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First Report on Better Functional Property of Black Chicken Meat from India

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ABSTRACT

Background: Indian domesticated chickens have a wide variety of phenotypes. Unlike other chicken breeds, Kadaknath chicken has black meat and is used by indigenous tribal people for its invigorating and therapeutic properties. To look for the functional traits that might be contributing towards the acclaimed benefits, free radical scavenging capacity and metal chelating ability of Kadaknath meat were explored for the first time in comparison to the commercial Cobb broiler.

Methods: During the period 2018-2020, breast and thigh meat were collected from chickens (n=20/ group) at their commercial slaughter age. Meat extract was used for qualitative evaluation of protein as well as the antioxidant capacity utilizing diverse *in vitro* methods corresponding to different antioxidation mechanisms.

Result: Protein concentration (g/100g of tissue) in Kadaknath breast (25.25 ± 0.31) and thigh (19.98 ± 0.29) meat was significantly (P<0.05) higher than the Cobb. Breast meat had better scavenging capacity than the thigh. The superiority of the antioxidant capacity of Kadaknath meat was explicitly established by more than one *in vitro* assay. Free radical scavenging assays *viz.* 2,2-azinobis (3-ethyl-benzothiazoline-6-sulfonic acid) (ABTS); 1,1-diphenyl-2-picrylhydrazyl (DPPH) and Cupric reducing antioxidant capacity (CUPRAC) had significantly (P<0.05) higher values for Kadaknath in comparison to the Cobb broiler meat. Ferric reducing antioxidant power (FRAP) values (mM Fe^{2+}/g of tissue) were also more in breast meat of Kadaknath (26.97±0.37) than the Cobb (15.24±0.40). Similarly, FRAP values were higher in Kadaknath (33.85±0.47) than the Cobb (19.2±0.31) thigh meat. Kadaknath had higher antioxidant capacity as reflected by metal chelation inhibition value. These findings help to explain the unique nutritional and functional characteristics of Kadaknath black-bone chicken and provide basic research data for exploring the commercial potential of its meat in the fields of functional foods, cosmeceuticals and nuutraceuticals.

Key words: Antioxidant capacity, Breast and thigh meat, Indian poultry, Kadaknath chicken.

INTRODUCTION

Chickens (*Gallus Gallus domesticus*) were the first domesticated bird species and were subjected for more than 8000 years to the combined effects of natural selection and human-driven artificial selection. Compared with their wild progenitors (red jungle fowl, *Gallus gallus*), chickens present many characteristics associated with domestication that impact behavior, morphology, physiology, egg production and skin color (Li *et al*., 2020). Kadaknath is the only all-black chicken among the 19 diverse chicken breeds of India (www.nbagr.res.in). Their internal organs show intense black coloration due to genetic condition, fibromelanosis (Dharmayanthi *et al*., 2017). There are only three breeds of chicken in the world known to have black meat these include Silky in China and Ayam cemani in Indonesia apart from Indian Kadaknath.

Most of the Indian native chicken breeds are facing threats due to the commercialization of poultry production. Kadaknath is one such breed of Indian chicken seriously endangered with extinction. It is bred by tribals in the Jhabua and Dhar districts of Madhya Pradesh state. Associating a breed to specific products or quality can be an effective way of increasing its value that can drive its conservation. Kadaknath is considered to have flavorful lean meat and, the gamey texture that is preferred by the consumers. It is supposed to be invigorating and full of medicinal properties. Black-bone chickens are commonly believed to have medicinal properties and have been used as remedies to enhance the human immune system, prevent emaciation, treat diabetes and cure conditions such as menstrual abnormalities and postpartum complications (Li *et al*., 2020). Thus, there is a dire need for unraveling the scientific basis for health-promoting properties proclaimed for Kadaknath meat.

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Free radicals are acknowledged to have a role in the pathophysiology of aging, degenerative and various non-communicable human diseases. Therefore, the antioxidant potential of chicken meat could be the key not only in forecasting the prospective health benefits but also the oxidative stability of the meat (Lengkidworraphiphat *et al*., 2020). Meat is a rich source of endogenous cytosolic (hydrophilic), as well as lipid-soluble (hydrophobic) antioxidants. Better antioxidative capacity of Kadaknath meat might be one of the factors contributing towards their use as traditional medicine by tribes. Numerous antioxidants present in heterogeneous food such as meat makes it difficult to decipher the role of each constituent. Moreover, natural antioxidants can utilize more than one way to exert their effect *viz*. decomposing peroxides, scavenging radicals, inhibiting free radical chain reactions, metal chelation and decreasing local oxygen concentration. Hence, the overall activity of antioxidants needs to be evaluated by more than one method. Based on the aforesaid factors, this study evaluated and compared the antioxidant potential of breast and thigh meat of all black Kadaknath chicken and that of a commercial broiler (Cobb) for elucidating functional properties of unique Indian poultry breed.

MATERIALS AND METHODS

This is an inter-institutional study that was carried out during the period from February 2018 to March 2020. Birds were raised at Nanaji Deshmukh Veterinary Science University, Jabalpur and subsequent biochemical analysis was carried out at two ICAR institutes at Karnal; National Bureau of Animal Genetic Resources and National Dairy Research Institute.

Birds and meat extract preparation

Twenty male birds, each of indigenous Kadaknath and commercial broiler (Cobb 400) were reared at the experimental poultry farm of College of Veterinary Sciences and Animal Husbandry, Jabalpur, India under a deep litter system. The experiment was approved by the Institutional animal ethics committee (O No. 4040/Dean/Vety/2018 dated 18.12.2018). Similar standard management conditions were followed in the open-sided poultry houses. Birds were sacrificed at the respective age of slaughter; 8 weeks for Cobb and 20 weeks for Kadaknath following standard scientific procedures. Surface fat and connective tissue were dissected away from the primal cuts; breast and thigh and were ground with a meat grinder. Minced samples were covered with an aluminum sheet to avoid exposure to light and frozen at - 20°C in PE plastic bags. Before analysis, samples were defrosted at 20°C in a thermostatic bath. Two grams of meat was homogenized in 20 mL of phosphate-buffered saline (pH, 7.4) in an ice bath using a homogenizer (Benchmark Scientific D1000, USA) for preparation of meat extract. The homogenate was extracted in dark for 20 min at 4°C followed by centrifugation at 2,346 g for 15 min at 4°C. The solid residue was discarded.

Estimation of the functional property

All the reagents were from Sigma Chemical (St. Louis, MO, USA) and solvents were of analytical grade. The protein content of meat extract was determined by the Lowry method (Lowry *et al*., 1951) with Bovine serum albumin (BSA) as a standard.

In vitro anti-oxidation assays

Ferric reducing antioxidant power (FRAP) assay was conducted using the kit (EIAFECL2) from Invitrogen, Thermo Fischer scientific following manufacturer's instructions. The reduction of iron in the FRAP reagent led to the appearance of a blue product that was read at 560 nm. FRAP value was derived using the $FeCl_2$ standard curve that was linear between 200 and 1000 µM (r^2 value = 0.998). Results were expressed in mM Fe^{2+} / g of meat.

ABTS (2,2-azinobis (3-ethyl-benzothiazoline-6-sulfonic acid) antioxidant capacity was estimated following the method of Re *et al*. (1999) with some modi-fications. $ABTS^+$ cation bleaching rate of the sample was supervised at 735 nm. To generate the $ABTS^+$ radical stock solution, equal volumes of $ABTS^+$ solution (14 mM) and potassium persulfate solution (5.9 mM) were mixed and kept in the dark for 12 h at 23 ± 1°C. It was diluted to an absorbance (735 nm) of 0.70 ± 0.02 with distilled water at 30°C. Trolox (0-600 µM), the hydrophilic homolog of vitamin E was used as a standard reference to estimate the Trolox equivalent antioxidant capacity (TEAC).

DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity was elucidated by measuring the scavenging of DPPH radicals by the antioxidants by the method of Brand-Williams *et al*. (1995) with some modifications. In brief, 1 mL of extract was diluted with 1 mL of water and 1 mL of ethanolic DPPH solution (0.2 mM). It was vortexed and incubated for 40 min at room temperature in the dark followed by centrifugation at 4,500 rpm for 10 min at 4°C. The absorbance was measured at 517 nm. Scavenging of DPPH radicals (%) was expressed as [(control absorbance-sample absorbance)/ control absorbance] x 100. Where, control has ethanol instead of the sample.

The ability of the sample to reduce Cu^{2+} ion to Cu^+ was estimated by Cupric reducing antioxidative capacity (CUPRAC) assay with a kit (MAK-187, Sigma-Aldrich, USA). 100 µL of Cu^{2+} working solution was added to the 40 µL of the sample followed by mixing on a horizontal shaker and incubation in the dark for 90 min at room temperature. Absorbance was recorded at 570 nm. The total antioxidant capacity of meat extract was derived using Trolox standard curve (r^2 value = 0.997) and was expressed in mM Trolox equivalents (TE)/ g of tissue.

Metal chelating activity

Iron chelation activity was determined by measuring the formation of Fe^{2+} - Ferrozine complex by using the method of Dinis *et al*. (1994) with slight modifications. The reaction was started by the addition of 0.2 ml ferrozine (5 mM) to 100µl of extract having 100µl ferrous chloride solution (2 mM).

It was incubated at room temperature for 10 minutes and then the absorbance of Fe^{2+} - Ferrozine complex is measured at 562 nm. The chemical metal chelator Ethylene diamine tetra acetic acid (EDTA) was used as a positive control and calibration curve was drawn with the concentration ranging from 25-200 µM. Results were expressed both as the EDTA equivalent chelating capacity (µM EDTA/ g of tissue) and the iron chelating activity (% inhibition) which was calculated using following equation:

$$\text{Iron chelation activity (\%)} = \frac{\text{Absorbance of the control - Absorbance of the sample}}{\text{Absorbance of the control}} \times 100$$

Statistical analysis

Tests of significant differences were determined by Duncans' Post hoc or *t* test at 95% confidence level (P<0.05) using SPSS 10.0 software package (SPSS Inc., Chicago, IL, USA). Analytical results are expressed as means ± standard error of triplicate independent measurements that were analyzed by one-way ANOVA.

RESULTS AND DISCUSSION

Endogenous antioxidant systems are composed of non-enzymatic hydrophilic and lipophilic compounds such as vitamin E, vitamin C, carotenoids, ubiquinols, polyphenols, cellular thiols and enzymes like superoxide dismutase, catalase and glutathione peroxidase. Together, enzymatic and non-enzymatic antioxidant systems counteract the action of pro-oxidants in muscle tissues both in living animals and also after slaughter (Li *et al*., 2020). Antioxidative capacity gives a valuable indication of the functional property of meat. Most natural antioxidant or neo-formed antioxidants upon processing are multifunctional and in complex heterogeneous foods such as meat and meat products, their activity cannot be evaluated by a single method. Thus numerous methods have been proposed over the decades to estimate the antioxidative action of a plethora of food matrices (Liu *et al*., 2016). However, to date, no single method can be considered to be universally accepted for adequately describing the overall antioxidative potential of the food including meat. Two or more radical scavenging capacity assays are required to investigate heterogeneous samples since each assay involves different chemical mechanism(s) and may reflect different aspect(s) of their antioxidant properties. Therefore, we selected five different commonly accepted and validated methods for a robust comparative evaluation of Cobb and Kadaknath meat. These corresponded to both hydrogen atom transfer (HAT) and single electron transfer (ET) reactions (ABTS and DPPH). DPPH allows evaluation of the hydrogen-donating potency, ABTS radical scavenging estimates single electron transfer capabilities, FRAP and CUPRAC assay measures the reductive antioxidant power.

The primary importance of meat in human nutrition is related to its high-quality proteins that provide essential amino acids upon digestion. Proteins play an important role in antioxidant activity of meat by scavenging free radicals and chelating pro-oxidative metals (Serpen *et al*., 2012). Kadaknath meat was found to be protein-dense in comparison to that of the Cobb broiler (Table 1). Average protein content (g/100 g of wet weight) of Kadaknath breast (25.21 ± 0.31) and thigh (19.98 ± 0.29) meat was higher (P<0.05) than the corresponding values of Cobb breast (21.81 ± 0.39) and thigh (18.31 ± 0.20) meat. The breast meat was significantly (P<0.05) proteinaceous than the thigh meat. Kadaknath had previously been reported to have the highest protein content among the Indian chicken breeds (Mohan *et al*., 2008). Protein concentration (g/100g) in Kadaknath breast meat (25.21) was even higher than the only other report on all black chicken, Silky fowl of China (22.8) (Wang *et al*., 2018). Concurrent to our findings, higher protein than the commercial broilers have been reported in Korean indigenous (Jayasena *et al*., 2013) and Brazilian free-range chicken (Da Silva *et al*., 2017) that was attributed to differences in their growth rate. Therefore, slow-growing Kadaknath chicken can be considered an excellent source of protein in the human diet.

The antioxidant potential of breast and thigh meat extracts has been summarized in Table 1. A comparison of the breast and thigh cuts has been depicted in Fig 5. Chicken breast constitutes mainly type-IIB glycolytic fiber (fast-twitch) whereas, the thigh has type I oxidative fibers (slow-twitch) (Intarapichet and Maikhunthod, 2005). Therefore, both the breast and thigh muscles as a typical representative of white and red muscles were investigated.

Table 1: Protein and antioxidant potential of breast and thigh meat extracts of Kadaknath and commercial broiler (Cobb 400).

Group	Protein (g/100 g of tissue)	FRAP (mM Fe^{2+} /g of tissue)	ABTS		DPPH (% inhibition)	CUPRAC (mM TE /g of tissue)	Metal chelation activity (% inhibition)
			% inhibition	TE (mM)/ g of tissue			
Breast meat							
Cobb broiler	21.81±0.39^{a}	15.24±0.40^{a}	43.78±1.47^{a}	6.06±0.26^{a}	70.56±0.59^{a}	9±0.24^{a}	53.63±1.79 ^{a}
Kadaknath Chicken	25.21±0.31^{b}	26.97±0.37^{b}	52.72±1.42^{b}	7.31±0.24^{b}	73.26±0.70^{b}	12.76±0.35^{b}	62.71±0.99 ^{b}
Thigh meat							
Cobb broiler	18.31±0.20^{a}	19.2±0.31^{a}	29.62±1.27^{a}	3.74±0.21^{a}	63.46±0.56^{a}	7.15±0.24^{a}	75.07±0.98^{a}
Kadaknath chicken	19.98±0.29^{b}	33.85±0.47^{b}	30.14±1.00^{a}	3.65±0.17^{a}	66.75±0.55^{b}	8.76±0.22^{b}	80.75±0.95^{b}

Values are mean ± Standard error. Values with different superscripts within the same column differ significantly (P<0.05).

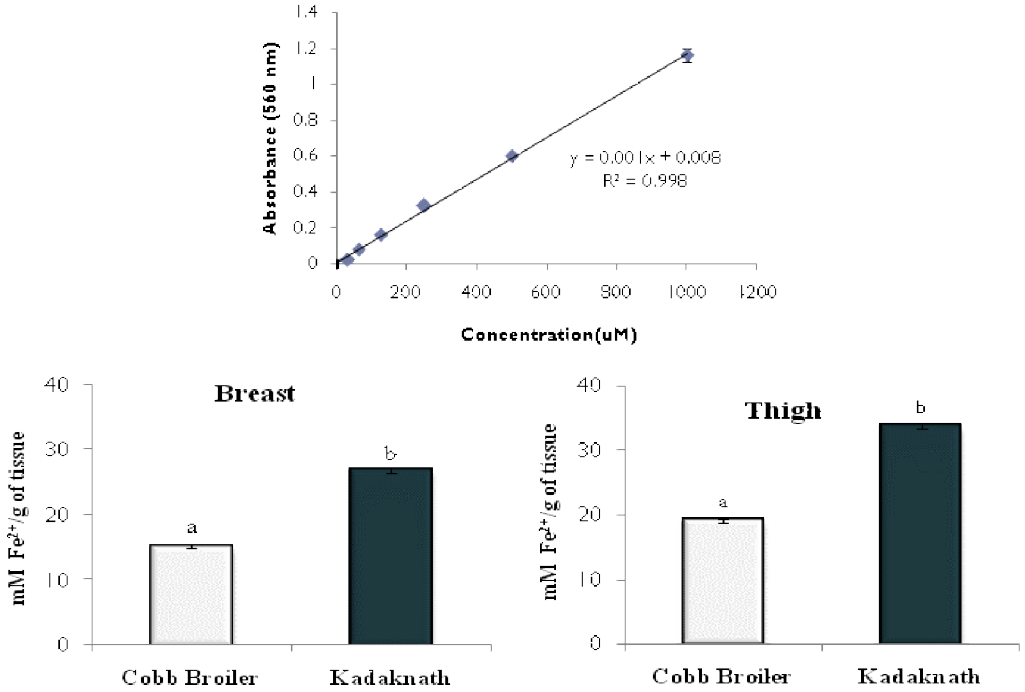

Fig 1: FRAP value of meat extracts from commercial Cobb broiler and Kadaknath chicken (N=20, each). A standard curve of $FeCl_2$ was used (0-1000 µM). Different superscripts within the same graph differ significantly (P<0.05).

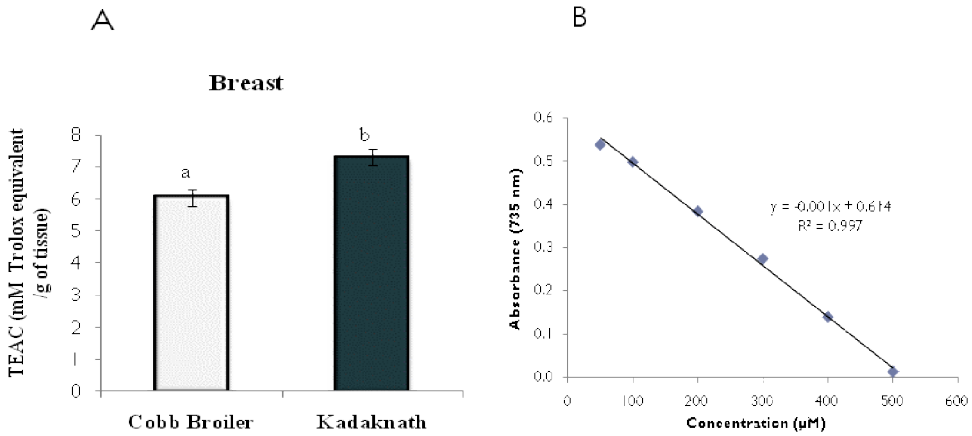

Fig 2: Trolox equivalent antioxidative capacity (TEAC) of meat extracts A) breast tissue TEAC values B) Trolox (100-500 µM) standard curve. Different superscripts within the same graph differ significantly (P<0.05).

Kadaknath meat extract showed a higher degree of Fe^{+3} reduction than the Cobb (P<0.05). Its FRAP value (mM Fe^{2+}/ g of tissue) for breast and thigh meat was 26.97 ± 0.37 and 33.85 ± 0.47, respectively. Corresponding values in Cobb broiler were 15.24 ± 0.40 (breast) and 19.20 ± 0.31 (thigh). The high values of FRAP could be attributed to the abundance of antioxidants in Kadaknath meat that reduce ferric ion to its ferrous form. Interestingly, thigh extract exhibited higher FRAP values than the breast (Table 1). The current findings are concordant with the work of Serpen *et al*. (2012) who reported FRAP value to be higher in chicken meat. It was superior to the values reported for the pork, fish and goat meat.

Similarly, $ABTS^{+}$ radical scavenging activity was also significantly (P<0.05) more in Kadaknath breast meat extract. The TEAC value (mM Trolox equivalents/ g of wet weight) were 6.06 ± 0.26 (43.78% inhibition) and 7.31 ± 0.24 (52.72% inhibition) in Cobb and Kadaknath, respectively (Fig 2). Once again, TEAC in the thigh was approximately half of the breast extract (Fig 5).

DPPH scavenging activity (% inhibition) was also significantly higher in Kadaknath breast (73.26 ± 0.7) as compared to that of the Cobb broiler (70.56 ± 0.59). The same trend was recorded in the thigh (Fig 3). Breast meat had significantly higher (P<0.05) potential for scavenging DPPH radical than the thigh meat (Fig 5). The observations

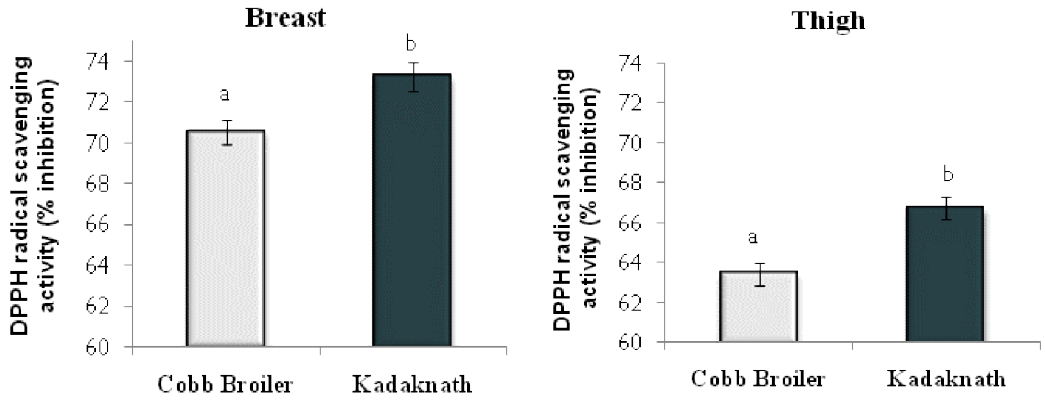

Fig 3: DPPH· free radical scavenging capacity of commercial Cobb broiler and Kadaknath chicken meat extracts (N=20, each). Different superscripts within the same graph differ significantly (P<0.05).

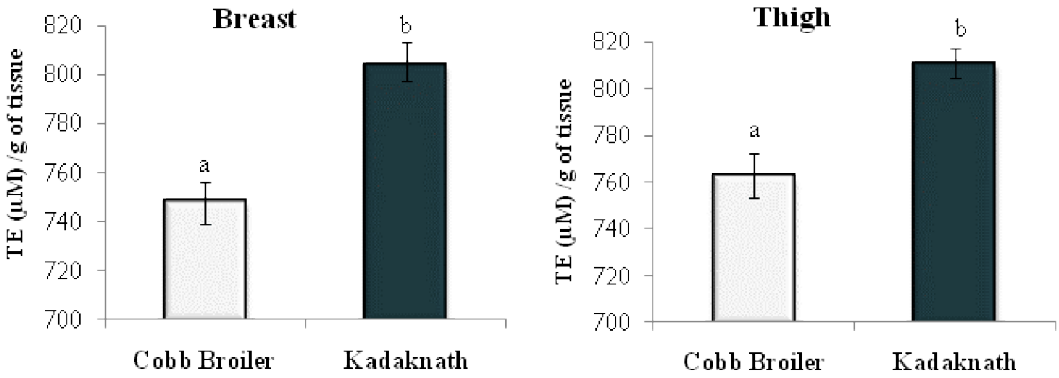

Fig 4: Total antioxidant capacity (CUPRAC) of breast and thigh meat extracts. Different superscripts within the same graph differ significantly (P<0.05).

were parallel to the previous report (71.0%) in breast and thigh extract of chicken (Huang and Kuo, 2000) and it was significantly superior to beef, pork and fish meat (Serpen *et al*., 2012).

CUPRAC value pointed towards the bigger total antioxidant capacity of Kadaknath breast, as well as, thigh meat extract in comparison to the respective extracts of Cobb broiler (Table 1). Among the two meat cuts, the breast was a more potent antioxidative agent (Fig 4).

The superior antioxidation capacity of Kadaknath meat in comparison to the commercial broiler can be a collective effect of different antioxidants. It has been demonstrated that proteins and peptides have an important antioxidant action in meats due to their ability to scavenge free radicals and chelate prooxidative metals (Mirzaee *et al*., 2017). Thus, high protein concentration could be one of the reasons for the high antioxidant capacity of Kadaknath meat. Various endogenous molecules including functional dipeptides such as carnosine and anserine and aromatic amino acids can interrupt the oxidative chain (Manihaini *et al*., 2013). Similarly, cysteine, a non-aromatic amino acid can directly interact with free radicals (Sarmadi and Ismail 2010) while, polyamines can also contribute towards radical scavenging activity (Sacchetti *et al*., 2008). Chicken meat is reported to be enriched in carnosine and anserine and has strong antioxidant activities, more than that of beef and pork (Serpen *et al*., 2012). Among different chicken breeds, dark chicken meat from all black silky fowl has been reported to have higher histidine dipeptides, thus higher antioxidant activity (Kojima *et al*., 2014).

In the current investigation, the antioxidant potential of breast meat was superior to the thigh meat extract for DPPH, ABTS and CUPRAC assays (Fig 5). These differences may be ascribed to the protein content that is higher in the breast. Moreover, breast and thigh meat have different concentrations of antioxidant functional dipeptides. The breast meat extract of Silky fowl had elevated carnosine (4.03 fold) and anserine (3.59 fold) than the thigh (Kojima *et al*., 2014). More fat in the thigh may also be responsible for low scavenging activity (Sacchetti *et al*., 2008). FRAP assay reflected the opposite trend. Iron and iron-binding proteins are associated with free radical abolition and inhibition (Kojma *et al*., 2014). It might be the reason for the better reducing ability (FRAP value) of the thigh meat. Anatomically the breast (white) and thigh (red) muscles are different. Myoglobin content and capillary density are also more abundant in the red muscles.

Chelation of pro-oxidant metal is among the significant mechanism of action of antioxidants. Iron enhances oxidation as it acts as the catalyst for free radical reaction. They catalyze the formation of radical oxygen species and stimulate lipid oxidation (Damgaard *et al.* 2014). Complex

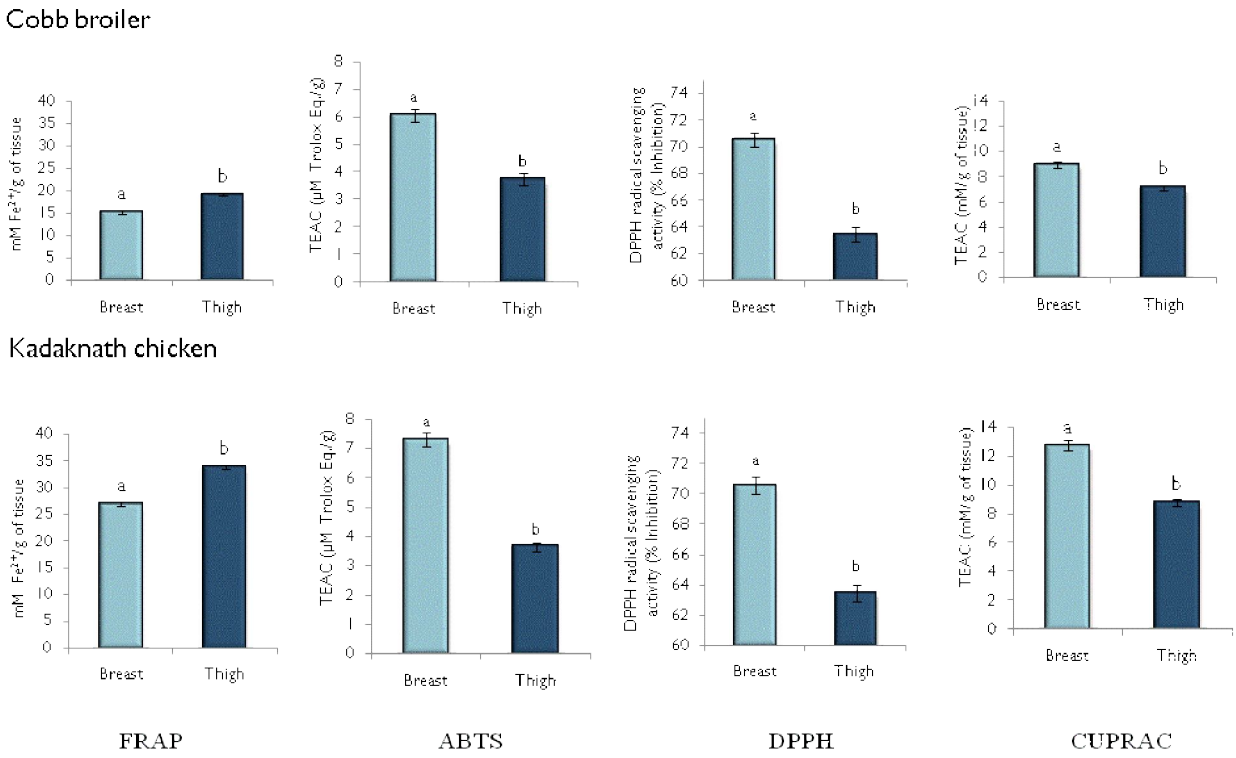

Fig 5: Comparative antioxidant potential of two types of meat cuts (breast and thigh). Values are mean ± Standard error. Different superscripts within the same graph differ significantly (P<0.05).

formation of iron with organic compounds decreases its pro oxidant impact by stabilizing oxidized form of the iron. Results (Table 1) equivocally supported the better antioxidant potential of Kadaknath breast and thigh over and above that of the Cobb due to high metal chelation capability. Inhibition was more than sixty percent in breast (62.71) and more than eighty percent (80.75) in the thigh extract of Kadaknath whereas; it was only 53.63% in breast of Cobb (Table 1).

From this research study, it emerged that Kadaknath chicken meat is a better candidate for the dietary antioxidants than the Cobb broiler to shield the human body from adverse effects of oxidative stress and hence the risk of various degenerative diseases. Black chicken is supposed to have medicinal utility and have been utilized to boost the human immune system, cure diabetes, check emaciation and treat female reproductive ailments (Li *et al*., 2020). Their meat is an enriched source of functional dipeptides (carnosine and anserine) that are considered to be the key components for the bioactivity of meat. Carnosine has antiaging, antiglycation, antioxidation and neurotransmitter functions (Jung *et al*., 2013). Anserine, an N-methylated derivative of carnosine, has similar biological activities. Silky fowl skeletal muscle carnosine content was 1.6 - 2.3 fold higher (P<0.05) and had more antioxidative capacity than the other chicken species (Kojima *et al*., 2014). The same may be the case with the Kadaknath black chicken that might be favorable for its restorative and medicinal functions and demands urgent in-depth scientific investigations. Indigenous breeds of chicken, raised under free-range backyard conditions such as Kadaknath, play a vital role in rural economies of developing countries like India. Moreover, they are an integral component of a sustainable farming system, a source of high-quality animal protein, remuneration and contribute to women empowerment in addition to the momentous role in the village socio-cultural life. Increasing scientific knowledge of breed characteristics and the quality of their products including nutritional characteristics will act as a fundamental step in the development of a brand name for Kadaknath products.

CONCLUSION

Kadaknath meat had a higher antioxidant capacity than the meat of commercial broiler. These findings valorize unique Kadaknath chicken by providing new knowledge about their meat quality and prospects of exploiting the ever-growing market potential of consumers that are increasingly interested in nutritional and therapeutic quality, animal welfare and environmental sustainability, which in turn, will also support Kadaknath breed conservation.

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