Effects of Dietary Supplementation with *Aspergillus Awamori* on Growth Performance and Antioxidative Status of Broiler Chickens Exposed to High Ambient Temperature

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This study was conducted to evaluate the effects of dietary supplementation with *Aspergillus awamori* on the growth performance and antioxidative status in male broiler chickens exposed to high ambient temperatures. Twenty-four 15-d-old male broiler chickens were randomly divided into 2 dietary groups fed a corn-soybean meal-based diet supplemented without or with 0.05% of *Asp. awamori*. Six birds of each dietary group were kept under a thermoneutral condition (22°C), and 6 birds were exposed to heat stress (35°C, 9 h/d) for 15 d. Dietary *Asp. awamori* decreased feed intake and improved the feed conversion ratio in chickens kept at 22°C. Body weight gain and feed intake were decreased in chickens exposed to heat stress. There was no beneficial effect of dietary *Asp. awamori* on growth performance under the heat stress condition. However, the malondialdehyde content in skeletal muscle, as an index of lipid peroxidation, was decreased by dietary *Asp. awamori* in chickens kept at 22°C. Although the malondialdehyde content increased under the heat stress condition, dietary *Asp. awamori* alleviated the increased malondialdehyde. In addition, the α-tocopherol content in skeletal muscle was negatively affected by the exposure to heat stress, and dietary *Asp. awamori* recovered the reduction in the α-tocopherol content. Moreover, mRNA expression of glutathione peroxidase in liver was increased by dietary *Asp. awamori* under both of the thermal conditions. In conclusion, this study shows that dietary *Asp. awamori* improves the diminished antioxidative status under the heat stress condition partially because of its effect on the expression of hepatic antioxidant enzymes in broilers.

**Key words:** antioxidant enzymes, *Aspergillus awamori*, broiler chicken, heat stress, malondialdehyde


**Introduction**

High temperature beyond the thermoneutral zone causes environmental heat stress (HS). Chickens are more vulnerable to HS compared with other domestic animals because of a lack of sweat glands and their higher body temperature (Ensminger *et al.*, 1990; Sahin *et al.*, 2009). Therefore, HS is a major stressor and causes a wide range of physiological alterations in chickens (Whitehead and Keller, 2003). As the heat load increases, the rise in body temperature results in tissue damage by HS-induced production of reactive oxygen species (ROS) (Khan *et al.*, 2012).

Reactive oxygen species, which are produced in mitochondria under physiological conditions, are essential for the body to function (Khan *et al.*, 2012); i.e., defense against infectious agents, functioning of cellular signaling systems, and induction of the mitogenic response (Valko *et al.*, 2007). However, ROS are neutralized by the antioxidant system in the physiological state because a higher level of ROS production is potentially harmful to the maintenance of homeostasis (Surai, 2002). Intracellularly, ROS are removed by antioxidant enzymes. The most important antioxidant enzymes, which act as catalysts in many important reactions related to antioxidant defense, are glutathione peroxidase (GPX), catalase (CAT), and superoxide dismutase (SOD). These enzymes are localized in the cytosol or mitochondria (Ercal *et al.*, 2001; Pinto *et al.*, 2003).

Several methods have been established to alleviate HS and
improve poultry performance under heat conditions. Environmental strategies include keeping birds in open-sided cages, increasing ventilation, establishing an intermittent light schedule, instituting an early heating condition, implementing early feed restriction, and lowering stocking density (Ensminger et al., 1990; Sahin et al., 2009). Genetic strategies include selecting heat-tolerant lines and using major genes (e.g., naked neck gene, frizzle gene, and dwarf gene) (Lin et al., 2006). However, because of high costs and impracticality, some of the aforementioned recommendations cannot be satisfactorily applied. Alternatively, nutritional manipulation has been a common approach in poultry production (Sahin et al., 2009); i.e., the use of other nutrients such as probiotics, trace elements, and vitamins has also been proven beneficial in alleviating the adverse effects of HS (Eid et al., 2003, 2008; Lin et al., 2006; Sahin et al., 2009). Kaminishi et al. (1999) reported that several strains of Aspergillus produce antioxidative substances. Yokoyama et al. (2001) described detailed identification, classification and phylogeny of the Aspergillus family. Aspergillus awamori, a variant of Aspergillus niger, which is called “koji” in Japan and has been used for the processing of shoucho (a major distilled liquor in Japan). The products containing Asp. niger have been given GRAS (Generally Recognized As Safe) status from the Food Drug Administration (Bigelis and Lasure, 1987). Recently, dietary administration of Asp. awamori was reported to improve growth performance in broiler chickens (Saleh et al., 2011). Saleh et al. (2011, 2012) reported that decreased lipid peroxidation in skeletal muscle was observed in broiler chickens fed a diet containing 0.05% of Asp. awamori for 12 d. These results raised the possibility that Asp. awamori may be used as an antioxidant in poultry production. However, because of a lack of information on the role of Asp. awamori in high ambient temperatures, the present study was undertaken to examine the effects of feeding Asp. awamori on growth performance, oxidative stress, and the antioxidant status of broiler chickens under an HS condition.

Materials and Methods

Animals and Experimental Design

The animal experiment was conducted in accordance with the guidelines of Kagoshima University, Japan. One hundred 1-d-old male broiler chicks (Chunky strain Ross 308) were supplied by a commercial hatchery (Kagoshima Chicken Food Company, Ltd., Kagoshima, Japan). The chicks were housed in an electrically heated battery brooder and provided with water and a commercial starter diet (22% crude protein and 3.0 Mcal/kg; Nichiwa Sangyou Company, Hyogo, Japan) until 12 d of age. On d 12, 24 chicks with similar body weights were selected from the 100 chicks and housed individually in wire-bottomed aluminum cages (50×20×60 cm). The chicks were preconditioned for 3 d before the treatment and were fed a basal diet. The experimental diets were formulated using mainly ground yellow corn and a soybean meal, as shown in Table 1. Aspergillus awamori (Biogenkoji Research Institute, Kirishima, Japan) was incorporated into the diet at a concentration of 0.5 g/kg (0.05%) of diet. The chicks were divided into 2 dietary treatment groups (n=12/group): the basal diet supplemented without and with Asp. awamori. Six birds of each dietary group were kept under a thermoneutral condition (22°C±1°C), and 6 birds were exposed to an HS condition (35°C±1°C). The chicks were fed the experimental diets from 15 to 30 d of age. The birds assigned to the HS group were subjected to acute HS every day in temperature-controlled rooms. Heating was started at 9:00, and within 1 hour the room temperature reached at 35°C, and it was kept at 35±1°C by 18:00. Then immediately the room temperature was cooled down to 22°C.

The control birds were kept at moderate ambient temperatures (22°C±1°C) with a relative humidity of 50% to 70% throughout the experiment. Body weight was recorded every 6 d and feed intake (FI) was recorded daily during the experimental period. At the end of the experimental period, the chickens were slaughtered and dissected to measure the weight of the breast muscle (pectoral superficial muscle), abdominal fat and liver. Muscle and liver tissue samples were frozen in liquid nitrogen then stored at −80°C for subsequent gene expression analysis. Blood samples were collected into heparinized test tubes, quickly centrifuged at 5,900×g for 10 min at 4°C to separate the plasma, and stored at −30°C until analysis.

Determination of Skeletal Muscle and Liver Malondialdehyde Content

Malondialdehyde (MDA) is one of the most frequently used indicators of lipid peroxidation. To evaluate lipid

Table 1. Composition of the basal diet

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Content, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn</td>
<td>55.10</td>
</tr>
<tr>
<td>Alfalfa meal</td>
<td>2.90</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>33.50</td>
</tr>
<tr>
<td>Corn oil</td>
<td>4.70</td>
</tr>
<tr>
<td>DL-Methionine</td>
<td>0.14</td>
</tr>
<tr>
<td>CaHPO4</td>
<td>2.00</td>
</tr>
<tr>
<td>CaCO3</td>
<td>0.66</td>
</tr>
<tr>
<td>NaCl</td>
<td>0.50</td>
</tr>
<tr>
<td>Mineral and vitamin mixture</td>
<td>0.50</td>
</tr>
<tr>
<td>Nutrient composition</td>
<td>0.21</td>
</tr>
</tbody>
</table>

1 Provided per kilogram of diet: 1,500 IU of vitamin A (all-trans retinol); 200 IU of vitamin D3; 9.1 IU of vitamin E (α-tocopherol acetate); 1.1 mg of vitamin K3 (menadione); 1.8 mg of thiamin; 3.6 mg of riboflavin; 11 mg of calcium D-pantothenate; 35 mg of nicotinic acid; 3.5 mg of pyridoxine; 0.15 mg of biotin; 0.55 mg of folic acid; 0.01 mg of cyanocobalamine; 1,498 mg of choline chloride; 600 mg of magnesium oxide (Mg); 60 mg of manganese sulfate (Mn); 40 mg of zinc sulfate (Zn); 80 mg of iron sulfate (Fe); 8 mg of copper sulfate (Cu); 0.35 mg of calcium iodate (I).

2 Calculated values.
peroxidation levels in the liver and skeletal muscle of chickens, the MDA content was determined colorimetrically as a 2-thiobarbituric acid-reactive substance according to the method described by Azad et al. (2010). In brief, liver and skeletal muscle were homogenized in 5 volumes of 154 mM KCl. Forty microliters of the homogenate was mixed with 40 μL of 8.1% SDS, 300 μL of 20% acetic acid (pH 3.5), and 300 μL of 0.8% 2-thiobarbituric acid. After vortexing, the samples were incubated at 95°C for 1 h and then cooled on ice. The samples were mixed by vortexing and centrifuged at 1,200 × g for 10 min after adding 1 mL of butanol:pyridine 15:1 (v:v). Absorbance of the supernatant, which comprised the butanol:pyridine layer, was measured at 532 nm. The content of 2-thiobarbituric acid-reactive substance was expressed as the MDA equivalent.

RNA Extraction and Real-Time PCR

Total RNA was extracted from a piece of liver or muscle (about 50 mg) using an RNA extraction reagent kit (ISOGEN II; Nippon Gene, Tokyo, Japan) according to the manufacturer’s protocol. The RNA concentration and purity were determined by a spectrophotometer (NanoDrop Lite S17 NNP0027; Thermo Scientific, Hudson, NH, USA). Complementary DNA was synthesized at 400 ng of RNA per 10 μL of the reaction solution with the PrimeScript RT Master Mix Kit (RR036A, Takara, Shiga, Japan) using the Program Temp Control System PC-320 (Astec, Fukuoka, Japan) with the following protocol: reverse transcription at 37°C for 15 min, inactivation of RT at 85°C for 5 s, and refrigeration at 4°C for 5 min. The primers used in this study are listed in Table 2. Gene expression was measured by real-time PCR using the 7300 Real-Time PCR system (Applied Biosystems, Foster City, CA, USA) with the SYBR Select Master Mix (4472918; Applied Biosystems). The thermal cycle was as follows: 1 cycle at 50°C for 2 min and 95°C for 2 min; and 60 cycles at 95°C for 15 s, 60°C for 15 s, and 72°C for 1 min. The expression of 18S ribosomal RNA was used as an internal standard and was not significantly different among the 4 groups. Results of gene expression are given as the percentage of the value of the control groups kept under the thermoneutral condition.

Determination of α-Tocopherol Content

The α-tocopherol concentration in the muscle and plasma were determined by the LC-2000Plus HPLC system (Jasco Co., Tokyo, Japan) with an Inertsil ODS-3 column (4.6 × 250 mm; GL Sciences, Inc., Tokyo, Japan) according to the method described by Faustman et al. (1989).

Statistical Analysis

The significance of differences was evaluated by ANOVA and Tukey’s multiple-range test. All statistical analyses were performed with the general linear model procedures of SAS (version 9.2, SAS Institute). A P-value of < 0.05 was considered statistically significant.

Results

Growth Performance

Growth performance and the relative weights of breast muscle, abdominal fat and liver are presented in Table 3. Under the thermoneutral condition, dietary Asp. awamori significantly decreased FI and improved the feed conversion ratio (FCR) (P < 0.05). Body weight gain and the relative weights of tissues were not different between the 2 dietary groups except for relative liver weight in which Asp. awamori significantly (P = 0.004, ANOVA) decreased relative liver weight under the two environmental condition. Conversely, BW gain and FI were significantly (P < 0.05) decreased in chickens exposed to the HS condition compared with chickens fed the diet without Asp. awamori under the thermoneutral condition. The resulting FCR was significantly (P < 0.05) increased in chickens exposed to the HS condition compared with those of chickens kept under the thermoneutral condition, the same trend was observed with relative liver weight.

Tissue MDA Content

Figure 1 shows the MDA content in the skeletal muscle (A) and liver (B) of broiler chickens. Under the thermoneutral condition, the MDA content in skeletal muscle was significantly (P < 0.05) decreased in chickens fed the diet with Asp. awamori compared with chickens fed the diet without Asp. awamori. Conversely, the MDA content was increased in chickens exposed to the HS condition compared

### Table 2. List of primer sequences used for qualitative real-time PCR

<table>
<thead>
<tr>
<th>Gene</th>
<th>Sequence</th>
<th>Position (5’ to 3’)</th>
<th>Product length (bp)</th>
<th>Accession no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>GPX</td>
<td>F: 5’-TTGTAACATCCAGGCAAAA-3’&lt;br&gt;R: 5’-TTGGCCTCTTCAGGAG-3’</td>
<td>165 to 304</td>
<td>140</td>
<td>NM_001163245.1</td>
</tr>
<tr>
<td>SOD</td>
<td>F: 5’-AGGGGTCTACCTACCC-3’&lt;br&gt;R: 5’-CCCATTTGTTTGTCCTCCAA-3’</td>
<td>50 to 171</td>
<td>122</td>
<td>NM_205064.1</td>
</tr>
<tr>
<td>CAT</td>
<td>F: 5’-GGGAGGTTTACTGCAAG-3’&lt;br&gt;R: 5’-CTTTGATGTGTTCTCAG-3’</td>
<td>819 to 956</td>
<td>138</td>
<td>AJ719360.1</td>
</tr>
<tr>
<td>18S</td>
<td>F: 5’-AAAGGCTACACATCCAG-3’&lt;br&gt;R: 5’-CCTCAATGGATCGGCTCT-3’</td>
<td>333 to 486</td>
<td>154</td>
<td>KC433410.1</td>
</tr>
</tbody>
</table>

1 Glutathione peroxidase (GPX), Cu/Zn-superoxide dismutase (SOD), and catalase (CAT).
2 F = forward; R = reverse.
with chickens kept at 22°C. There was a significant ($P=0.001$, ANOVA) reducing effect of Asp. awamori and a significant ($P=0.001$, ANOVA) increasing effect of heat stress on the MDA content in the skeletal muscle. A similar tendency was seen in the MDA content in the liver among these groups, and the effect of heat stress was significant ($P=0.046$, ANOVA).

**Gene Expression of Antioxidant Enzymes in Liver and Muscle**

Figure 2 shows the mRNA expression of antioxidant enzymes [GPX (A), SOD (B), and CAT (C)] in liver. Under the thermoneutral condition, chickens fed the diet with Asp. awamori showed a 3-fold increase in GPX mRNA expression compared with the dietary control chickens. When chickens were exposed to the HS condition, the GPX mRNA expression tended to decrease; this decrease was recovered by dietary Asp. awamori. There was a significant ($P=0.001$, ANOVA) increasing effect of Asp. awamori and a significant ($P=0.001$, ANOVA) reducing effect of heat stress on the GPX mRNA expression. A similar tendency was seen in both the SOD and CAT mRNA expression in the liver between the thermoneutral and heat stress groups, but was not statistically significant. In addition to liver, effects of HS and Asp. awamori on GPX, SOD, and CAT mRNA expressions in muscle were examined; however, there were no changes in these expressions (data not shown). In the present study, a linear negative correlation ($R^2=0.201$, $P=0.028$) was found between liver GPX mRNA expression and liver MDA content (Figure 4A), and a linear negative correlation ($R^2=0.317$, $P=0.004$) was also found between the GPX expression and muscle MDA content (Figure 4B).

**Muscle and Plasma α-Tocopherol**

Figure 3 shows the α-tocopherol content in the plasma (A) and skeletal muscle (B) of broiler chickens. The plasma α-tocopherol concentration was increased by dietary Asp. awamori under both of the thermal conditions. There was a significant ($P=0.002$, ANOVA) increasing effect of dietary Asp. awamori on the plasma concentration. The skeletal muscle content of α-tocopherol was negatively affected by exposure to the HS condition, resulting in reduced α-tocopherol content. Dietary Asp. awamori recovered the reduction in the α-tocopherol content. The effect of Asp.
awamori on the α-tocopherol content was statistically significant ($P=0.050$, ANOVA).

**Discussion**

In the present study, both FI and the FCR of broiler chickens fed a diet containing 0.05% of Asp. awamori were significantly decreased under the thermoneutral condition. This is in agreement with the results of Saleh et al. (2011), who evaluated the influence of dietary Asp. awamori (0.05% and 0.2%) on FI and the FCR of broiler chicks. Although birds have no genetic ability to produce enzymes such as cellulase and xylanase, which are required for the digestion of soluble nonstarch polysaccharides, Aspergillus can produce these enzymes (Hajati, 2010). In addition, Aspergillus may help to digest and increase metabolizable energy for broiler chickens, resulting in an improved FCR (Mohan et al., 1996). This may be the reason for the improvement of FI and the FCR in broiler chickens fed the diet with Asp. awamori. In addition, similar effect of Asp. awamori on a decrease in food consumption was also found in rats (Saleh et al., 2013), indicating that the effect is independent on species. It is considered that butoxybuty alcohol, a growth promoter found in fermentation products processed by Asp. awamori (Kamizono et al., 2013), are also involved in the appetite regulation at the central nervous system (Kamisoyama et al., 2009).

Furthermore, HS has negative effects on BWG, FI, the FCR, and carcass traits (Smith and Teeter, 1987; Sahin et al., 2003). In the present study, these negative effects were confirmed in control broiler chickens exposed to the HS condition. The FCR and the abdominal fat weight were
improved by dietary *Asp. awamori* under the HS condition; however, the ameliorating effect in the HS condition seemed to be weaker than that in the thermoneutral condition.

In this study, a lower MDA content in skeletal muscle and liver was observed in broiler chickens fed the diet containing *Asp. awamori* compared with chickens fed the basal diet alone under the thermoneutral condition. This is in agreement with a previous study by Saleh *et al.* (2011) showing that the MDA content in skeletal muscle was lower in broiler chickens fed a diet containing 0.05% of *Asp. awamori* than in chickens fed a diet without *Asp. awamori*. Although the mechanism by which dietary *Asp. awamori* decreases the MDA content in the tissues of broiler chickens remains unclear, one possible explanation involves the antioxidant substance produced by *Asp. awamori*. It has been well documented that feeding an antioxidant-containing diet results in improvement of the oxidative status in broiler chickens. For example, Mujahid *et al.* (2009) reported that an olive oil-supplemented diet alleviated both mitochondrial ROS production and lipid peroxidation in the skeletal muscle of broiler chickens kept under a thermoneutral condition. In addition, Kaminishi *et al.* (1999) reported that *Aspergillus* has the ability to produce antioxidative substances. Lee *et al.* (2007) and Bhanja *et al.* (2009) refer the increase in the antioxidant activity of *Asp. awamori* to the increase of total phenolic and anthocyanin contents during the fermentation process, on the other hand Kanaiuchi *et al.* (2008) showed that a natural material called feruloyl esterase could contribute in enhancing the antioxidative proprieties of *Asp. awamori*. Therefore, it was suggested that feeding *Asp. awamori* might result in a decreased MDA content in the skeletal muscle and liver of broiler chickens because of its antioxidative substance-producing ability. Another possible explanation is the increased mRNA expression of genes encoding antioxidant enzymes such as GPX, SOD, and CAT observed in broiler chickens fed a diet with *Asp. awamori*. In this study, the expression of genes encoding antioxidant enzymes in the liver was decreased in broiler chickens during 15 d of heat exposure. High temperatures impair transcription, RNA processing, and translation involved in genes encoding antioxidant enzymes (Mager and De Kruijff, 1995; Iwagami, 1996). In rats, a positive relationship between antioxidant enzyme gene expression and antioxidant enzyme activity (Tiedge *et al.*, 1997) and a negative correlation between mRNA expression of antioxidant enzymes and liver MDA levels (El-Beshbishy *et al.*, 2012) have been shown. The negative linear correlation shown in Figure 4 A and 4B suggest that decreased gene expression encoding antioxidant enzymes may minimize the antioxidative defense ability in broiler chickens exposed to HS conditions. However, dietary *Asp. awamori* increased the expression of genes encoding antioxidant enzymes in the liver of broiler chickens and cancelled out the negative effects of HS on the expression of these genes under the HS condition. In the present study, mRNA expressions of antioxidant enzymes in muscle tissue were also measured; however, there were no effect of heat stress nor *Asp. awamori* on muscle GPX, SOD, and CAT mRNA expressions (data not shown). It can be therefore suggested that *Asp. awamori* might suppress oxidative damages in skeletal muscle under heat stress conditions, thereby leading to the decrease in the MDA levels without changes in the gene expressions of antioxidant enzymes in the muscle. Although the molecular mechanisms by which dietary *Asp. awamori* increased the mRNA expression of these genes are unclear, these data suggest that increased mRNA expression of hepatic antioxidant enzymes such as GPX might affect the decreased MDA content.

![Correlations between liver GPX mRNA expression and liver MDA content (A) and muscle MDA content (B).](image-url)

**Fig. 4.** Correlations between liver GPX mRNA expression and liver MDA content (A) and muscle MDA content (B) in broilers under both of the thermal conditions.
temperatures despite the fact that their metabolism and energy consumption are increased under these conditions (Gómez et al., 2002). The reduced FI may result in mobilization of lipids from stored fat. In terms of this increased lipid mobilization, a high degree of tissue damage arises from increased lipid peroxidation (Whittow, 2002; Khan et al., 2012), while fat deposition is enhanced as an adaptive regulation under heat conditions (Lu et al., 2007). In this study, a decrease in FI was observed in chickens fed Asp. awamori even under the thermoneutral condition, which is in agreement with the results of our previous study (Saleh et al., 2011). Although the reason why Asp. awamori did not alleviate oxidative stress in broiler chickens remains unclear, it is suggested that the decreased FI seen in the chickens fed Asp. awamori may have resulted in higher levels of lipid peroxidation, which could have cancelled out the effect of the higher antioxidant activity of Asp. awamori. Saleh et al. (2011) reported that FI did not change in broiler chickens fed a diet containing 0.2% of Asp. awamori compared with chickens fed a diet containing 0.05% of Asp. awamori. These results allow us to postulate that an increase in the additive amount might be more effective in overcoming the negative effect of HS on either growth performance or lipid peroxidation in broiler chickens.

Heat stress is also known to depress carcass weight and meat quality in poultry (Aksit et al., 2006). Heat stress-induced ROS production negatively affects the integrity of muscle cell membranes, causing an increase in drip loss during storage, while α-tocopherol preserves this negative effect of HS by inhibiting the passage of sarcoplasmic fluid and acting as a radical-quenching antioxidant (Asghar et al., 1991; Gray et al., 1996; Faustman et al., 1998). In this study, although HS increased the MDA content ($P<0.001$, ANOVA) and decreased the α-tocopherol content ($P=0.001$, ANOVA) in the skeletal muscle, both the MDA and the α-tocopherol in chickens fed the diet with Asp. awamori under the HS condition returned to the basal levels of control chickens kept under the thermoneutral condition. Dietary Asp. awamori significantly ($P=0.002$, ANOVA) increased the plasma concentration of α-tocopherol regardless of the ambient temperature. In addition, a significant linear negative correlation ($R^2=0.449$, $P<0.001$) was found between the MDA content and the α-tocopherol content in the skeletal muscle. It is indicated that the cellular consumption of α-tocopherol is reduced due to the antioxidative effect of Asp. awamori. As a result, it might be possible to keep the cellular concentration of α-tocopherol high in peripheral tissues by feeding Asp. awamori-containing diet.

Rowe et al. (2004a, b) reported an increase in proteolysis in steaks from α-tocopherol-fed animals, indicating that the use of antioxidants in meat could improve tenderness. These results suggest that the presence of Asp. awamori in the diets of broiler chickens might improve meat qualities such as drip loss or tenderness or both during storage. The improvement in the antioxidative status of chickens fed Asp. awamori may be effective to maintain high meat quality after processing and distributing on the market. Further studies are needed to gain more information about meat quality in broiler chickens fed an Asp. awamori-containing diet under normal and HS conditions.

In conclusion, this study shows that dietary Asp. awamori improves the diminished antioxidative status under the heat stress condition partially through its effect on the expression of antioxidant enzymes. These results suggest that Asp. awamori could be used as an effective feed additive to reduce oxidative stress in broilers.

Acknowledgments

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References


Ensminger ME, Oldfield JE and Heineman WW. Pages 8–110 in Feeds and Nutrition, 2nd ed. 1990.


Faustman C, Cassens RG, Schaef DM, Buege DR, Williams SN and Scheller KK. Improvement of pigment and lipid stability in...


