Effect of dietary probiotic, *Bacillus coagulans*, on growth performance, chemical composition, and meat quality of Guangxi Yellow chicken

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ABSTRACT The effect of *Bacillus coagulans* ZJU0616 with different concentrations supplemented as probiotic was evaluated on growth performance, feed conversion ratio, survival rate, breast chemical composition, and meat quality of Guangxi Yellow chicken. Birds were randomly segregated into 12 groups so that 3 replicates were available for each of the 3 treatments (T-1, T-2, and T-3) and control groups. The control groups were fed a basal diet without any probiotic and other groups were fed the diets that consisted of 3 probiotic levels at initial concentrations of $1.0 \times 10^6$ cfu·g$^{-1}$ (T-1), $2.0 \times 10^6$ cfu·g$^{-1}$ (T-2), and $5.0 \times 10^6$ cfu·g$^{-1}$ (T-3). The lowest final weight and daily weight gain were found in control groups ($P < 0.05$) and there were no significant differences among probiotic-treated groups. Significantly lower feed conversion ratio and higher survival rate were observed in T-2 and T-3 than that of the control. There were no significant differences ($P > 0.05$) in contents of breast chemical composition including moisture, CP, crude fat, and crude ash among all groups. For the meat quality parameter assays, the pH, shear force, and drip loss were used as indicators. As for pH values of breasts, no significant difference was found across all treatments. Higher shear force was observed ($P < 0.05$) in T-2 compared with T-1 and the control. Similar significant difference was also observed in T-1 compared with the control. However, there was no significant difference in shear force between T-3 and the other probiotic treatment groups (T-1 and T-2). As for probiotic-treated groups, there was significant difference ($P < 0.05$) in breast drip loss compared with that of the control (6.22 ± 0.18%), with the lowest drip loss found in T-3 (5.38 ± 0.25%). However, no significant difference was observed in drip loss between T-2 and T-3.

INTRODUCTION

Guangxi Yellow chicken meat is one of the most common used local animal protein sources because of its nutritional value and relative reasonable prices in China. However, the abuse of antimicrobial drugs, pesticides, and disinfectants in farm chickens has led to the evolution of resistant strains of bacteria and questions from society (Witte, 2000). Human health can be affected directly through residues of antibiotic in related food (Boerlin and Reid-Smith, 2008). At the same time, there has been a considerable increase in demand for better safety chicken due to the improved standard of living in recent years (Kleter and Marvin, 2009). Thus, we need to avoid foodborne illness from antibiotic-resistant bacteria and produce antibiotic-free chicken by using probiotics.

A probiotic, which means “for life” in Greek (Gibson and Fuller, 2000), has been defined as “a live microbial feed supplement which beneficially affects the host animal by improving its intestinal balance” (Fuller, 1989). There are many reports concerning the effect of using probiotics including *Lactobacillus*, *Bifidobacterium*, *Bacillus*, *Streptococcus*, *Pediococcus*, *Enterococcus*, and yeast such as *Saccharomyces cerevisiae* on chickens (Endo and Nakano, 1999; Mahajan et al., 2000; Casula and Cutting, 2002; Patterson and Burkholder, 2003; Huang et al., 2004; Kabir et al., 2004; Karaoğlu et al., 2004; Aksu et al., 2005; Ahmad, 2006; Mountzouris et al., 2007). No study has been carried out on spore-forming lactic acid-producing bacteria such as *Bacillus coagulans* as probiotics in Guangxi Yellow chicken.

Many factors make *B. coagulans* a good candidate for probiotic use; it produces organic acids, possesses...
the capacity to sporulate, and is easily cultured in bulk (Hyronimus et al., 2000). In addition, in the spore form, it is more resistant to heat, which facilitates the pelleting process used in the mass production of probiotic chicken feeds. Therefore, this study attempted to investigate the effect of probiotic, \( B. \ coagulans \) ZJU0616, on growth performance and meat quality of Guangxi Yellow chicken, which was one of the most valuable local chicken species farmed in China. For the meat quality parameter assays, the \( \text{pH} \), shear force, and drip loss were used as indicators. Furthermore, the percentage of moisture, CP, crude fat, and crude ash of Guangxi Yellow chicken was also determined.

**MATERIALS AND METHODS**

**Probiotic and Diets**

The probiotic bacterium used in this study was \( B. \ coagulans \) ZJU0616 kept in our laboratory. It was cultured and counted on normal nutrient agar by spore staining with the spread plate technique (Marshall and Beers, 1967). Stock cultures of probiotics were stored at \(-70^\circ\text{C} \) (Forma 702, Thermo Fisher Scientific, Waltham, MA) in powdered skim milk suspension with 25% glycerol before use (Cabo et al., 1999). The strains of \( B. \ coagulans \) ZJU0616 were grown in culture medium in a shaking incubator at \( 37^\circ\text{C} \). After incubation, the cells were harvested by centrifugation (\( 2,000 \times \text{g} \)), washed 3 times with PBS (pH 7.2, Sangon, Shanghai, China), and resuspended in the same buffer until use. The chemical composition of the basal diets used in the experiment was according to the NRC (1994) and the related ingredients were obtained from Yixing Co. in Haiyan, China.

**Experimental Design**

Four treatment groups were carried out with healthy 1-d-old Guangxi Yellow chickens (\( n = 360 \)) in an environmentally controlled isolation facility for 90 d. All chickens with similar initial weights (29.28 ± 0.75 g) were randomly segregated into 12 groups (30 chickens for each group) so that 3 replicates were available for each of 3 treatments and control groups. The control groups were fed a basal diet without any probiotic and the 3 treatments groups were fed the diets consisting of 3 \( B. \ coagulans \) ZJU0616 levels at initial concentrations of \( 1.0 \times 10^6 \text{ cfu·g}^{-1} \) (T-1), \( 2.0 \times 10^6 \text{ cfu·g}^{-1} \) (T-2), and \( 5.0 \times 10^6 \text{ cfu·g}^{-1} \) (T-3). To reach these final concentrations, strains were slowly applied into the diets, mixing part by part in a drum mixer. The amount of probiotic strains in each diet was determined and modified by plate counting on medium agar.

The feeding trial was conducted under the supervision of the Animal Care and Use Committee of Zhejiang University. The experimental chickens were provided with continuous lighting from incandescent lamps in the ceilings of each room, but each brood-grow battery provided an area of subdued light for sleeping and resting. Fresh water was provided on a daily basis during the first week of the experiment to all of the broilers and then every other day thereafter. Remaining water from the previous day was discarded before adding fresh water and the water intake was not measured. Body weight, feed intake, and mortality were recorded. The feeding trial process was according to the commercial producer.

**Sampling and Analytical Methods**

Weight of all collected chickens determined at 1 and 90 d of age was treated as initial weight and final weight, respectively. The daily weight gain (DWG; g·d\(^{-1}\)) was calculated as follows: (final weight – initial weight)/90 (g·d\(^{-1}\)). The feed conversion ratio (FCR) used the following formula: total feed consumption/(total final weight – total initial weight + total mortality weight).

At the end of the feeding period, 18 birds were selected at random from each treatment group. The birds were held without feed for 12 h and slaughtered by severing the jugular vein. After standard cutting, breasts were obtained from the 4 treatment groups (T-1, T-2, T-3, and control). The \( \text{pH} \) value of breasts was then immediately determined using a \( \text{pH} \) meter (\( \text{pH} 211 \), Hanna, Padua, Italy). The \( \text{pH} \) value was measured using a direct probe by thrusting the probe into the muscle. Meat shear force of breast muscle samples was measured according to Shu et al. (2006) using a texture analyzer (C-LM3, Northeast Agricultural University, Harbin, China). The drip loss from 0 to 72 h was assessed from the breast meat packaged in a transparent polythene bag and stored in a chilling room at 4°C for 72 h, after which the excess moisture was wiped out and the breast samples were weighed (at 72 h), and the drip loss was calculated as percentage of breast meat yield in grams measured at 0 h. The chicken breast samples were stored immediately at \(-70^\circ\text{C} \) (Forma 702, Thermo Fisher Scientific) for the biochemical analysis including CP, crude fat, crude ash, and moisture according to AOAC (1995) methods. Crude protein was determined using the Kjeltec Analyzer Unit (2300, Foss, Höganäs, Sweden) and crude fat was measured using the Soxtec Auto Extraction Unit (2050, Foss).

**Statistical Analysis**

Data were analyzed by the GLM procedures for ANOVA (SAS Institute, 1997). The means were used to evaluate chicken growth performance and meat quality. Significant differences among treatment means were separated using the multiple range tests with a 5% probability.
RESULTS

Growth Performance

Initial weight, final weight, DWG, FCR, and survival rate of Guangxi Yellow chickens given diets containing different probiotic levels were shown in Table 1. There was no significant difference in initial weight (\(P > 0.05\)) among treatments groups. The lowest final weight and DWG were found in control groups (\(P < 0.05\)) and there were no significant differences among probiotic-treated groups. Lower FCR in T-2 (2.87 ± 0.04) and T-3 (2.87 ± 0.06) were observed than that of the control groups (3.04 ± 0.06). However, there was no significant difference (\(P < 0.05\)) in FCR between T-1 and the other treatment groups. The groups that received the probiotic with a final concentration of 2.0 × 10^6 cfu·g\(^{-1}\) (T-2) and 5.0 × 10^6 cfu·g\(^{-1}\) (T-3) showed higher survival rate (98.14 ± 1.70% and 97.40 ± 1.28%, respectively) than that in T-1 and control groups (95.55 ± 2.22% and 91.48 ± 2.80%, respectively) and there was no significant difference between T-2 and T-3. No statistical differences (\(P > 0.05\)) were observed in survival rate between T-1 and the control.

Biochemical Analysis

Table 2 showed the contents of moisture, CP, crude fat, and crude ash in Guangxi Yellow chicken breasts treated with (T-1, T-2, and T-3) and without (control) probiotic. No significant differences were found in the contents of moisture and crude ash among treatment groups, although probiotic supplementation showed an increasing trend in CP (T-1, 23.77 ± 1.60%; T-2, 24.24 ± 1.39%; and T-3, 24.11 ± 2.04%, respectively) compared with the control (23.19 ± 1.35%). However, there was no significant difference (\(P > 0.05\)) in CP content among treatments groups. The crude fat percentage in chicken breast ranged from 5.85 to 6.56%. Relative lower crude fat percentage was observed in T-3 in this study. However, there were also no significant differences (\(P > 0.05\)) among the T-1, T-2, T-3, and control groups.

Meat Quality

No difference was found in meat pH of Guangxi Yellow chickens in groups treated with and without \(B.\) coagulans ZJU0616 (data not shown). A concentration response of probiotic strains in T-1, T-2, and T-3 was also not observed in the present research. As shown in Figure 1, lower shear force was observed (\(P < 0.05\)) in T-2-supplemented probiotic with a final concentration of 2.0 × 10^6 cfu·g\(^{-1}\) compared with T-1 and the control. Similar significant difference was also observed in T-1 compared with the control. However, there was no significant difference in shear force between T-3 and the other probiotic treatment groups (T-1 and T-2, Figure 2). As for \(B.\) coagulans ZJU0616-treated groups, there was significant difference (\(P < 0.05\)) in breast drip loss compared with that of the control. In addition, lower drip loss was found in T-2 and T-3 (5.28 ± 0.18% and 5.38 ± 0.25%, respectively) than that of T-1 (5.73 ± 0.22%). No significant difference was observed in drip loss between T-2 and T-3.

### Table 1. Daily weight gain (DWG), feed conversion ratio (FCR), and survival rate of Guangxi Yellow chickens treated with (T-1, T-2, and T-3) or without (control) probiotic

<table>
<thead>
<tr>
<th>Item</th>
<th>Control</th>
<th>T-1</th>
<th>T-2</th>
<th>T-3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial weight (g)</td>
<td>29.3 ± 0.7a</td>
<td>29.3 ± 0.9a</td>
<td>29.3 ± 0.8b</td>
<td>29.3 ± 0.8a</td>
</tr>
<tr>
<td>Final weight (g)</td>
<td>1,028.8 ± 35.3a</td>
<td>1,086.0 ± 31.8b</td>
<td>1,124.5 ± 60.1b</td>
<td>1,119.7 ± 68.6b</td>
</tr>
<tr>
<td>DWG (g·d(^{-1}))</td>
<td>11.1 ± 0.4a</td>
<td>11.7 ± 0.4b</td>
<td>12.2 ± 0.7b</td>
<td>12.1 ± 0.8b</td>
</tr>
<tr>
<td>FCR</td>
<td>3.04 ± 0.06a</td>
<td>2.94 ± 0.05ab</td>
<td>2.87 ± 0.04b</td>
<td>2.87 ± 0.06b</td>
</tr>
<tr>
<td>Survival rate (%)</td>
<td>91.48 ± 2.80a</td>
<td>95.55 ± 2.22b</td>
<td>98.14 ± 1.70b</td>
<td>97.40 ± 1.28b</td>
</tr>
</tbody>
</table>

a,bMeans in the same row with different superscripts are significantly different (\(P < 0.05\)).

\(1\)T-1, T-2, and T-3 were fed with diets containing different concentrations of viable \(B.\) coagulans ZJU0616 (with a final concentration of 1 × 10^6 cfu·g\(^{-1}\), 2 × 10^6 cfu·g\(^{-1}\), and 5 × 10^6 cfu·g\(^{-1}\), respectively). Results are presented as mean ± SE (n = 90).

### Table 2. Percentage of moisture, CP, crude fat, and crude ash of Guangxi Yellow chicken meat treated with (T-1, T-2, and T-3) or without (control) probiotic

<table>
<thead>
<tr>
<th>Item</th>
<th>Control</th>
<th>T-1</th>
<th>T-2</th>
<th>T-3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture (%)</td>
<td>68.00 ± 2.48</td>
<td>67.21 ± 3.20</td>
<td>66.79 ± 2.11</td>
<td>67.15 ± 2.28</td>
</tr>
<tr>
<td>CP (%)</td>
<td>23.19 ± 1.35</td>
<td>23.77 ± 1.60</td>
<td>24.24 ± 1.39</td>
<td>24.11 ± 2.04</td>
</tr>
<tr>
<td>Crude fat (%)</td>
<td>6.56 ± 0.61</td>
<td>6.02 ± 0.23</td>
<td>5.91 ± 0.42</td>
<td>5.85 ± 0.49</td>
</tr>
<tr>
<td>Crude ash (%)</td>
<td>1.23 ± 0.05</td>
<td>1.23 ± 0.06</td>
<td>1.27 ± 0.05</td>
<td>1.26 ± 0.05</td>
</tr>
</tbody>
</table>

\(1\)T-1, T-2, and T-3 were fed with diets containing different concentrations of viable \(B.\) coagulans ZJU0616 (with a final concentration of 1 × 10^6 cfu·g\(^{-1}\), 2 × 10^6 cfu·g\(^{-1}\), and 5 × 10^6 cfu·g\(^{-1}\), respectively). Results are presented as mean ± SE (n = 90).
DISCUSSION

The effect of probiotic, *B. coagulans* ZJU0616, on Guangxi Yellow chicken growth performance was evaluated in this study. It was clear that the administration of probiotic via the basal diet had beneficial effects on final weight and DWG. The previous studies showed that supplementation of the adherent *Lactobacillus* cultures to chickens, either as a single strain of *Lactobacillus acidophilus* or as a mixture of 12 *Lactobacillus* strains, increased significantly (*P* < 0.05) the BW of broilers after 40 d of feeding (jin et al., 2000). Similar findings were obtained in other reports (Noh, 1997; Zulkifli et al., 2000; Lan et al., 2003; Timmerman et al., 2006) and showed that the probiotic strains promoted the growth performance of birds. On the contrary, Kumprechtova et al. (2000) investigated the effect of probiotic, *S. cerevisiae* Sc47, on chicken broiler performance and showed that the bacteria strains could not improve the live weight at 21 and 42 d of age. No positive effect on the growth performance of chickens was also found in another study (Priyankarage et al., 2003). However, it was difficult to directly assess different studies using probiotics because the efficacy of a probiotic application depended on many factors (Patterson and Burkholder, 2003) such as species composition and viability, administration level, application method, frequency of application, overall diet, bird age, overall farm hygiene, and environmental stress factors. In addition, there was no significant difference among the treatment groups (T-1, T-2, and T-3) with different concentrations of probiotic bacteria. This indicated that the quantity of *B. coagulans* ZJU0616 was only one of the factors improving final weight and DWG of the local chickens in China.

Findings of this study showed that the use of the probiotic *B. coagulans* at a certain concentration (2.0 × 10⁶ cfu·g⁻¹ and 5.0 × 10⁶ cfu·g⁻¹) in diet could significantly reduce FCR of Guangxi Yellow chickens. Similar improvements in feed efficiency had been reported for poultry receiving probiotic *Lactobacillus* strains (Mohan et al., 1996; Lan et al., 2003). The work investigating the efficacy of a new multibacterial species probiotic containing *Lactobacillus*, *Bifidobacterium*, *Enterococcus*, and *Pediococcus* strains in male Cobb broilers had shown inconsistent results (Mountzouris et al., 2007). In contrast, there was no significant difference in FCR between probiotic treatments and the control. It could be associated with the different species of birds and probiotic.

The *Lactobacillus* strains that were isolated from fresh digesta and intestinal tissue samples of healthy chickens had improved the survival rates of broilers in controlled trials by the addition of probiotics to the drinking water (Timmerman et al., 2006). A similar result was also observed by Vicente et al. (2007) with *Lactobacillus* spp.-based probiotic in broiler chicks. In the present research, a higher survival rate was observed in T-2 and T-3 compared with T-1 and the control. It coincided with the result of FCR and growth performance.

The effect of probiotic in broiler chicks (Hubbard Classic) with age ranging from 1 to 35 d was studied by Mahmood et al. (2005) and the results showed that there were significant differences in chemical composition including moisture percentage, CP, crude fat, and crude ash between the probiotic-treated groups and the control. However, our results showed no significant difference among the groups with and without probiotics in the above chemical composition of Guangxi Yellow chickens. It might be associated with the culture days, which was 90 and much higher than the above research (Mahmood et al., 2005).

Tenderness is the sum of the mechanical strength of skeletal muscle tissue after rigor mortis and the weakening of the structure during the postmortem storage (Takahashi, 1996). Shear force, defined as the force acting on a substance in a direction perpendicular to the...
extension of the substance, is the most popular indicator for meat texture or tenderness and it has been widely used to estimate tenderness or texture quality of poultry breast fillets, with high Warner-Bratzler shear values associated with less tender poultry breast fillets (Yang and Jiang, 2005; Zhuang et al., 2008). It was clear from our studies that the administration of probiotic, *B. coagulans* ZJU0616, via the basal diet had beneficial effects on shear force of chicken. It was not in agreement with the results determined by Zhang et al. (2005), who observed no significant difference in shear force in raw breast meats of male broilers (Ross strain) among all treatments with or without probiotic, *S. cerevisiae*, for 35 d. It might be explained by the different probiotic strains and culture days. The drip loss was one of the important indicators of water-holding capacity and reflected the juiciness of meat (Rasmussen and Andersson, 1996). In our study, there was significant difference (*P* < 0.05) between the control groups and probiotic, at 2.0 × 10⁶ cfu·g⁻¹. It coincided with the results in our study, where *Lactobacillus* Sc47 on chicken broiler performance (Lan, P. T. N., L. T. Binh, and Y. Benno. 2003. Impact of two probiotic *Bacillus* and *Lactobacillus* strains feeding on fecal lactobacilli and weight gains in chicken. *J. Gen. Appl. Microbiol.* 49:29–36). Mahajan, P., J. Sahoo, and P. C. Panda. 2000. Effect of probiotic (*Lacto-Sacc*) feeding and season on poultry meat quality. *Indian J. Food Microbiol.* 631.

Overall, the results in this work showed that probiotic administration in feed with a certain concentration displayed a growth-increasing effect and promoted FCR, survival rate, shear force, and drip loss. Based on these results, use of a 2.0 × 10⁶ cfu·g⁻¹ supplement of *B. coagulans* ZJU0616 in Guangxi Yellow chickens diet was recommended to stimulate productive performance and meat quality. The addition, however, of probiotics to local chickens in general requires further research to clearly understand the functional mechanism between probiotic and native microorganisms and how the probiotics work in the digestive tract of chickens.

### ACKNOWLEDGMENTS

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### REFERENCES


