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Modulation of Meat Quality by Capsaicin in Neuropathic White Leghorn Poultry

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ABSTRACT

An experimental study was carried out to evaluate the effect of capsaicin on meat quality attributes and associated biochemical alterations in chicken. The birds were divided into three experimental groups: control, neuropathic, and capsaicin-treated neuropathic groups following a sciatic nerve crush–induced neuropathic pain model. Eaten chicken was randomly allocated equally among the three groups. Neuropathic pain was induced by surgically exposing and crushing the right sciatic nerve. Birds in the treated group received local infiltration of capsaicin (1 mg/kg) at the site of nerve injury, whereas the control and untreated neuropathic groups received only saline infiltration. After four weeks, plasma malondialdehyde levels were estimated as an indicator of oxidative stress, while Thio barbituric acid and trimethylamine contents were analysed to assess meat quality. Sensory evaluation revealed a decline in meat quality parameters in neuropathic birds, indicating the adverse effect of pain on meat quality. Significant differences were observed between control and neuropathic duck meat samples for various sensory attributes. Administration of capsaicin markedly attenuated sciatic nerve crush–induced pain and reduced oxidative stress markers. It can therefore be concluded that the anti-inflammatory and antioxidative effects of capsaicin following sciatic nerve injury in chicken contribute to improved meat quality compared with untreated neuropathic birds.

Introduction

Globally, duck meat production has shown a steady increase and was estimated at 3.5 million metric tons in 2005 (FAO, 2007). In India, egg production has increased rapidly by 67%, reflecting the growing consumer demand for poultry products. According to the Livestock Census (2007), the duck population in India is approximately 27.43 million, accounting for 8.52% of the total poultry population. The distribution of chicken indicates that they are predominantly reared in the eastern, northeastern, and southern regions of the country. In recent years, duck meat has been recognized for its high nutritional value and desirable flavour (Omojola, 2007), and its popularity continues to rise in many Asian countries. Furthermore, chicken are highly adaptable to free-range production systems and exhibit faster growth rates than chickens. Duck meat is characteristically darker and redder in colour than chicken meat due to its higher phospholipid content and elevated levels of polyunsaturated fatty acids (PUFA), which may constitute up to 60% of total fatty acids. The darker coloration of duck meat is primarily attributed to its high myoglobin content. The proximate composition of duck meat includes 76.41% moisture, 20.06% protein, 1.84% fat, and 0.92% ash (Ali *et al*., 2007). Owing to its relatively lower fat content compared with chicken and other meats, consumer interest in duck meat consumption has increased.

In India, duck farming is largely practiced by small and marginal farmers as well as nomadic communities. Duck rearing is gaining prominence due to its economic advantages and nutritional benefits. However, under intensive indoor or cage housing systems, overcrowding often induces pain and stress in birds, leading to substantial economic losses for farmers. Therapeutic interventions are seldom adopted, and reliance on traditional management practices remains common. This situation provides an opportunity to explore indigenous therapeutic agents that may benefit both animal welfare and farm productivity. Accordingly, the present investigation was designed to induce pain in chicken and evaluate the effects of capsaicin. There exists considerable scope to assess the effectiveness of indigenous products in the management of neuropathic pain (Quintass *et al*., 2013). Capsaicin is an exogenous agonist of the transient receptor potential vanilloid 1 (TRPV1) receptor (Caterina *et al*., 1997), which has emerged as a novel target for pain therapy and represents a promising pharmacological approach for pain management (Jara-Oseguera *et al*., 2008).

In the present study, the efficacy of capsaicin in alleviating pain in a duck model was evaluated, along with its influence on various meat quality parameters. The study aimed to determine whether pain adversely affects meat quality and to assess the therapeutic potential of this indigenous compound in improving meat quality outcomes.

Materials and Methods

The experiment was conducted following approval from the Institutional Animal Ethical Committee (IAEC), College of Veterinary Science & Animal Husbandry, Odisha University of Agriculture and Technology (OUAT), Bhubaneswar. White Pekin chicken of either sex, aged four weeks and weighing between 700 and 800 g at the time of surgery, were used in the present study. The chicken were housed on floor space measuring 0.279 m^2 (3 sq. ft.) with soft bedding under a 12 h light and 12 h dark cycle for at least three days prior to experimentation to allow acclimatization. Feed and water were provided ad libitum. All efforts were undertaken to minimize animal suffering and to reduce the number of animals used. During the experimental period, the chicken were regularly examined for any signs of disease or parasitic infestation, and no health abnormalities were observed. Bedding materials were appropriately maintained and cleaned daily.

Prior to surgery, the chicken were deprived of feed for 12 hours. Anaesthesia was induced using xylocaine (2%) administered intramuscularly at a dose of less than 4 mg/kg. Following induction of anaesthesia, feathers around the surgical site were cleaned and shaved. The common sciatic nerve was exposed at the mid-thigh level by blunt dissection through the biceps femoris muscle. Approximately 7 mm of the nerve proximal to the sciatic trifurcation was carefully freed from surrounding connective tissue. The nerve was then mobilized and subjected to a 30-second crush injury using a serrated haemostat approximately 7 mm distal to its point of emergence. After completion of the procedure, the incision was closed using 3-0 silk sutures. Betadine® solution was applied externally using a cotton swab, followed by topical application of Nebasulf® powder (bacitracin 250 IU, neomycin 5 mg, and sulphacetamide sodium 60 mg; Pfizer, India) at the surgical site. Immediately after surgery, the birds were placed in soft-bedded cages with feed provided at ground level to enable feeding without bearing weight on the hind limbs (Naik *et al*., 2012).

Local infiltration of capsaicin (1 µg/ml dissolved in normal saline, pH 7.2) was administered at the site of injury in one of the treated groups comprising six birds. In the control group, normal saline was administered as the vehicle in the same manner as used in the treated group.

Three types of sausages were prepared from control, neuropathic, and capsaicin-treated duck meat. The meat was weighed, cut into small pieces, and minced using a meat mincer fitted with a 10-mm plate after adequate thawing. The formulation for 1 kg of duck sausage batter consisted of minced meat (600 g), vegetable fat (200 g), ice flakes (100 g), salt (25 g), sugar (10 g), monosodium glutamate (0.5 g), sodium nitrate and sodium nitrite (0.1 g each), condiments (45 g), spice mix (15 g), and wheat flour (34.3 g). The sausage emulsion was prepared using a bowl chopper. After collecting samples (n = 6) for analysis, the emulsions were kept at air-conditioned temperature (15–18 °C) for 4 hours to ensure uniform setting. The sausages were then steam-cooked until a core temperature of 85 °C was attained, followed by cold showering for 10 minutes. Cooked sausage samples (n = 6) from each group were collected for analysis and sensory evaluation. The cooked samples were packed appropriately and used for sensory assessment.

Blood samples from crush-injury-induced and sham-operated chicken were collected on the 28th day post-surgery from the wing vein. The samples were centrifuged to obtain plasma. Malondialdehyde (MDA), an indicator of lipid peroxidation and free radical generation, was estimated following the method described by Ohkawa *et al*. (1979). The reaction mixture was centrifuged, the organic layer separated, and absorbance measured at 532 nm. Plasma MDA levels were expressed as nmol/ml.

Meat homogenate samples were transferred to disposable test tubes containing thiobarbituric acid–trichloroacetic acid (TBA-TCA) reagent (Acharya *et al*., 2002). The mixture was vortexed, heated in a water bath for 15 minutes, cooled in a cold-water bath for 10 minutes, and centrifuged at 2900 rpm for 15 minutes. The absorbance of the supernatant was recorded at 531 nm using a microsample spectrophotometer (Hoyland, 1991). Thiobarbituric acid values were expressed as mg MDA/kg of meat.

Fresh minced meat samples were blended with 7.5% trichloroacetic acid solution. The homogenized mixture was centrifuged at 2000–3000 × g until a clear supernatant was obtained. Aliquots of the supernatant were pipetted into test tubes along with standard trimethylamine (TMA) solutions and blank samples. The reagents were mixed thoroughly and transferred to a spectrophotometric cell, and absorbance was measured at 410 nm against the blank (Horsfall *et al*., 2006).

Trimethylamine content was expressed as mg TMA per 100 g of sample.

Samples for sensory evaluation were collected from each treatment group after cooking to the desired internal temperature. A panel of seven trained evaluators aged between 20 and 30 years assessed the cooked meat samples. Uniform bite-sized portions from each treatment were coded, replicated three times, and served in odourless plastic plates. Each sample was evaluated independently using an eight-point hedonic scale (1 = disliked extremely; 8 = liked extremely) for appearance, flavour, tenderness, juiciness, and overall acceptability (Safiudo *et al*., 1998).

Data obtained for oxidative stress markers, thiobarbituric acid, and trimethylamine were analyzed using GraphPad QuickCalcs, and the Student's *t*-test was employed to determine statistical significance. A *p* value of less than 0.001 was considered statistically significant. Sensory evaluation attributes were analyzed using paired *t*-tests with IBM® SPSS Statistics software (Version 23.0), and differences were considered significant at $p < 0.05$.

Results and Discussion

For the assessment of meat quality, a significant difference ($p < 0.001$) in thiobarbituric acid (TBA) values was observed between the control group and neuropathic pain (NP)–induced chicken. Furthermore, a significant ($p < 0.001$) increase in TBA value was noted in the capsaicin-treated group [(0.18 ± 0.01) mg MDA/kg] compared with neuropathic chicken [(0.10 ± 0.01) mg MDA/kg] (Table 1). A significant ($p < 0.001$) elevation in trimethylamine (TMA) levels was recorded in NP-induced birds [(3.42 ± 0.18) mg TMA-N/100 g] relative to the control group [(1.42 ± 0.09) mg TMA-N/100 g]. Conversely, a significant ($p < 0.001$) reduction in TMA concentration was observed in the capsaicin-treated group [(2.36 ± 0.09) mg TMA-N/100 g] when compared with neuropathic chicken [(3.42 ± 0.18) mg TMA-N/100 g] (Table 1).

Plasma TBARS levels measured after four weeks were significantly elevated ($p < 0.001$) in NP-induced birds (8.41 ± 0.20 nmol MDA/mg protein) as well as in the capsaicin-treated group (6.77 ± 0.17 nmol MDA/mg protein) when compared with the control group (5.51 ± 0.26 nmol MDA/mg protein) (Table 2). Additionally, noteworthy correlations among these variables were identified (Table 2).

Sensory evaluation of meat products by panelists revealed significant differences ($p < 0.05$) between control and neuropathic duck meat samples (Table 3). Significant differences ($p < 0.05$) in appearance and texture were observed between the control group and neuropathic duck meat. The juiciness score of control meat samples (7.28 ± 0.09) differed significantly ($p < 0.05$) from neuropathic duck meat (6.33 ± 0.07) and also from capsaicin-treated duck meat (6.95 ± 0.04). Similarly, flavour and overall acceptability scores were significantly lower ($p < 0.05$) in neuropathic duck meat (6.76 ± 0.10 and 6.85 ± 0.09, respectively) compared with control (7.28 ± 0.10 and 7.38 ± 0.09) and capsaicin-treated duck meat samples (7.07 ± 0.09 and 7.30 ± 0.08, respectively) (Table 3).

Chicken was selected as the experimental model due to its poikilothermic nature and adaptability to experimental conditions. Capsaicin (8-methyl-*N*-vanillyl-6-nonenamide), the bioactive component of chili peppers (*Capsicum frutescens*), is known to induce a burning sensation accompanied by flare and wheal responses. These effects occur rapidly, while a poorly localized, prolonged dull pain persists due to the activation of slow-conducting C nerve fibers through TRPV1 receptor stimulation. Capsaicin also induces the release of substance P and calcitonin gene-related peptide from peripheral and central nerve terminals, contributing to neurogenic inflammation (Winter *et al.*, 1995). As capsaicin does not affect large nerve fibers responsible for motor and sensory functions such as touch and vibration, and systemic absorption remains minimal when applied topically, localized administration represents a promising approach for neuropathic pain management. Recent developments include higher concentrations of capsaicin and related capsaicinoids, which are being investigated for their antioxidant, anticarcinogenic, and analgesic properties (Reyes-Escogido *et al.*, 2011).

Table.1 Assessment of Meat Quality Parameters (n = 6)

Meat quality parameter	Control	Neuropathic pain	Treated
TBA (mg MDA/kg)	0.32 ± 0.01	0.11 ± 0.01[a]	0.19 ± 0.01[a]
TMA (mg N/100 g)	1.42 ± 0.09	3.42 ± 0.18[a]	2.36 ± 0.09[a]

[a] $p < 0.001$ compared with normal control group.

Table.2 Effect of Oxidative Stress on Lipid Peroxidation Antioxidant Enzyme Levels (n = 6)

Plasma parameter	Control	Neuropathic pain	Treated
TBARS (nmol MDA/mg protein)	5.51 ± 0.26	8.42 ± 0.20[a]	6.78 ± 0.18[a]

[a] $p < 0.001$ compared with normal control group.

Table.3 Sensory Quality of Duck Sausages Prepared from Control, Neuropathic, and Treated Birds (n = 6)

Sensory attribute	Control	Treated	Neuropathic pain
Appearance	7.33 ± 0.08[a]	7.28 ± 0.09	7.07 ± 0.06[a]
Flavour	7.28 ± 0.10[a]	7.07 ± 0.09	6.76 ± 0.10[a]
Juiciness	7.28 ± 0.09[a]	6.95 ± 0.04	6.33 ± 0.07[a]
Texture	7.26 ± 0.10[a]	7.14 ± 0.09	6.90 ± 0.09[a]
Overall acceptability	7.38 ± 0.09[a]	7.30 ± 0.08	6.85 ± 0.09[b]

[a] $p < 0.05$ compared with control group. [b] $p < 0.05$ compared with control group.

Topical application of capsaicin has been shown to be more effective than placebo in the management of chronic neuropathic and musculoskeletal pain, although some individuals experience transient burning or stinging sensations that may influence perceived efficacy (Mason *et al.*, 2004). Capsaicin has an approximate half-life of 24 hours (Hayman and Kam, 2008). It has been observed that neuropathic pain resulting from peripheral nerve injury can be attenuated more effectively with early intervention rather than treatment after pain becomes chronic. Therefore, in the present study, capsaicin was administered as a single local dose (1 µg/ml per bird) immediately following nerve injury (Naik *et al.*, 2012). In vitro studies using human skin have demonstrated slow biotransformation of capsaicin, with most of the compound remaining unchanged (Chanda *et al.*, 2008). Given the limited reports on capsaicin use in avian neuropathic pain models, the present study explored the effects of local drug infiltration in chicken.

In the current investigation, plasma malondialdehyde (MDA) levels increased following sciatic nerve crush injury, consistent with findings reported by Tamaddonfard *et al*. (2013) in rats. Similar elevations in MDA levels have been documented in ischemia–reperfusion injury of the sciatic nerve (Bagdatoglu *et al*., 2002) and following sciatic nerve crush injury in rats (Senoglu *et al*., 2009). Mitochondrial reactive oxygen species have been implicated in the mechanisms underlying denervation-induced skeletal muscle atrophy (Muller *et al*., 2007). Elevated MDA levels are indicative of oxidative stress and contribute to lipid membrane damage through rearrangement of double bonds in unsaturated fatty acids, ultimately leading to tissue injury (Cheesman, 1993). Increased lipid peroxidation therefore serves as a reliable marker of oxidative stress.

The present findings demonstrated that neuropathic birds exhibited lower TBA concentrations (19%) compared with control birds (30%), indicating that pain adversely affects meat quality. In contrast, capsaicin-treated birds showed higher TBA concentrations (51%) than neuropathic birds (19%). These results suggest a potent antioxidative effect of capsaicin, leading to reduced malondialdehyde formation, mitigation of stress-related damage, and subsequent improvement in meat quality.

Trimethylamine (TMA) is a well-recognized indicator of meat spoilage (Längkvist *et al*., 2013), and meat quality has been reported to be strongly associated with TMA concentration (Ward *et al*., 2009). TMA formation is commonly linked with fish spoilage, and polyphenolic compounds such as 6-gingerol have been shown to suppress TMA formation (Suryanti *et al*., 2014). In the present study, TMA levels in neuropathic birds (49%) were significantly higher than those in control birds (19%) (Fig. 1), indicating a deterioration in meat quality. Notably, capsaicin-treated birds exhibited a significant reduction in TMA concentration (32%) compared with neuropathic birds, suggesting that capsaicin may play a role in inhibiting TMA formation. However, further investigations are required to elucidate the underlying mechanisms.

The present study indicates that capsaicin may contribute to the attenuation of neuropathic pain in birds. The findings identify malondialdehyde as an important determinant associated with neuropathic pain. Sensory evaluation of duck meat demonstrated improved meat quality parameters in capsaicin-treated birds compared with neuropathic duck meat. Furthermore, the investigation highlights the need for additional approaches to validate the therapeutic potential of capsaicin, particularly through molecular-level studies related to neuropathic pain.

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