0-10, 10-20, 20-30cm³ layer, and put it into the aluminum box, measuring the weight.

Plant samples: After recovery, the plant fresh weight was weighed with an electronic balance (accuracy of 0.01), and then dried in an oven at 65 °C until constant weight was taken.

Samples of underground roots: the roots were rinsed at a temperature of 65 DEG C at the oven and baked to a constant weight. The root dry weight was measured by an electronic balance (accuracy 0.01).

Soil bulk density / water content: the aluminum box was placed at 105 DEG C for drying and the dry weight was measured.

Soil organic matter: sieved soil samples were measured by chemical methods.

Plant nitrogen content: the plant was dried and ground, and the 0.25mm sieve was removed. The H₂SO₄-H₂O₂ method was used to measure the plant nitrogen content.

Soil enzyme activity: enzyme activities were measured by microplate microplate assay.

3.4 Data analysis

Spss19.0 statistical analysis software was used to analyze the variance and significance of the data, and Excel was used to map the data.

4. Results and analysis

4.1 Effect of antimicrobial peptides on growth

As a grassland construction species, *Leymus chinensis* nutrient content is high. It is a kind of high quality forage. Therefore, the growth of *Leymus chinensis* is also an important indicator to determine the productivity of pasture. Typically, to a certain extent, the growth height of the plant can reflect the potential growth of the plant. The number and yield of plants are the most persuasive indicators of land productivity. The height of *Leymus chinensis* under different fertilization treatments in 2014 and 2015 is shown in Figure 1.

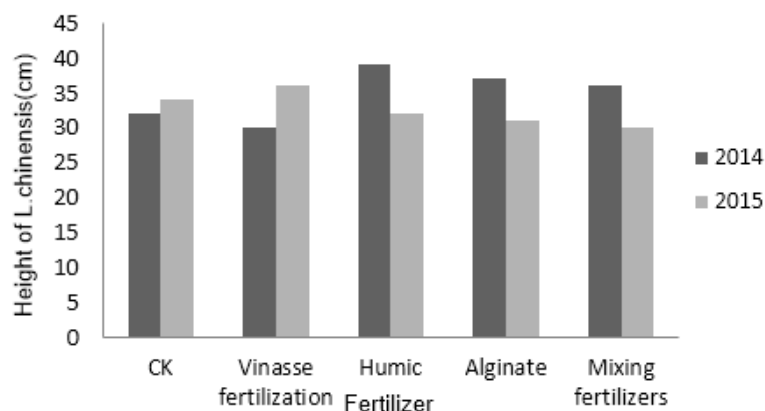


Figure 1: The height of *Leymus chinensis* under different fertilization treatments in 2014 and 2015

As can be seen from Figure 1, there was no significant difference in the height of *Leymus chinensis* between the treatments in the same year, indicating that there was no significant influence on the height of *Leymus chinensis* in the same year in different fertilization treatments. In 2014, the concentrations of humic acid, alginic acid and mixed fertilizer were higher than that of CK. The height of *Leymus chinensis* treated with molasses was slightly lower than CK. In 2015, the height of *Leymus chinensis* treated with molasses was slightly higher than that of CK, and the height of *Leymus chinensis* in other treatments was slightly lower than CK. Compared with 2014, the concentration of humic acid, alginic acid and mixed fertilizer showed a downward trend in 2015.

Density of *Leymus chinensis* under different fertilization treatments in 2014 and 2015 is shown in Figure 2.

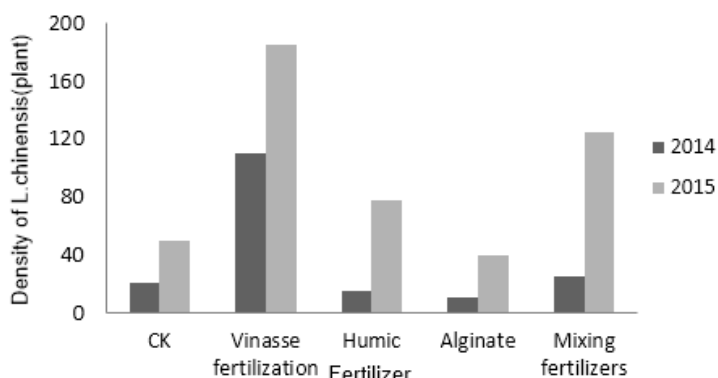


Figure 2: Density of *Leymus chinensis* under different fertilization treatments in 2014 and 2015

As shown in Figure 2, the density of CK in *Leymus chinensis* is relatively low in 2014, which may be due to the lack of precipitation to limit the growth of *Leymus chinensis*. After treatment with molasses, it significantly increased the density of *Leymus chinensis*. Compared with CK, it increased by 59%. After mixed fertilizer treatment, the density of *Leymus chinensis* also increased by 49% than that of CK. However, it did not reach a significant level. After humic acid and alginate treatment, the density of *Leymus chinensis* was reduced by 18% and 31.25% compared with CK. There is no significant difference between it and CK. In 2015, the density of *Leymus chinensis* increased by 360% compared with CK after treatment with molasses. After treatment with mixed fertilizer and humic acid, the density of *Leymus chinensis* increased by 105% and 43% compared with that of CK, but it was not significant. After treated with alginic acid, the density of *Leymus chinensis* was 30% lower than that of CK. It did not achieve significant difference with CK. Compared with the increase in 2014 in 2015, the growth rate of molasses treatment, humic acid treatment and mixed fertilizer treatment was higher than that of CK. Therefore, the effects of molasses treatment and mixed fertilizer treatment on growth of *Leymus chinensis* density were not obvious. Humic acid treatment had no significant effect on the density of *Leymus chinensis*, and alginate treatment could inhibit the density of *Leymus chinensis*. By comprehensive comparison, molasses fermentation has the best effect on promoting the density of *Leymus chinensis*. The biomass of *Leymus chinensis* under different fertilization treatments in 2014 and 2015 is as shown in Figure 3.

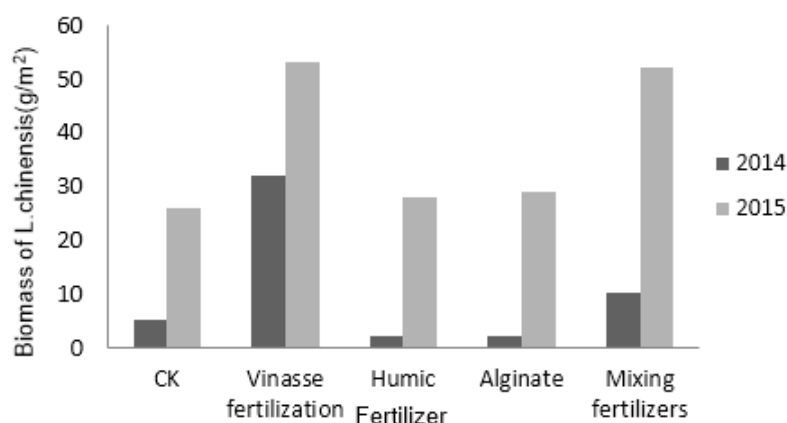


Figure 3: The biomass of *Leymus chinensis* under different fertilization treatments in 2014 and 2015

Figure 3 reflects the difference between the treatments of *Leymus chinensis* biomass. The results showed that the biomass of *Leymus chinensis* was increased by 560% compared with CK after 2014. The biomass of *Leymus chinensis* was increased by 32% compared with CK. After treatment with humic acid and alginic acid, the biomass of *Leymus chinensis* decreased by 12% and 21% compared with CK, which did not reach the significant level. In 2015, the yield of *Leymus chinensis* was significantly higher than that of CK after treatment with molasses and mixed fertilizer. They were increased by 133.18% and 125.18% respectively. The yield increment of *Leymus chinensis* is 29.30g/m² and 27.54g/m². The results showed that the biomass of *Leymus*

chinensis was increased by molasses fermentation every year, but the effect of mixed fertilizer treatment in 2014 was not significant. The treatment effect was remarkable in 2015, and this method could be delayed.

4.2 Influence of microbial fertilizer on soil enzyme activity

All biochemical reactions in the soil are carried out under the participation of soil enzymes. The level of soil enzyme activity can reflect the extent of soil biological activity and biochemical reaction. Enzyme activities in soil affect the soil nutrient cycle and plant growth and absorption. Therefore, soil enzyme activity is an important aspect of soil fertility determination. Different fertilizers and methods of soil improvement can have different effects on different enzymes. In this experiment, 8 kinds of enzymes in soil were selected and their activities were analyzed.

Table 2: Effects of antimicrobial peptides on the weight gain, specific growth rate, fatness and feed coefficient of cyprinus carpio

Enzymatic activity	CK	Molasses	Humic acid	Alginic acid	Mixed fertilizer
Phosphatase	1665.5±458.55a	1380.54±399.55a	1781.25±247.65a	1714.95±345.39a	1771.00±89.61a
β- Glucosidase	654.76±118.83abc	534.88±83.21c	821.84±102.35ab	879.80±229.49a	739.21±206.09abc
β- Cellobioglycosidase	139.24±39.53bc	131.97±10.98c	146.91±16.98bc	190.48±38.06ab	222.98±32.70a
Acetylglucosidase	103.82±6.16b	145.58±35.17ab	111.29±31.85b	133.01±35.62ab	184.41±38.74a
β- Xylosidase	240.22±72.58ab	253.10±70.44ab	268.88±62.71ab	185.51±30.78	326.62±87.14a
α- Glucosidase	80.13±10.64b	122.73±22.32	124.23±8.88a	128.04±23.09	105.16±33.00ab
Polyphenol oxidase	2.62±0.89b	3.79±0.14a	2.24±1.03b	2.67±0.63b	3.93±0.66a
Peroxidase	3.46±0.84a	3.82±0.22a	3.88±0.11a	3.77±0.14a	3.62±0.11a

As can be seen from Table 2, the soil enzyme activities of *Leymus chinensis* were different under different treatments. β-glucosidase treatment in all treatments except for the application of molasses fermentation is lower than CK (control), the other treatments have different degrees of increase. Among them, alginic acid fertilizer treatment was significantly higher than CK, which increased by 34.40% than CK.

β-cellobiose enzyme mixed fertilizer treatment significantly increased compared with CK, and it increased by 60.14%. The application of alginic acid fertilizer was also significantly higher than that of CK. Compared to the control, it also increased by 35%. Molasses fermentation treatment has shown a downward trend.

Acetylglucosidase mixed fertilizer treatment was significantly higher than CK. Compared to CK, it increased by 77.62%. There was also a significant increase in the treatment of molasses fermented with CK. Alginic acid treatment also has a small increase. However, none of them reached a significant level. Humic acid treatment and CK were essentially flat, and they were no significant differences.

β-xylosidase is the highest in mixed fertilizer treatment. Compared with CK, it grew 35.97%, but it did not reach a significant level. Alginic acid treatment was significantly lower than other treatments. Compared to CK, it is down, but its change is not obvious. There was no significant difference in the activity of p-xylose from other treatments.

α- glucosidase molasses fermented, humic acid and alginic acid were significantly higher than CK (control). Growth rate can reach more than 50%. Only mixed fertilizer treatment growth is relatively low, and it does not reach a significant level.

The treatment of polyphenol oxidase, molasses fermentation and mixed fertilizer was significantly higher than CK, which increased by 44.66% and 50% respectively. Humic acid treatment was lower than CK.

There was no significant difference between phosphatase and peroxidase in each treatment. However, the phosphatase treated by molasses decreased slightly compared with other treatments, and the treatment of peroxidase had a small increase. It can also be explained that fertilization treatment of *Leymus chinensis* grassland has great influence on soil enzyme activity.

5. Conclusions

In this study, the effects of microbial manure on herbage growth and soil chemical properties were analyzed and studied. First, under the molasses fermentation, the population density of *Leymus chinensis* was increased by 64.90%. *Leymus chinensis* production was increased to 5.41m². The biomass was increased by 13% and 296.47%, respectively. Molasses fermentation also improves a variety of enzyme activities, forage yield and soil fertility. It is a kind of improved cultivation of grassland microbial fertilizer. In addition, under the humic acid treatment, the yield and community yield of *Leymus chinensis* were reduced by 2.03 g / m² and

19.61 g/m², respectively. Humic acid reduces grassland productivity, which is not suitable for grassland conservation and improvement. At the same time, alginic acid was not beneficial to the growth of *Leymus chinensis* in *Leymus chinensis* grassland. After comprehensive consideration, the improvement of alginic acid to grassland is not a good scheme. In addition, three kinds of fertilizer mixed fertilizer in *Leymus chinensis* grassland community yield was increased by 6.01g/m², and the yield of *Leymus chinensis* was increased by 27.54g/m². The results showed that mixed fertilizer could improve grassland productivity.

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