Probiotics are live microorganisms, which have been defined as 'live microorganisms when administered in adequate amounts, and confer health benefit the host,(FAO/WHO (2002). Currently, in the poultry industry, probiotics seem to be a good alternative to the use of antibiotics as growth promoters (Tomasik and Tomastik 2003), which are being offered to poultry and livestock in an attempt to improve growth rate, feed conversion ratio (FCR) and decrease mortality rate in these animals (Tannock 1999, Mohan et al. 1996, Edens 2003, Koenen et al. 2004, Mountzouris et al. 2007, Knap et al. 2010, Simon 2011).

A variety of microbial species have been used as probiotics including Lactobacillus, Bifidobacterium, Bacillus, Streptococcus, Pediococcus, Enterococcus and yeast, which are widely used to prevent poultry pathogens and disease so as to improve broiler performance (Tortuero 1973, Owings et al. 1990, Cavazzoni et al. 1998, Jin et al. 1998, Zulkifli et al. 2000, Kalavathy et al. 2003, Patterson and Burkholder 2003, Kabir et al. 2004, Osipova et al. 2005, Timmerman et al. 2006, and Mountzouris et al. 2007).

The ideal probiotic should be an image of the indigenous strains resistant to feed processing, acidity, bile salts and digestive enzymes. The most well known group of probiotics is Lactobacillus consisted of 7 Lactobacillus species isolated from the digestive tract of chickens, which had the beneficial effects on broiler performance. (Jin 1998, Patterson and Burkholder 2003, Timmerman et al. 2004, 2005, 2006, Chaucheyras-Durand et al. 2008, Mountzouris et al. 2007, A. Alkhalf 2010). Although there have been many investigations in the effect of Lactobacillus on poultry, little information is provided on the effect of chicken-specific Lactobacillus on the growth performance and meat quality of broiler chickens.

To further qualify the potential of chicken-specific Lactobacillus CSL-13 isolated from the digestive tract of chickens, we investigated the effect of CSL-13 that was administered with the drinking water on the growth performance and meat quality in broilers. Meanwhile, broiler performance parameters of body weight gain (BWG), feed intake (FI), FCR and mortality of chicken (MC) were determined. Furthermore the effect of CSL-13 on qualitative traits of chicken breast fillet that may influence the

Present address: 1Engineer, happylvle@163.com; 2Professor, haiyan@ustb.edu.cn (Correspondence); 3Engineer, xiaolu19@yahoo.com.cn; 4Engineer, lmian68@163.com; 5Engineer, chyin@ustb.edu.cn; Department of Biological Science and Engineering, School of Chemical and Biological Engineering.
consumers’ acceptance was also studied.

MATERIALS AND METHODS

Probiotic strains

A bacterial strain of chicken-specific *Lactobacillus* isolated from the digestive tract of chickens was cultured at 37°C for 1 d. The cells were harvested with the centrifugation at 10000 rpm for 10 min and the cell solution containing 1.0×10^10 colony-forming units per ml in water was used to feed broiler chicks by mixing with drinking water.

Birds and dietary treatments

All practice in the process of this study concerning animal care followed principles required by the university. Day-old broiler chicks (900) with original average body weight of 50 g for each with no significant differences (P>0.05) were assigned at random to 3 experimental groups with each group consisted of 3 replications of 100 birds (50 cocks and 50 hens), and raised floor pens (0.90 m²/bird) for 6 weeks. Chicks were vaccinated at hatch for Marek, infectious bronchitis and Newcastle disease. Feed and water were supplied for consumption *ad lib*. Chicks were fed a commercial maize-soybean diet without any antibiotics or growth promoters, which was formulated for starter (0–3 weeks) and grower (3–6 weeks) growth periods (Table 1). The temperature was set at 32°C on the first day, gradually reduced to 24°C by the third week, and then maintained at 24°C to the end of the experiment. The drinking water was untreated well water with following specifications: pH 7.5, electrical conductivity 570 mmohs/cm and free residual chlorine 0 mg/l. CSL-13 was provided through drinking water and prepared daily. Birds in the control group received no probiotic in either water or feed. Two treatment groups took in chicken-specific *Lactobacillus* via drinking water from dosage pump starting immediately after the arrival of the chicks. The 1ml CSL-13 treatment had probiotic at a concentration of daily based on an expected growth curve to achieve an average dose of 1.0×10^10 cfu/kg of BW while 2 ml CSL-13 supplemented group received probiotic prepared the same way at a dose rate of 2.0×10^10 cfu/kg of drinking water.

Growth performance traits

All birds in each group were weighed individually at hatch (0 week) and every week. Body weight was assessed by dividing the total weight per experimental flock by the number of chicks alive. The amounts of added feed to each pen were recorded daily, and feed residues in each pen were weighed weekly. Feed consumption was therefore calculated on a weekly basis. In the controlled trial, body weight and feed intake were recorded for 6 growth stages, which are starter: the first (hatch- 7d), second (8-14d) and third (14-21d), and grower: fourth (21-28d), fifth (28-35d) and sixth (35-42d). Daily weight gain and feed convention ratio (FCR) of each week, as well as starter period (3–6 weeks) and overall (0–6 weeks) individually at hatch (0 week) and every were calculated. In all trials mortality was recorded daily and weekly and reported as a cumulative percentage. All dead birds were removed daily in the morning.

Chemical composition of chicken breast

At 42 days of age, 10 randomly chosen male chickens from each pen were slaughtered, and the breast muscles were dissected and stored at 20°C prior to analysis. Protein and moisture content in chicken breast fillet was determined by the AOAC Official Method (AOAC, 1980). Fat content was determined with Soxhiet extraction (AOAC, 1980). Extraction residue was dried in an oven at 105°C for 24 h and cooled in a desiccator before being weighed to obtain dry solid content.

Free amino acid analysis

Samples were dissolved in water with methanol (1: 1) for 30 min and centrifuged at 10000 g for 5 min. The supernatant was filtered through glass wool and stored at –80°C, until use. After centrifugation to separate soluble from insoluble material, 40 ml of the supernatant were labeled with iTRAQ...
reagents as recommended by the manufacturer and analyzed on an biosystems equipped with a column (150mm length, 4.6mm diameter, 5 mm particle size).

**Sensory evaluation**

Three groups of semi-trained students formed the sensory panel for the chicken breast fillet sensory evaluation. Each group consisted of 15 students with approximately equal number of males and females whose age was between 20–28 years. Chicken breast fillet samples were sliced into 1 cm thick pieces and grilled for about 45 s to reach an internal temperature of 71–75°C, after which samples were provided to the sensory panel using a coded identifier. Before tasting, panelists were well instructed on the assessment criteria, meat attributes to be rated, and how to properly complete the questionnaire. Each treated sample was tasted by at least 3 different panelists. Drinking water was provided to cleanse off flavor from the last taste. Panelists used a 9-point hedonic scale to assess meat quality attributes. Sensory- textual attributes being scored were: meat colour (from extremely light to extremely dark), juiciness (from extremely dry to extremely juicy), aroma strength (from very weak to very strong), stickiness to the upper mouth cavity (from extremely gooey to extremely smooth)-which is very important, texture- the experience during chewing, and overall satisfaction (disagreeable to enjoyable).

**Statistical analysis**

Data were subjected to a one-way analysis of variance using the general linear models (GLM) procedure of SPSS 19.0.0 (SPSS2010). When significant treatment differences (*P*<0.05) were detected, means were separated using Duncan test.

**RESULTS AND DISCUSSION**

**Growth performance**

CSL-13 tested in this trial significantly improved the body weight of the cocks (Table 2), and the cocks fed on CSL-13 2 ml exhibited best daily weight gain than those of 1 ml as well as control group. The 2 ml CSL-13 treatment group showed a significant increase in the daily weight gain at 1, 3, 4, 5, 6, weeks of age (*P*<0.05). In addition, the average daily weight gain over weeks 3–6 and 0–6, the chicks fed on the CSL-13 showed higher daily weight gain than those in control group (*P*<0.05). Also, it can be noticed that the treatment groups showed significant increase in the initial average BWG of cocks compared with those in control group at 1 week of age.

The average daily weight gains of the hens are summarized in Table 3. The 1ml CSL-13 treatment group had no differences in growth performance compared with the control group during the entire experimental period except of 4 weeks of age (*P*<0.05), but had a decrease of BWG compared with controls on 5 week (*P*<0.05). Whereas, the hens fed on the 2 ml CSL-13 showed a significant increase in the daily weight gain compared with those in control group at 3, 4, 5 weeks of age. Moreover it had a greatest average daily weight gain than 1 ml treatment group as well as control hens over weeks 0–3, 3–6, and 0–6 (*P*<0.05).

Feed conversion rate and mortality rate concerning the feed consumption over 0–3, 3–6, and 0–6 weeks, the CSL-13 treatment groups consumed slightly more feed than control group, furthermore, 2 ml CSL-13–supplemented treatment had the higher feed consumption than control group over grower period, whereas, there was no obviously differences among three groups at all time of this trial. The means of feed conversion ratios (FCR) over 0–3, 3–6 and 0–6 weeks are summarized in Table 4. It can be noticed that FCR were affected by supplement CSL-13 treatments, as well as the birds fed on 2 ml CSL-13 had lowest FCR than the control group and 1 ml CSL-13 treatment group (*P*<0.05). Moreover,  

<table>
<thead>
<tr>
<th>Age in week</th>
<th>Daily weight gain of cock treatment groups (g)</th>
<th>Lactobacillus supplementation (cells/ml water)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>L1*</td>
</tr>
<tr>
<td>0–1</td>
<td>20.26±0.250a</td>
<td>21.88±0.249b</td>
</tr>
<tr>
<td>1–2</td>
<td>30.81±0.585a</td>
<td>32.82±0.592a</td>
</tr>
<tr>
<td>2–3</td>
<td>73.41±0.802a</td>
<td>74.36±0.817a</td>
</tr>
<tr>
<td>3–4</td>
<td>94.87±0.899a</td>
<td>86.56±0.896a</td>
</tr>
<tr>
<td>4–5</td>
<td>93.76±1.540a</td>
<td>96.86±1.548a</td>
</tr>
<tr>
<td>5–6</td>
<td>82.38±1.927a</td>
<td>113.78±1.941a</td>
</tr>
<tr>
<td>0–3</td>
<td>41.49±0.523a</td>
<td>43.02±0.512ab</td>
</tr>
<tr>
<td>3–6</td>
<td>90.34±1.318a</td>
<td>99.07±1.306b</td>
</tr>
<tr>
<td>0–6</td>
<td>65.91±0.889a</td>
<td>71.05±0.904b</td>
</tr>
</tbody>
</table>

*L1: 1 ml Chicken-Specific Lactobacillus CSL-13 supplemented treatment; L2: 2 ml Chicken-Specific Lactobacillus CSL-13 supplemented treatment.

<table>
<thead>
<tr>
<th>Age in week</th>
<th>Daily weight gain of hen treatment groups (g)</th>
<th>Lactobacillus supplementation (cells/ml water)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>L1*</td>
</tr>
<tr>
<td>0–1</td>
<td>21.42±0.249ab</td>
<td>20.74±0.251a</td>
</tr>
<tr>
<td>1–2</td>
<td>28.49±0.490a</td>
<td>30.18±0.487a</td>
</tr>
<tr>
<td>2–3</td>
<td>58.07±0.691a</td>
<td>57.78±0.687a</td>
</tr>
<tr>
<td>3–4</td>
<td>68.41±0.838a</td>
<td>76.65±0.835ab</td>
</tr>
<tr>
<td>4–5</td>
<td>74.89±1.286a</td>
<td>66.55±1.270a</td>
</tr>
<tr>
<td>5–6</td>
<td>75.00±1.984a</td>
<td>82.17±1.973a</td>
</tr>
<tr>
<td>0–3</td>
<td>36.00±0.459a</td>
<td>36.23±0.456a</td>
</tr>
<tr>
<td>3–6</td>
<td>72.77±0.977a</td>
<td>75.12±0.973b</td>
</tr>
<tr>
<td>0–6</td>
<td>54.38±0.708a</td>
<td>55.68±0.714a</td>
</tr>
</tbody>
</table>

*L1: 1 ml Chicken-Specific Lactobacillus CSL-13 supplemented treatment; L2: 2 ml Chicken-Specific Lactobacillus CSL-13 supplemented treatment.
The effect of 2 ml CSL-13 on FCR started at growing periods, and persisted until 6 weeks of age. In this study, administration of both 1 ml and 2 ml CSL-13 preparation had no effect on mortality during the whole process.

Chemical composition

With regard to the influence of CSL-13 on meat basal biochemical investigated in the experiments, no significant changes on fat content and moisture content among three groups at all time of this trial as illustrated in Figure 1 A-C. Meat of chickens with 2 ml CSL-13-supplemented treatment had the higher protein content (88.49%) than those of control (84.90%) (P < 0.05) and 1 ml CSL-13 supplemented treatment (88.06%). Unexpectedly, the two levels (1 ml and 2 ml) of CSL-13 groups showed slight increase (8.36% and 8.34%) in fat content of chicken breast fillet compared with that in control (7.93%). In addition, the moisture content of chicken breast fillet was not affected by two levels of CSL-13 supplementation in this study, and had a slight decrease compared with that in control.

Amino acids

Fig. 1 D-F clearly demonstrated that the probiotic tested in the study significantly improved the amino acid content of chicken breast fillet. It is noticed that meat of chickens fed on water without CSL-13 had the lowest total free amino acid content which was significantly different from those of the two probiotic-supplement treatments (P < 0.05). Moreover, 2 ml CSL-13 supplemented treatment had the highest amino acid content and no differences were found between two treatments. Similarly, there were more essential amino acids in two probiotic-supplemented treatments than that in control. Especially, the essential and flavour amino acid composition in chicken breast fillet from the 2 ml CSL-13 supplemented treatment was found to be higher than the other two groups (P < 0.05), and the control group had the lowest essential and flavour amino acid content.

Sensory attributes

With regard to the influence of CSL-13 on meat quality, we accessed the meat colour, juiciness, flavour, aroma strength, and texture of chicken breast fillet between control and CSL-13-supplemented treatments. Figure 2 clearly demonstrated that the control group had lower values of meat colour and juiciness than CSL-13-supplemented treatments (P < 0.05). It was also observed that CSL-13-supplemented treatments had significantly higher flavour pleasantness scores and aroma strength scores (P < 0.05) than that of control. No significant difference was observed for the texture sensory attributes among treatments, suggesting that the texture of chicken breast fillet might not be related to the

Table 4. Feed conversion ratios of chickens fed on rations containing different concentration of Lactobacillus

<table>
<thead>
<tr>
<th>Age in week</th>
<th>Feed conversion ratio of treatment groups (g)</th>
<th>Control</th>
<th>L1*</th>
<th>L2*</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–3</td>
<td>1.61±0.022b</td>
<td>1.61±0.021a</td>
<td>1.52±0.022b</td>
<td></td>
</tr>
<tr>
<td>3–6</td>
<td>1.91±0.023b</td>
<td>1.87±0.024b</td>
<td>1.76±0.024b</td>
<td></td>
</tr>
<tr>
<td>0–6</td>
<td>1.81±0.021a</td>
<td>1.74±0.022ab</td>
<td>1.69±0.022b</td>
<td></td>
</tr>
</tbody>
</table>

*L1: 1 ml Chicken-Specific Lactobacillus CSL-13 supplemented treatment; L2: 2 ml Chicken-Specific Lactobacillus CSL-13 supplemented treatment.*

Fig.1. Comparison of moisture content, protein content, fat content and amino acid content between the control group and Chicken-Specific Lactobacillus CSL-13 treated groups (C0: control L1: 1 ml Chicken-Specific Lactobacillus CSL-13 supplemented treatment; L2: 2 ml Chicken-Specific Lactobacillus CSL-13 supplemented treatment.)
Assessed by the sensory panel using a hedonic (1–9) scale, colour, flavour, juiciness, aroma strength, and texture were different doses of CSL-13. However, the statistically significant effect of the CSL-13 on the improvement of overall satisfaction was found (P < 0.05).

Each column represents the mean of 15 observations; meat colour, flavour, juiciness, aroma strength, and texture were assessed by the sensory panel using a hedonic (1–9) scale where the meat colour ranged from extremely light to extremely dark, flavour from extremely unpleasant to extremely enjoyable, juiciness from extremely dry to extremely juicy, aroma strength from very weak to very strong, and texture from extremely gooey to extremely smooth. Error bars represent standard deviations (n=15).

DISCUSSION

It showed that the administration of chicken-specific *Lactobacillus* via the drinking water had beneficial effects on broiler performance. Compared with those of control, Cocks and hens fed on CSL-13 of 2 ml significantly improved the BW of broilers over weeks 0–3, 3–6 and 0–6, however, hens fed on CSL-13 of 1 ml had no effects on broiler performance. This finding is in agreement with several previous studies (Maiorka et al. 2001, Khaksefidi et al. 2006, Mountzouris et al. 2007, Awad et al. 2009, Karimi Torshizi et al. 2010). Feed conversion data showed that the increase in BWG was not a simple consequence of increased feed intake, since improvement in efficiency of feed utilization might also be involved. Edens et al. (1997) showed that in ovo and ex ovo administration of *Lactobacillus reuteri* resulted in an increased villus height, indicating that probiotics are potentially able to enhance nutrient absorption and thereby improve growth performance and feed efficiency. Data analysis also suggested that probiotic treatments caused a statistically significant improvement of feed conversion. Meanwhile, improvement of FCR was evident in probiotic groups during grower phases (P < 0.05). The effect of 2 ml CSL-13 on FCR started at growing periods, and persisted until 6 weeks of age.

Appetite stimulation effects by probiotic have been reported in chickens, including increased lipid, protein, and mineral retention (Nahanshon et al. 1994). Several studies indicated that probiotic exert trophic effects restoring the intestinal homeostasis. Oral administration of *Lactobacillus* in rats enhanced the activity of brush border membrane enzymes, e.g., sucrase-isomaltase, lactase, maltase-glucoamylase, α-glucosidase and alkaline phosphatase, which have a positive influence on nutrient degradation and absorption (Buts et al. 1986, Jahn et al. 1996). The supplementation of broilers with CSL-13 increased the BWG and FCR, which indicated that the addition of CSL-13 may promote the growth of beneficial bacteria and thus provide a healthier intestinal system for better absorption of nutrients (Kelly et al. 1994, Rada et al. 1995, Line et al. 1998, Salminen et al. 1998, Pascual et al. 1999). In addition, the higher concentration of lactic acids in the gut contents when the birds fed on CSL-13, which should be expected to decrease pH in the gut (Högberg et al. 2006), resulting in better nutrient digestibility (Lyberg et al. 2006). Moreover, the increase in lactic acid concentration in ileum and colon may exert antibacterial effects on gut enterobacteria, which will add to the beneficial effects on nutrient utilization. Based on results
from the determination of chemical composition in chicken breast fillet, we deduced that feeding of CSL-13 appeared to have improved nutrient digestion. However it remained unclear whether there was any added benefit in the gut microflora composition, which is needed to further study.

Although the term 'meat quality' has different definitions among different areas (Dikeman 1994), it is possible to have uniformity for delimited areas of production and only considering the muscular development and the amount of fat cover of carcass level (Nardonea and Valfre 1999). The improvement of protein and amino acid content could be considered as positive factors, and the main negative effect of nutrition has been the excess of fat deposition (Larbier, 1991). Furthermore, increasing the ratio of protein, essential amino acids and flavour amino acids to energy may improve feed conversion and nutrient digestion and absorption (Maybray et al. 1981, Smith et al. 1998). Here we found that the chicken breast fillet of CSL-13 treatments appeared to have higher protein, essential amino acids, flavour amino acids and total amino acid content (Fig. 1A, D-F), which indicated that CSL-13 intakes made the main contribution to chemical, nutritional and sensorial quality improvements.

It was reported that Lactobacillus could involve in the decomposition of food to improve digestion and nutritional absorption, and produce lactic acid and vitamins include VK, VB2, VB6, VB12 and so on, which might be beneficial to enhance digestion and improve host nutrition (Salminen me et al. 1999; Ichikawa et al. 1999; Dock et al. 2004; Rastall et al. 2005). Consumer usually expresses their preference of meat according to organoleptic quality (Nardonea et al. 1999). Results of sensory evaluation showed significant variations in the overall satisfaction ratio between CSL-13 treated groups and the control group, and the degree of satisfaction in CSL-13-supplemented treatments was higher than that in control. Therefore, it was fair to conclude that CSL-13 intake could result in improved growth performance and meat quality of broiler chickens.

CONCLUSION

The supplementation of chicken-specific lactobacillus to broilers displayed a growth-promoting effect, which significantly increased bw and decreased feed gain ratios. The dose amount of 2 ml CSL-13 was found better than control and 1 ml CSL-13 treatment groups, but no impact on the mortality was found. Moreover, chicken breast fillet from CSL-13 supplement treatment broilers had increased the contents of protein, essential amino acid, flavor amino acid and total amino acid. The chicken-specific lactobacillus may be a promising new growth promoter of broiler, which has a remarkable potential in improving poultry production and meat quality.

ACKNOWLEDGEMENTS

This research was supported by the Fundamental Research Funds for the Central Universities, China (FRF-AS-09-003A, FRF-AS-10-001B).

REFERENCES


