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Solid-state fermentation of corn-soybean meal mixed feed with *Bacillus subtilis* and *Enterococcus faecium* for degrading antinutritional factors and enhancing nutritional value

Changyou Shi, Yu Zhang, Zeqing Lu and Yizhen Wang*

Abstract

Backg o nd: Corn and soybean meal (SBM) are two of the most common feed ingredients used in pig feeds. However, a variety of antinutritional factors (ANFs) present in corn and SBM can interfere with the bioavailability of nutrients and have negative health effects on the pigs. In the present study, two-stage fermentation using *Bacillus subtilis* followed by *Enterococcus faecium* was carried out to degrade ANFs and improve the nutritional quality of corn and SBM mixed feed. Furthermore, the microbial composition and in vitro nutrient digestibility of inoculated mixed feed were determined and compared those of the uninoculated controls.

Re l : During the fermentation process, *B. subtilis* and lactic acid bacteria (LAB) were the main dominant bacteria in the solid-state fermented inoculated feed, and fermentation produced a large amount of lactic acid (170 mmoL/kg), which resulted in a lower pH (5.0 vs. 6.4) than the fermented uninoculated feed. The amounts of soybean antigenic proteins (β-conglycinin and glycinin) in mixed feed were significantly decreased after first-stage fermentation with *B. subtilis*. Inoculated mixed feed following two-stage fermentation contained greater concentratioin of crude protein (CP), ash and total phosphorus (P) compared to uninoculated feed, whereas the concentrations of neutral detergent fiber (NDF), hemicellulose and phytate P in fermendted inoculated feed declined ($P < 0.05$) by 38%, 53%, and 46%, respectively. Notably, the content of trichloroacetic acid soluble protein (TCA-SP), particularly that of small peptides and free amino acids (AA), increased 6.5 fold following two-stage fermentation. There was no difference in the total AA content between fermented inoculated and uninoculated feed. However, aromatic AAs (Phe and Tyr) and Lys in inoculated feed increased, and some polar AAs, including Arg, Asp, and Glu, decreased compared with the uninoculated feed. In vitro dry matter and CP digestibility of inoculated feed improved ($P < 0.05$) compared with the uninoculated feed.

Concl ion : Our results suggest that two-stage fermentation using *B. subtilis* followed by *E. faecium* is an effective approach to improve the quality of corn-soybean meal mixed feed.

Ke o d : Antinutritional factors (ANFs), In vitro digestibility, Mixed feed, Nutritional value, Two-stage fermentation

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Background

C (BM)

C . H ,

- BM

(ANF),

, , ,

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1.

,

, 2, 3. P

4. F ,

, , ,

5.

P

ANF 6.

I C ,

(F),

ANF ,

7., 8.,

9.

F. H ,

F

. F

(FLF)

. A

FLF -FLF

,

H FLF

10. I , BM

Bacillus subtilis

.

ANF . , *Enterococcus faecium*

H .

Methods

Mic oo gani m and ba al b a e

B. subtilis J12-1

( ). *B subtilis* J12-1

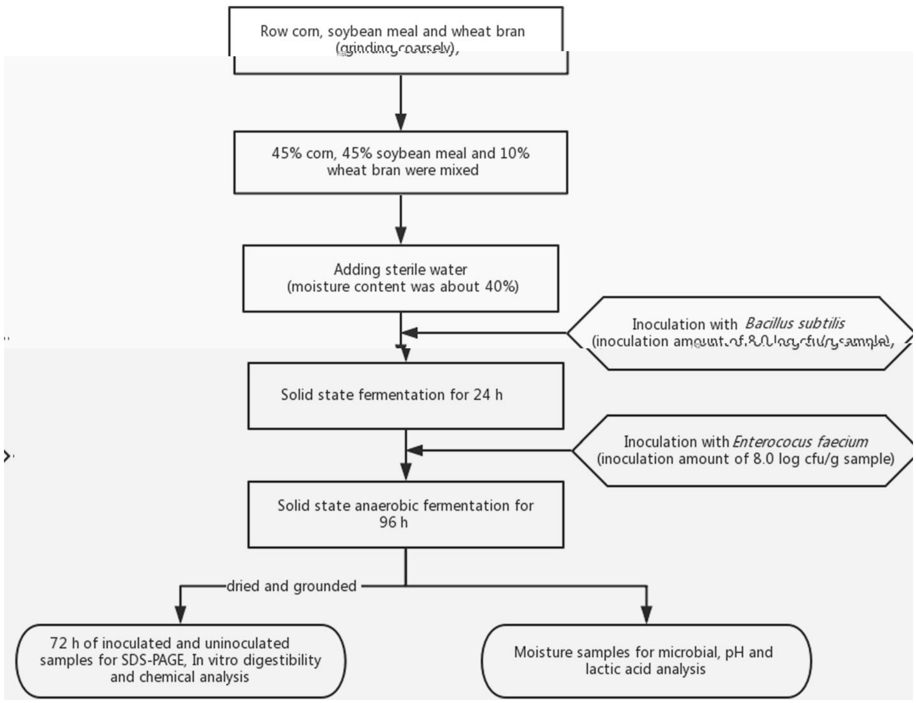

Fig. 1 Schematic outline of the manufacturing process for two-stage fermented feed

O L D, B , H , E) 30 C 2 . N B *Bacillus* . 37 C 1 . .

Chemical anal i

(24 *Bacillus subtilis* 48 *Enterococcus faecium*) (DM), (CP), , (NDF), (ADF), , (C) (P) AOAC I (2005). (CA- P) O . 11.. N D 12.. P - P 28.2% . (L-8900; H , , J). B , 6 /L HC 110 C 24 . M M . H ( ) HI 99163 H (H , , I, A) 2 18 L .

(N - J B C ., N , C) . β- (ELI A) (L B C ., B , C). .

Sodi m dodec l lfa e-pol ac lamide gel elec opho e i (SDS-PAGE)

E 13. . 60- , 1.5 L –HC (20 /L, H 8.3), 0.1% D , 5 /L 5 μ / L 0.1 , 30 . 14,000 *g* 10 4 C (5804 , E , G), E . B - P A K (B - , A). D -PAGE 14.. 4 - 12% 0.1% D . A 20 μ 65 120 . A

26616 - (10–170 D)
. A ,
C B B -250 (B -
, A) 45 - 7%. .

In i o dige ibili

A -
. 15,
. I ,
(3) 150 L
E . 10,000 / L -
(: 3,000 / ,) (0.05 /L
KC -HC , H 2.0)
39 C 150 ( ) 4 . H
7.0 1 /L N OH, 150
(: 250 / ,)
, ,
39 C 150 4 . A
, 5 L 20%
30 .
3,000 15 ,
.
105 C 4 CP
AA . I (%) = (
–) /
100%.

S a i ical anal i

G L M A
(A , 1999). A 0.05
.
.

Results

Mic obial compo i ion, pH and lac ic acid concen a ion d ing SSF

F 2 , H
F. -
, *B. bacillus* LAB
-
(F . 2). *B. subtilis*
8.0 / . A 24 ,
9.6 / ,
. LAB
(<3.0 /),
LAB 24 8.1 / -
E. faecium; 9.6 /
48 . D
, LAB
B. subtilis. N ,
Enterobacteriaceae ( )
- ,
(8.3 /) 24 . H ,
Enterobacteriaceae
,
(<3.0 /).
B. subtilis *E. faecium*
H
(F . 2).
H
B. subtilis (6.8 24 -
. 6.4 0
). A *E. faecium,* H
6.8 5.0. A
-
,
31 170 / -
- .

Biodeg ada ion of o bean an igenic p o ein of mi ed feed af e fe men a ion

O -
23–80 D
(F . 3). -
, β- α, α
β,
. F - *B. subtilis*
. α α β-
F. I ,
(<25 D)
. H ,
- *E. faecium* -
.
1. B β-
, -
- .

Chemical compo i ion

-
72
2. C ,
CP, , P,
NDF,
P ($P < 0.05$) 39%, 53%, 46%, -
. N , CA- P (<10 D)
1.18%, 6.5-
. I *B. subtilis*
E. faecium AA
(A ,
; A , G) , (P
) L . C

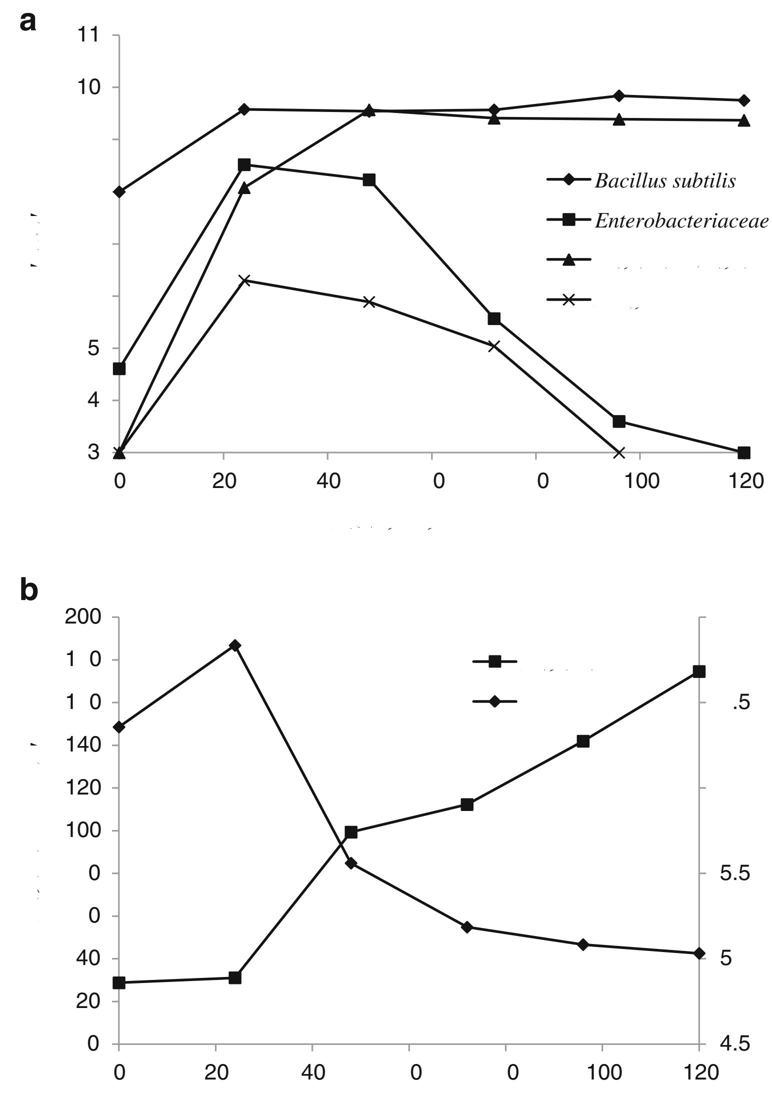

Fig. 2 Microbial composition (log cfu/g) **a**, pH, and lactic acid concentration (mmol/kg) (**b**) in inoculated feed during solid-state fermentation, on a DM basis

, AA 7%; , .

In i o amino acid dige ibili of he fe men ed ample

AA - - 3. I CP DM - (P < 0.05) 8% 11%, , . I , 11 , (H , I , L , M , P), (P < 0.05). N , (H , P C) 10%.

Discussion

I E. 16, 17. FLF - >9 / LAB (>150 /L), - (GI) , *Salmonella* 18. A FLF - 19., 20., 21. F FLF 22.,

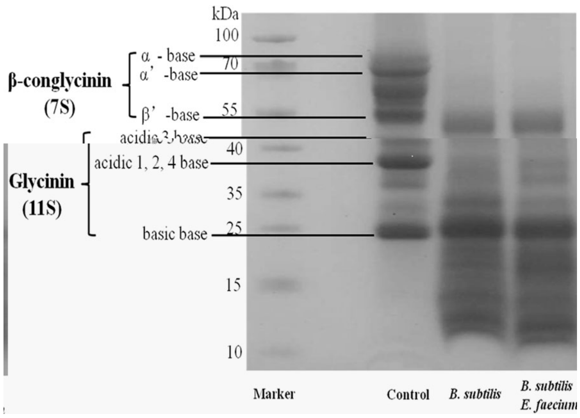

Fig. 3 Distribution of peptides in inoculated and uninoculated feed. Marker: protein molecular weight markers (10–100 kDa); control: uninoculated mixed feed (corn 45%, soybean meal 45%, wheat bran 10%) was incubated at 37 °C for 72 h; *B. subtilis*: the mixed feed was inoculated with *B. subtilis* and fermented at 37 °C for 24 h; *B. subtilis* and *E. faecium*: first-stage fermented (*B. subtilis*) mixed feed was inoculated with *E. faecium* and fermented under anaerobic conditions at 37 °C for 48 h

Table 1 Effect of fermentation on the concentration of soybean antigenic protein, as air-dry basis

Item	Glycinin		β-conglycinin	
	Content, mg/g	Degradation[a], %	Content, mg/g	Degradation, %
Raw mixed feed	63.74	-	31.76	-
Uninoculated feed[b]	61.02	-	32.15	-
B. subtilis[c]	7.97	86.94	6.98	78.28
B. subtilis and *E. faecium*[d]	8.47	86.12	7.12	77.53

[a]Degradation rate = soybean antigenic protein content in uninoculated feed – soybean antigenic protein content in inoculated feed) / soybean antigenic protein content in uninoculated feed × 100%

[b]Uninoculated feed: sterile medium was added to mixed feed (45% corn, 45% soybean meal and 10% wheat bran) instead of inoculated bacteria, other experimental procedures were the same as those of inoculated mixed feed

[c]*B. subtilis*: mixed feed was inoculated with *B. subtilis* and fermented at 37 °C for 24 h

[d]*B. subtilis* and *E. faecium*: first-stage fermented (*B. subtilis*) mixed feed was inoculated with *E. faecium* and incubated under anaerobic conditions at 37 °C for 48 h

Table 2 Analyzed nutrient composition of fermented inoculated and uninoculated feed, as air-dry basis[1]

Item	Inoculated feed	Uninoculated feed[2]	AA compositon	Inoculated feed	Uninoculated feed
DM,%	88.06 ± 1.02	89.09 ± 1.67	Indispensable AA, %		
CP,%	27.61 ± 2.73[a]	24.03 ± 1.93[b]	Arg	1.01 ± 0.15[b]	1.17 ± 0.19[a]
TCA-SP,%	8.85 ± 1.19[a]	1.18 ± 0.12[b]	His	0.58 ± 0.08	0.52 ± 0.11
Fat,%	3.37 ± 0.65	3.67 ± 0.73	Ile	0.78 ± 0.13	0.78 ± 0.16
NDF,%	8.33 ± 0.95[b]	13.64 ± 0.99[a]	Leu	1.50 ± 0.18	1.46 ± 0.23
ADF,%	3.58 ± 0.40	3.49 ± 0.76	Lys	1.17 ± 0.08[a]	0.99 ± 0.10[b]
Hemicellulose[3],%	4.75 ± 0.87[b]	10.15 ± 0.56[a]	Met	0.26 ± 0.05	0.23 ± 0.07
Ash,%	4.71 ± 0.51[a]	3.77 ± 0.38[b]	Phe	1.78 ± 0.26[a]	0.86 ± 0.13[b]
Ca,%	0.18 ± 0.03	0.17 ± 0.02	Thr	0.79 ± 0.12	0.75 ± 0.09
Total P,%	0.55 ± 0.05	0.49 ± 0.07	Val	1.06 ± 0.12	1.04 ± 0.17
Phytate P,%	0.21 ± 0.04[b]	0.39 ± 0.04[a]	Dispensable AA,%		
			Asp	1.68 ± 0.10[b]	1.92 ± 0.17[a]
			Ser	0.79 ± 0.14	0.75 ± 0.16
			Glu	3.23 ± 0.58	3.49 ± 0.44
			Gly	0.85 ± 0.18	0.80 ± 0.13
			Ala	0.98 ± 0.16	0.94 ± 0.10
			Cys	0.48 ± 0.05[a]	0.38 ± 0.06[b]
			Tyr	1.40 ± 0.21[a]	0.67 ± 0.09[b]
			Pro	1.09 ± 0.17	1.17 ± 0.21
			Total AA	19.56 ± 2.33	18.12 ± 2.47

[1]Values are means of three replicates per treatment. Means in a row without common superscript differ significantly ($P < 0.05$)
[2]Uninoculated feed: sterile medium was added to mixed feed (45% corn, 45% soybean meal and 10% wheat bran) instead of inoculated bacteria, other experimental procedures were the same as those of inoculated feed
[3]Hemicellulose = NDF-ADF

14, 27. ELI A … β- … 78 88%, … . H , … B. subtilis … . B. subtilis … , … , … , … 30. , … B. subtilis … ( ). I … CP, , P … , … 31., … 14, 32., 33, 34. … 35. ( )

36. B … CP , AA … . H … , … , … , A G … , , P , L … . … 14. … B. bacillus 62.93%. … A , AA L , … L , P , 37., … . … F. H , … . F , … CA- P (8.8%) … (1.2%). A … CA- P

Table 3 In vitro CP and AA digestibility (%) of fermented inoculated feed and uninoculated[1]

Item	Inoculated feed	Uninoculated feed[2]
DM,%	70.60 ± 2.87[a]	59.33 ± 2.32[b]
CP,%	86.28 ± 2.23[a]	78.36 ± 2.04[b]
Indispensable AA,%		
Arg	82.50 ± 4.65	82.72 ± 3.87
His	84.91 ± 3.70[a]	74.85 ± 3.46[b]
Ile	80.49 ± 3.42[a]	75.62 ± 2.44[b]
Leu	77.30 ± 3.04[a]	69.71 ± 2.81[b]
Lys	84.59 ± 3.91	81.44 ± 3.60
Met	85.30 ± 3.96[a]	70.31 ± 2.74[b]
Phe	81.99 ± 4.25[a]	65.64 ± 3.63[b]
Thr	78.73 ± 4.12	75.03 ± 3.83
Val	80.74 ± 3.77[a]	74.49 ± 3.48[b]
Mean	81.29 ± 4.09[a]	74.80 ± 3.21[c]
Dispensable AA,%		
Asp	83.14 ± 5.32	78.54 ± 4.97
Ser	77.86 ± 3.13	74.29 ± 3.74
Glu	85.13 ± 2.47[a]	80.25 ± 3.02[b]
Gly	80.78 ± 4.21	76.70 ± 4.08
Ala	84.53 ± 3.38[a]	75.51 ± 3.66[b]
Cys	79.74 ± 3.64[a]	67.90 ± 3.87[b]
Tyr	81.86 ± 3.43[b]	72.28 ± 3.10[c]
Pro	79.31 ± 4.28	75.41 ± 3.94
Mean	82.72 ± 3.11[a]	77.16 ± 3.04[b]
Total AA,%	82.15 ± 3.43[a]	76.07 ± 3.35[c]

[1]Values are means of three replicates per treatment. Means in a row without common superscript differ significantly ($P < 0.05$)
[2]Uninoculated feed: sterile medium was added to mixed feed (45% corn, 45% soybean meal and 10% wheat bran) instead of inoculated bacteria, other experimental procedures were the same as those of inoculated feed

( ). CA- P (2–20). AA 38. D- CA- P , AA , AA 39. F , NDF, , FMF . , (N P)- , . F *Aspergillus niger* . 33., NDF, , 14.45%, 43.72%, 86.08%, , F. P P

34. , NDF - . - *B. subtilis* *E. faecium* - BM .

Conclusions

B. subtilis *E. faecium* ANF (, NDF,) - BM CA- P CP . F , H *Enterobacteriaceae.* DM CP . , , . O F .

Additional file

Additional file 1: Strain identification information. (DOCX 1027 kb)

Abb e ia ion

AA: Amino acid; ANFs: Antinutritional factors; Ca: Calcium; CP: Crude protein; FLF: Fermented liquid feed; LAB: Lactic acid bacteria; NDF: Neutral detergent fiber; NSP: Non-starch polysaccharide; P: Phosphorus; SBM: Soybean meal; SDS-PAGE: Sodium dodecyl sulfate – polyacrylamide gel electrophoresis; SSF: Solid state fermentation; TCA-SP: Trichloroacetic acid soluble protein

Ackno ledgmen

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F nding

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A ailabili of da a and ma e ial

The datasets generated and analyzed during the current study are not publicly available. Please contact the authors for data requests.

A ho ' con ib ion

YZW and CYS conceived and designed the experiment. CYS and YZ carried out the experiment, including the solid-state fermentation, chemical analysis, and determination of in vitro digestibility. CYS analyzed the data and wrote the manuscript. ZQL verified the validity and checked the results. All authors read and approved the final version of this manuscript.

Compe ing in e e

The authors declare that they have no competing interests.

Con en fo p blica ion
Not applicable.

E hic app o al and con en o pa icipa e
Not applicable.

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