Growth performance, carcass characteristics, meat and egg quality, and intestinal microbiota in Beijing-you chicken on diets with inclusion of fresh chicory forage

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ABSTRACT
The aim of this study was to evaluate the effects of fresh chicory forage in chicken diets on growth performance, carcass characteristics, meat and egg quality, as well as intestinal microbiota. A total of 600 healthy 16 weeks female Beijing-you chickens were randomly assigned to four dietary treatments containing 0%, 5%, 8% and 10% chicory forage with six replicate pens and 25 chickens in each replicate. Chickens were raised in a free-range system for 13 weeks after seven days of adaptation. High-throughput sequencing was used to characterise the microbiota in three different intestinal sections (duodenum, ileum and caecum). The results showed that dietary supplementation of chicory forage increased ($p < .05$) egg weight, yolk colour, total amino acids and delicious amino acids in muscles, and decreased ($p < .05$) abdominal fat yield compared to the control. However, increased ($p < .05$) average daily feed intake, increased ($p < .05$) feed conversion ratio, increased ($p < .05$) mortality and decreased ($p < .05$) eggshell strength were observed in chickens fed chicory forage than in those fed a basal diet. Dietary supplementation of chicory forage stimulated the proliferation of the Lactobacillus in ileum and the Bacteroides in caecum, and depressed pathogenic microbes including the Rikenella, thereby further improved intestinal health and nutrient utilisation. It was concluded that 8% chicory forage could be considered as a beneficial feed additive in Beijing-you chicken diets. In addition to the good nutritional profile, medicinal properties were associated with the beneficial effects of chicory forage on chickens.

HIGHLIGHTS
• Dietary supplementation of chicory forage improved muscle nutritional value and flavour.
• Dietary supplementation of chicory forage affected egg quality.
• Dietary supplementation of chicory forage regulated the intestinal microbiota.

Introduction
The demand for animal protein for human nutrition in the developing world is still increasing, especially for poultry products, and the cost of feed concentrates for poultry is increasing. In this regard, there is a growing interest in using novel feed resources, and the utilisation of forage for poultry diet could be a sustainable and natural alternative (Tufarelli et al. 2018). Forage and forage meal could be valuable alternative sources of protein for poultry where they are easily available and not expensive (Mourao et al. 2008). Forage available to poultry showed a relatively high level of protein with a desirable amino acids profile, especially lysine, methionine and other sulphur-amino acids, which for poultry adequately balances the limitations of cereal proteins (Luscher et al. 2014). The primary benefit of forage consumption is that dietary fibre in plant matter can stimulate the development of the gastrointestinal tract (GIT), gut motility and nutrient utilisation (Liu et al. 2011). In addition, some medicinal plants were found to have prebiotic effects to modulate intestinal microbiota through favouring a quick proliferation of beneficial strains and inhibiting the growth of pathogenic microbes (Ali 2011). It is well established that a large range of forages, such as alfalfa, ryegrass, clover and...
Chicory (Cichorium intybus L.), which is native to Europe, many parts of Asia, Africa and America, is a perennial herb that can produce nutritious and high-quality forage (Li and Kemp 2005; Liu et al. 2018a). The nutritional profile of chicory is good because it has certain amount of protein, metabolic energy, vitamins, minerals and different types of bioactive compounds (Saeed et al. 2017). In addition, all parts of this plant possess great medicinal importance due to the presence of a number of medicinally important compounds such as alkaloids, inulin, sesquiterpene lactones, coumarins, chlorophyll pigments, unsaturated sterols, flavonoids, saponins and tannins (Shad et al. 2013). Chicory has been used as a forage for livestock in many parts of the world, and the results showed that animal performance on chicory is similar to that on legumes and superior to grass-based pastures (Li and Kemp 2005). Recently, chicory was reported as a beneficial feed ingredient for poultry. Liu et al. (2011) suggested that chicory forage had potential to be used as a valuable fibre source for broiler chickens and could be included at 6% without any major negative effects on diets digestibility and growth performance. In one of our previous studies, Beijing-you chickens had a better growth performance, meat and egg quality when grazed on chicory pasture rather than on bare land (Meng et al. 2016).

Microbial community in the GIT plays an important role in overall health and function of host, be it in human or in animals (Shaufi et al. 2015). During the processes of nutrition, metabolism, physiology and immunity, the intestinal microbiota can promote digestion and absorption of nutrients, stimulate the immune response of the host and enhance resistance to infection (Wang et al. 2017). In addition to animal age, hygiene and environmental factors, diet is a major factor that can influence the microbial population in the intestinal tract (Dong et al. 2017). Previous studies suggested that dietary supplementation of forage had an impact on poultry performance by modifying the gut microbial structure to a bacterial community that is more conducive to host growth (Jiang et al. 2014; Saeed et al. 2017). Extensive studies have been conducted to disclose the most abundant microbial community in the chicken GIT by using culture-based approach, DNA fingerprinting method and polymerase chain reaction (PCR)-based sequencing (Xiao et al. 2017). Although these studies have expanded our knowledge of the intestinal microbiota, they only identified a few of the most abundant operational taxonomic units (OTUs) present, because of their poor limits of detection (Zheng et al. 2017).

Recent advancements in molecular tools, especially for high-throughput sequencing, have enabled us to perform a comprehensive assessment of the intestinal microbiota. One of our former studies showed that dietary supplementation of alfalfa meal stimulated the proliferation of beneficial bacteria, such as the Lactobacillus and Bacteroides, and inhibited potential pathogens including the Clostridium (Zheng et al. 2019). However, limited information is available regarding the metagenomic analysis of the intestinal microbiota affected by dietary chicory forage supplementation. In this study, therefore, we aim to perform a comprehensive assessment of the intestinal microbiota in chickens with and without dietary supplementation of chicory forage by high-throughput sequencing. We also provide data on growth performance, carcass characteristics, meat and egg quality of chickens. This approach enables us to obtain information on intestinal microbiota correlated with performance measures and provides base information for designing high-efficiency feed formula with chicory forage supplementation.

**Materials and methods**

**Experimental design and dietary treatments**

Beijing-you chicken is one of the most famous Chinese local breeds with superior meat and egg quality (Fu et al. 2015). A total of 600 healthy 16 weeks female Beijing-you chickens were selected and randomly allocated into four dietary treatments for a period of 13 weeks. As shown in Table 1, the chickens received isoenergetic and isonitrogenic diets supplemented with 0% (control group), 5%, 8% and 10% fresh chicory forage (on dry matter base, treatment groups) that were formulated to meet or exceed nutrient requirement estimates (NRC 1994). Chicory (Cichorium intybus cv. Grassland Puna) forage that was used in this study contained 10.3% dry matter (DM), 23.5% crude protein (CP), 22.3% crude fibre, 3.2% crude fat and 10.7% crude ash. Chicory forage was chopped to about 1 cm using a forage cutter and mixed with the basal diet thoroughly. Each dietary treatment consisted of six replicates with 25 chickens per replicate. Chickens were raised in a free-range system, in which chickens were fed in an indoor house (20 m²) with free access to an outdoor paddock as a playground (30 m²). Chickens were allowed ad libitum access to feed and water throughout the experiment,
and fed daily at 7:00 and 16:00. This study was carried out at Shunyi District (Beijing, China) during the period from 13 July to 11 October 2017, with ambient temperature from 8.9 to 32.3°C.

Data collection and sampling
Residual feed was weighed daily to determine average daily feed intake (ADFI). Mortalities and health status were visually observed and recorded daily, and ADFI was adjusted for dead chickens. Egg numbers and weight per pen were recorded daily. Feed conversion ratio (FCR) was calculated as grams of total feed consumption per grams of total egg mass. Individual body weight (BW) was recorded after 12 h of feed deprivation at the start and the end of the trial to determine average daily gain (ADG). Three healthy chickens of average BW from each replicate pen were chosen and slaughtered at the end of the trial. After exsanguination by cutting the carotid arteries and jugular veins, chickens were scalded in water at 60°C for 45 s before defeathering, eviscerating, tissue and intestinal content sample collection. Intestinal contents were scraped aseptically from duodenum, ileum (2 cm from Merkel’s diverticulum and 2 cm from caecum junction) and caecum (both pairs) by sterile glass slides, and pooled into sterile centrifuge tubes to reduce individual variation. Intestinal contents were then flash-frozen in liquid nitrogen and stored at −80°C until DNA isolation. The defeathered carcass, including head and feet, was eviscerated manually and weighed as eviscerated weight (EW). Eviscerated yield was calculated as the percentages of BW. Breast muscle, thigh muscle and abdominal fat pad including leaf fat surrounding the cloaca and gizzard were separated and weighed. Breast and thigh muscle yields were calculated as the percentages of EW. Abdominal fat percentage was calculated by abdominal fat weight/(abdominal fat weight + EW). Muscle samples were minced using a food mixer and then freeze-dried for further analysis.

Meat quality measurements
The DM, CP, crude fat and ash contents of breast and thigh muscle samples were determined by the standard procedures of the Association of Official Analytical Chemists (AOAC 1990). The inosine monophosphate (IMP) concentration in breast and thigh muscles was determined by high-performance liquid chromatography (LC-10A, Shimadzu Corporation, Tokyo, Japan) according to the method of Jung et al. (2013). Free amino acids were determined by automatic amino acid analyser (L-8800, Hitachi Ltd., Tokyo, Japan) as described by Yan et al. (2018).

Egg quality measurements
At the end of the experiment, 36 saleable eggs (no shell defects, cracks or double yolks) per treatment (six eggs per replicate) were collected and used to determine egg quality within 24 h after collection. The egg shape index was calculated as the ratio of vertical and horizontal diameter of the egg using Egg Form...
Quality control and assignment of Illumina MiSeq PE300 platform (Illumina Corporation, San Diego, CA) and sequenced on an Illumina MiSeq (EMT-5200, Robotmation Co., Ltd., Tokyo, Japan). Yolk was separated from albumen to determine yolk weight. Yolk and albumen samples were dried using a freeze dryer (Modulyod-230, Thermo Fisher Scientific Inc., MA) and then ground for protein, lecithin and cholesterol determination (Trupia et al. 2016; Liu et al. 2018b).

**DNA extraction and 16S rRNA gene amplification**

Genomic DNA was extracted using the QIAamp DNA Stool Mini Kit (Qiagen Inc., Valencia, CA) by following the manufacturer instructions. The normalised concentration of purified genomic DNA was used as a template to analyse microbial communities. The V3-V4 region of the 16S rRNA gene was amplified using eubacterial primers (338F: 5’-ACTCCTACGGGAGG CAGCA-3’ and 806R: 5’-GGACTACHVGGGTWTCTAAT-3’). The forward primer contained 12-bp barcodes unique to each sample, in order to enable the pooling of all PCR products for sequencing and the subsequent assignation of sequence reads to their respective samples. PCR reactions were performed in 25 µL volumes containing 30 ng of template DNA, 1 µL of each primer (5 µmol/L), 3 µL of BSA (2 ng/µL), 12.5 µL of 2 × Taq PCR Master Mix (Takara Bio Inc., Shiga, Japan), and double-distilled water was added to obtain a final volume of 25 µL. Thermocycling conditions were as follows: 95 °C for 5 min to denature the DNA, with amplification proceeding for 28 cycles at 95 °C for 45 s, 55 °C for 50 s and 72 °C for 45 s; a final extension of 10 min at 72 °C was added to ensure complete amplification.

**High-throughput sequencing and data processing**

PCR products were purified with GeneJET Gel Extraction Kit (Thermo Fisher Scientific Inc., Carlsbad, CA) and mixed in equidensity ratios. Sequencing libraries were generated using NEB Next Ultra DNA Library Prep Kit for Illumina (New England Biolabs Inc., Ipswich, MA) and sequenced on an Illumina MiSeq PE300 platform (Illumina Corporation, San Diego, CA). Quality control and assignment of Illumina MiSeq sequences to samples based on their barcodes were done following the standard QIIME pipeline (ver. 1.7.0, http://qiime.org/index.html). The sequence reads were then clustered into OTUs by de novo OTU picking at a 97% level of sequence similarity. A single representative sequence from each clustered OTU was assigned to different taxonomic levels (phylum and genus) at a cut-off of 97% comparing with the SILVA bacteria reference database (ver. 1.8, http://www.arb-silva.de). Alpha diversity and Good’s coverage analyses consist of community diversity (Shannon) and richness (OTUs number observed and Chao1) were performed using Mothur software (ver. 1.30.1, http://www.mothur.org/wiki/Classify.seqs) based on summary single command. Principal coordinate analysis (PCoA) was performed at the genus level using Mothur software.

**Statistical analysis**

Data on the growth performance, carcass, meat and egg traits were subjected to one-way ANOVA to test the effect of dietary chicory forage content as a completely randomised design using the GLM procedure of SAS (SAS Institute Inc., Cary, NC). Significant differences among the means were determined using Tukey’s multiple range test. Treatment differences were considered significant at \( p < .05 \).

**Results**

**Growth performance**

The effects of dietary supplementation of chicory forage on growth performance, egg production and FCR of Beijing-you chickens are presented in Table 2. An increase \( (p < .05) \) in ADFI, FCR and mortality was observed with increasing supplementation of chicory forage in the diet. The egg weight increased \( (p < .05) \) by 10.87%, 10.89% and 7.94% in chickens fed 5%, 8% and 10% chicory forage diets compared to those given no chicory forage. However, no diet effects \( (p > .05) \) on BW, ADG or egg-laying rate were detected.

**Carcass characteristics and meat quality**

Yields of eviscerated carcass, breast muscle and thigh muscle were increased because of dietary supplementation of chicory forage compared to the control, but no significant differences \( (p > .05) \) were observed among treatments (Table 3). However, dietary supplementation of 5%, 8% and 10% chicory forage decreased \( (p < .05) \) abdominal fat yield by 75.30%, 65.38% and 73.12% compared to the control.
Dietary supplementation of 5% and 8% chicory forage increased (p > .05) DM, CP, IMP and essential amino acids contents of breast muscle compared to the control. Irrespective of the amount of chicory forage added, there was an increase (p < .05) in crude fat, total amino acids, non-essential amino acids and delicious amino acids contents of breast muscle in chickens fed chicory forage diets compared to the control. The highest DM, CP, crude fat, IMP, total amino acids, non-essential amino acids and delicious amino acids contents of breast muscle were observed in chickens fed 8% chicory forage diet, which increased by 1.08%, 1.41%, 79.46%, 4.03%, 12.46%, 16.84% and 22.77% compared to the control. An increase (p < .05) in total amino acids, non-essential amino acids and delicious amino acids contents of thigh muscle was also observed because of dietary supplementation of chicory forage. Dietary supplementation of 8% chicory forage resulted in the highest thigh muscle contents of CP, crude ash, total amino acids, essential amino acids, non-essential amino acids and delicious amino acids, which increased by 0.41%, 2.38%, 12.42%, 4.32%, 18.52% and 22.88% compared to the control.

**Egg quality**

A slight improvement in albumen height, Haugh unit, yolk weight, eggshell weight, yolk protein, yolk lecithin and albumen protein was observed in chickens fed diets containing chicory forage compared to those fed

| Item | C0 | C1 | C2 | C3 | SEM | p value |
|------|----|----|----|----|-----|--------|
| BW, g | 1924.83 | 2011.84 | 1984.87 | 1904.16 | 27.33 | .53 |
| ADG, g/d | 8.61 | 9.85 | 9.67 | 7.66 | 0.48 | .38 |
| ADFI, g/d | 66.70d | 106.30c | 145.90b | 157.10a | 13.34 | <.001 |
| FCR, g of feed/g of egg | 3.68d | 5.73c | 7.96b | 9.07a | 0.29 | .01 |
| Egg laying rate, % | 48.35 | 45.79 | 45.84 | 43.45 | 0.82 | .76 |
| Egg weight, g | 40.30b | 44.68a | 44.69a | 43.90a | 0.50 | .002 |
| Mortality, % | 3.33b | 3.33b | 6.67ab | 9.33a | 2.93 | .003 |

**Table 2.** Effects of dietary treatments on performance and feed efficiency of Beijing-you chickens.

| Item | C0 | C1 | C2 | C3 | SEM | p value |
|------|----|----|----|----|-----|--------|
| Carcass characteristics, % | | | | | | |
| Eviscerated carcass yield | 56.32 | 57.58 | 58.49 | 56.64 | 0.63 | .68 |
| Breast muscle yield | 7.82 | 7.95 | 8.60 | 8.06 | 0.16 | .34 |
| Thigh muscle yield | 9.95 | 11.13 | 11.03 | 10.48 | 0.26 | .39 |
| Abdominal fat yield | 4.13a | 1.02b | 1.43b | 1.11b | 0.49 | .05 |
| Breast muscle chemical compositions, % | | | | | | |
| DM | 26.00 | 26.09 | 26.28 | 25.78 | 0.121 | .59 |
| CP | 95.43 | 95.90 | 96.78 | 95.34 | 0.45 | .72 |
| Crude fat | 1.85b | 2.24ab | 3.32a | 2.30ab | 0.20 | .03 |
| Crude ash | 4.71 | 4.65 | 4.69 | 4.71 | 0.05 | .98 |
| IMP | 1.49 | 1.52 | 1.55 | 1.50 | 0.01 | .58 |
| Total amino acids | 66.27b | 70.03a | 74.53a | 71.28b | 1.01 | .01 |
| Essential amino acids | 24.53 | 26.02 | 25.76 | 27.22 | 0.41 | .12 |
| Non-essential amino acids | 41.74b | 44.01b | 48.77a | 44.06b | 0.87 | .01 |
| Delicious amino acids | 28.99b | 30.92a | 35.59a | 30.28b | 0.85 | .01 |
| Thigh muscle chemical compositions, % | | | | | | |
| DM | 25.00 | 25.55 | 24.72 | 23.97 | 0.32 | .42 |
| CP | 93.66 | 93.29 | 94.04 | 92.33 | 0.36 | .41 |
| Crude fat | 10.75 | 11.91 | 10.14 | 10.83 | 0.43 | .60 |
| Crude ash | 5.46 | 5.36 | 5.59 | 5.40 | 0.04 | .08 |
| IMP | 1.14 | 1.15 | 1.13 | 1.15 | 0.03 | .99 |
| Total amino acids | 70.05b | 76.69ab | 78.75a | 74.53c | 1.22 | .02 |
| Essential amino acids | 26.17 | 26.64 | 27.30 | 26.62 | 0.28 | .63 |
| Non-essential amino acids | 43.41b | 50.51a | 51.45a | 45.83b | 1.11 | .003 |
| Delicious amino acids | 33.91b | 41.03a | 41.67a | 36.38b | 1.09 | .004 |

**Table 3.** Effects of dietary treatments on carcass characteristics and meat quality of Beijing-you chickens.

**#C0: control; C1, C2 and C3: dietary supplementation of 5%, 8% and 10% fresh chicory forage (on dry matter base), respectively.**

**DM: dry matter; CP: crude protein; IMP: inosine monophosphate.**

**SEM: standard error of the mean.**

**†Essential amino acid including threonine, valine, methionine, isoleucine, phenylalanine, lysine and leucine.**

**§Delicious amino acids including aspartic acid, serine, glutamic acid, glycine, alanine and arginine.**

**Means in the same row with different superscript letters are significantly different by Tukey’s multiple comparison method (p < .05).**
Table 4. Effects of dietary treatments on egg quality of Beijing-you chickens.

| Item                        | C0   | C1   | C2   | C3   | SEM | p value |
|-----------------------------|------|------|------|------|-----|---------|
| Eggshell strength, kg/cm²   | 4.28 | 3.68 | 3.72 | 3.70 | 0.08 | .02     |
| Eggshell thickness, mm      | 0.32 | 0.31 | 0.31 | 0.30 | 0.01 | .51     |
| Albumen height, mm          | 6.43 | 6.50 | 6.99 | 6.52 | 0.18 | .70     |
| Haugh unit                  | 82.60| 86.52| 87.97| 85.49| 0.99 | .27     |
| Yolk colour                 | 7.18 | 8.44 | 9.84 | 8.20 | 0.34 | <.001   |
| Egg shape index             | 1.33 | 1.31 | 1.33 | 1.27 | 0.01 | .11     |
| Yolk weight, g              | 11.47| 12.35| 12.19| 11.50| 0.16 | .10     |
| Eggshell weight, g          | 5.50 | 5.76 | 5.61 | 5.76 | 0.07 | .49     |
| Yolk protein, mg/g yolk     | 633.50| 684.70| 697.30| 684.10| 4.99 | .43     |
| Yolk lecithin, mg/g yolk    | 255.30| 262.10| 264.30| 269.90| 1.60 | .96     |
| Yolk cholesterol, mg/g yolk | 27.10 | 25.00 | 25.80 | 25.30 | 0.23 | .20     |
| Albumen protein, mg/g albumen| 815.3 | 835.00| 854.20| 835.90| 3.97 | .55     |

*CO: control; C1, C2 and C3: dietary supplementation of 5%, 8% and 10% fresh chicory forage (on dry matter base), respectively.

*SEM: standard error of the mean.

**Means in the same row with different superscript letters are significantly different by Tukey’s multiple comparison method (p<.05).

In terms of eggshell thickness and egg shape index, the values were also statistically similar (p > .05) for all chickens on experimental diets. Eggshell strength decreased (p < .05) by 14.02%, 13.08% and 13.55%; yolk colour increased (p < .05) by 17.55%, 37.05% and 4.80% and 6.64%, respectively, in chickens receiving 5%, 8% and 10% chicory forage diets than in those given no chicory forage.

Table 5. Effects of dietary treatments on alpha diversity of the intestinal microbiota of Beijing-you chickens.

| Samples | Reads | Length | OTUs | Shannon | Chao1 | Coverage |
|---------|-------|--------|------|---------|-------|----------|
| Duodenum|       |        |      |         |       |          |
| C0      | 19466 | 405    | 30   | 0.99    |       |          |
| C1      | 19466 | 425    | 38   | 0.99    |       |          |
| C2      | 19466 | 425    | 35   | 0.99    |       |          |
| C3      | 19466 | 425    | 35   | 0.99    |       |          |
| Ileum   |       |        |      |         |       |          |
| C0      | 19466 | 425    | 30   | 0.99    |       |          |
| C1      | 19466 | 430    | 35   | 0.99    |       |          |
| C2      | 19466 | 430    | 35   | 0.99    |       |          |
| C3      | 19466 | 430    | 35   | 0.99    |       |          |
| Caecum  |       |        |      |         |       |          |
| C0      | 19466 | 430    | 30   | 0.99    |       |          |
| C1      | 19466 | 430    | 35   | 0.99    |       |          |
| C2      | 19466 | 430    | 35   | 0.99    |       |          |
| C3      | 19466 | 430    | 35   | 0.99    |       |          |

*CO: control; C1, C2 and C3: dietary supplementation of 5%, 8% and 10% fresh chicory forage (on dry matter base), respectively.

*Length: average read length (base pair); OTUs: number of operational taxonomic units; coverage: Good’s coverage.

Regardless of the dietary treatments, the Firmicutes was the most abundant phylum in both duodenum (with the relative abundance of 76.20% to 90.28%) and ileum (40.44% to 70.72%), while the dominant phylum in caecum was identified as the Bacteroidetes (50.71% to 59.61%). The Firmicutes (26.42% to 30.31%) and Bacteroidetes (22.91% to 41.83%) were also commonly found in caecum and ileum, respectively. Compared to the microbial community in the control, the relative abundance of the Bacteroidetes increased in caecum, while it decreased in caecum because of dietary supplementation of chicory forage compared to the control. Good’s coverage was around 0.99 in all samples, indicating that the sampling depth had adequately captured most of the microbial community.

The Firmicutes, Bacteroidetes, Actinobacteria and Proteobacteria were the major phyla common to the three GIT sections of Beijing-you chickens (Figure 1).

The genus Lactobacillus accounted for more than 67% of the sequences obtained from duodenum of chickens with all dietary treatments, while the relative abundance of other detected genera was less than 3%. The Lactobacillus (10.32% to 62.59%) was also observed as the dominant genus in ileum, followed by the genus Bacteroides (17.42% to 22.04%). However, in caecum, the dominant genus was replaced by the Bacteroides (25.81% to 37.20%), the genera Desulfovibrio (2.45% to 3.93%), Ruminococcus (5.56% to 7.31%), Alloprevotella (1.67% to 2.35%) and Rikenella...
(3.44% to 5.52%) were also commonly identified. For the microbial community in ileum, the relative abundance of the *Lactobacillus* increased by more than 35%, while the *Ruminococcus*, *Romboutsia*, *Phascolarctobacterium* and *Rikenella* decreased because of dietary supplementation of chicory forage compared to the control. In caecum, dietary supplementation of chicory forage resulted in an increase of more than 10% in the relative abundance of the *Bacteroides*, and a decrease of the *Anaerobium* and *Rikenella* compared to the control.

The similarity and difference of microbial community composition in the three GIT sections of chickens with different dietary treatments are shown in the PCoA plot (Figure 2). The microbial community of caecum formed a distinct cluster separated from that of duodenum and ileum, and there were overlaps among duodenum and ileum groups. In addition, the microbial community in ileum and caecum of chickens fed 8% chicory forage diet separated from that of chickens given other three diets.

**Discussion**

Various dietary strategies have been used to improve the poultry growth performance and feed efficiency,
such as probiotics including *Bacillus subtilis* and *Lactobacillus* sp. (Guo et al. 2017; Fathi et al. 2018), prebiotics including fructooligosaccharides and galactooligosaccharides (Park et al. 2017), forage products including alfalfa meal (Zheng et al. 2019) and other natural products including green tea (Xia et al. 2018). In this study, however, chickens fed diets containing chicory forage had a lower feed efficiency compared to those fed a basal diet. The increased FCR because of dietary supplementation of chicory forage was associated with the increased ADFI, which was in agreement with Almeida et al. (2012). The increased ADFI because of dietary supplementation of chicory forage might be due to the following reasons: (1) feed intake was weighed based on fresh chicory forage rather than dried sample; and (2) chickens have compensated for a comparatively smaller availability of the commercial feed by consuming more chicory forage. The prebiotic effects of chicory forage or its extracts (such as inulin and oligofructose) on chicken health have been reported previously (Swiatkiewicz et al. 2011; Shad et al. 2013; Liu et al. 2018a). In this study, in addition to high moisture content of fresh chicory forage, low temperature of the rearing system might contribute to the increased mortality and decreased egg-laying rate of chickens fed chicory forage diets compared to the control.

Carcass yield is one of the most important indicators of the overall performance of modern broiler chicken lines (Kwiatkowska et al. 2017). In this study, however, dietary supplementation of chicory forage had no effect on some carcass traits, such as yields of eviscerated carcass, breast muscle and thigh muscle. Lee et al. (2003) suggested that the use of highly digestible feed ingredients in the diet and hygienic conditions in research studies could mask the beneficial effects of forage on growth performance and carcass traits. A series of animal studies demonstrated that chicory fructans could affect the metabolism of lipids primarily by decreasing triglyceridemia (Van Loo 2007; Lin et al. 2014). In this study, dietary supplementation of chicory forage significantly decreased abdominal fat yield compared to the control, which confirmed other reports (Yusrizal and Chen 2003; Ali 2011; Saeed et al. 2017).

In this study, the nutritional value of breast and thigh muscles was improved by dietary supplementation of chicory forage, as indicated by the higher contents of total amino acids and CP in chickens receiving chicory forage diets than in those fed a basal diet. In addition to the abundant essential amino acids, the low concentration of condensed tannins in chicory forage might be partly responsible for the improved efficiency of protein utilisation (Li and Kemp 2005; Saeed et al. 2017). Flavour is another important factor that determines meat quality, and the active flavour components in chicken meat are mainly composed of free glutamic acid, 5'-inosinic acid and potassium ion. In this study, delicious amino acids (Yan et al. 2018) contents in breast and thigh muscles increased significantly because of dietary supplementation of chicory forage compared to the control. Therefore, dietary supplementation of chicory forage could improve the taste of meat by increasing some of the delicate flavours produced by amino acids. In agreement with our findings, improved muscle flavour was obtained when chickens were grazed on chicory pasture (Azcona et al. 2008; Meng et al. 2016).

Measurements on eggshell parameters showed that forage crops were sufficient to meet the calcium and phosphorus requirements of hens (Horsted et al. 2006). It seems surprising, however, the results of this study showed that dietary supplementation of chicory forage decreased eggshell strength. It could not be excluded that chicory forage supplementation changed the balance of feed nutrition, which resulted in insufficient absorption and utilisation of calcium and phosphorus. In agreement with previous findings (Van Loo 2007; Saeed et al. 2017), dietary supplementation of chicory forage had good effects on laying hen health status, reducing eggs cholesterol levels. These beneficial effects might be due to the inulin and oligofructose amount provided by chicory forage. Chen et al. (2005) suggested that inulin and oligofructose prebiotic dietary supplementations increased concentrations of unabsorbable cholesterol in the jejunum contents and cholesterol excretion, therefore resulting in lowered concentrations of cholesterol in the yolk and in serum. Large amount of cholesterol in egg yolk once has been a primary health concern for consumers (Xia et al. 2018). However, recent epide-miologic data have clearly demonstrated that cholesterol is an essential constituent of animal cells and increasing concentrations of dietary cholesterol are not correlated with increased risk for coronary heart disease (Secci et al. 2018). Yolk colour is another important egg quality parameter for the consumer, and it has been demonstrated to become dark with increasing dietary supplementation of forage crops (Hammersho and Steenfeldt 2005). Quantity of xanthophyll contained in chicory forage might be related to the dark egg yolk from hens with access to chicory forage (Horsted et al. 2006).
Previous studies demonstrated that feed additives (prebiotics and probiotics) modulate the microbial ecology of the poultry GIT, and improve gut functionality and consequentially the health status (Jiang et al. 2014; Borrelli et al. 2017). In this study, pyrosequencing results indicated that dietary supplementation of chicory forage might have benefited intestinal health by increasing the microbial community diversity in caecum of chickens. Several studies, in fact, revealed that a rich microbial community is associated with a healthy status as a more diverse microbial community shows stronger homeostasis of the intestinal microbial community and resistance to pathogens (Borrelli et al. 2017; Li et al. 2017). On the contrary, the stimulation of predominant bacteria might have led to a fall in the microbial diversity in duodenum and ileum of chickens fed chicory forage diets compared to the control. In agreement with Liu et al. (2018a), the microbial richness in duodenum and ileum was decreased because of dietary supplementation of chicory forage compared to the control. The results of PCoA suggested that microbial community in caecum formed a distinct cluster and separated from that in duodenum and ileum, confirming that microbial compositions in caecum were different from those in duodenum and ileum. Xiao et al. (2017) suggested that variance in the microbiota among different GIT sections might be attributed to different GIT functions. As reported, the small intestine (duodenum, jejunum and ileum) is the important site for nutrient digestion and absorption, while intestinal microbiota in caecum carries many important roles such as fermentation and breaking undigested substrates (Shaufi et al. 2015).

In agreement with our findings, plenty of studies indicated that the Firmicutes was the dominant phylum in duodenum and ileum of chickens, while caecum was inhabited mostly by the Bacteroidetes (Li et al. 2017; Xiao et al. 2017; Zheng et al. 2019). At the genus level, the Firmicutes and Bacteroidetes were primarily composed of the Lactobacillus and Bacteroides, respectively. Our findings showed that the Lactobacillus was more common in duodenum and ileum, indicating the Lactobacillus contributes to the intestinal functions related to nutrient digestion and absorption. The Lactobacillus has also been widely used as probiotics in poultry feed to reduce egg yolk cholesterol concentration (Ramasamy et al. 2009), improve eggshell thickness (Gallazzi et al. 2008), stimulate the immune system (Fong et al. 2015), improve growth performance (Peng et al. 2016) and regulate the intestinal microbiota (Li et al. 2017). In this study, compared to the control, dietary supplementation of chicory forage significantly increased the relative abundance of the Lactobacillus in ileum, and thereby further improved intestinal health and nutrient utilisation.

The Bacteroides plays an important role in breaking down complex molecules to simpler compounds which are essential for the growth of host and gut microbiota (Shaufi et al. 2015). Therefore, the Bacteroides showed significant negative correlations with FCR and feed intake, and a significant positive correlation with body weight gain (Crisol-Martinez et al. 2017). In this study, the increased populations of the Bacteroides in caecum of chickens fed chicory forage diets are further indications for a chicory-induced stimulation of intestinal fermentative activity. As we suggested previously, dietary fibre in diet might stimulate the Bacteroides proliferation (Zheng et al. 2019). In addition, pathogenic microbes, such as the Rikenella, were depressed because of dietary supplementation of chicory forage. This study showed that dietary supplementation of chicory forage promoted the growth of the Lactobacillus and Bacteroides, whereas it could inhibit the growth of the Rikenella. The inulin content in chicory forage might serve as substrate for the intestinal microbiota, influencing the composition and the microbial fermentation metabolites (Liu et al. 2018a). Our findings strongly corroborate the hypothesis of possible beneficial effects of chicory forage-based diet on global health of hens, even though further studies are necessary to decipher its impact on bacterial metabolites production.

Conclusions

Fresh chicory forage appeared to be palatable and could be supplemented in Beijing-you chicken diets at 8% (on dry matter base) with some beneficial effects on growth performance, carcass characteristics, meat and egg quality, and intestinal microbiota. Dietary supplementation of chicory forage increased the relative abundance of the Lactobacillus in ileum and the Bacteroides in caecum, while depressed pathogenic microbes including the Rikenella. Therefore, productive performance increased with shifts in the intestinal microbiota. In addition to the good nutritional profile, medicinal properties were associated with the beneficial effects of chicory forage on chickens.

Disclosure statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this paper.
Ethical approval

All study procedures were approved by the Animal Care and Use Committee of Beijing Academy of Agriculture and Forestry Sciences and were in accordance with the Guidelines for Experimental Animals established by the Ministry of Science and Technology (Beijing, China). All efforts were made to minimize the suffering of the animals.

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