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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 combinationsș 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).

Oxidatation 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.

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Azadirachata indica A. Juss, commonly known as neem, is extensively used in Ayurveda, Unani as homoeopathic 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, Vijayawda) 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 (Balasubramanian *et al.,* 1988) and protein carbonyls (Levine *et al*., 1990) and antioxidant marker SOD (Madesh and Balasubramanian, 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 (Trout *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 done as per 9 point Hedonic scale that ranged from 1-dislike extremely to 9-extremely like with semi trained taste panel regarding to the aspects of color, flavor, juiciness, tenderness and overall acceptability.

Score	Interpretation
9	Excellent (like extremely)
8	Good (like very much)
7	Moderately good (like moderately)
6	Just good (like slightly)
5	Fair (neither like nor dislike)
4	Just poor (dislike slightly)
3	Moderately poor (dislike moderately)
2	Poor (dislike very much)
1	Extremely poor (dislike extremely)

Statistical analysis

The data was subjected to statistical analysis by applying one way ANOVA using statistical package for social sciences (SPSS) version 17. Differences between the means were tested using Duncan's multiple comparision test and significance was set at $P<0.05$.

Results

Peroxidation and antioxidant markers

By the end of the seventh week of experimental period, the levels of TBARS were found significantly decreased in all groups compared to the control group. Maximum decrease in TBARS was observed in group V followed by group II as indicated in Table 1. Protein carbonyls indicated a significant ($P<0.05$) decrease in all the treated groups compared to control. In kidney 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).

Physico-chemical quality parameters of the meat

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, juciness, 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 thiobarbituric 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 (Lawrie, 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), Sithisaran *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 antioxidant properties, can improve the shelf-lifeand quality of meat and meat products (Liu *et al.*, 1992; Botsoglou *et al.*, 2002). Lipid and hydroxyl radicals were neutralized by herbal phenolic components (Yanishlieva-Maslarova, 2001). A significant antioxidant activity in lamb meat was observed during a dietary treatment with oregano essential oil which is rich in phenols (Simitzis *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. Antioxidant profile of different tissues from birds fed with neem leaf and/or spirulina substituted diet

Group	TBARS (μM of MDA/mg of protein)			Protein carbonyls (nM/100mg protein)			SOD (U/mg of protein)		
	Liver	Kidney	Muscle	Liver	Kidney	Muscle	Liver	Kidney	Muscle
I	4.50 ± 0.14^{C}	3.27 ± 0.17^{C}	2.49 ± 0.07^{C}	22.45 ± 0.55^{B}	13.51 ± 1.30^{C}	6.04 ± 0.35^{C}	9.84 ± 0.29^{A}	7.34 ± 0.35^{A}	6.49 ± 0.21^{A}
II	2.79 ± 0.17^{A}	2.01 ± 0.31^{A}	1.37 ± 0.21^{A}	19.34 ± 0.95^{A}	10.65 ± 0.95^{A}	4.69 ± 0.51^{A}	12.75 ± 0.48^{C}	8.87 ± 0.41^{B}	7.85 ± 0.31^{B}
III	3.34 ± 0.08^{B}	2.65 ± 0.27^{B}	1.85 ± 0.13^{B}	20.27 ± 0.89^{A}	11.43 ± 1.01^{B}	5.01 ± 0.41^{B}	11.76 ± 0.25^{B}	8.12 ± 0.39^{B}	7.21 ± 0.28^{B}
IV	3.09 ± 0.16^{B}	2.35 ± 0.14^{B}	1.78 ± 0.17^{B}	19.89 ± 1.21^{A}	11.09 ± 1.20^{B}	4.85 ± 0.35^{A}	12.01 ± 0.37^{C}	8.35 ± 0.35^{B}	7.45 ± 0.22^{B}
V	2.38 ± 0.13^{A}	1.90 ± 0.12^{A}	1.25 ± 0.09^{A}	18.79 ± 1.02^{A}	10.13 ± 0.75^{A}	4.36 ± 0.47^{A}	13.36 ± 0.57^{D}	9.02 ± 0.47^{C}	8.03 ± 0.26^{C}
VI	3.76 ± 0.21^{B}	2.75 ± 0.15^{B}	1.96 ± 0.11^{B}	20.84 ± 1.09^{A}	11.87 ± 0.45^{B}	5.43 ± 0.27^{B}	11.14 ± 0.39^{B}	7.98 ± 0.27^{A}	7.11 ± 0.36^{B}

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

Table 2. Physico-chemical profiles of tissues of fresh and preserved broiler chicken meat fed with neem leaf and/or spirulina substituted diet

Group	ERV (ml)		WHC (%)		pH	
	Fresh	1st week	Fresh	1st week	Fresh	1st week
I	22.00 ± 0.61^{A}	21.00 ± 0.60^{A}	59.39 ± 0.31^{A}	57.49 ± 0.66^{A}	5.69 ± 0.04^{D}	5.72 ± 0.03^{D}
II	26.00 ± 0.58^{BC}	25.00 ± 0.56^{CD}	63.98 ± 0.47^{CD}	62.68 ± 0.34^{D}	5.51 ± 0.02^{AB}	5.55 ± 0.02^{B}
III	24.00 ± 0.66^{B}	23.00 ± 0.60^{B}	61.85 ± 0.65^{B}	60.81 ± 0.46^{B}	5.60 ± 0.03^{C}	5.63 ± 0.02^{C}
IV	25.50 ± 0.70^{BC}	24.50 ± 0.60^{C}	62.70 ± 0.53^{C}	61.40 ± 0.32^{C}	5.53 ± 0.01^{BC}	5.58 ± 0.01^{B}
V	27.50 ± 0.86^{C}	26.00 ± 0.47^{D}	64.68 ± 0.37^{D}	63.49 ± 0.31^{E}	5.47 ± 0.02^{A}	5.51 ± 0.01^{A}
VI	23.50 ± 0.62^{B}	22.50 ± 0.60^{B}	61.40 ± 0.40^{B}	60.10 ± 0.36^{B}	5.62 ± 0.02^{C}	5.65 ± 0.02^{C}

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

Table 3. Organoleptic characteristics of fresh meat in different groups of broiler chicks fed with neem leaf and/or spirulina substituted diet

Group	Tenderness	Juiciness	Flavor	Color	Overall acceptability
I	7.14 ± 0.27	6.84 ± 0.26	6.83 ± 0.31	7.29 ± 0.36	6.85 ± 0.26
II	7.24 ± 0.26	7.14 ± 0.26	7.00 ± 0.26	7.57 ± 0.20	7.14 ± 0.26
III	8.29 ± 0.29	7.86 ± 0.40	7.83 ± 0.31	7.29 ± 0.29	8.00 ± 0.39
IV	7.43 ± 0.37	7.57 ± 0.30	7.67 ± 0.33	7.57 ± 0.37	7.57 ± 0.20
V	7.14 ± 0.26	7.14 ± 0.34	7.50 ± 0.43	7.57 ± 0.37	7.43 ± 0.37
VI	8.00 ± 0.31	7.17 ± 0.27	7.33 ± 0.21	7.57 ± 0.20	7.43 ± 0.20

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 pigs 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). Egbeyale *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 effecting carcass charecterstics. 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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