Oxidative Stability and Physico-chemical Properties of Meat from Broilers Fed with Dietary Neem Leaf Powder, Spirulina and their Combination

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Abstract

This 6 week study was conducted to evaluate the antioxidant potential, maintaining quality and sensory properties of broiler meat from birds fed on dietary neem leaf powder (NLP). A total of 90 Vencobb broiler chicks were randomly allotted to 6 groups of 15 birds in each. Dietary treatments consisted of normal diet (control Group I), feed containing terramycin-200 (TM-200*) at the concentration of 0.05% (Group II), feed containing NLP of 0.2% (Group III), feed containing NLP of 0.2% and spirulina of 1% (Group IV), feed containing TM-200 at 0.05% and spirulina of 1% (Group V) and feed containing spirulina of 1% (Group VI). At the end of the experiment liver, kidney and muscle samples were collected to evaluate the tissue peroxidation (TBARS and protein carbonyls) and antioxidant markers (SOD). Physico-chemical quality determinants of both fresh and preserved meat viz. extract release volume (ERV), water holding capacity (WHC) and pH were also studied. TBARS protein carbonyls indicated a significant (P < 0.05) decrease in all the treated groups when compared to control. Superoxide dismutase levels were found to be significantly increased in all the treated groups, in all the tissues collected. Compared to control group, favorable physico-chemical quality determinants were recorded in all the treated groups. The sensory attributes did not show significant (P < 0.05) differences for color, flavor, juiciness, tenderness and overall acceptability. This study indicates enhanced stress tolerance levels, improved meat quality with unaffected consumer acceptance levels of the meat observed in the study, from broilers fed with neem and spirulina either alone or in combinations, this points out that neem at 0.2% level can be used in poultry diets instead of antibiotic growth promoters (AGP).

Abbreviations: AGP-Antibiotic growth promoters; NLP-Neem leaf powder; TBARS-Thiobarbituric acid reactive substances; SOD-Superoxide dismutase; ERV-Extract release volume; WHC-Water holding capacity; NADPH-Nicotinamide adenine dinucleotide phosphate

Keywords: neem leaf powder, oxytetracycline, physico-chemical quality, spirulina

Introduction

Antibiotic growth promoters (AGP) at sub-therapeutic level improve overall performance of poultry (Falcão-e-Cunha et al., 2007). In recent years the usage of many antimicrobial drugs was banned due to their possible harmful effects on consumers (Black, 1984; Donoghue, 2003). The ban on the use of antibiotic feed additives has triggered intensive research to find and develop alternative strategies to maintain health and performance in poultry production. There is also an increasing public and governmental pressure in several countries to search for natural alternatives to antibiotics (McCartney, 2002). As a consequence, plant products are emerging as potential replacements for AGP (Charis, 2000). Plant derived products have proven to be neutral, less toxic, residue free and are thought to be ideal feed additives in food animal production (Hashemi et al., 2008). Generally, plant products do not develop or aid in the development of drug resistance (Tipu et al., 2006). Further, broilers fed on herbal feed additives are well accepted by the consumers (Hernandez et al., 2004). Oxidation is a very general process that affects lipids, proteins and carbohydrates (Kanner, 1994). Environmental stress diminishes in vivo antioxidant status and results in oxidative damage (Klasing, 1998; Sahin et al., 2001). Muscle tissue oxidation continues after slaughter and determines the shelf-life of the meat. Lipid oxidation and oxidative rancidity are often decisive factors in determining the useful storage life of food products. To maximize the oxidative stability of meat, antioxidants, mostly synthetic, are added to animal feed.
**Azadirachta indica** A. Juss, commonly known as neem, is extensively used in Ayurveda, Unani as homeopathic medicine and has become a cynosure for modern medicine (Koul and Opender, 1990; Subapriya and Nagini, 2005). Neem leaves contain a vast array of chemically diverse and active ingredients (Devakumar and Skutt, 1993). A wide range of medicinal utilities and wide range of pharmacological activities have been described for neem leaf (Durrani et al., 2008).

*Spirulina platensis*, a blue green algae rich in proteins, essential amino acids, antioxidants like Vitamin C, β-carotenes and tocopherols (Abd-El-Baky et al., 2003; Ciferri and Tiboni, 1985; Khan et al., 2005) inhibits the lipid peroxidation more significantly than chemical antioxidants (Manoj et al., 1992).

The present study was conducted to study the effect of dietary inclusion of neem leaf powder, alone and in combination with spirulina, on maintaining quality of broiler chicken meat and their effect on meat sensory parameters.

**Materials and Methods**

**Experimental design and management**

A total of 90, day-old Vencobb broiler chicks (M/s Srinivasa Hatcheries, Vijayawada) were randomly distributed to 6 groups of 15 birds and were maintained in battery cages. Birds were maintained with 24 hours of light in the first week, followed by 20 hours lighting from the second week through the end of the experimental study. The ambient temperature ranged between 32-35 °C with a relative humidity of 65%. All the birds were kept under uniform managemental conditions with *ad libitum* feed and water. Standard vaccination and management practices were followed throughout the experiment. Neem leaves collected locally were shade dried and grounded to powder with willey mill. *Spirulina platensis* powder (M/s Parry Neutraceuticals, Chennai) and oxytetracycline were obtained from commercial sources.

The feed given to different groups consisted of normal feed (Group I), feed containing oxytetracycline-200 (TM-200*+) at the concentration of 0.05% (Group II), feed containing NLP of 0.2% (Group III), feed containing NLP of 0.2% and spirulina of 1% (Group IV), feed containing TM-200 of 0.05% and spirulina of 1% (Group V) and feed containing spirulina of 1% (Group VI).

At the end of 42 days all the birds were sacrificed by decapitation. Liver, kidney and muscle samples were collected to evaluate the tissue peroxidation markers viz. TBARS (Balsubramanian et al., 1988) and protein carbonyls (Levine et al., 1990) and antioxidant marker SOD (Madesh and Balsubramanian, 1998).

Physico-chemical quality determinants of both fresh and preserved meat viz. extract release volume (ERV) (Jay and Kalliopi, 1964), water holding capacity (WHC) (Whiting and Jenkins, 1981) and pH (T rout et al., 1992) using digital pH meter were also studied.

Organoleptic evaluation of oil cooked meat as judged by color, flavor, juiciness, tenderness and overall acceptability was found unaltered with respect to fresh meat in all the treated groups compared to control. Maximum decrease in TBARS was observed in group V followed by group II as indicated in Table 1. Protein carbonyls indicated a significant (P<0.005) decrease in all the treated groups compared to control. In kidney dye the decrease was highly significant (P<0.05) in group II and V while in muscle tissue, maximum decrease was noticed in group II, IV and V (Table 1). Superoxide dismutase levels were found to be significantly increased in all the treated groups in all the tissues collected (Table 1).

**Peroxidation and antioxidant markers**

The extract release volume and water holding capacity values were found to be significantly increased in all the treated groups both in fresh as well as in preserved meat. In contrast, the pH values were found significantly lowered in fresh meat and in preserved meat in all the treated groups (Table 2).

**Organoleptic characteristics**

Organoleptic characteristics like tenderness, juiciness, flavor, color and overall acceptability were found unaltered with respect to fresh meat in all the treated groups compared to control (Table 3).

**Discussion**

In all the treated groups, a significant decrease in tissue peroxidation markers (TBARS and protein carbonyls) and a significant increase in tissue antioxidant marker (SOD) were observed in liver, kidney and muscle. Overall comparison of the antioxidant status profiles in various treated groups revealed their highest levels in antibiotic plus spirulina group followed by antibiotic group, neem plus spirulina group, neem group and spirulina group in descending order. This revealed that the treated birds had higher stress tolerance levels or otherwise lowered physiological stress compared to control birds. This can be attributed to different mechanisms of action of treatments.

Generally oxidative damage occurs in *in vivo* conditions due to altered equilibrium between the production of reactive oxygen or nitrogen species and the constituent defense mechanisms. It is generally accepted that lipid oxidation is one of the primary mechanism of quality deterioration in meat (Morrissey et al., 1998). The extent of lipid oxidation in meat can be measured by...
estimating the thiosulfuric acid reactive substance concentration (Raharjo and Sofos, 1993)

As a major component of muscle tissue, proteins play a
decisive role in meat and meat products (Laurie, 1998). Muscle
protein oxidation produces deleterious effects on meat quality
(Decker et al., 1993; Mercier et al., 1995). Protein oxidation
is known to affect meat quality including texture (Kemp et al.,
2010), color (Kazemi et al., 2011), flavor (Toldra, 1998) and
WHC (Van Laack et al., 2000).

Subapriya et al. (2005), Sithisan et al. (2005), Ghimeray et
al. (2009) and Kiranmai et al. (2011) reported the antioxidant
potential of various parts of neem in both in vivo and in vitro
experimental studies. Subapriya et al. (2005) stated that
ethanolic extract of neem contains a number of antioxidants
including terpinoids, limonoids, quercetin and sitosterols.

The results observed according to spirulina in respect to
increasing antioxidant status in the present study are akin to
the earlier reports that come from in vitro, animal and human
studies (Miranda et al., 1998 and Won-Loy Chu et al., 2010).
Hayashi et al. (2009) stated that phycocyanobilin a
phytochemical richly present in spirulina, reportedly inhibits
NADPH oxidase activity and promotes glutathione synthesis
along with a marked production of antioxidant enzyme having
potential for management of oxidative stress.

It is known that many active components in herbs slow
down the lipid peroxidation by activation of enzymes like SOD,
catalase and glutathione peroxidase (Botsoglou et al.,
2003). Herbs containing polyphenolic substances, that possess
SOD, catalase and glutathione peroxidase (Botsoglou
et al., 2008). Several researchers have reported the possibility of antioxidative effects for some
traditional herbs (Park and Yoo, 1999; Liu et al., 2006). Decker
(2000) opined that oxidative stability of meat can be improved
by managing animal feeds.

Dietary supplementation with garlic bulb also resulted in
significantly lower TBARS value in chicken thigh muscle
compared with muscle from birds fed non supplemented diets
(Kim et al., 2009). In a similar study, Jang et al. (2008) observed
increased antioxidant activity in the breast meat as evidenced
by decreased TBARS compared to control birds in broiler
chicks fed with dietary medicinal herb extract mix. Increased
antioxidant activity results in increased protection against the
stresses caused by lipid oxidation. The decreased TBARS and
protein carbonyls and increased SOD activity implies that the
shelf-life of the meat can be improved by dietary
supplementation of neem, spirulina and their combination.

Good quality meat with a relatively low bacterial count
releases large volumes of extract (ERV) (Jay and Kalliopi, 1964)
and will have higher water holding capacity (WHC) (Jay,
1965). A lower WHC in muscle can induce liquid outflow,
loss of soluble nutrients and flavor and thereby reducing the
meat quality since the muscle become hard and tasteless
(Barbut, 1996). In the present study it was observed that
WHC and ERV at the time of slaughter were significantly
higher in all the treated groups compared to the control.
Similar trend was observed even after one-week storage.

The ultimate pH of meat is highly dependent on the
amount of glycogen present in the muscle. This glycogen is

### Table 1. Physico-chemical profiles of tissues of fresh and preserved broiler chicken meat fed with neem leaf and/or spirulina substituted diet

<table>
<thead>
<tr>
<th>Group</th>
<th>TBARS (µM of MDA/mg of protein)</th>
<th>Protein carbonyls (nm/mg of protein)</th>
<th>SOD (U/mg of protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Liver</td>
<td>Kidney</td>
<td>Muscle</td>
</tr>
<tr>
<td>I</td>
<td>4.50 ± 0.14</td>
<td>3.27 ± 0.17</td>
<td>2.49 ± 0.08</td>
</tr>
<tr>
<td>II</td>
<td>2.79 ± 0.03</td>
<td>0.17 ± 0.31</td>
<td>1.27 ± 0.24</td>
</tr>
<tr>
<td>III</td>
<td>3.15 ± 0.08</td>
<td>2.65 ± 0.27</td>
<td>3.35 ± 0.18</td>
</tr>
<tr>
<td>IV</td>
<td>3.89 ± 0.16</td>
<td>2.35 ± 0.46</td>
<td>2.07 ± 0.17</td>
</tr>
<tr>
<td>V</td>
<td>2.38 ± 0.13</td>
<td>1.90 ± 0.12</td>
<td>1.25 ± 0.09</td>
</tr>
<tr>
<td>VI</td>
<td>3.76 ± 0.21</td>
<td>2.75 ± 0.59</td>
<td>1.96 ± 0.11</td>
</tr>
</tbody>
</table>

Note: Values are Mean ± S.E (n=15) one way ANOVA (SPSS). Means with different superscripts differ significantly (P<0.05)

### Table 2. Organoleptic characteristics of fresh meat in different groups of broiler chicks fed with neem leaf and/or spirulina substituted diet

<table>
<thead>
<tr>
<th>Group</th>
<th>Tenderness</th>
<th>Juiciness</th>
<th>Flavor</th>
<th>Color</th>
<th>Overall acceptability</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>7.4 ± 0.17</td>
<td>6.84 ± 0.26</td>
<td>6.43 ± 0.31</td>
<td>7.29 ± 0.36</td>
<td>6.85 ± 0.26</td>
</tr>
<tr>
<td>II</td>
<td>7.4 ± 0.28</td>
<td>7.14 ± 0.24</td>
<td>7.09 ± 0.25</td>
<td>7.57 ± 0.20</td>
<td>7.14 ± 0.26</td>
</tr>
<tr>
<td>III</td>
<td>7.2 ± 0.28</td>
<td>7.86 ± 0.20</td>
<td>7.83 ± 0.31</td>
<td>7.29 ± 0.29</td>
<td>8.00 ± 0.39</td>
</tr>
<tr>
<td>IV</td>
<td>7.4 ± 0.28</td>
<td>7.57 ± 0.30</td>
<td>7.57 ± 0.37</td>
<td>7.57 ± 0.20</td>
<td>7.43 ± 0.20</td>
</tr>
<tr>
<td>V</td>
<td>7.4 ± 0.28</td>
<td>7.14 ± 0.34</td>
<td>7.57 ± 0.37</td>
<td>7.57 ± 0.20</td>
<td>7.43 ± 0.20</td>
</tr>
<tr>
<td>VI</td>
<td>7.4 ± 0.28</td>
<td>7.17 ± 0.27</td>
<td>7.33 ± 0.21</td>
<td>7.57 ± 0.20</td>
<td>7.43 ± 0.20</td>
</tr>
</tbody>
</table>

Values are Mean ± S.E (n=15) one way ANOVA (SPSS)
depleted in the muscles of birds that have been exposed to stress (Ngoka and Froning, 1982). Since pH is an important determinant of microbial growth, it is obvious that ultimate pH of meat is significant for its resistance to spoilage. Post mortem pH of the meat is determined by the amount of lactic acid produced from glycogen during anaerobic glycolysis and this will be curtailed if glycogen is depleted by fatigue, inanition or fear in the animal before slaughter (Lawrie, 1998). pH of the meat at slaughter and after storage in treated groups remained significantly lower compared to control. This indicated better muscle glycogen reserves in all treated groups which indirectly reflect reduced stress on birds.

Thus, favorable WHC, ERV and pH values of the meat from neem and spirulina treated birds suggest that both the agents enhance the shelf-life and quality of the meat. This is in accordance with the results of the study made by Chen et al. (2008) where the pork from pig fed with garlic powder showed increased WHC with no effect on color, marbling and firmness. WHC also increased in pork of pigs fed on 2 g of fresh spirulina with forage (Almantas et al., 2013). Young et al. (2003) attributed the increase in WHC of broiler breast muscle in favour of the improvement of the antioxidant activity via inhibition of TBARS.

To know the general consumer acceptance of the meat from the treated birds, meat sensory attributes in terms of color, flavor, tenderness, juiciness and overall acceptability on fresh basis were studied using sensory panel. It was observed that none of the sensory attributes were adversely affected indicating no negative impact on the general acceptance of the meat from neem and spirulina fed birds. The inclusion of neem at rates up to 2% in feed did not alter the aroma, color, flavor, texture, juiciness and overall acceptability of broiler meat as stated by Bonsu et al. (2012). Egbeyle et al. (2014) reported that inclusion of neem leaf meal at the rate of 0.5% in broiler diets produced better results in terms of growth without affecting carcass characteristics. Also there was no modification in organoleptic properties of chicken meat fed on diets containing spirulina at 1%, 2.5% and 5% level as reported by Raach-Moujahed et al. (2011).

Conclusions

Enhanced stress tolerance levels and improved meat quality with unaffected consumer acceptance levels of the meat observed in the study, with neem and spirulina either alone or in combinations, indicated that neem at 0.2% level can be used in poultry diets instead of AGPs. However, much favorable results were obtained in the present study when neem was used in combination with spirulina than when it was used alone.

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References


