**INTRODUCTION**

*Phaffia rhodozyma* is a strain of yeast (Miller et al., 1976) discovered to be strikingly different from other pigmented yeasts, specifically regarding the production of the carotenoid pigment, astaxanthin (AST; 3,3′-dihydroxy-β,β′-carotene-4,4′-dione; Andrewes et al., 1976). Astaxanthin is one of a group of natural pigments, known as xanthophyll carotenoids, and exhibits a wide variety of biological activities, including antioxidative, anti-*Helicobacter pylori*, anticancer, and antiinflammatory effects in mammals (Jyonouchi et al., 1996; Hussein et al., 2006; Pashkow et al., 2008). Animals cannot synthesize carotenoids; hence, they must obtain these pigments from algae and plants. Before *Haematococcus* algae meal became commercially available, natural sources of AST included krill oil and meal, crawfish oil, and *Phaffia* yeast. However, these sources have low AST concentrations ranging from 0.15% in the oils to 0.40% in *Phaffia* yeast. As a result, the quantities required in the feeds for efficient pigmentation add deleterious bulk and ash to the final feeds. By contrast, *Haematococcus* contains between 1.5 and 3.0% astaxanthin and has gained acceptance in aquaculture and other markets as a concentrated form of natural AST (Lorenz and Cysewski, 2000). Astaxanthin has been extensively used in the aquaculture feed industry for its pigmentation characteristics, but has yet to be approved for use in feed for food animals.

There have been a few previous studies performed to evaluate feeding AST to animals (Lei and Kim, 2014). However, the primary source of the AST originated from *Haematococcus* algae for most studies. Inborr and Lignell (1997) and Inborr (1998) suggested that broilers fed on an algae meal diet containing 133 or 266 mg of *Haematococcus* algae meal/kg of feed for 35 d gained weight quicker and had significantly greater breast muscle weight and higher feed efficiency. However, Inborr and Waldenstedt (2000) reported that experimental diets supplied with 5 and 25 mg of *Haematococcus* algae AST/kg presented higher feed efficiency, not growth performance. In a study performed by An et al. (2004), feeding 22.5 mg of synthetic or *Xanthophyllomycetes dendrorhous* yeast derived AST/kg of feed to broilers for 4 wk delayed peroxidation during storage.

**ABSTRACT** A prospective alternative to antibiotics currently being evaluated is yeast and its derivative products. *Phaffia rhodozyma* is a species of yeast that produces the carotenoid pigment, astaxanthin (AST), which exhibits a wide variety of biological activities, including antioxidation in animals. A total of 432 one-day-old male broilers (Arbor Acres) were used in a 4-wk feeding experiment and each dietary treatment consisted of 9 replicate cages, with 16 broilers per replicate. Birds were randomly allotted to 1 of 3 corn-soybean meal-based diets supplemented with 0 mg (CON, basal diet), 1,000 mg (CON + AST production 0.1%), or 2,000 mg (CON + AST production 0.2%) of *P. rhodozyma* yeast per kg of feed, giving an intake of approximately 0, 2.3, and 4.6 mg of AST/kg of feed, respectively. The inclusion of AST linearly improved weight gain in the finisher period (linear, \(P = 0.0264\)) and during the overall experimental period (linear, \(P = 0.0194\)) and linearly decreased feed conversion ratio in the finisher period (linear, \(P = 0.0422\)) and tended to decrease during the overall experimental period (linear, \(P = 0.0568\)). No significant effects were observed with red blood cell, white blood cell, and lymphocyte numbers in response to 2.3 or 4.6 mg of AST/kg of feed (\(P > 0.05\)). The ammonia emission from samples treated with 2.3 and 4.6 mg of AST/kg was significantly lower than that of CON (linear, \(P = 0.0110\)). Taken together, these results indicate that supplementation with AST could improve BW gain and decrease feed conversion ratio and fecal noxious gas emission of ammonia in broilers.
Accumulation of AST in animal tissues has been shown after feeding to rats (Ranga Rao et al., 2010; Stewart et al., 2008) and increases linearly with increasing levels of algal meal inclusion (Inborr and Lignell, 1997; Waldenstedt et al., 2003). In fact, AST can deposit in tissues such as the crest, egg yolk, and in various tissues in the domestic chicken (Fletcher, 1989; Lorenz and Cysewski, 2000). The AST shows high antioxidant activity compared with other carotenoids (Lawlor and O’Brien, 1995) and has also been reported to stimulate the immune system (Jyonouchi et al., 1996). Lastly, AST has been reported to reduce Helicobacter pylori (Wang et al., 2000), Campylobacter spp. (Blaser et al., 1993), and Clostridium perfringens (Waldenstedt et al., 2003) numbers, and therefore appears capable of modulating intestinal bacterial growth of broilers, which would indirectly alter noxious gas emission.

Therefore, to accurately establish the value of AST produced by P. rhodozyma as an alternative feed additive for enhancing broiler production, we conducted experiments to evaluate its effect on growth performance, blood profile, meat quality, and noxious gas emission in broilers to compare with known findings from previous studies using AST was originating from Haematococcus algae.

**MATERIALS AND METHODS**

The experimental protocols used in this study were approved by the Animal Care and Use Committee of Dankook University (Anseodong, Cheonan, Choongnam, Korea).

**Yeast Strain and AST Production**

For the production of biological AST, Phaffia rhodozyma strain 67–210 (natural isolate) was used in this study. Natural isolates of P. rhodozyma were kindly provided by the Sun Bio Company (Cheonan, Korea). Yeasts were maintained on slants of liquid medium (composed of 20 g/L of glucose, 20 g/L of corn steep liquor, 0.5 g/L of MgSO4, and 0.2 g/L of MnCl2). In fermenter liquid batch culture, growth of P. rhodozyma began after a 2-d lag (6.2 × 104 cfu/mL) and a constant growth of yeast was reached after about 4 d (1.3 × 107 cfu/mL) at 27°C. For high cell mass cultivation, steep liquor with 40% moisture. In fermenter solid state fermentation, growth of P. rhodozyma began after a 1-d lag (1.1 × 104 cfu/mL) and a constant growth of yeast was reached after about 5 d (1.4 × 108 cfu/mL) at 25°C with 30 min/d aeration to give a steady concentration of dissolved oxygen.

The AST production by P. rhodozyma was fermented in a jar and then freeze-dried with the concentration of AST being 2,305 mg/kg of medium, which was suggested by Lorenz and Cysewski (2000). The AST production was analyzed by HPLC (Agilent 1100 series, Palo Alto, CA) and detected by a UV-visible detector at 474 nm. Chromatographic separation was achieved by Nova-Pak C18 column (4 μm, 4.6 μm × 150 mm) equipped with a C-18 guard column (4 μm, 4.6 μm × 20 mm) from Waters (Taunton, MA), at 20°C using isocratic elution. The mobile phase consisted of methanol/acetonitrile (75:25, vol/vol). The flow rate was 1.0 mL/min, whereas the injection volume was 10 μL. For quantification, AST (98%; Sigma catalog A-9335, St. Louis, MO) was used as standard.

**Experimental Design, Birds, Housing, and Diets**

A total of 432 conventionally healthy 1-d-old male Arbor Acres broilers (BW of 47.94 ± 0.10 g) was fed in a 4-wk trial. Birds were randomly allotted to 1 of 3 treatment groups in a completely random block design. Phaffia rhodozyma was administered by replacing the same amount of corn in the treatment diets. Each dietary treatment consisted of 9 replicate cages, with 16 broilers per replicate. Dietary treatments included: I) control (CON, basal diet), II) treatment 1 (CON + 1,000 mg of AST production/kg of feed), and III) treatment 2 (CON + 2,000 mg of AST production/kg of feed), giving an intake of approximately 0, 2.3, and 4.6 mg of AST/kg of feed, respectively. The basal diet was formulated to meet all the nutrient requirements of broilers (NRC, 1994), and supplied in mash form during starter (wk 0 to 2) and finisher (wk 3 to 4) phases. Ingredients and calculated nutrient composition of the basal diet are shown in Table 1. All diets were fed with feed and water provided ad libitum throughout the experimental period. All the chicks were kept in a battery brooder with 3 levels of stainless-steel cages (124 cm width × 64 cm length × 40 cm height) with 8 adjacent cages per level. Each cage was equipped with 2 drinker nipples and 2 open trough feeders. The temperature of the battery brooder was maintained at 33 ± 1°C during the 1st week and decreased by 3°C per week until reaching 24°C. Artificial light was provided 24L:0D with fluorescent lights.

**Sampling and Measurements**

All feed samples were ground to pass through a 1-mm screen, after which they were analyzed for N (method 920.40; AOAC, 2000), Ca (method 984.01; AOAC In-
Table 1. Ingredient and composition of the basal diet (as-fed basis)1

<table>
<thead>
<tr>
<th>Ingredient (%)</th>
<th>Starter (0–2 wk)</th>
<th>Finisher (3–4 wk)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn</td>
<td>55.42</td>
<td>62.98</td>
</tr>
<tr>
<td>Soybean meal (CP 48%)</td>
<td>28.25</td>
<td>24.61</td>
</tr>
<tr>
<td>Corn gluten meal (CP 60%)</td>
<td>6.50</td>
<td>3.50</td>
</tr>
<tr>
<td>Soybean oil</td>
<td>5.50</td>
<td>4.89</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
<td>2.46</td>
<td>2.29</td>
</tr>
<tr>
<td>Limestone</td>
<td>0.89</td>
<td>0.75</td>
</tr>
<tr>
<td>Salt</td>
<td>0.20</td>
<td>0.20</td>
</tr>
<tr>
<td>DL-Methionine (98%)</td>
<td>0.17</td>
<td>0.17</td>
</tr>
<tr>
<td>L-Lysine-HCl (78%)</td>
<td>0.21</td>
<td>0.21</td>
</tr>
<tr>
<td>Vitamin premix2</td>
<td>0.20</td>
<td>0.20</td>
</tr>
<tr>
<td>Trace mineral premix3</td>
<td>0.20</td>
<td>0.20</td>
</tr>
<tr>
<td>Total</td>
<td>100.00</td>
<td>100.00</td>
</tr>
</tbody>
</table>

Nutrient composition

- ME (kcal/kg): 3,140 vs. 3,200
- CP (%): 22.00 vs. 20.09
- Lys (%): 1.10 vs. 1.05
- Met (%): 0.54 vs. 0.41
- Met + Cys (%): 0.93 vs. 0.93
- Ca (%): 1.00 vs. 0.87
- Total P (%): 0.80 vs. 0.75
- Crude fat (%): 4.32 vs. 5.87
- Crude fiber (%): 4.71 vs. 6.21

1 The experimental diets were formulated by replacing soybean meal with 0% (control), 0.1%, and 0.2% astaxanthin (Sumbio Co., Soongnam-City, Korea).

2 Provided per kilogram of complete diet: 15,000 IU of vitamin A, 3,750 IU of vitamin D3, 37.5 mg of vitamin E, 2.25 mg of vitamin K3, 3 mg of thiamine, 7.5 mg of riboflavin, 4.5 mg of vitamin B6, 24 μg of vitamin B12, 51 mg of niacin, 1.5 mg of folic acid, 0.2 mg of biotin, and 13.5 mg of Ca-pantothenate.

3 Provided per kilogram of complete diet: 37.5 mg of Zn (as ZnSO4); 37.5 mg of Mn (as MnO2); 37.5 mg of Fe (as FeSO4·7H2O); 3.75 mg of Cu (as CuSO4·5H2O); 0.83 mg of I (as KI); and 0.23 mg of Se (as Na2SeO3·5H2O).

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The effect of dietary AST on the emission of ammonia, total mercaptan, hydrogen sulfide, and acetic acid from fecal samples was determined and analyzed. Prior to measurement, the fecal samples were shaken manually for approximately 30 s to mix thoroughly and disrupt any surface crust formation. The adhesiveness of the pressed sample and the expressed moisture were delineated and then determined with a digitzing area line sensor (MT-10S, M.T. Precision Co. Ltd., Tokyo, Japan). The ratio of water:meat area was then calculated, giving a measure of WHC (a smaller ratio indicates increased WHC). Drip loss was measured with approximately 2 g of heat sample according to the plastic bag method described by Honikel (1998). The 2-TBA reactive substances (TBARS) were measured by the method described by Witte et al. (1970). The TBARS values were expressed in terms of milligrams of malondialdehyde per kilogram of muscle. Trichloroacetic acid solution (20% wt/vol) was used for extraction. The chromium concentration was determined by spectrophotometry (UV-1201, Shimadzu, Kyoto, Japan).

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Statistics

All data were analyzed using MIXED procedures of SAS (2003, SAS Institute Inc., Cary, NC) with the following statistical model of $Y_{ijk} = \mu + t_i + r_k + e_{ijk}$, where $Y_{ijk}$ was an observation on the dependent variable $ij$, $\mu$ was the overall population mean, $t_i$ was the
fixed effect of AST treatments, \( r_k \) was the pen as a random effect, and \( e_{ijk} \) was the random error associated with the observation \( ijk \). Significant difference level of 0.05 was used to determine statistical significance, and a level of 0.10 was considered a trend. In addition, orthogonal comparison were conducted using polynomial regression to measure the linear and quadratic effects of increasing dietary concentrations of supplemental AST production by \( P. rhodozyma \).

**RESULTS**

**Growth Performance**

The inclusion of AST improved BWG in the finisher period (linear, \( P = 0.0264 \)) and during the overall experimental treatment period (linear, \( P = 0.0194 \)), but not in the starter period (\( P > 0.05; \) Table 2). However, AST treatment had no effect on FI as compared with CON. Consequently, FCR in the finisher period decreased significantly (linear, \( P = 0.0422 \)) and tended to decrease during the overall experimental period as compared with CON (linear, \( P = 0.0568 \)).

**Blood Profiles**

The AST supplementation did not affect RBC, WBC, lymphocyte percentage, or IgG concentration (\( P > 0.05; \) data not shown).

**Meat Quality and Relative Organ Weight**

Broilers fed the AST supplemental diet did not differ in breast muscle color (\( L^*, a^*, \) and \( b^* \)) or pH value as compared with those fed CON (\( P > 0.05; \) Table 3). The TBARS and WHC values were not different across AST treatments (\( P > 0.05 \)). Moreover, the inclusion of AST treatments decreased the relative spleen weight (linear, \( P = 0.0137 \)) and tended to increase abdominal fat weight (linear, \( P = 0.0517 \)) in relation to the BW as compared with CON. No differences were observed in other relative organ weights among treatments (\( P > 0.05 \)).

**Fecal Noxious Gas Emission**

The effect of dietary AST on the emission of ammonia, total mercaptan, hydrogen sulfide, and acetic acid is shown in Table 4. The ammonia emission from samples obtained from broilers fed AST was significantly lower than that of CON (linear, \( P = 0.0110 \)). No significant differences were observed in the total mercaptan, hydrogen sulfide, and acetic acid of samples obtained from broilers throughout the experimental period (\( P > 0.05 \)).

**DISCUSSION**

Previous studies have suggested that diet supplementation with AST improves growth performance and feed efficiency (Inborr and Lignell, 1997; Inborr, 1998); however, Akiba et al. (2001) and Waldenstedt et al. (2003) reported conflicting results. In the current study, AST supplementation at 2.3 and 4.6 mg of AST/kg in feed, linearly improved BWG and FCR over the finisher and overall periods, respectively. It is possible that AST might maintain and promote the growth of a beneficial microbial population in fast-growing broilers because an active microflora may have a decreased energy requirement for maintenance and an increased efficiency of nutrient utilization as compared with CON (Yang et al., 2009). This is in partial agreement with Chesson (1994), who suggested that several factors including carotenoid/AST utilization, the metabolism between hybrids, the age of the broilers, the dose of AST, diet composition, feed form, and interaction with other dietary feed additives can be attributed to these various results, indicating that AST may not be the main factor behind increased growth performance. Furthermore, the quantity and rapidity of free-form AST being released from the conjugated form in feed may affect the amount digested and absorbed. This could

**Table 2. Effect of astaxanthin (AST) supplementation on growth performance in broilers**

<table>
<thead>
<tr>
<th>Item 1</th>
<th>0</th>
<th>2.3</th>
<th>4.6</th>
<th>SEM</th>
<th>Linear</th>
<th>Quadratic</th>
</tr>
</thead>
<tbody>
<tr>
<td>BWG (g)</td>
<td>408</td>
<td>412</td>
<td>414</td>
<td>7</td>
<td>0.5386</td>
<td>0.9410</td>
</tr>
<tr>
<td>FI (g)</td>
<td>587</td>
<td>592</td>
<td>596</td>
<td>5</td>
<td>0.2382</td>
<td>0.9227</td>
</tr>
<tr>
<td>FCR</td>
<td>1.439</td>
<td>1.437</td>
<td>1.440</td>
<td>0.019</td>
<td>0.9015</td>
<td>0.9558</td>
</tr>
<tr>
<td>BWG (g)</td>
<td>969</td>
<td>989</td>
<td>1,024</td>
<td>16</td>
<td>0.0264</td>
<td>0.7183</td>
</tr>
<tr>
<td>FI (g)</td>
<td>1,599</td>
<td>1,585</td>
<td>1,599</td>
<td>16</td>
<td>0.9767</td>
<td>0.4924</td>
</tr>
<tr>
<td>FCR</td>
<td>1.650</td>
<td>1.603</td>
<td>1.562</td>
<td>0.028</td>
<td>0.0422</td>
<td>0.9898</td>
</tr>
<tr>
<td>BWG (g)</td>
<td>1,377</td>
<td>1,401</td>
<td>1,439</td>
<td>17</td>
<td>0.0194</td>
<td>0.7407</td>
</tr>
<tr>
<td>FI (g)</td>
<td>2,186</td>
<td>2,177</td>
<td>2,194</td>
<td>16</td>
<td>0.7228</td>
<td>0.5122</td>
</tr>
<tr>
<td>FCR</td>
<td>1.588</td>
<td>1.554</td>
<td>1.525</td>
<td>0.021</td>
<td>0.0568</td>
<td>1.0000</td>
</tr>
</tbody>
</table>

1BWG, BW gain; FI, feed intake; FCR, feed conversion ratio.
20, only basal diet; 2.3, basal diet with 0.1% AST; and 4.6, basal diet with 0.2% AST (Sumbio Co., Seongnam-City, Korea).
be affected by the presence of polar ends of AST; thus, additional studies are needed to clarify this response.

With regards to meat quality, water loss reduces the nutritional value of meat because some nutrients may be lost in the leachate, resulting in tougher and flavorless meat (Hamm, 1985), which was not the case observed in this study because WHC did not differ between AST and CON treatments. The WHC is influenced by muscle structure, activity of enzymes, and by properties of the muscle proteins, which in turn is affected by pH, in the range of 5.0 to 6.5 of the muscle, and is used to assess the eating quality of the food product (Hamm, 1985; Fennema, 1990). The meat color and pH of breast muscle was unaffected by AST treatments in the current study. Generally, meat color is closely associated with the meat pH (Fletcher et al., 2000). Therefore, the lack of any response to meat color changes might be explained by the same pH value among treatments.

There was no difference ($P > 0.05$) across treatments for TBARS, although both AST treatments resulted in lower values, suggesting AST has some effect on oxidation. Overall, the results were consistent with antioxidant activity of AST, which is important to protect membranous phospholipids and other lipids against peroxidation (Palozza and Krinsky, 1992). This may be attributed to the distinctive structure of AST, due to the presence of hydroxyl and keto moieties on both ends, allowing it to align into the phospholipid bilayer of cell membranes. Perhaps at higher concentrations, the antioxidant effect of AST might be more apparent and lead to statistical significance in TBARS.

Airborne pollutants in livestock production can increase the susceptibility to common and important respiratory diseases, and have to be considered in terms of environmental risk assessments. Ammonia is considered the most harmful gas to livestock, and can reduce daily weight gains and feed utilization (Kalich, 1980; Carlile, 1984). In the current study, AST treatments linearly decreased fecal ammonia. One possible explanation for this lies with the possibility of AST being capable of altering intestinal microfloral populations. In a recent study, Yonei et al. (2013) examined the effects of AST on intestinal microflora in mice fed a normal and high-fat diet and determined that administration of AST had suppressive effects on enteric flora derangement induced by imposing a high-fat diet on mice. In particular, administration of AST was able to suppress the rapid growth of gram-negative bacteria while increasing the count of Lactobacillus and Streptococcus groups, which belong to the group of lactic acid bacteria. Chiang and Hsieh (1995) have reported that probiotic bacteria supplementation in feed can reduce...
the concentration of ammonia in the excreta and improve BWG and FCR of broilers. Coincidentally, many probiotic bacteria are derived from lactic acid bacteria, especially from the Lactobacillus group. Therefore, we speculate that if AST can induce an improvement in the intestinal microbial flora balance, a resulting subsequent shift in the type and amount of fermentation acids produced by the microflora might occur (Yang et al., 2009) with increased enzyme activities (Balcazar et al., 2006; Lara-Flores et al., 2010), which in turn, might lead to better absorption quality, and more degradation of higher molecular weight protein to lower molecular weight peptides and amino acids (De Schrijver and Olleveir, 2000). Moreover, the stimulating growth by probiotics, containing lactic acid bacteria strains, has been associated with improved FCR and protein efficiency ratio attributed to an increase in lactic acid and cellulolytic and amylolytic enzyme production (Kesarcodi-Watson et al., 2008). All these factors may contribute toward optimizing the digestion and use of protein for growth, possibly resulting in more efficient protein utilization and less excreta nitrogen content in broilers.

Lastly, because airborne pollutants in livestock can increase the susceptibility to common and important respiratory diseases, it is important for the immune system to be fully functional. Astaxanthin has been purported to have a positive immunomodulatory property. Takimoto et al. (2007) suggested that dietary supplementation of AST from *P. rhodozyma* in 1-wk-old chicks for 14 d can increase lymphocyte proliferation and IgG production as a part of acquired immunity. In addition, AST has been reported to stimulate splenocyte function in mice (Chew et al., 1999) and modulate cytokine release by splenocytes in humans (Bennedsen et al., 1999). However, in this study, AST had no effect on WBC, RBC, lymphocyte, and IgG amount, possibly indicating that low AST concentrations, such as 2.3 and 4.6 mg/kg of diet, may be insufficient to stimulate the immune system as compared with a higher concentration of 100 mg/kg of diet used (Kurihara et al., 2002; Park et al., 2013). Interestingly, AST decreased the relative weight of the spleen to BW as compared with CON, which is in agreement with a previous study by Pardue et al. (1985). Further studies are needed to clarify the effect of low AST concentrations on spleen atrophy.

The findings of this study demonstrate that feed supplementation of finisher broilers with AST (2.3 and 4.6 mg/kg of feed) has a positive effect on BWG and FCR in the finisher period and the overall experimental period. Moreover, there was an effective reduction in the fecal ammonia in response to treatment with AST.

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