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Full Length Research Paper

Comparison of broiler performance, carcass yields and intestinal microflora when fed diets containing transgenic (Mon-40-3-2) and conventional soybean meal

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This study was conducted to analyze the effects of transgenic glyphosate-tolerant soybeans on the performance, carcass yields and intestinal microflora of broiler chickens. Three hundred and sixty one-day-old Abor Aerec broilers were randomly divided into two dietary treatments, adding genetically modified (GM) glyphosate-tolerant soybean meal or conventional soybean meal, respectively. Broiler body weight and feed intake were recorded at regular intervals (day 0, 21 and 42). Chickens were slaughtered at day 42 for carcass yield measurement and sampling. Diversity of the ileum and cecum microflora was determined by denaturing gradient gel electrophoresis (DGGE) technique and DNA sequencing. No treatment differences (P > 0.05) were detected among dietary treatments for any measured performance and carcass parameters. The microbial population in ileum and cecum also had no significant difference between the two treatments (*P*>0.05). The similarity of the total ileum and cecum microflora between the two treatments was about 62 and 58%, respectively. The DNA-DGGE electrophoresis pattern bands of intestine microbe were divided into two groups because of the different diet. Fifteen DGGE DNA bands were identified, of which five of them were identified as known bacteria. The current study showed that there were no adverse effects of the transgenic soybean meal on the intestinal microflora of broilers.

Key words: Broiler, glyphosate-tolerant soybean meal, intestinal microbiota, feed safety.

INTRODUCTION

With the development of the transgenic technology, great contributions have been made to the famine and poverty alleviation by increasing crop production and lowering the cost of food consumption. However, the wide application of this technology has also caused many biosafety problems (Lu et al., 2010; Yoshimura et al., 2006). Therefore, attention must be paid to assessing its potential safety problems. In 2010, the cultivated area of the glyphosate-tolerant transgenic soybean was about 7330 hectares, nearly the 50% of the total cultivated area of transgenic plant (Adenle, 2011). The by-product of the transgenic soybean has been extensively used in husbandry industry. Its feed safety is not only associated with the health of livestock, but with the human's health. At present, the safety assessment of transgenic crops is mainly focused on the feeding value evaluation (Jiao et al., 2010; Taylor et al., 2007), the fate of the transgenic DNA and protein in the animal products (Jennings et al., 2003), and the immunity and breed parameters of animals (Brake and Evenson, 2004; Finamore et al., 2008).

However, the effects of transgenic crops on the intestinal health have been less studied. The gut is the

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Table 1. Diet composition and nutrient level.

Ingredient	CSM group		TSM group	
	Starter (0 - 21 day)	Grower (22 - 42 day)	Starter (0 - 21 day)	Grower (22 - 42day)
Corn (Crud protein 7.8%)	53.33	56.97	54.63	57.45
Conventional soybean meal (GSM)	36.00	34.00	-	-
Transgenic soybean meal (TSM)	-	-	36.00	34.00
Corn protein powder (Crud protein 55%)	3.30	1.71	2.00	1.30
Corn oil	2.50	3.00	2.50	3.00
limestone	1.30	1.27	1.30	1.21
Calcium hydrophosphate	1.90	1.57	1.90	1.57
L-Lysine-HCL	0.06	0.004	0.06	0.00
Methionine	0.26	0.126	0.26	0.12
Choline chloride (50%)	0.05	0.05	0.05	0.05
Salt	0.30	0.30	0.30	0.30
Premix (1%)	1.00[1]	1.00[2]	1.00[1]	1.00[2]
Total	100	100	100	100
Nutrition level				
ME (Mcal/kg)[3]	2.95	3.01	2.95	3.01
Crude protein (%)	21.78	19.93	21.78	19.93
Calcium (%)	1.02	0.90	1.02	0.90
Available phosphorus (%)	0.46	0.41	0.46	0.40
Lysine (%)	1.16	1.00	1.16	1.02
Methionine (%)	0.58	0.40	0.58	0.42
Methionine+ cysteine (%)	0.92	0.78	0.92	0.77

[1]The premix provides the following per kg diet: Vitamin A. 8000 IU; vitamin D_3, 1000 IU; vitamin E, 20 mg; vitamin K, 0.5 mg; vitamin B_1, 2.0 mg; vitamin B_2, 8.0 mg; D-pantothenic acid, 10.0 mg; nicotinic acid, 35.0 mg, VB_6, 3.5 mg; biotin, 0.18 mg, folic acid, 0.55 mg; vitamin B_{12}, 0.01 mg; choline, 1300 mg; Fe (as ferrous sulfate), 100 mg; Cu (as copper sulfate), 8.0 mg; Zn (as zinc sulfate), 100 mg; Mn, 120 mg; I, 0.7 mg; Se, 0.3 mg; flavophospholipol, 6 mg. [2] The premix provides the following per kg diet: Vitamin A, 6000 IU; vitamin D_3, 750 IU; vitamin E, 10.0 IU; vitamin K_3, 0.5 mg; vitamin B_1, 2.0 mg; vitamin B_2, 5.0 mg; D-pantothenic acid, 10.0 mg; nicotinic acid, 30.0 mg; vitamin B_6, 3.0 mg; biotin, 0.15 mg, folic acid, 0.55 mg; vitamin B_{12}, 0.01 mg; choline, 1000 mg; Fe (as ferrous sulfate), 80 mg; Cu (as copper sulfate), 8.0 mg; Zn (as zinc sulfate), 80 mg; Mn, 100 mg; I, 0.7 mg; Se, 0.3 mg; flavophospholipol, 4 mg. [3]ME is the calculated value. Other nutrient levels are measured values.

first defensive line to protect the animal body from the damage by the harmful matters from feed. If the transgenic soybean meal is harmful to broilers, it may first damage the intestinal protection system, especially of the intestinal mucosal immune system and the intestinal microflora of broilers. Gut microbe is an important constituent part of the animal digestive organ, which can enhance host metabolic function, protect against pathogens, enhance adaptive immunity and modulate gut physiology (Lee and Mazmanian, 2010; Zoetendal et al., 2004). Nevertheless, there is little data about the effects of transgenic crops on the animal intestinal microflora. Therefore, the aim of the present study was to evaluate the feed biosafety of the glyphosate-tolerant soybean meal on commercial broilers based on detecting the effects of glyphosate-tolerant on the intestinal microflora of 42-day-old broilers by PCR-DGGE technique.

MATERIALS AND METHODS

Birds, diets and treatments

A total of 360 one-day-old Arbor Acres broilers were randomly assigned to two dietary treatments, conventional soybean meal (CSM) group and transgenic soybean meal (TSM) group. Each treatment has 5 replicates (cages) with 36 broilers (18 males and 18 females each cage). Broilers were housed in the cages with 3 layers (length 100 cm × width 50 cm × height 45 cm) with a 24-h constant-light schedule and room temperature was maintained at 34°C during the first 3 days and gradually reduced to 28°C at day 12.

A 2-phase feeding schedule (starter, day 0 to 21; grower, day 22 to 42) was used in this study, and feeds in pellet form were offered *ad libitum*. Each phase diet was formulated to meet the nutrition requirements by NY/T33-2004 (China). The composition and nutrient level of diets are shown in Table 1. Non-transgenic control soybean was produced in Heilongjiang province of China, and the transgenic soybean (Monsanto 40-3-2) was imported from America.

All soybean sources were processed into their respective meal and oil fractions under similar conditions at China Agricultural University.

Sample collection and preparation

At 42 days, 3 broilers from each replicate were selected according to the average body weight (1/layer), and euthanized for carcass yield measurement and sampling. Then the ileal and cecum digesta was collected from each bird, and an equal amount of the digesta from each bird was mixed to create pooled samples from the broilers in the same replicate. The pooled digesta was placed in a 1.5-ml tube and stored at -80℃ until DNA extraction.

Performance and carcass yields

Broilers were observed three times daily for general health, all broilers that died or eliminated due to their unhealthy condition were weighed and necropsied; the probable cause of death and reason for eliminated were recorded. Broilers were individually weighed by pen at 1, 21 and 42 days of age. Meanwhile, feed intake per pen for calculating the feed conversion ratio (FCR) during the experiment (1 to 21, and 22 to 42 days of age) were recorded. The adjusted FCR were calculated by using total feed consumption minus the assumed feed consumption of the dead or eliminated broilers in a pen divided by the total weight gain of surviving broilers at 42 days of age. At the end of the experiment, carcass measurements (chill eviscerated weight and abdominal fat, wing, thigh, breast muscle, and thigh muscle weights) were taken.

DNA isolation and polymerase chain reaction (PCR) amplification of bacterial 16s rDNA

The DNA was extracted from digesta samples using the QIAamp DNA Stool Mini Kit, according to its protocols. The concentration of DNA was measured by absorbance value at 260 nm. The DNA was stored at -20℃. The primers used in this study were described by Muyzer et al. (1993), which target the V3 region of the 16S rRNA gene of the whole intestinal microbe. These primers include: the forward primer, 375F-GC,GC-clamp-5′-CCTACGGGAGGCAGCAG-3′(GC-clamp:5′-CGCCCGCCGCGCGCGGCGGGCGGGGCGGG-GGCACGGGGGG-3′) and the reverse primer, 517R, 5′-ATTACCGCGGCTGCTGG-3′.

The PCR mixture (50 µL) contained 1µL of template DNA (40 to 50 ng/µL), 1 µL of each primer (10 pmol/µL), 5 µL of the 10X Taq buffer, 1 µL of dNTP mixture (10 pmol/µL), 0.5 µL of Taq DNA polymerase (5 U/µL), 40.5 µL of ddH_2O [containing 0.1% bovine serum albumin (BSA)]. The PCR program was as follows: initial denaturation for 5 min at 95℃, followed by 35 cycles of denaturation (30 s at 95℃), annealing (30 s at 65℃), and extension (40 s at 72℃), with a final extension at 72℃ for 10 min. The expected size of amplified fragments was approximately 200 bp and was verified on a 1.5% (wt/vol) agarose gel for 25 min at 110 V.

Denaturing gradient gel electrophoresis (DGGE), cloning and sequencing

The DGGE was performed by a Bio-Rad DCode Universal Mutation Detection System (Bio-Rad, USA) using a 16 cm × 16 cm × 1 mm 8% (wt/vol) polyacrylamide gel containing a 40 to 65% linear denaturant gradient (a 100% denaturing solution consisting of 7.0 M urea and 40% (vol/vol) deionized formamide). The electrophoresis was performed in 0.5% tris-acetate-EDTA (TAE) buffer at 60℃ for 20 min at a constant voltage of 150 V, then for 16 to 18 h at a constant voltage of 75 V. The PCR-DGGE bands were visualized by ethidium bromide (EB) staining for 30 min. Cluster analysis of PCR-DGGE fingerprint was conducted by Quantity one software package (Version 4.2.1, BioRad). The band matching of fingerprint was normalized with a 1.0% position tolerance. The Pearson correlation coefficients were calculated between every two lanes, student's t-test was used to test the correlation coefficients among pool groups.

Bands of high luminance were excised aseptically from the PCR-DGGE gel into TE buffer (pH = 8) and incubated overnight at 4℃. The aforementioned primer (without GC-clamp) was used to amplify the above eluant, with the PCR program as earlier described. The PCR product was purified by the QIAquick PCR purification kit and cloned into pGEM-T Easy; all clones were checked by DGGE (using primer: 375F-GC and 517R). After confirmation, the DGGE bands migrated as the original, and the cloned plasmid were sequenced by Beijing Sunbiotech Co. The retrieved sequences were compared with the Genbank database by BLAST (http://www.ncbi.nlm.nih.gov).

Statistical analysis

Data for growth performance, carcass yield and bands number were statistically analyzed to determine the differences between transgenic and conventional soybean meal diets. Statistical analysis was performed according to the GLM procedure of SAS, version 9.1.3(SAS Institute Inc., Cary, NC). The treatment means were compared using Student's *t*-test. Values were presented as mean ± standard error, and $P<0.05$ was considered as significant difference for all statistical comparisons.

RESULTS

Bird performance and carcass yields

Data for growth performance and carcass yield at day 0 to 42 are summarized in Table 2. The growth performance did not differ significantly among two treatments. In addition, no differences in the relative carcass weights (Half-eviscerated yield, abdominal Fat, wing, thigh, breast muscle, and thigh muscle) were detected among two treatments.

Analysis of the PCR-DGGE Profiles

The PCR-DGGE fingerprint of the V3 region gene of 16S rDNA of the bacteria from the ileum and cecum of broilers at day 42 is shown in Figure 1. The different bands represented different kinds of bacteria, and the luminance of the bands indicated their abundance. It could be concluded from the fingerprint that different samples had many differences in the band location and abundance, and the abundance in the transgenic group was obviously higher than convention group. Different samples had many common bands, but with difference in the abundance. As shown in Table 4, there was no significant difference in the number of PCR-DGGE bands within the ileum and cecum ($P>0.05$), indicating that the transgenic soybean meal had no significant effect on the intestine

Table 2. Performance and carcass yield comparison of broilers fed diets containing transgenic and conventional soybean meal.

Parameter	CSM group	TSM group	P value
Performance			
Weight gain (kg) 0 - 42 days	2.14 ± 0.03	2.15 ± 0.03	0.832
Feed intake (kg) 0 – 42 days	3.80 ± 0.05	3.84 ± 0.04	0.575
Feed conversion (g/g) 0 - 42 day	1.77 ± 0.04	1.78 ± 0.03	0.820
Carcass yield			
Half-eviscerated yield (%)	91.2 ± 0.20	91.1 ± 0.64	0.473
Abdominal fat (%)	2.20 ± 0.18	2.48 ± 0.08	0.210
Wing (%)	5.52 ± 0.02	5.7 ± 0.02	0.077
Thigh (%)	27.05 ± 0.52	27.66 ± 0.33	0.353
Breast muscle (%)	25.06 ± 0.44	25.06 ± 0.44	0.999
Thigh muscle (%)	20.92 ± 0.63	21.64 ± 0.29	0.328

CSM, Conventional soybean meal; TSM, transgenic soybean meal.

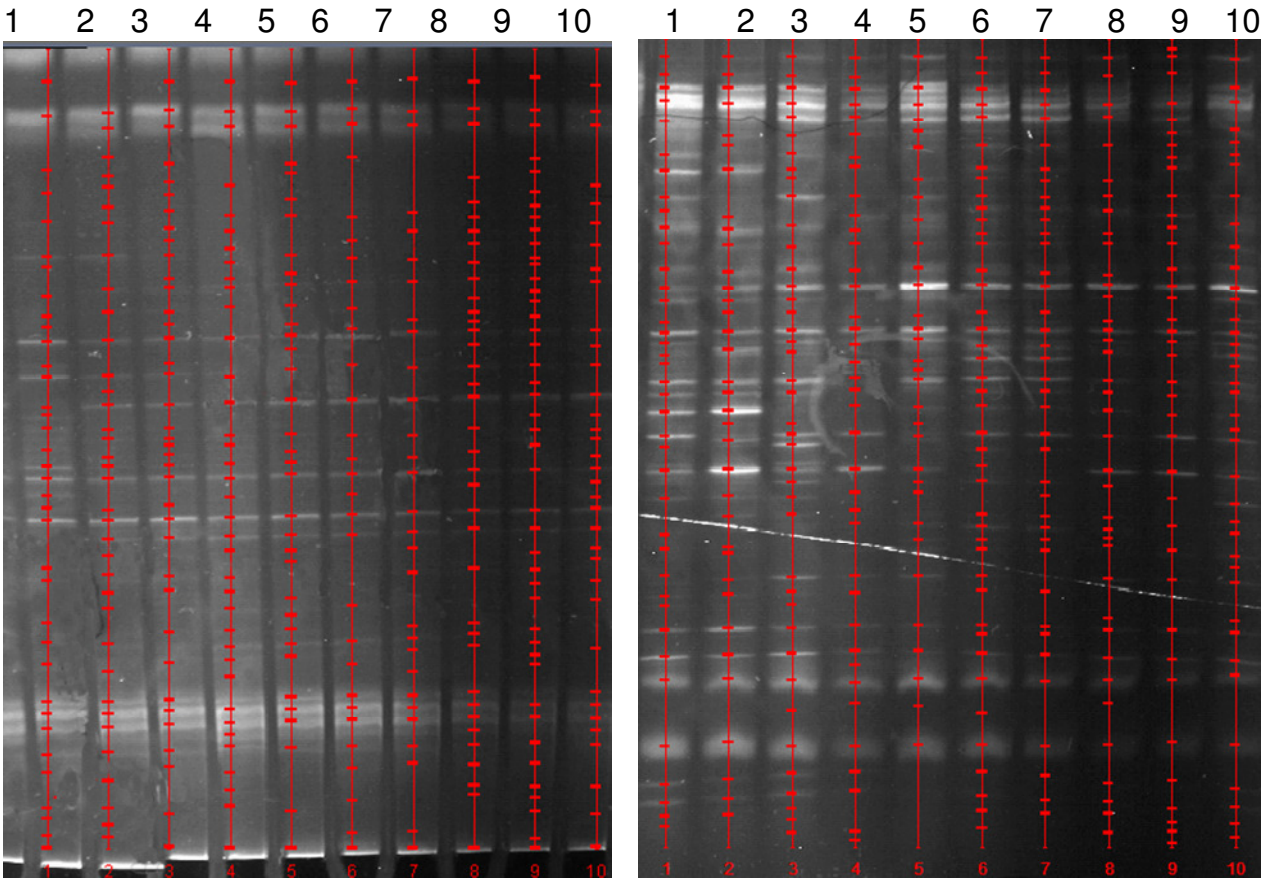


Figure 1. The PCR-DGGE profile of the V3 region gene of 16S rDNA of the bacteria from the ileum (left) and cecum (right) of broilers; lanes 1-5 is the transgenic soybean meal group, lanes 6-10 is the conventional soybean meal group. Every band was marked by red ticks.

microbial population of broilers.

Furthermore, the cluster analysis result of microbial PCR-DGGE fingerprint bands in the two treatments is shown in Figure 2. Except for the cecum sample 6 and 7,

Table 3. Effect of TSM on the band number of PCR-DGGE fingerprint of the intestine microbe in broilers.

Intestine	CSM group	TSM group	P Value
Ileum	33.6 ± 0.68	32.4 ± 1.08	0.373
Cecum	38.6 ± 2.14	38.8 ± 1.32	0.938

CSM, Conventional soybean meal; TSM, transgenic soybean meal.

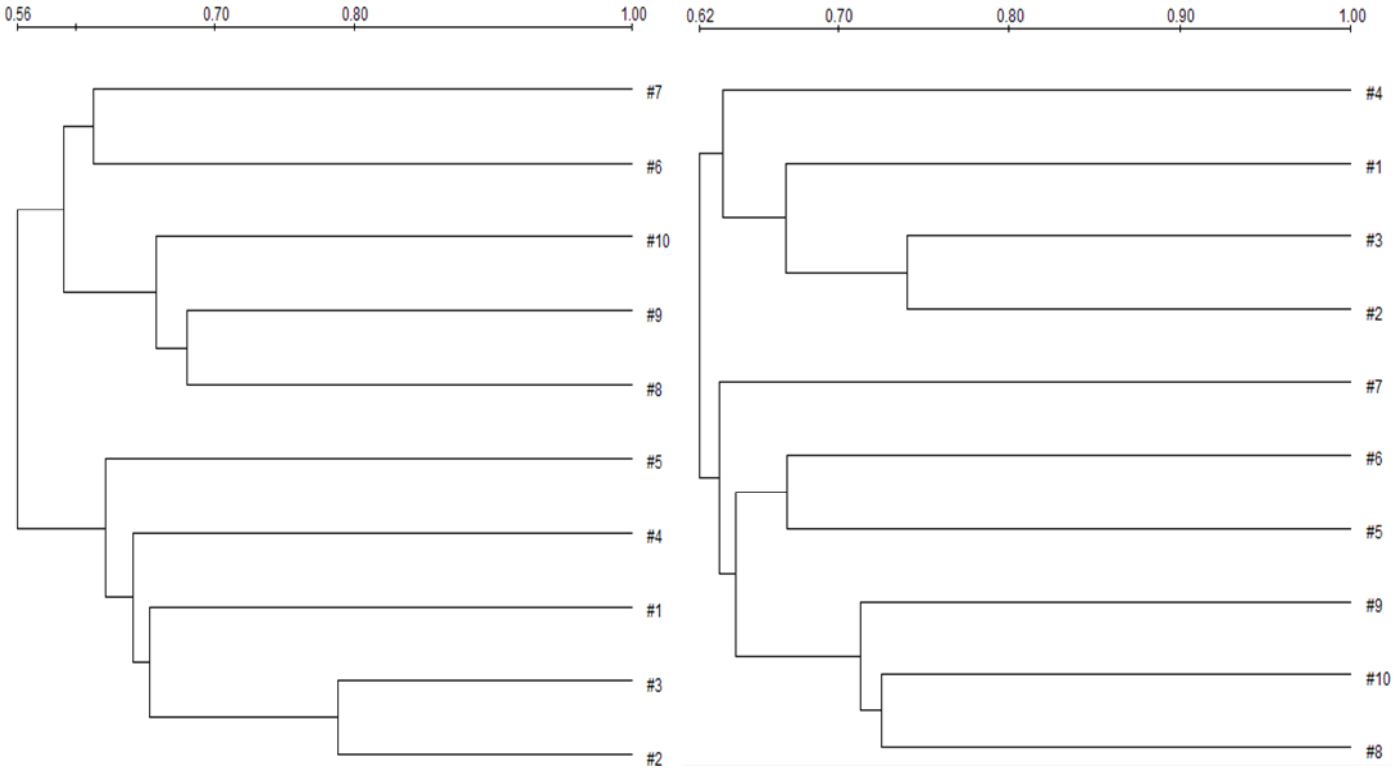


Figure 2. Cluster analysis of the PCR-DGGE fingerprint of the V3 region gene of 16S rDNA of the bacteria from the ileum (left) and cecum (right) of broilers.

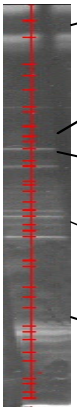
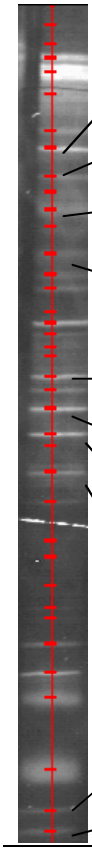
the similarity index of the other samples is relatively low. The within-group similarity index of the ileum and cecum samples is 62 and 58%, respectively which indicated that the microbial community of every broiler had a great individual difference. As to cluster analysis results of PCR-DGGE fingerprint bands of the ileum and cecum bacterial, it was found that the microbial community of the ileum was separated into two groups due to the difference of the diet, and the similarity intra and intergroup were very low.

Putative bacterial species identified by cloning and sequencing

After the separation of the intestinal bacterial 16S rDNA V3 region by DGGE, some advantage bands were collected for the putative species identification and confirmation by further PCR-DGGE analysis, cloning and sequencing. Among them, 15 bands were analyzed. The result shows that the similarity between the analyzed bands and their putative bacterial species was significantly high. Eleven of the fifteen bands had a 100% similarity with their putative species. The sequence result is shown in Table 4; most clones were closely related to uncultured bacteria.

Among the 15 bands sequenced, the closest putative cultured bacterial of C-3 was *Barnesiella viscericola* DSM 18177, while the closest putative cultured bacterial of I-4 was *Clostridiales* bacterium 21-4c. And C-5 and I-2 have a common closest putative cultured bacterial *B. viscericola* DSM 18177. Compared to the closest putative bacterial of advantage bands in the ileum and cecum microbial PCR-DGGE fingerprint, the closest putative

Table 4. The advantage PCR-DGGE band sequence analysis of the Ileum (up) and cecum (below) microbial 16S rDNA V3 Region

	Band number	Sequence size (bp)	Closest relative (species)	Identification (%)	GenBank accession number
	I-1	189	Uncultured bacterium clone HFV09_448	100	GU107887
	I-2	189	Barnesiella viscericola DSM 18177	100	AB267809
	I-3	168	Uncultured bacterium clone 100	100	GU060400
	I-4	189	Uncultured bacterium clone 16slp47-01c02.p1k	99	FJ505998
	I-5	189	Uncultured bacterium clone YO00045C05	97	EU198767
	C-1	189	Uncultured bacterium clone cc_269	100	GQ175524
	C-2	189	Uncultured bacterium clone NO50	98	AY916251
	C-3	192	Uncultured bacterium clone WD3_aak03c01	93	EU510227
	C-4	172	Clostridiales bacterium 21-4c	100	HQ452858
	C-5	189	Barnesiella viscericola DSM 18177	100	AB267809
	C-6	174	Uncultured bacterium clone 22Day21treat1colorado	100	GU171177
	C-7	189	Uncultured bacterium clone HFV09_448	100	GU107887
	C-8	171	Uncultured bacterium clone 426F05	100	HQ237250
	C-9	195	Uncultured bacterium clone BioVDRday3577	100	GU171020
	C-10	189	Uncultured bacterium clone HFV06_481	100	GU106362

bacterial of I-1 and C-7 was uncultured bacterium clone HFV09_448, and I-2 and C-5 have the same closest putative bacterial *B. viscericola* DSM 18177, which implied that there were many common bacteria in ileum and cecum.

DISCUSSION

Genetic engineering has enabled significant innovations in agricultural field; however, it got a violent opposition for its frame due to lack of biological coherence in the recombinant DNA technology (Herring, 2008). Broilers are sensitive to small nutritional or harmful changes in diet during its rapid growth period; comparative feeding studies with broilers have always been used to detect potential unintended effects resulting from the diet. This study detected no biologically relevant difference in performance and carcass yield that was consistent with previous report (Cromwell et al., 2002). The results from

this feeding experiment indicate that the transgenic soybean meal (Mon 40-3-2) is equivalent in nutritional value to conventional soybean meal.

The trillions of bacteria in broilers intestine play a very important role in the maintenance of the host health because of its prevention of pathogens colonization in the gastrointestinal track via its competitive exclusion. An imbalance of the intestinal microbiota could lead to inflammation and immune response in intestinal mucosa (Kelly and Conway, 2005; Neish, 2009), and various bacteria-associated disease. Previous reports showed that the composition change of the intestinal microbiota may be associated with obesity and weight loss of animals (Duncan et al., 2008; Ley et al., 2006), and the modulation of gut microbiota could improve the feed conversion and growth performance of broilers (Torok et al., 2011). So the stability of the broiler intestinal microbiota was closely linked with the economical profit. Previous study showed that the transgenic maize significantly influenced the intestinal immunity of weaning and old mice (Finamore et al., 2008). Hence, it is necessary to characterize the effects of transgenic crops on the intestinal microbiota of broilers.

The PCR-DGGE analysis of the ileum and cecum content bacterial between transgenic and conventional treatments suggested that there were no significant difference in the band number of the DGGE profile of the intestine microbe, which indicated that transgenic soybean meal had little effect on the species number of broiler intestinal bacteria. The band number of cecum bacterial DGGE profile was also more than ileum bacterial, which demonstrated that the bacterial population in cecum was more abundant than ileum. Meanwhile, the sequencing result showed that ileum and cecum had many common bacteria, but its proportion needed further research. In fact, the composition of the ileal and cecum microbiota is nearly the same in the first 3 days after hatch, and along with the broiler growing, the microbiota become complex. After 14 days old, microbiota composition in the ileum and cecum was significantly different (Lu et al., 2003; Wielen et al., 2002). The present study therefore provides further evidence on intestinal region-specific microbiota of commercial broiler, which is a successive system and with an increasing complexity along the GI tracts.

Several investigators (De Filippo et al., 2010; Sonnenburg et al., 2010) studied the effect of diet on gut microbiota shaping, it was demonstrated that the diet could significantly influence the intestinal microbiota (Geier et al., 2009). In this study, we detected the grouping of the microbiota in the ileum and cecum associated with the different diet, and the results indicated that the experiment diet had a significant effect on the microflora of broilers. Except for the soybean meal, the other ingredient in diet was the same between two treatments, so the transgenic soybean meal might have impact on the microbiota of ileum. The gut microbiota is influenced by the nutrient levels of fat, protein, carbohydrate, and even the physical size (Zhou et al., 2007). A similar study also showed that the transgenic technique caused some unintended compositional changes in transgenic rice seed (Jiao et al., 2010). So, the groping of the ileum microbiota is attributed to the compositional difference of soybean meal.

In this study, a significant individual variation in microflora was found within groups, even though they were in the same feeding conditions. Such individual variations were observed in several previous studies (Gong et al., 2007; Wielen et al., 2002). Community membership and function of the intestinal microbiota can change for many variables, such as the use of antibiotic (Dumonceaux et al., 2006) and prebiotics (Gibson and Roberfroid, 1995), diet (De Filippo et al., 2010), environment (Turnbaugh et al., 2008), and intestinal immune response (Frank et al., 2007; Hooper and Macpherson, 2010). The intestinal microbiota is an immensely diverse ecosystem (Eckburg et al., 2005), and no core microbiota can be shared by all individuals in animals (Yin et al., 2009). Hence, it is difficult to research the intestinal microbiota of broilers due to the individual variation. Moreover, Zhou (2007) suggested that pooling digesta from at least 5 broilers could provide a proper sample size for detecting the gut microbiota changes in broiler (Zhou et al., 2007).

The signs highlighted in the intestinal health could be the onset of a chronic process; however, no minimal length for feed safety assessment was established. Therefore, a prolonged study, even a multigenerational study should be conducted on feed safety evaluation of transgenic crops (Seralini et al., 2011). It is necessary to establish a standard method to evaluate the safety of transgenic crops. More deep safety assessment is needed to ensure that consumption of transgenic crops would not provoke any health problem, which could build confidence in the acceptance of the use of transgenic crops in animal production. This study detected a slight change in intestinal microbiota of broilers, but did not have significant effect on the growth performance and carcass yield. These data therefore support the conclusion that the glyphosate-tolerant soybean meal is as wholesome and nutritious as conventional soybean meal.

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