Paper Type: Original Article

Effects of Fermentation with Different Bacterial Strains on Complete Feed for Finishing Pigs

Yongben Wang[1], Guofang Wu[1,2,3], Lei Wang[1*]

1. Academy of Animal Science and Veterinary Medicine, Qinghai University, Xining 810016, China;

2. Qinghai Provincial Key Laboratory of Plateau Livestock Genetic Resource Protection and Innovative Utilization, Xining 810016, China;

3. Key Laboratory of Livestock Genetic Breeding on Qinghai-Tibet Plateau, Ministry of Agriculture and Rural Affairs, Xining 810016, China.

Corresponding Author: Lei Wang , E-mail: 1040601346@qq.com

Abstract

Microbial fermented feed enhances the nutritional and fermentation quality of feed through microbial technology, making it suitable for livestock, poultry, and aquaculture, particularly for young animals and antibiotic-free farming needs. This study utilized Bacillus to ferment complete pig feed to investigate the effects of Bacillus on the nutritional and fermentation quality of the feed post-fermentation. The complete feed was mixed with water at a 1:1 ratio, and 5% Bacillus was added, thoroughly mixed, and then bagged (with an exhaust valve) for fermentation at room temperature for 10 days. A control group was set up with the same proportion of sterile water added to unfermented feed. The nutritional and fermentation quality of the fermented feed were subsequently measured. The results indicated that compared to unfermented feed, the contents of dry matter, crude fiber, neutral detergent fiber, ADF, ammonia nitrogen, and soluble sugar in fermented feed were significantly lower than those in the control group ($P < 0.05$), while the contents of crude protein and starch were significantly higher than those in the control group ($P < 0.05$). The lactic acid, acetic acid, and propionic acid in experimental group I and experimental group II were significantly higher than those in the control group ($P < 0.05$), whereas the pH and butyric acid content in the control group were significantly higher than those in experimental group I and experimental group II ($P < 0.05$). The acetic acid, propionic acid, and butyric acid contents in experimental group II were significantly higher than those in experimental group I, and the pH was significantly lower than that in experimental group I. Fermented feed with different Bacillus strains improved the nutritional quality and fermentation quality of the feed.

Keywords: Bacillus amyloliquefaciens, Bacillus licheniformis, fermented feed, lactic acid.

1 Introduction

With the large-scale development of the global pig farming industry, the shortage of feed resources and rising costs have become core issues constraining the sustainable development of

the industry. The annual production of agricultural waste such as straw is enormous, but its high crude fiber content (30%-50%) and complex lignin structure result in poor palatability and low digestibility when directly fed. Traditional physical or chemical pretreatment methods are associated with high energy consumption and environmental pollution risks. In recent years, microbial fermentation technology, centered on probiotics, has provided a new pathway for the high-value utilization of straw resources due to its green, economical, and efficient characteristics. Chen Xinzhu .[1] used lactic acid bacteria for silage fermentation of broad bean straw, which not only better preserved the nutritional value of the broad bean straw silage but also improved fermentation quality and microbial diversity. The microbial fermentation of animal feed can enhance its nutritional quality by eliminating anti-nutritional factors and improving the bioavailability of nutrients[2]. Studies have shown that Bacillus strains (such as Bacillus subtilis and Bacillus licheniformis) exhibit significant advantages during straw fermentation: the cellulase, xylanase, and lignin peroxidase they secrete can specifically decompose the cell wall structure, while their metabolites (such as organic acids and antimicrobial peptides) can inhibit the proliferation of pathogenic bacteria and improve the sanitary quality of the feed [3, 4].

This experiment compares the effects of single-strain fermentation by different strains on the nutritional components and fermentation quality of complete feed for fattening pigs, providing technical references for the subsequent development and application of fermented feed.

2 Materials and Methods.

2.1 Test materials and methods

The strains were Bacillus amyloliquefaciens YR3.2 and Bacillus licheniformis YR3.1 screened in the pre-test.The complete diet consisted of corn, soybean meal, bran, straw, vegetable oil, premix, premix was purchased from Cargill Feed (Yichun) Co. Ltd. and the rest of the feed ingredients were provided by Qinghai Yufu Livestock Development Co. The basal diet composition and nutrient levels are shown in Table 1.

Table 1　Basal dietary composition and nutrient level (dry matter base)

Ingredient	Content	Nutrient levels	Content
Corn	63%	DE，MJ/Kg	13.46
Soybean meal	17%	CP，%	14.28
Bran	5%	Ca，%	0.53
Straw	4%	TP，%	0.72
Edible oil	3%	Lys，%	0.96

Premix	8%	

Note: The premix provided per kilogram of the diets: VA 44000 IU, VD3 7000 IU, VE 200 mg, VK3 15 mg, VB2 35 mg, VB5 88 mg, Cu 120 mg, Fe 770 mg, Zn 2800 mg, Mn 300 mg, I 5 mg, Se 2 mg.

2.2 Test methods

(1) Seed solution

Take 100 μL of activated Bacillus spp. respectively and inoculate them into 1L of LB liquid medium, and incubate them at 37℃ with constant temperature and shock for 36 h, so that their viable bacterial count reaches 1×107 CFU/mL, and then obtain the seed liquid of Bacillus spp.

(2) Fermentation of full-value material

After mixing the whole material with water at the ratio of 1:1 and adding 5% of Bacillus sp., mix well and bag (with exhaust valve) to ferment at room temperature for 10 days, and then determine the nutritional quality and fermentation quality of the fermented feed. The CK group was unfermented feed (1:1 feed to water), test group I was Bacillus amyloliquefaciens YR3.2 fermented feed, and test group II was Bacillus licheniformis YR3.1 fermented feed.

2.3 Measurement indexes

Dry matter (DM) content was determined by drying method (GB/T 6435-2014), crude protein (CP) content was determined by Kjeldahl method (GB/T 6432-2018), crude fibre (CF) content was referred to according to the national standard GBT5009.10-2003 (1), and neutral detergent fibre (NDF) content was referred to the (GT/T 20806-2022), acid detergent fibre (ADF) content was determined according to the national standard (NY/T 1459-2022), and soluble sugars (SS), ammonia nitrogen (NH3-N), and amylose (AM) were determined using a test kit, which was purchased from Shanghai Enzyme Link Biotechnology Co. Lactic acid (LA), acetic acid (AA), propionic acid (PA) and butyric acid (BA) were determined by gas chromatography. pH was determined by hand-held pH meter. Three parallel samples were taken for each sample, and the relative deviation must be within the permissible range.

2.4 Results and analysis

The experimental data were pre-processed by Excel-2019 and analysed by one-way ANOVA (one-wayANONA) using SPSS-26.0 statistical software, and multiple comparisons were performed by Duncan's method, with $P<0.05$ indicating significant differences.

3 Results and analyses

3.1 Changes in nutrient composition before and after feed fermentation

The analyses of chemical composition of fermented feeds are shown in Table 2. Compared with unfermented feeds, the contents of dry matter, crude fibre, neutral detergent fibre, ADF, ammoniacal nitrogen, and soluble sugar in fermented feeds were significantly lower than those in the control group ($P < 0.05$), while the contents of crude protein and starch were significantly higher than those in the control group ($P < 005$).

Table 2 Changes in nutrient composition of fermented feed

Item	CK	Test Group I	Test Group II
DM，%	43.62±0.02	44.22±0.03	46.58±0.02
CP，%	14.29±0.02c	21.93±0.45a	16.32±0.25b
CF，%	23.26±0.21a	17.60±0.10b	17.16±0.21c
NDF，%	48.85±0.06a	44.90±0.15c	45.16±0.45b
ADF，%	20.16±0.15a	17.48±0.21c	18.88±0.26b
NH3-N，μg/g	5971.52±11.75 a	1457.73±10.72c	2855.78±9.68b
SS，mg/g	16.17±0.18a	9.33±0.23c	10.60±0.46b
AM，mg/g	144.91±2.25b	201.56±5.80a	203.64±6.85a

Note: The different letters is significant ($P <0.05$) and the same letter is not significant ($P> 0.05$).

3.2 Feed fermentation quality

As can be seen from Table 3, the volatile fatty acid content of fermented feeds of different strains differed, with lactic acid, acetic acid and propionic acid in test group I and test group II being significantly higher than that of the control group ($P < 0.05$), while pH and butyric acid content in the control group were significantly higher than that of test group I and test group II ($P < 0.05$). Test group II contained significantly higher levels of acetic, propionic and butyric acids than test group I and had significantly lower pH than test group I.

Table 3 The pH and volatile fatty acid content of the fermented feed

Item	CK	Test Group I	Test Group II

pH	6.26±0.01a	4.96±0.02c	5.24±0.01b
LA，mg/g	22.19±0.19b	41.09±0.34a	40.99±0.31a
AA，mg/g	0.74±0.04c	5.85±0.12b	8.75±0.19a
PA，mg/g	0.22±0.01c	0.61±0.03b	0.70±0.03a
BA，mg/g	2.81±0.06a	1.15±0.03c	2.11±0.03b

4 Discussion.

In the present study, it was found that the nutritional composition of feeds was significantly changed after fermentation treatment of Bacillus amyloliquefaciens YR3.2 with Bacillus licheniformis YR3.1. The CP content of Bacillus amyloliquefaciens YR3.2 fermented feed reached 21.93%, which was significantly higher than that of the unfermented group and Bacillus licheniformis YR3.1, probably due to the fact that Bacillus amyloliquefaciens has a strong non-protein nitrogen conversion ability, which can be used to convert the nitrogen source in the substrate into the bacterial protein through the secretion of proteases [5], and meanwhile, during the fermentation process, Bacillus amyloliquefaciens inhibited the growth of other stray bacteria that reduced the nitrogen source damage.

In terms of structural carbohydrates, both groups of fermentation treatments significantly reduced CF, NDF and ADF contents ($P<0.05$), with the highest reduction of ADF in the Bacillus amyloliquefaciens YR3.2 fermented feeds, which was presumably related to the higher activity of cellulase and xylanase secreted by Bacillus amyloliquefaciens [6].The magnitude of the reduction of NDF indicated that the fermentation treatments effectively damaged the plant cell wall structure, which may enhance the nutrient release rate of the feed in the digestive tract of the animals, which is in line with the description of the mechanism of fermentation to improve feed digestibility by Chang Juan [7] .

Regarding nitrogen metabolism, the NH3-N content in the Bacillus amyloliquefaciens YR3.2 fermented feed group was only 24.4% of that in the unfermented group, suggesting that B. amyloliquefaciens could convert free ammonia into bacterial proteins more efficiently and reduce nitrogen loss, and this difference might be related to the urease activity and nitrogen assimilation efficiency of the strain [8]. Carbon source utilisation characteristics showed that fermentation treatments significantly reduced SS content while AM content was significantly elevated to 201.56-203.64 mg/g, suggesting that Bacillus preferentially utilised soluble sugars as a carbon source, while possibly releasing bound starch or promoting extracellular polysaccharide synthesis through enzymatic degradation [9].

Although both Bacillus species improved feed quality, Bacillus amyloliquefaciens performed

better in elevating CP, reducing ADF and NH3-N, while Bacillus licheniformis was more advantageous in starch accumulation. This functional difference may be related to strain-specific metabolic pathways: Bacillus amyloliquefaciens may have a stronger protein synthesis system, while Bacillus licheniformis has a more prominent α-amylase secretion capacity [10-12].

In the present study, we found that fermentation of feed by Bacillus amyloliquefaciens YR3.2 and Bacillus licheniformis YR3.1 significantly altered the pH and organic acid profile of the feed ($P<0.05$). The pH values decreased to 5.24 and 4.96 after fermentation treatment, respectively, which were 1.02-1.30 units lower than that of the unfermented group, and showed strain-specific gradient differences, which were closely related to the type and concentration of organic acids they produced. Lactic acid, as the main metabolite, was significantly elevated to the level of 41 mg/g in both treatment groups, which is in line with the characteristics of heterozygous fermentation of Bacillus [13]. the significant decrease in pH was strongly correlated with the accumulation of lactic acid ($P<0.05$), and such an acidic environment not only inhibits the proliferation of spoilage bacteria (e.g., butyric acid-producing bacteria) but also activates the endogenous enzyme system of the feed. The butyric acid content of the Bacillus amyloliquefaciens YR3.2 fermented group was 59.1% lower than that of the unfermented group, which, in combination with its higher lactate/butyric acid ratio, suggests that the fermented feed inhibited butyric acid-producing bacterial activity through competitive metabolism, a mechanism that is in agreement with the findings of Lonneke O [14]. Whereas the relatively higher butyric acid content of the Bacillus licheniformis YR3.1 fermented group may be related to its metabolite diversity, it was still 24.9% lower overall than the unfermented group.

Analysing the organic acid composition, the acetic acid/propionic acid ratio was significantly higher in the Bacillus licheniformis YR3.1 fermentation group than in the Bacillus amyloliquefaciens YR3.2 fermentation group, and this difference may affect the palatability and metabolizable energy value of the fermented feeds. Acetic acid, as a major volatile fatty acid, promotes milk fat synthesis in ruminants, while propionic acid is an important raw sugar precursor, a property that suggests that Bacillus licheniformis-fermented feeds may be more suitable for lactating ruminants [15]. Meanwhile, an acidic system dominated by lactic acid was formed in both treatment groups, and this environment is conducive to maintaining feed storage stability, and its pH has been lower than the optimal growth range of most pathogenic bacteria (e.g., Escherichia coli) (pH 6.0-7.0), which is similar to that of the experimental results of Zhang Fan [16].

5 Conclusion

This study revealed that Bacillus amyloliquefaciens and Bacillus licheniformis significantly increased the crude protein content and decreased the crude fibre content of feed, in which the effect of Bacillus amyloliquefaciens YR3.2 in decreasing NDF and ADF was better than that of Bacillus licheniformis YR3.1, and the two strains of fermented feeds also produced a large

amount of organic acids, which improved the nutritional quality of the feeds and the quality of fermentation.

Funding

This study was sponsored by Probiotics Application and Demonstration in the Eight-brow Pig Industry Chain (2023-NK-137).

References

[1] CHEN Xinzhu, LIN Pingdong, YUE Jian, et al. Effects of different additives on silage quality and microbial diversity of broad bean straw[J]. Journal of Grass Industry,2025,34(04):164-174.

[2] Zhang Y, Shi C, Wang C, et al. Effect of soybean meal fermented with Bacillus subtilis BS12 on growth performance and small intestinal immune status of piglets[J]. Food and Agricultural Immunology, 2018, 29(1): 133-146.

[3] WANG Zongshen,JIANG Hailong. Optimisation of fermentation conditions of DDGS by Bacillus subtilis strain BS2 and Bacillus licheniformis strain BL1[J]. Feed Industry,2021,42(20):18-25.

[4] Chukwuma O B, Rafatullah M, Tajarudin H A, et al. Lignocellulolytic enzymes in biotechnological and industrial processes: a review[J]. Sustainability, 2020, 12(18): 7282.

[5] YANG Dingyong,XIONG Yan. Evaluation of in vitro feed protein degradation capacity of Bacillus subtilis[J]. Heilongjiang Animal Husbandry and Veterinary Medicine,2017,(19):164-167.

[6] ZHANG Lulu,WEI Xubiao,S Dayong,et al. Screening and identification of cellulase-producing Bacillus cereus[J]. Feed Industry,2016,37(18):45-50.

[7] CHANG Juan,YIN Qingqiang,REN Tianbao,et al. Effects of steam blasting pretreatment and microbial fermentation on the degradation rate of corn stover[J]. Journal of Agricultural Engineering,2011,27(04):277-280.

[8] ZHANG Zhixiao, ZE Sangzi, TU Lianrong, et al. Phylogenetic study of soil urease activity regulatory factors and urease-active bacteria[J]. Western Forestry Science,2018,47(01):65-73.

[9] Tian Xiaoqun. Preliminary study on alkaliphilic Bacillus sp (Bacillus sp) NT-39 and the alkaline amylase it produces[J]. Journal of Wuhan University (Natural Science Edition),1990,(02):105-111.

[10] Wu Q. Analysis of cytidine metabolic pathway in Bacillus amyloliquefaciens and construction of efficient gene knockout system[D]. Ningxia:Ningxia University,2016.

[11] Niu Dandan,Shi Guiyang,Wang Zhengxiang. Bacillus licheniformis secreting highly efficient proteins and its industrial applications[J]. Biotechnology Bulletin,2009,(06):45-50.

[12] CHEN Yu Kun, LI Qing Hua, ZHOU Jing Wen, et al. High-throughput screening of α -amylase-producing Bacillus licheniformis based on droplet microfluidics[J]. Food and Fermentation Industry,2021,47(17):41-46.

[13] Slattery L , O'Callaghan J , Fitzgerald G F ,et al.Invited review: lactobacillus helveticus--a thermophilic dairy starter related to gut bacteria. [J].Journal of Dairy ence, 2010, 93(10):4435-4454.

[14] Onrust L, Ducatelle R, Van Driessche K, et al. Steering endogenous butyrate production in the intestinal tract of broilers as a tool to improve gut health[J]. Frontiers in veterinary science, 2015, 2: 75.

[15] Chung Y H , Ii H G B , Williams C C ,et al. Effects of methionine and lysine on fermentation in vitro and in vivo, nutrient flow to the intestine, and milk production.[J].Journal of Dairy Science, 2006, 89(5):1613-1620.

[16] ZHANG Fan,HAN Shumin,LI Jingchun,et al. Effects of different additives on the fermentation quality of maize seeds[J]. Herbology,2018,(S1):14-15.