Effects of Flammulinavelutipes Stem Base on Microflora and Volatile Fatty Acids In Caecum of Growing Layers under Heat Stress Condition

ABSTRACT

The present study was undertaken to investigate the effects of Flammulinavelutipes stem base (FVS) on growth performance, microbial flora and volatile fatty acids of growing layers under heat stress condition. A total of 72 ISA Brown hens were randomly divided into six treatments: thermoneutral temperature control group (CON), heat stress control group (HS), heat stress antibiotic group (ANT) as positive control and heat stress FVS groups (20, 40 or 60 g/kg FVS). The experimental period had a duration of 28 d (days 84-112). On day 98, daily gain average was significantly higher (p<0.05) in the FVS groups than in the HS group. The number of bands in the FVS groups were higher (p<0.05) than in the HS group on day 98. The microbial similarity between the 60 g/kg FVS group and the HS group were the lowest on day 98. FVS group's specific bacteria were mainly Coprococcus comes, [Clostridium] papyrosolvens, Butyricicoccuspullicaecorum on day 98. Whereas on day 112, the FVS groups specific bacteria were mainly Parabacteroides distasonis, Coprobacterfastidiosus, Elusimicrobiumminutum. The content of acetic acid and butyric acid were higher (p<0.05) in 20 g/kg FVS group than in the CON group on day 112. In conclusion, FVS can lighten the adverse effect of heat stress by increasing the diversity of intestinal flora in growing layers.

INTRODUCTION

The condition of the gut system affects the nutrients utilization for organ development, tissue growth and immune system maturation in the host. Cecal microbial populations are the indication of gut health in animals (Mahfuz et al., 2017). The cecum is a complex ecosystem of microbial colonization in poultry. Volatile fatty acids (VFA) usually is produced through bacterial fermentation in the cecum that is necessary for the intestinal function and intestinal integrity (Meimandipour et al., 2010). In recent years, high-through put sequencing has been used by many researchers to investigate the gut microbial diversity in animals (Wang et al., 2017). Intestinal flora has an important influence on host health (Chang et al., 2016). The composition and activities of intestinal microflora can be altered by dietary patterns, such as feed additives (Maesschalck et al., 2015) and antibiotics (Kalter et al., 2010; Zou et al., 2016).

Since the last few decades, antibiotic shave been used in the poultry to promote growth performance. These antibiotics products include bambermycin, avilamycin, and the flavomycin (Butaye et al., 2003). The product flavomycin (synonyms: moenomycin, flavophospholipol and bambermycin) is a glycolipid antibiotic produced by Streptomyces species including S. bambergiensis, S. ghanaensis, S. geysirensis, and S. ederensis (Huber et al., 1965; Wallhauser et al., 1965). In addition,
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MATERIALS AND METHODS

Test materials, experimental condition and feeding management

Experimental chickens (ISA Brown) were purchased from Changchun Octavia Farms and FVS was collected from the local domestic mushroom farm in Changchun City, Jilin, China.

The experiment was carried out at the Animal Unit, College of Chinese Medicine Materials, Jilin Agricultural University, and all the procedures were approved by the animal care and use committee of Jilin Agricultural University. A total of 72 hens, aged 84d derived from ISA Brown strain were divided into 6 groups, with 3 replications having 4 chickens each. The birds were housed into a wire cage (100cm, 60cm, 50cm, length, width, height) and an average homogeneous not significant body weight (1192±15.32 g; Table 2) was considered for each replication. Dietary treatment included thermoneutral temperature control group (CON, basal diet, 28±1°C), heat stress control group (HS, basal diet, 38±1°C), heat stress antibiotic group (ANT, basal diet supplemented with 5 mg/kg flavomycin, 38±1°C) as positive control and heat stress FVS group (basal diet supplemented with 20, 40, and 60 g/kg FVS, 38±1°C). Heat stress was not constant throughout the experimental period. During the experimental period, room temperature was maintained at 38±1°C from 8:00-18:00, after the heat stress time, the room temperature was 28±1°C, until the next morning. The incandescent lamps with room heater were used to maintain the heat stress and the spray method was used to control the relative humidity at 50%-60%. A wet and dry bulb thermometer were used to record the temperature and humidity throughout the experimental period. The trial lasted for 28 days from day 84 to day 112. All procedures were applied for heat stress, only. Feed and water were provided ad libitum throughout the whole period. Mushroom and antibiotics were mixed with the growing layers diet formulated according to NRC (1994) specification in Feed Mill (Jilin Hanghong Animal Husbandry Co. Ltd, China). The analyzed nutritional composition of the experimental diet and FVS are presented in Table 1.
Sample collection

Mushroom stem was harvested from a domestic mushroom farm at Changchun city, and sun dried properly. Then the sub sample was grinded and was prepared (0.01mm) for proximate component analysis. Feed sample and FVS were analyzed (n=6) following the method of AOAC (2000). Dry matter, ether extract, crude fiber, and total ash were analyzed according to the procedures of AOAC (2000). Nitrogen was determined using an FP528 nitrogen determinator (LECO Corporation, Joseph, MI, USA). The analyzed results were presented in Table 1. Analyzed compositions of Flammulina velutipes mushroom stem were dry matter=88.50±0.80g/kg, crude protein=13.55±0.42g/kg, crude fiber=21.05±0.11g/kg, ether extract=2.3±0.014g/kg, Ash=11.4±0.085g/kg, calcium=4.0±0.1 g/kg and phosphorus=6.2±0.28g/kg. A total of 36 birds (two from each replicate, n=6) were slaughtered on day 98 and day 112 to collect cecum and samples were kept in a freezer(-80°C) for further analysis.

Growth performance

Body weight and feed intake per pen were recorded from day 84 to day 98 and day 98 to 112 and used to calculate average daily gain (ADG), average daily feed intake (ADFI), and feed conversion ratio (FCR).

Analysis of the cecal digesta microbial flora

Cecal chime were taken to extract total bacterial DNA by using AxyPrep genomic DNA mini kit following the instructions of the manufacture Co. The 16SrDNA V3 region of the total bacterial DNA was amplified by PCR. JY-TD331A PCR-DGGE (denaturing gradient gel electrophoresis) and Vilber gel scanning imaging system were used for DGGE test. The common and specific bands in the DGGE map were recovered by gel cutting and amplified where DGGE electrophoresis was carried out according to the above PCR method to confirm the correctness of the retrieved target fragments. DNA was amplified and purified by universal primers (without GC clamps). The PCR product of the target template was connected by

Table 1 – Experimental diet with nutritional composition

| Item          | CON | HS  | ANT  | 20 g/kgFVS | 40 g/kgFVS | 60 g/kgFVS |
|---------------|-----|-----|------|------------|------------|------------|
| Ingredient    |     |     |      |            |            |            |
| Corn          | 673.50 | 674.00 | 674.50 | 670.00     | 645.00     | 615.00     |
| Soybean meal  | 256.50 | 256.00 | 255.00 | 240.00     | 245.00     | 255.00     |
| FVS           |      |     |      |            |            |            |
| Lysine        | 2.00 | 2.00 | 2.00 | 2.00       | 2.00       | 2.00       |
| Methionine    | 2.50 | 2.50 | 2.50 | 2.50       | 2.50       | 2.50       |
| Dicalcium     | 30.00 | 30.00 | 30.00 | 30.00      | 30.00      | 30.00      |
| Limestone     | 31.00 | 31.00 | 31.00 | 31.00      | 31.00      | 31.00      |
| Common salt   | 2.50 | 2.50 | 2.50 | 2.50       | 2.50       | 2.50       |
| Vit-mineral premix | 2.00 | 2.00 | 2.00 | 2.00       | 2.00       | 2.00       |
| Antibiotics   |      |     |      |            |            |            |
| Total         | 1000 | 1000 | 1000 | 1000       | 1000       | 1000       |
| Chemical analysis |     |     |      |            |            |            |
| DM (g/kg)     | 914.70 | 913.40 | 914.40 | 913.60     | 915.50     | 914.50     |
| CP (g/kg)     | 166.40 | 167.80 | 166.90 | 168.70     | 168.60     | 167.60     |
| Ca (g/kg)     | 11.20 | 11.10 | 11.10 | 11.20      | 11.20      | 11.10      |
| P (g/kg)      | 5.40  | 5.50  | 5.60  | 5.60       | 5.50       | 5.70       |
| EE (g/kg)     | 28.30 | 28.30 | 28.60 | 28.68      | 28.59      | 28.30      |
| CF (g/kg)     | 25.50 | 25.40 | 25.60 | 27.70      | 32.10      | 37.30      |
| Calculated analysis (g/kg) |     |     |      |            |            |            |
| ME (g/kg)     | 11.55 | 11.54 | 11.57 | 11.60      | 11.59      | 11.63      |
| Lysine        | 10.00 | 9.90  | 10.00 | 9.98       | 10.01      | 10.10      |
| Methionine    | 4.80  | 4.80  | 5.10  | 4.90       | 4.80       | 5.00       |
| Cystine       | 2.80  | 2.82  | 2.90  | 2.88       | 2.81       | 2.81       |

1. CON=thermoneutral temperature control group (basal diet, 28±1°C), HS=heat stress control group (basal diet, 38±1°C), ANT=antibiotic group (basal diet supplemented with 5 mg/kg flavomycin, 38±1°C), FVS=FVS group (basal diet supplemented with 20, 40 or 60 g/kg FVS, 38±1°C).
2. Provided per kg of the complete diet: retinyl acetate, 4500 IU; cholecalciferol, 1200 IU; DLα-tocopheryl acetate, 2500 IU; thiamin, 5000 mg; riboflavin, 20000 mg; phylloquinone, 10000 mg; niacin, 45000 mg; pantothenic acid, 35000 mg; biotin, 1500 mg; folic acid, 3000 mg; cyanocobalamin, 40 mg; zinc, 45 mg; copper, 4 mg; cobalt, 100 μg; iodine, 1 mg; selenium, 100 μg.
3. DM=dry matter; CP=crude protein; Ca=calcium; P=phosphorus; EE=ether extract; CF=crude fiber; ME=metabolisable energy.
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Statistical analysis
The experimental data were processed by Microsoft Excel, and then subjected to one-way analysis of variance using SPSS software. Multiple comparisons were performed using Tukey-kramer’s test. Cluster analysis was performed using Quantity one. p<0.05 indicates significant difference.

RESULTS
Effect of FVS on growth performance
The effects of FVS on growth performance growing layers were presented in Table 2. On day 98, the ADG in the FVS groups were higher (p<0.05) than that of the HS group. On day 112, the ADG of FVS groups was lower (p<0.05) than that of the HS group. There was no significant difference in FCR among groups throughout the test period.

Effect of FVS on the microbial diversity
The number of microbial flora bands was shown in Table 3. On day 98, the band number was higher (p<0.05) in the FVS groups than in the HS group, and

Table 2 – Effect of Flammulinavelutipes stembase on growth performance of growing layers1,2

| Item                      | CON    | HS     | ANT    | 20 g/kg FVS | 40 g/kg FVS | 60 g/kg FVS | SEM   | p-value |
|---------------------------|--------|--------|--------|-------------|-------------|-------------|-------|---------|
| Day 98                    |        |        |        |             |             |             |       |         |
| ADG (g/d per bird)        | 9.18a  | 7.06a  | 8.73a  | 8.23b       | 8.42b       | 8.67b       | 0.259 | 0.002   |
| ADFI (g/d per bird)       | 33.25a | 31.58b | 34.48a | 34.07a      | 32.92b      | 30.08a      | 0.525 | 0.001   |
| FCR %                     | 3.45   | 4.72   | 3.95   | 4.14        | 3.91        | 3.47        | 0.209 | 0.106   |
| Day 112                   |        |        |        |             |             |             |       |         |
| ADG (g/d per bird)        | 11.87ab| 10.87a | 12.1ab | 12.03ab     | 12.36ab     | 12.55b      | 0.248 | 0.037   |
| ADFI (g/d per bird)       | 62.37c | 60.91bc| 61.97bc| 59.79bc     | 59.04c      | 59.18b      | 0.562 | 0.021   |
| FCR %                     | 5.27   | 5.61   | 5.12   | 4.97        | 4.79        | 4.72        | 0.159 | 0.199   |
| IBW                       | 1187   | 1182   | 1210   | 1183        | 1178        | 1212        | 15.32 | 0.984   |

1CON=thermoneutral temperature control group (basal diet, 28±1°C), HS=heat stress control group (basal diet, 38±1°C), ANT=antibiotic group (basal diet supplemented with 5 mg/kg flavomycin, 38±1°C), FVS=FVS group (basal diet supplemented with 20, 40 or 60 g/kg FVS, 38±1°C).
2data represented the mean value of 12 birds per treatment. a, b, c, d, e-means in the same row with different letters are significantly different at p<0.05
SEM-pooled standard error of the means.

Table 3 – Effect of Flammulinavelutipes stem base on microbial flora bands number(Fig. 1) in caecum of growing layers1,2

| Item                      | CON     | HS      | ANT     | 20 g/kg FVS | 40 g/kg FVS | 60 g/kg FVS | SEM   | p-value |
|---------------------------|---------|---------|---------|-------------|-------------|-------------|-------|---------|
| Day 98                    |         |         |         |             |             |             |       |         |
| microbial flora bands number | 47.33c  | 40.33a  | 45.33b  | 49.33c      | 51.33c      | 44.67h      | 1.299 | 0.001   |
| Day 112                   | 38.0    | 38.0    | 38.0    | 42.67       | 36.33       | 42.0        | 1.330 | 0.311   |

1CON=thermoneutral temperature control group (basal diet, 28±1°C), HS=heat stress control group (basal diet, 38±1°C), ANT=antibiotic group (basal diet supplemented with 5 mg/kg flavomycin, 38±1°C), FVS=FVS group (basal diet supplemented with 20, 40 or 60 g/kg FVS, 38±1°C).
2data represented the mean value of 12 birds per treatment. a, b, c, d, e-means in the same row with different letters are significantly different at p<0.05
SEM-pooled standard error of the means.
there was no significant difference in the number of bands both in the FVS groups with the CON group. The number of the bands in the 20 g/kg FVS groups and 60 g/kg had no significant difference with the ANT group. On day 112, there was no significant difference among the groups.

The results of sequence alignment of bands in DGGE maps (Fig. 1) are shown in Table 4. Under heat stress on day 98, the specific bacteria in the CON group were *Ruminococcus lactaris*, *Alistipes senegalensis* (1, 3). The specific bacteria in the HS group were *Helicobacter pullorum*, *Stomatobaculum longum* (5, 7). The specific bacteria in the FVS groups were *Selenomonas ruminantium* strain, *Coprococcus comes*, *Intestinimonas butyriciproducens* strain, *Merdimonas faecis* strain, *[Clostridium] papyrosolvens*, *Butyricicoccus pullicaecorum*, *Terasakiellapusilla* (16, 18, 22, 23, 28, 29, 32, 33, 34). All the groups contain

Table 4 – Sequencing results of microbial florabands in DGGE map (Fig. 1)

| Stripe number | Sequence length (bp) | The closest name of the spawn in the GenBank database (login number) | Similarity % |
|---------------|----------------------|---------------------------------------------------------------------|--------------|
| 1             | 171                  | *Ruminococcus lactaris* ATCC 29176 Scflid_02_7 (NZDS990170.1)       | 99           |
| 2, 15, 20, 25, 31 | 170                | *[Clostridium] saccharolyticum* strain AN168 contig_53 (NZNFKU01000053.1) | 100          |
| 3             | 190                  | *Alistipes senegalensis* JC50 (NZCAH01000040.1)                       | 97           |
| 4, 13, 24, 26, 35, 41, 51, 55, 68 | 191          | *Bacteroides uniformis* ATCC 8492 Scflid_3.0.1_32(NZ_DS362249.1)        | 99           |
| 5             | 171                  | *Helicobacter pullorum* MIT 98-5489 supercont2.11 (NZDS990451.1)        | 100          |
| 6, 10, 11, 14, 17, 30 | 191                     | *Alistipesimonensis* JC136 strain DSM 25383 (NZFNRI01000015.1)         | 97           |
| 7, 62         | 172                  | *Stomatobaculum longum* strain ACC2 supercont1.5 (NZHJ958065.1)         | 87           |
| 8, 9          | 172                  | *Hatthewayaproteolytica* DSM 3090(NZ_FRAD01000002.1)                    | 89           |
| 12, 27        | 191                  | *Alistipesinsonis6tain strain 627 contig00044(NZJRGF01000044.1)         | 97           |
| 16            | 197                  | *Selenomonas ruminantium* strain WCT3(NZFNMC10000034.1)                 | 93           |
| 18            | 170                  | *Coprococcus comes* ATCC 27758 Scflid1(NZGG662006.1)                     | 99           |
| 19            | 173                  | *[Clostridium] terridits* CT1112 Ct_contig00106(NZARov01000077.1)        | 93           |
| 21            | 172                  | *Acholeplasmaaequifetale* ATCC 29724 T434DRAFT_scaffold00026.26_C(NZIH XL01000026.1) | 79           |
| 22            | 174                  | *Intestinimonasbutyriciproducens* strain AF211(NZCPO011307.1)          | 94           |
| 23, 28        | 171                  | *Merdimonas faecis* strain BR31_L001_R1_001_paired_contig_46 (NZMIEHO1000046.1) | 100          |
| 29            | 172                  | *[Clostridium] papyrosolvens* DSM 2782 ctg56 (NZACXX020000011.1)         | 90           |
| 32            | 191                  | *Butyricicoccus pullicaecorum* 1.2 acBRa-supercont1.4 (NZKB976106.1)      | 99           |
| 33, 34        | 171                  | *Terasakiellapusilla* DSM 6293 Q397DRAFT_scaffold000068.68_C(NZHYO01000068.1) | 89           |
| 36            | 190                  | *Olivibacterisistentis* DSM 17696 A375DRAFT_scaffold13.14_C(NZ_ATZA01000014.1) | 83           |
| 37            | 191                  | *Leeuwenhoekella sp.* MAR_2009_132 P164DRAFT_scf7180000000008_quiver.2_C(NZPOL01000002.1) | 90           |
| 38, 48        | 174                  | *Intestinimonas* sp. GD2 genomeassemblyIntestinimonasmassiliensis, scaffold00006 (NZLN869528.1) | 94           |
| 39, 50, 53, 56, 61, 65 | 191                | *Arenibacteralgicola* strain TG409 U735DRAFT_scf71800000000011_quiver.3_C(NZPO0010000003.1) | 90           |
| 40            | 192                  | *Bacteroides coprophilus* DSM 18228=JCM 13818 strain DSM 18228 Scflid2 (NZEQ973630.1) | 100          |
| 42            | 191                  | *Prevotelladentasini* JCM 15908 (NZBAKG01000039.1)                      | 94           |
| 43            | 170                  | *Alistipesinegoldii* DSM 17242, complete genomeSequence ID:(NC018011.1)   | 99           |
| 44, 46, 52    | 190                  | *Bacteroides sterconis* ATCC 43183 Scflid_02_15 (NZ_DS499676.1)         | 94           |
| 45            | 197                  | *Desulfitobacterium dichloroeliminans* LMG P-21439 (NC019903.1)          | 89           |
| 47            | 191                  | *Parabacteroides johnsonii* CL02T12C29 supercont1.5 (NZIHJ976469.1)      | 93           |
| 49            | 172                  | *Ruminococciirculans* chromosome II (NZHF545617.1)                      | 88           |
| 54            | 191                  | *Odoribacterisplanchnicus* DSM 20712 (NC015160.1)                       | 92           |
| 57            | 173                  | *Oscillibacter sp.* KLE 1745 Scaffold82 (NZK1271778.1)                  | 97           |
| 59            | 191                  | *Bacteroides distasonis* ATCC 8503 (NC009615.1)                         | 95           |
| 59            | 191                  | *Bacteroides coprocola* DSM 17136 Scflid_02_75 (NZDS981502.1)            | 95           |
| 60            | 191                  | *Rikenellamicrofusus* DSM 15922 RikmiDRAFT_RMD.2 (NZ_KJE58688.1)         | 97           |
| 63            | 171                  | *Tyzzerellaneelixis* DSM 1787 Scflid665 (NZDS995667)                     | 99           |
| 64            | 191                  | *Coprobacterfastidiosus* NSB1 scaffoldcontig19 (NZK440788.1)            | 92           |
| 66            | 191                  | *Bacteroides ovatus* strain ATCC 8483 (NZCPO12938.1)                     | 92           |
| 67            | 174                  | *Elusimicrobiurninuminutum* Pei191 (NC010644.1)                         | 92           |

1DGGE=denaturing gradient gel electrophoresis.
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Effects of FVS on the microbial similarity

As shown in Fig. 2 (a), on day 98, the highest similarity between the 20 g/kg FVS group and the ANT group was 70.47%. The lowest similarity between the 60 g/kg FVS group and the HS group was 55.13%. Among the heat stress groups, the highest similarity was in the 40 g/kg FVS group with the CON group. As shown in Fig. 2 (b), on day 112, the highest similarity between the 20 g/kg FVS group and the ANT group was 62.58%. Among the heat stress groups, the ANT group had the highest results, similar to the CON group and followed by the 60 g/kg FVS group.

Effects of FVS on VFA content

As shown in Table 5, on day 98, the content of VFA in the FVS groups was lower (p<0.05) than in the HS group. The content of acetic acid and butyric acid in the 20 g/kg FVS group and the 60 g/kg FVS group had no significant differences with the CON group on day 98. There were no significant differences in the content of propionic acid in the FVS groups with the CON group on day 98. On day...
The content of acetic acid and butyric acid in the 40 g/kg FVS group and the 60 g/kg FVS group had no significant difference with the CON group and the HS group respectively. The content of VFA in the 20 g/kg FVS group was higher (p<0.05) than in the CON group.

**Table 5** – Effects of *Flammulinavelutipes* stem base on volatile fatty acids content in caecum of growing layers\(^1,2\)

| Item            | CON  | HS   | ANT  | 20 g/kg FVS | 40 g/kg FVS | 60 g/kg FVS | SEM     | p-value |
|-----------------|------|------|------|-------------|-------------|-------------|---------|---------|
| **Day 98**      |      |      |      |             |             |             |         |         |
| Acetic acid     | 30.06\(^a\) | 85.95\(^c\) | 53.95\(^b\) | 44.57\(^a\) | 53.45\(^b\) | 28.52\(^a\) | 7.027   | 0.001   |
| Propionic acid  | 15.57\(^ab\) | 38.06\(^c\) | 20.59\(^b\) | 16.59\(^ae\) | 19.11\(^b\) | 10.81\(^a\) | 3.047   | 0.001   |
| Butyric acid    | 11.78\(^a\) | 43.46\(^d\) | 23.77\(^c\) | 16.29\(^ae\) | 19.14\(^bc\) | 11.1\(^a\)  | 3.846   | 0.001   |
| **Day 112**     |      |      |      |             |             |             |         |         |
| Acetic acid     | 30.38\(^a\) | 48.37\(^d\) | 63.78\(^b\) | 55.5\(^e\)  | 32.18\(^a\) | 29.56\(^a\) | 5.265   | 0.002   |
| Propionic acid  | 19.26\(^a\) | 19.64\(^a\) | 20.8\(^e\)  | 24.41\(^e\) | 13.6\(^a\)  | 14.85\(^a\) | 1.377   | 0.001   |
| Butyric acid    | 9.18\(^a\)  | 12.37\(^e\) | 21.95\(^c\) | 15.19\(^b\) | 9.14\(^a\)  | 10.82\(^a\) | 1.605   | 0.001   |

\(^1\)CON=thermonutral temperature control group (basal diet, 28±1°C), HS=heat stress control group (basal diet, 38±1°C), ANT=antibiotic group (basal diet supplemented with 5 mg/kg flavomycin, 38±1°C), FVS=FVS group (basal diet supplemented with 20, 40 or 60 g/kg FVS, 38±1°C).

\(^2\)Data represented the mean value of 12 birds per treatment. a, b, c-means in the same row with different letters are significantly different at p<0.05

SEM-pooled standard error of the means.
DISCUSSION

Effect of FVS on growth performance

Heat stress is a common problem, especially in most of the tropical countries. Heat stress is a hazard to commercial poultry production in most areas of China, especially in the summer season. This study highlighted that FVS could alleviate the effect of heat stress on the growth performance in laying hens. This may be related to FVS rich in crude fiber speeding up the intestinal peristalsis of the chicken under heat stress and promoting digestion and absorption. He et al. (2014) reported that heat stress could reduce the egg weight, growth performance, digestive enzyme activities, beneficial bacteria and increase harmful bacteria in the cecum of chicken. Similarly, this study found that heat stress can lead to the decline of growth performance of growing layers. The study of Ai (2008) showed that the early heat acclimatization to poultry could effectively alleviate the decline in production performance at the later stage of growth. On day 112, there were no significant differences in the ADG, ADFI and FCR between the HS group and CON group, which may be the result of heat stress adaptation in laying hens. Garriga et al. (2006) showed that stress can cause intestinal villus injury on the intestinal mucosa associated with poor nutrient absorption. This may be the reason for the decrease of ADG in the HS group.

Effect of FVS on the microbial similarity

This study found that FVS could alleviate the effect of heat stress on the similarity of intestinal flora, and the effect was similar to that of antibiotics. This study showed that the highest similarity in results were between the 20 g/kg FVS group and the ANT group. This ensured that the role of the 20 g/kg FVS group and the ANT group were the most similar to the composition of the intestinal flora. On day 98, the lowest similarity was found between the 60 g/kg FVS group and the HS group. The 40 g/kg FVS group was the highest similarity among the heat stress groups with the CON group. These indicated that feeding FVS may alleviate the effect of heat stress on the composition of the intestinal flora. On day 112, among the heat stress groups, the ANT group was the highest similar to the CON group and followed by the 60 g/kg FVS group. This showed that feeding high doses of FVS could alleviate the effect of heat stress on the composition of the intestinal flora. These results were similar to the study by Guo et al. (2004), who reported that the cecal viscosity and microbial populations were significantly improved by feeding mushroom extracts.

Effect of FVS on VFA content

VFA and other organic acids (such as lactic acid and succinic acid) are the key metabolites of carbohydrate fermentation in the large intestine. Previous studies have shown that acetate and propionate have a good therapeutic effect on colitis (Tedinlind et al., 2007). Van Der Wielen et al. (2000) stated that VFA are responsible for the reduction of Enterobacteriaceae in the ceca of broiler chickens during growth. Among the VFA, butyric acid stands out as a preferred energy source for enterocytes and takes part in cellular differentiation and proliferation within the intestinal mucosa (Rinttilä et al., 2013). In addition, the butyrogenetic effect of different prebiotics in the broiler cecum has been previously reported (Rehman et al., 2008). This study found that FVS could alleviate the effect of heat stress on the content of VFA in the intestine. This may be due to the fact that the FVS groups contained specific bacterial flora that were different from the other groups and resulted in changes in the VFA content. The concentration of VFA was related to the composition of feed, number and type of anaerobic bacteria (Rehman et al., 2007).
The FVS groups contained a lot of beneficial bacteria that could increase the VFA concentration directly and indirectly. *Coprococcus comes* produces butyric acid primarily. *Intestinimonas butyriciproducens* strain is mainly responsible for the production of acetic acid and butyric acid. *Alistipesisopnops* strain mainly produces acetic acid and succinic acid. *Merdimonas faecis* strain can ferment glucose to produce acetic acid. 

*Intestinimonas faecalis*, *C. papyrosolvens*, *A. butyriciproducens*, and *B. barnesiae* belong to the genus *Clostridium* and has the potential of probiotics whose metabolites are acetic acid and butyric acid.

**CONCLUSIONS**

FVS could alleviate the effect of heat stress on growth performance, intestinal flora and VFA in growing layers. In conclusion, this study provides a new way to find environmentally friendly alternatives to antibiotics, by the utilization of edible and medicinal fungi wastes, which has great significance for alleviating heat stress in poultry production. In order to achieve better performance and sound gut health, FVS may be considered an alternative potential feed supplement for growing layers under heat stress condition.

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**REFERENCES**

Abidin Z, Khatoon A. Heat stress in poultry and the beneficial effects of ascorbic acid (vitamin C) supplementation during periods of heat stress. World Poultry Science Journal 2013;69:135-152.

Ai Q. Effect of early age thermal acclimation on adaptability acquisition in broilers during heat exposure. Beijing: Chinese Academy of Agricultural Sciences; 2008.

AOAC - Association of Official Analytical Chemistry. Official methods o analysis of AOAC international. 17th ed. Maryland; 2000.

Butaye P, Devriese LA, Haesebrouck F. Antimicrobial growth promoters used in animal feed:effects of less well known antibiotics on gram-positive bacteria. Clinical Microbiology Reviews 2003;16:175.

Chang CL, Chung CY, Kuo CH, Kuo TF, Yang CW, Yang WC. Beneficial effect of Bidentispilaosa on body weight gain, food conversion ratio, gut bacteria and coccidiosis in chickens. Plos One 2016;11:e146141.

Chen P, Yong Y, Gu Y, Wang Z, Zhang S, Lu L. Comparison of antioxidant and antiproliferation activities of polysaccharides from eight species of medicinal mushrooms. International Journal of Medicinal Mushrooms 2015;17:287-295.
Effects of Flammulinavelutipes Stem Base on Microflora and Volatile Fatty Acids In Caecum of Growing Layers under Heat Stress Condition

NRC- National Research Council. Nutrient requirements of poultry. 9th ed. Washington: National Academy Press; 1994.

Osweiler GD, Jagannatha S, Trampel DW, Imerman PM, Ensley SM, Yoon I, et al. Evaluation of XPC and prototypes on aflatoxin-challenged broilers. Poultry Science 2010;89:1887-1893.

Quinteiro-Filho WM, Ribeiro A, Ferraz-de-Paula V, Pinheiro ML, Sakai M, Sa LR, et al. Heat stress impairs performance parameters, induces intestinal injury, and decreases macrophage activity in broiler chickens. Poultry Science 2010;89:1905-1914.

Ramnath V, Rekha PS, Sujatha KS. Amelioration of heat stress induced disturbances of antioxidant defense system in chicken by bhringaraj rasayana. Evidence-based Complementary and Alternative Medicine 2008;77-84.

Rehman H, Bohm J, Zentek J. Effects of differently fermentable carbohydrates on the microbial fermentation profile of the gastrointestinal tract of broilers. Journal of Animal Physiology & Animal Nutrition 2008;92:471-480.

Rehman HU, Vahjen W, Awad WA, Zentek J. Indigenous bacteria and bacterial metabolic products in the gastrointestinal tract of broiler chickens. Archives of Animal Nutrition 2007;61:319-335.

Rinttilä T, Apajalhti J. Intestinal microbiota and metabolites-Implications for broiler chicken health and performance.1. Journal of Applied Poultry Research 2013;22:647-658.

Steinbrueckner B, Haerter G, Pelz K, Weiner S, Rump JA, Deissler W, et al. Isolation of Helicobacter pullorum from patients with enteritis. Scandinavian Journal of Infectious Diseases 1997;29:315-318.

Tedelind S, Westberg F, Kjerrulf M, Vidal A. Anti-inflammatory properties of the short-chain fatty acids acetate and propionate: A study with relevance to inflammatory bowel disease. World Journal of Gastroenterology 2007;13:2826-2832.

Zou F, Zeng D, Wen B, Sun H, Zhou Y, Yang M, et al. Illumina MiSeq platform analysis caecum bacterial communities of rex rabbits fed with different antibiotics. AMB Express 2016;6:100.