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RESEARCH ARTICLE

Antioxidant and Oxidant Profiles in Thigh and Breast Meat of Pakistani Domestic Chicken Breeds

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ABSTRACT

Chicken meat is preferred due to its low-fat content, and superior protein value. The increasing awareness of consumers about food health benefits has served as driving force to identify nutritionally enriched poultry breeds. In this view, the objectives of the current study were to identify better domestic backyard chicken breeds through comparative antioxidant and oxidant profiling of most consumed thigh and breast meat. Twenty healthy birds (10 male and 10 female) of each chicken breed i.e., Aseel (As), Misri Gold (MG), Fayoumi (Fa) and Naked Neck (NN) were reared as scavengers till 6 months of age and then slaughtered. A boneless chunk/cube of meat from both breasts (white cut/pectoralis major) and thigh muscles (dark cut/biceps femoris) were used for analysis. The mean live weight (1423.40±26.0g) bleeding weight (1401.50±22.70g) and carcass weight (925.20±08.39) was maximum in male MG birds. While in female birds, live weight (1144.10±48.70g) and bleeding weight (1115.90±49.20g) was highest in As and defeathered weight in MG (899.60±10.90). Breast (pectoralis major) and thigh (Biceps femoris) meat from male and female birds was compared for biochemical profiles. Male birds breast meat depicted significantly higher superoxide dismutase (SOD) (145.99±4.01), total flavonoids (TF) (150.86±1.28) in MG, catalase (385.00±5.00) peroxidase (POD) (2972.20±41.80) in NN. While ascorbate peroxidase (APX) (2075.00±75.00) and total antioxidant capacity (TAC) (12.93±0.33) and lowest TOS (1170.00±10.00) value was observed in Fa and MDA content (197.55±2.45) in NN. Female birds breast meat, had highest SOD (121.93±3.07), POD (6723.60±69.60) and APX (967.50±7.50) in MG, CAT (495.00±5.00), TF (129.31±0.94) and TAC (15.70±0.43) in Fa. While least TOS (1230.00±15.00) was in As and minimum MDA (279.53±53) in MG. In male birds thigh meat, SOD was found highest (168.16±1.84) in MG, CAT (505.00±5.00) in Fa, POD (4074.00±78.00) and TF (128.18±1.93) in As, APX (807.50±7.50) in NN. However, lowest TOS (1295.00±5.00) was in Fa and MDA in As (176.71±3.29). Female birds thigh meat, had highest SOD (167.76±2.24), APX (1507.50±12.50) in Fa, CAT (467.00±7.00) in As, POD (2204.70±59.70), TF (132.81±2.32) in MG, TAC (14.24±0.32) in NN and lowest TOS (1052.50±7.50) and MDA (171.39±2.61) in MG. Chicken breeds MG and Fa can prove to be a potential source of cheap protein and provide substantial health benefits along with cheap income generation for small rural households in developing countries.

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INTRODUCTION

Poverty is an intricate and multidimensional phenomenon which can be subjected to a variety of socio-economic components (Chishti, Rehman, and Murshed, 2021). To conquer the socio-economic advancement, one of the recognized key factors is poverty alleviation (Szirmai, 2015). Over 805 million inhabitants of the world

does not have adequate food supply at their disposal (The World Bank, 2021) where poultry plays a vital role in resource-limited rural and peri-urban areas of developing countries like Pakistan.

As per recommendations of the World health organization (WHO), a single person requires at least 27g of animal proteins on daily basis out of which 5g comes from poultry in Pakistan (Memon, 2012; Hussain *et al.*, 2015). Chicken meat consumption is moving higher, reaching ~31% globally thanks to being cost effective and higher nutritional value with low saturated fats, high proteins and low caloric content, making it a superior rather more desirable choice (Muhlisin *et al.*, 2016b; Kralik *et al.*, 2018).

High quality proteins are obtained mainly from meat which not only provides essential amino acids but vitamins, minerals, and unsaturated fatty acids (Serpen, Gökmen, and Fogliano, 2012). During 1960's commercial poultry production started in Pakistan and has been providing a noteworthy portion of daily proteins to the indigenous population (Hussain *et al.*, 2015). At times, when COVID-19 has struck hard the whole world's economy and skyrocketing prices of red meat, developing countries like Pakistan are in a dire need to study and exploit the potential of native/domestic chicken breeds using the available resources. To cope up, identification of native backyard poultry potential can be a mean of, not only the provision of cheap and quality protein but also helpful in poverty alleviation through cheap backyard farming due to their higher disease resistance and better adaptability to local climate conditions (Usman *et al.*, 2014).

Government of Pakistan is already focusing on designing cheap backyard poultry farming projects for small household living below national poverty line. Provision of cheap and quality protein is a major concern for developing countries like Pakistan. Lipid oxidation plays a major role in affecting not only the flavour, texture, colour, and aroma of meat but also its nutritional value causing harmful effects to human body (Reitznerová *et al.*, 2017). Present study is designed to investigate physiochemical properties and provide a comparative analysis among both male and female birds of commonly reared domestic poultry breeds. Histochemical studies have revealed that meat can be categorized metabolically into two types based on their muscle fibers i.e., oxidative (red/dark) and glycolytic (white) (Lawrie and Ledward, 2006). The present study intended to identify better domestic backyard chicken breeds through comparative antioxidant and oxidant profiling of thigh and breast meat from both genders. We also tried to shed light whether domestic backyard poultry breeds (DBPB) have the potential for low-cost antioxidant enriched protein source.

MATERIALS AND METHODS

Animals and husbandry: The four most famous domestic chicken breeds i.e., Aseel (As), Fayoumi (Fa), Misri Gold (MG) (Fa x RIR) and Naked Neck (NN), reared as backyard poultry in Pakistan, were selected for this study. Birds were collected from hatchery and were reared in an open system in a village house located in rural area of Faisalabad, Punjab, Pakistan. Birds were allowed to move freely, and no feed was given manually except water in utensils to mimic the environment provided by typical rural small household. A total of 20 clinically healthy birds (10 males and 10 females) of each breed were collected after 6 months of age since hatching and were brought to the anatomy lab, Faculty of Veterinary Science, University of Agriculture, Faisalabad, Pakistan.

Sample collection: Birds were slaughtered in compliance to the guidelines and recommendations of the Office of Research, Innovation and Commercialization and Animal Ethical Committee of University of Agriculture, Faisalabad. The initial weight of each alive bird was recorded as live weight and after slaughtering, letting heart pump out all the blood, weight of each bird was again observed, referring to bled weight. Feathers with skin were then removed and weighed again to see defeathered weight and after removing all inedible parts, carcass/dressed weight was recorded for each bird. A chunk/cube of (1cm x 1cm x 1cm) meat from both chest (white cut/pectoralis major) and thigh muscles (dark cut/biceps femoris) was collected, without bony tissue, immediately after slaughtering of bird in labeled zipper bags and was stored in refrigerator at -20℃ to determine its antioxidant enzyme activity.

Antioxidant enzyme extraction: Enzymes were extracted from thigh and breast muscles using the following procedure. The procedure was performed twice for each sample. Each sample (0.5g) was mixed with 2 mL of ice-cold phosphate buffer (extraction solvent, pH 7.0,50mM; disodium phosphate heptahydrate (Na2HPO4.7H2O) and KH2PO4). Samples were homogenized and then centrifuged (4500g, 40min, 4°C) and the supernatant was recovered. The resulting extract was used to analyze the activities of different enzymes being discussed here.

Superoxide Dismutase (SOD) Activity: Samples were estimated for superoxide dismutase activity assayed by measuring its ability to inhibit the photochemical reduction of nitro blue tetrazolium (NBT) according to the method of (Giannopolitis and Ries, 1977). The reaction solution (3mL) contained 50µM NBT, 1.3µM riboflavin, 13mM methionine,75nM EDTA, 50mM potassium phosphate buffer (pH 7.8) and 50µL sample. The photo-induced reaction was performed in an aluminum foil lined box fitted with a 15W fluorescent lamp. The absorbance of the irradiated solution at 560nm was determined with a spectrophotometer (Hitachi U-2800, Tokyo, Japan). One unit of SOD activity was defined as the amount of enzyme which caused 50 % inhibition of photochemical reduction of NBT.

Catalase (CAT) activity: Catalase activity in samples was assayed by a method described by (Beers and Sizer, 1952).For measurement of CAT activity, the assay solution contained 50mM phosphate buffer (pH 7.0), 59mM H2O2, and 0.1mL enzyme extract. The decrease in absorbance of the reaction solution at 240nm was recorded after every 20s. The absorbance change of 0.01 min-1 was defined as 1 U of CAT activity.

Peroxidase (POD) activity: POD activity was determined by using method described by (Chance and Maehly, 1955) with few amendments. For measurement of peroxidase activity, the assay solution contained distilled water (545µ L), 200 mM phosphate buffer (pH 7.0), 200mM guaiacol, 400 mM H_2O_2, and 15µL sample. The reaction was started after adding the sample. The increase in absorbance of the reaction solution at 470nm was recorded after every 20 seconds. One unit of POD activity was defined as an absorbance change of 0.01min-1.

Ascorbate peroxidase (APX) activity: APX activity was measured using the following method. Assay buffer was prepared by mixing 200mM potassium phosphate buffer (pH 7.0),10mM ascorbic acid and 0.5M EDTA. For measurement of APX, the activity assay solution contained assay buffer made up of 10mM ascorbic acid, 0.5M EDTA and 200mM potassium phosphate buffer, H2O2 (1mL), and supernatant 50µL. The oxidation rate of ascorbic acid was estimated by following the decrease in absorbance at 290nm after every 30seconds (Chen and Asada, 1989).

Non-enzymatic antioxidants

Total Flavonoid Content (TFC): The total flavonoid content was determined according to the aluminum chloride colorimetric method (Lin and Tang, 2007). The samples were mixed with 0.1mL of 10% aluminum chloride hexahydrate, 0.1mL of 1M potassium acetate and 2.8mL of deionized water. After the 40minutes incubation at room temperature, the absorbance of the reaction mixture was determined spectrophotometrically at 415 nm. Rutin (a flavanol) was used as a standard (concentration range: 0.005 to 0.1mg/mL) and the total flavonoid content was expressed as milligram RE per g of samples. The absorbance at 415nm = 14.171rutin (mg/mL) + 0.0461, R2 = 0.9991.

Total Antioxidant Capacity (TAC): The reduction of 2, 2-azino-bis (3-ethylbenzothiazoline-6-sulfonate) radical cation (ABTS•+ that is blue to green in color) by antioxidants to its original colorless ABTS form is the basis of the ABTS assay. The ABTS•+is decolorized by antioxidants according to their antioxidant content (Nenadis, Lazaridou, and Tsimidou, 2007). The assay mixture contained reagent R1 (mixture of sodium acetate buffer solution and glacial acetic acid, pH5.8), sample extract and reagent R2 (mixture of sodium phosphate buffer solution, glacial acetic acid, hydrogen peroxide and ABTs). The contents of the tubes were mixed and allowed to stand for 6 min. Absorbance was measured at 660nm. The ascorbic acid was used to develop a calibration curve. The TAC values were expressed as milli-molar ascorbic acid equivalent to L-1.

Other biochemical parameters

Total Oxidant Status (TOS): Total oxidant status (TOS) was determined by referring the method of (Erel, 2005) in which the oxidation of ferrous ion into ferric ion by oxidants present in the sample in an acidic medium and the measurement of ferric ion by xylenol orange (Hameed *et al.*, 2005). The assay mixture contained reagent R1, reagent R2 and sample extract. After 5 min, the absorption was measured at 560nm by using spectrophotometer. A standard curve was prepared using hydrogen peroxide. The results were expressed in µM H2O2 equivalent/L.

Malondialdehyde (MDA) content: The level of lipid peroxidation in the samples was measured in terms of malondialdehyde (1,3-propanedial) or MDA (a product of lipid peroxidation) content determined by the thiobarbituric acid (TBA) reaction using method of (Heath and Packer, 1968) with minor modifications as described by (Dhindsa, Plumb-dhindsa, and Thorpe, 1981). A 25µL sample was homogenized in 0.1% TCA. The homogenate was centrifuged at 14,462 × g for 5min. To 1 m aliquot of the supernatant 20% TCA containing 0.05% TBA were added. The mixture was heated at 95℃ for 30min and then quickly cooled in an ice-bath. After centrifuging at 14,462 × g for 10 min, the absorbance of the supernatant at 532 nm was read and the value for the non-specific absorption at 600 nm was subtracted. The MDA content was calculated by using extinction coefficient of 155 mM−1 cm−1.

Statistical analysis was performed using XL-STAT software version 2014.1.02 (Copyright Addinsoft 1995–2012) as described previously (Kausar *et al.*, 2023; Noreen *et al.*, 2024). To analyze and organize the resulting data, descriptive statistics were applied. Data were subjected to analysis of variance (ANOVA) with three replications. Tukey HSD test at a p-value of <0.05, and ANOVA was used to test the significance of the data. The values presented are mean±SE.

RESULTS

Comparative gross weight: The means (±SEM) of gross weight parameters i.e., live, bleeding, defeathered, and carcass weight of male and female groups are presented in table 1 and table 2 respectively.

Live weight: The mean live weight was recorded maximum (1423.40±26.0) in MG while minimum (1068.50±69.00) in NN was recorded in male birds while no significant (P≥0.05) difference was observed between live weight of Fa (1290.00±42.1) and As (1281.00±101.00). In female birds, mean live weight was observed highest in As (1144.10±48.70) followed by MG (1103.20±16.90), Fa (1037.40±42.30) and lowest in NN (585.40±20.00).

Bleeding weight: The bleeding weight was reported maximum (1401.50±22.70) in MG while minimum (1044.90±66.30) in NN among male birds where non-significant (P≥0.05) difference was observed between bleeding weight of Fa (1256.70±37.10) and As (1255.20±96.40). Among female birds, mean bleeding weight was observed highest in As (1115.90±49.20) followed by MG (1079.00±16.00), Fa (1005.80±38.50) and lowest in NN (568.40±18.30).

Defeathered weight: Statistically non-significant (P≥0.05) difference was observed in defeathered weight in As (1024.00±68.90g), Fa (992.40±40.70g), NN (873.60±59.80g) and MG (1065.00±19.50g) among male birds. Among females, highest defeathered weight was

Table 1: Comparative values for different weight parameters (Mean±SEM) among male chicken of studied breeds.

Groups	Live Weight	Bled Weight	Defeathered Weight	Carcass Weight	Dressing %age
	(g)	(g)	(g)	(g)	(%)
Aseel	1281.00±101.00^{ab}	1255.20±96.40^{ab}	1024.00±68.90^{a}	811.80±59.50^{ab}	63.37
Fayoumi	1290.00±42.10^{ab}	1256.70±37.10^{ab}	992.40±40.70^{a}	812.20±41.80^{ab}	62.96
Misri Gold	1423.40±26.00^{a}	1401.50±22.70^{a}	1065.00±19.50^{a}	925.20±08.39^{a}	64.99
Naked Neck	1068.50±69.00^{b}	1044.90±66.30^{b}	873.60±59.80^{a}	711.40±57.60^{b}	66.58

Different superscripts in same column indicates their statistically significant difference from each other at 5% level of significance.

Table 2: Comparative values for different weight parameters (Mean±SEM) among female chicken of studied breeds.

Groups	Live Weight	Bled Weight	Defeathered Weight	Carcass Weight	Dressing %age
	(g)	(g)	(g)	(g)	(%)
Aseel	1144.10±48.70^{a}	1115.90±49.20^{a}	868.80±36.00^{a}	683.00±36.40^{a}	59.70
Fayoumi	1037.40±42.30^{a}	1005.80±38.50^{a}	842.70±32.5^{a}	647.70±21.90^{a}	62.43
Misri Gold	1103.20±16.90^{a}	1079.00±16.00^{a}	899.60±10.90^{a}	651.70±32.10^{a}	59.07
Naked Neck	585.40±20.00^{b}	568.40±18.30^{b}	472.10±18.20^{b}	345.20±14.20^{b}	58.97

Different superscripts in same column indicates their statistically significant difference from each other at 5% level of significance.

observed in MG (899.60±10.90g) followed by As (868.80±36.00g) and Fa (842.70±32.5g) and lowest (472.10±18.20g) in NN.

Carcass weight: In male birds, carcass weight of MG (925.20±08.39g) was recorded maximum followed by Fa (812.20±41.80g), As (811.80±59.50g) and minimum in NN (711.40±57.60) while in female group, statistically non-significant (P≥0.05) difference was observed between As, MG and Fa where As recorded highest (683.00±36.40) carcass weight followed by MG (651.70±32.10) and Fa (647.70±21.90). NN recorded the lowest (345.20±14.20) carcass weight among female groups of all breeds.

Pectoralis major/Breast/White Meat Cut analysis among male groups: Biochemical analysis of PM of each male group is presented in table 3.

Significant (P≤0.05) difference was found between all groups in their mean enzymatic biochemical analysis of breast muscle meat. Superoxide Dismutase (SOD) activity was observed highest (145.99±4.01) in MG followed by As (113.08±1.92), Fa (85.57±4.43) and NN reported the lowest (83.47±1.53) mean SOD activity. Non-significant (P≥0.05) difference was observed between Fa (85.57±4.43) and NN (83.47±1.53).

Mean catalase activity among males differed significantly (P≤0.05) between all studied chicken breeds. Maximum (385.00±5.00) catalase activity was observed in NN followed by As (245.50±5.50), MG (217.50±2.50) while Fa reported the minimum (185.00±5.00).

Mean peroxidase (POD) activity differed significantly (P≤0.05) among all studied chicken breeds. NN recorded highest (2972.20±41.80) mean peroxidase activity followed by Fa (2714.30±16.30), MG (1946.50±51.50) and the lowest (1703.00±38.00) reported by As.

Mean ascorbate peroxidase (APX) activity among males was reported highest (2075.00±75.00) in Fa followed by MG (992.50±7.50), As (565.00±5.00) and lowest (445.00±5.00) mean activity was reported in NN. Non-significant (P≥0.05) difference was observed between As (565.00±5.00), and NN (445.00±5.00) mean APX activity.

Total Flavonoid Content (TFC) in PM of male groups were reported highest in MG (150.86±1.28) followed by Fa (135.59±0.86), As (118.80±1.56) and lowest (112.33±0.92) in NN. A Non-significant (P≥0.05) difference was observed between As (118.80±1.56) and NN (112.33±0.92) in their relative TF content.

Total antioxidant capacity (TAC) of Fa (12.93±0.33) and NN (12.86±0.60) differed non-significantly (P≥0.05) between both groups but differed significantly (P≤0.05) from the remaining groups. Maximum (12.93±0.33) TFC was recorded in Fa while minimum (1.89±0.32) in As.

Other biochemical parameters like total oxidant status (TOS) differed significantly (P≤0.05) among all studied chicken breeds with maximum (1255.00±5.00) value recorded by MG while minimum (1170.00±10.00) recorded by Fa.

Non-significant (P≥0.05) difference was observed in Malondialdehyde (MDA) content of all studied chicken breeds. As reported highest (306.52±46.52) while NN reported lowest (197.55±2.45) MDA content in their PM meat among male groups.

Pectoralis major (Breast) meat analysis among female groups: Biochemical analysis of PM of each female group is presented in table 4.

Significant (P≤0.05) difference was found between all groups in their mean enzymatic biochemical analysis of breast muscle meat. Superoxide Dismutase (SOD) activity was observed highest (121.93±3.07) in MG followed by As (120.63±1.36) and Fa (61.91±3.09) while NN reported the lowest (58.40±1.60) mean SOD activity.

Mean CAT activity among females differed significantly (P≤0.05) between studied chicken breeds. Maximum (495.00±5.00) CAT activity was observed in Fa followed by NN (337.50±2.50) and MG (242.50±2.50) while As reported the minimum (242.50±2.50). Non-significant (P≥0.05) difference in mean CAT activity was observed between MG (242.50±2.50) and As (242.50±2.50).

Mean peroxidase (POD) activity differed significantly (P≤0.05) among all studied chicken breeds. MG recorded highest (6723.60±69.60) peroxidase activity followed by Fa (2231.20±33.20) and As (2198.90±1.10) while the lowest (1709.50±44.50) was reported by NN. Non-significant (P≥0.05) difference was observed in the mean POD activity of Fa (2231.20±33.20) and As (2198.90±1.10).

Mean ascorbate peroxidase (APX) activity among females was reported highest (967.50±7.50) in MG followed by Fa (845.00±5.00), As (570.00±10.00) and lowest (565.00±5.00) in NN. Non-significant (P≥0.05) difference was observed between As (570.00±10.00) and NN (565.00±5.00) mean APX activity.

Table 3: Comparative profiles of oxidants and antioxidants in breast (pectoralis major) meat among male chicken of studied breeds.

Groups	Enzymatic				Non-enzymatic	Oxidants		
	SOD	Catalase	POD	APX	TF	TAC	TOS	MDA
	(Units/g f. wt.)	(µM/g f. wt.)	(µM/g f. wt.)	(µM/g f. wt.)	(Rutin equl. µg/ml)	(µM/g f. wt.)	(µM/g f. wt.)	(µM/g f. wt.)
Aseel	113.08±1.92^{b}	245.50±5.50^{b}	1703.00±38.00^{d}	565.00±5.00^{c}	118.80±1.56^{c}	1.89±0.32^{c}	1175.00±10.00^{bc}	306.52±46.52^{a}
Fayoumi	85.57±4.43^{c}	185.00±5.00^{d}	2714.30±16.30^{b}	2075.00±75.00^{a}	135.59±0.86^{b}	12.93±0.33^{a}	1170.00±10.00^{c}	277.81±2.19^{a}
Misri Gold	145.99±4.01^{a}	217.50±2.50^{c}	1946.50±51.50^{c}	992.50±7.50^{b}	150.86±1.28^{a}	4.55±0.07^{b}	1255.00±5.00^{a}	262.56±2.43^{a}
Naked Neck	83.47±1.53^{c}	385.00±5.00^{a}	2972.20±41.80^{a}	445.00±5.00^{c}	112.33±0.92^{c}	12.86±0.60^{a}	1217.50±2.50^{ab}	197.55±2.45^{a}

Different superscripts in same column indicates their statistically significant difference from each other at 5% level of significance.

Table 4: Comparative profiles of oxidants and antioxidants in breast (pectoralis major) meat among female chicken of studied breeds.

Groups	Enzymatic				Non-enzymatic	Other		
	SOD	Catalase	POD	APX	TF	TAC	TOS	MDA
	(Units/g f. wt.)	(µM/g f. wt.)	(µM/g f. wt.)	(µM/g f. wt.)	(Rutin equl. µg/ml)	(µM/g f. wt.)	(µM/g f. wt.)	(µM/g f. wt.)
Aseel	120.63±1.36^{a}	242.50±2.50^{c}	2198.90±1.10^{b}	570.00±10.00^{c}	116.02±1.43^{b}	7.23±0.09^{b}	1230.00±15.00^{a}	354.97±5.03^{a}
Fayoumi	61.91±3.09^{b}	495.00±5.00^{a}	2231.20±33.20^{b}	845.00±5.00^{b}	129.31±0.94^{a}	15.70±0.43^{a}	1292.50±7.50^{a}	342.23±7.77^{a}
Misri Gold	121.93±3.07^{a}	242.50±2.50^{c}	6723.60±69.60^{a}	967.50±7.50^{a}	109.51±0.75^{c}	13.99±0.53^{a}	1287.50±12.50^{a}	279.53±5.47^{b}
Naked Neck	58.40±1.60^{b}	337.50±2.50^{b}	1709.50±44.50^{c}	565.00±5.00^{c}	116.25±0.59^{b}	8.02±0.44^{b}	1252.50±7.50^{a}	294.77±5.23^{b}

Different superscripts in same column indicates their statistically significant difference from each other at 5% level of significance.

TF content of PM among female groups was observed highest in Fa (129.31±0.94) followed by NN (116.25±0.59), As (116.02±1.43) and lowest (109.51±0.75) in MG. A Non-significant (P≥0.05) trend of difference was observed between NN (116.25±0.59) and As (116.02±1.43) in their relative TF content.

Maximum (15.70±0.43) total antioxidant capacity (TAC) was reported in Fa and minimum (7.23±0.09) in As whereas non-significant (P≥0.05) difference was observed between Fa (15.70±0.43) and MG (13.99±0.53) as well as between NN (8.02±0.44) and As (7.23±0.09).

TOS in PM of female groups differed non-significantly (P≥0.05) among all groups. Fa reported maximum (1292.50±7.50) while As reported minimum (1230.00±15.00) TOS.

Maximum (354.97±5.03) Malondialdehyde (MDA) content was reported in As while minimum (279.53±53) in MG. A Non-significant (P≥0.05) difference was observed between As (354.97±5.03) and Fa (342.23±7.77) as well as between NN (294.77±5.23) and MG (279.53±5.47).

Biceps femoris (Thigh) meat analysis among male groups: Biochemical analysis of BF of each male group is presented in table 5.

Significant (P≤0.05) difference was reported between all groups in their mean enzymatic biochemical analysis of thigh muscle meat. Superoxide Dismutase (SOD) activity was observed highest (168.16±1.84) in MG and lowest (48.93±1.06) in As. Non-significant (P≥0.05) difference was observed between NN (88.61±1.39) and Fa (87.80±2.20).

Mean CAT activity among females of all studied chicken breeds differed significantly (P≤0.05). Maximum (505.00±5.00) activity was observed in Fa followed by MG (483.00±3.00) and As (282.50±2.50) while NN reported the minimum (217.50±2.50) catalase activity.

Mean peroxidase (POD) activity differed significantly (P≤0.05) among females of all studied chicken breeds. As recorded highest (4074.00±78.00) POD activity followed by MG (3407.60±55.60) and Fa (3149.40±47.40) while the lowest (2972.20±41.80) was reported by NN.

Mean ascorbate peroxidase (APX) activity among females was reported highest (807.50±7.50) in NN and lowest (530.00±10.00) in MG. Non-significant (P≥0.05) difference was observed in mean APX activity between NN (807.50±7.50), As (805.00±5.00) and Fa (797.50±2.50).

TF content of BF among male groups was reported highest in As (128.18±1.93) and lowest (114.12±1.12) in NN. Total antioxidant capacity (TAC) of BF was observed maximum (15.12±0.12) and minimum (4.01±0.12) in Fa and As, respectively.

Non-significant (P≥0.05) difference was observed in total oxidant status of all male studied chicken breeds where As (1320.00±5.00) reported maximum TOS followed by MG (1315.00±5.00), NN (1300.00±10.00) and lowest in Fa (1295.00±5.00).

Non-significant (P≥0.05) difference was observed in Malondialdehyde (MDA) content of BF of all male studied chicken breeds. NN reported the highest (187.13±2.87) MDA content in their thigh muscle meat followed by MG (183.85±1.14), Fa (177.48±2.52) and lowest in As (176.71±3.29).

Biceps femoris (Thigh) meat analysis among female groups: Biochemical analysis of BF of each female group is presented in table 6.

Mean Superoxide Dismutase (SOD) activity of Fa (167.76±2.24) and MG (165.73±4.27) differed significantly (P≤0.05) from the rest of the groups among females. Maximum (167.76±2.24) and minimum (47.72±2.28) mean SOD activity was observed in Fa and NN, respectively.

Mean catalase activity among females of all studied chicken breeds differed significantly (P≤0.05). Maximum (467.00±7.00) and minimum (345.00±5.00) CAT activity was observed in As and Fa, respectively. Non-significant (P≥0.05) difference was reported between NN (426.00±6.00) and MG (408.50±7.50).

Mean peroxidase (POD) activity differed significantly (P≤0.05) among females of all studied chicken breeds. MG recorded highest (2204.70±59.70) peroxidase activity followed by NN (1819.60±21.40) and As (1400.30±1.70) while the lowest (1191.90±6.90) was reported by Fa.

Mean ascorbate peroxidase (APX) activity differed significantly (P≤0.05) among females of all studied chicken breeds. Fa recorded highest (1507.50±12.50) ascorbate peroxidase activity followed by NN (965.00±5.00) and As (847.50±7.50) while the lowest (365.00±5.00) was reported by MG.

Table 5: Comparative profiles of oxidants and antioxidants in thigh (bicep femoris) meat among male chicken of studied breeds.

Groups	Enzymatic				Non-enzymatic		Oxidants	
	SOD	Catalase	POD	APX	TF	TAC	TOS	MDA
	(Units/g f. wt.)	(µM/g f. wt.)	(µM/g f. wt.)	(µM/g f. wt.)	(Rutin equl. µg/ml)	(µM/g f. wt.)	(µM/g f. wt.)	(µM/g f. wt.)
Aseel	48.93±1.06^{c}	282.50±2.50^{c}	4074.00±78.00^{a}	805.00±5.00^{a}	128.18±1.93^{a}	4.01±0.12^{c}	1320.00±5.00^{a}	176.71±3.29^{a}
Fayoumi	87.80±2.20^{b}	505.00±5.00^{a}	3149.40±47.40^{bc}	797.50±2.50^{a}	118.16±1.98^{b}	15.12±0.12^{a}	1295.00±5.00^{a}	177.48±2.52^{a}
Misri Gold	168.16±1.84^{a}	483.00±3.00^{b}	3407.60±55.60^{b}	807.50±7.50^{a}	123.56±1.55^{ab}	14.16±0.36^{a}	1315.00±5.00^{a}	187.13±2.87^{a}
Naked Neck	88.61±1.39^{b}	217.50±2.50^{d}	2972.20±41.80^{c}	530.00±10.00^{b}	114.12±1.12^{b}	7.01±0.11^{b}	1300.00 10.00^{a}	183.85±1.14^{a}

Different superscripts in same column indicates their statistically significant difference from each other at 5% level of significance.

Table 6: Comparative profiles of oxidants and antioxidants in thigh (bicep femoris) meat among female chicken of studied breeds.

Groups	Enzymatic				Non-enzymatic		Oxidants	
	SOD	Catalase	POD	APX	TF	TAC	TOS	MDA
	(Units/g f. wt.)	(µM/g f. wt.)	(µM/g f. wt.)	(µM/g f. wt.)	(Rutin equl. µg/ml)	(µM/g f. wt.)	(µM/g f. wt.)	(µM/g f. wt.)
Aseel	98.68±1.32^{b}	467.00±7.00^{a}	1400.30±1.70^{c}	847.50±7.50^{c}	114.39±0.86^{bc}	12.04±0.42^{b}	1140.00±5.00^{b}	227.26±2.74^{c}
Fayoumi	167.76±2.24^{a}	345.00±5.00^{c}	1191.90±6.90^{d}	1507.50±12.50^{a}	109.24±1.01	2.91±0.10^{c}	1335.00±10.00^{a}	293.05±1.95^{b}
Misri Gold	165.73±4.27^{a}	408.50±7.50^{b}	1819.60±21.40^{b}	365.00±5.00^{d}	132.81±2.32^{a}	13.98±0.23^{a}	1052.50±7.50^{c}	171.39±2.61^{d}
Naked Neck	47.72±2.28^{c}	426.00±6.00^{b}	2204.70±59.70^{a}	965.00±5.00^{b}	119.64±0.81^{b}	14.24±0.32^{a}	1150.00±10.00^{b}	388.55±1.45^{a}

Different superscripts in same column indicates their statistically significant difference from each other at 5% level of significance.

Non-enzymatic antioxidants like TFC (Total Flavonoid Content) of BF among female groups differed significantly. Maximum (132.81±2.32) and minimum (109.24±1.01) TF content was reported in MG and Fa, respectively.

Total antioxidant capacity (TAC) of NN (14.24±0.32) and MG (13.98±0.23) differed significantly (P≤0.05) from the rest of the members of female group. Maximum (14.24±0.32) and minimum (2.91±0.10) total antioxidant capacity in BF was observed in NN and Fa, respectively.

Non-significant (P≥0.05) difference was observed in the total oxidant status of NN (1150.00±10.00) and As (1140.00±5.00). Maximum (1335.00±10.00) and minimum (1052.50±7.50) mean (±SEM) was observed in Fa and MG, respectively.

Significant (P≤0.05) difference was observed in the mean (±SEM) Malondialdehyde (MDA) content of BF in female studied chicken breeds where NN reported the highest (388.55±1.45) MDA content followed by Fa (293.05±1.95), As (227.26±2.74) and lowest in MG (171.39±2.61).

DISCUSSION

Constant growth in chicken meat consumption is on the rise. Relative low-fat content and high protein values of chicken meat to its counterpart red meat, makes it more pleasing and desired (Kralik *et al.*, 2018). Current study revealed that MG attained significantly (P≤0.05) higher weight gain among all studied chicken breeds of male birds, while NN weighed the lowest. This suggests that in conditions provided by local small households of rural areas where no supplementation or support is given to studied breeds of domestic poultry birds, MG (Fa x RIR) provides better growth performance which is in line with the findings of Azharul *et al*., (2005) who demonstrated that said breed has the tendency to attain higher body weights among native breeds. Live body weight of Fa is recorded higher to that of observed by (Khawajaa *et al.*, 2012) who studied the growth performance of Fa in deep litter system with manual feeding and all time fresh water availability at 20 weeks of age which is expected as compared to the age (~26 weeks) of birds in current study. Live weight of As and NN observed in current study is lower than the findings of (Yakubu, Ogah, and Barde, 2008) and (Richard Churchil *et al.*, 2019), respectively. This may be due to difference in rearing systems implemented in current study as explained by (Pathak *et al.*, 2015). Selection along with supplementation can improve the productive traits of native chicken breeds in a concomitant way (Khan and Sardar, 2005; Singh *et al.*, 2014). Although dressing percentage (DP) among all studied chicken breeds differed non significantly but it may be pertinent to note that NN among male groups while Fa among female groups represented the highest dressing percentage i.e., 66.58 and 62.43 respectively. Current study DP of MG and Fa is in line with the findings of Azharul *et al*. (2005) and as near to the findings of Khan (2020) in Broiler.

Most of the stresses, at cellular level, in poultry are oxidative in nature where superoxide dismutase (SOD) seems to be the very first level in antioxidant defence system of living organism (Surai, 2016). The antioxidant enzymatic activities like Superoxide Dismutase (SOD) and Catalase (CAT) are developed by living organisms to respond against oxidative effects (Min *et al.*, 2008). SOD is the most powerful natural antioxidant which plays major defensive role in oxidative stress associated diseases like cancer, heart and inflammatory diseases, ischemia, rheumatoid arthritis, diabetes and aging at cellular level (Kim *et al.*, 2002; Masini *et al.*, 2002: Mahajan and Tandon, 2004;Oberley, 2004; Yasui *et al.*, 2005; Bafana *et al.*, 2011: Yan, 2014). SOD removes $O_2^{\bullet-}$, resultant is H_2O_2 and Oxygen. Catalase will then further transforms this H_2O_2 into water and molecular oxygen, hence, the antioxidant activity (Mark, 2006; Domínguez Lorenzo, 2017; Domínguez *et al.*, 2019). Efforts are made to exploit SOD activity as a therapeutic measure to treat said diseases (Younus, 2018).

Antioxidants like Ascorbate peroxidase (APX) plays crucial starring role in hydrogen peroxide detoxification and its activity enhances even further with antioxidants like SOD and CAT which in succession are responsible for less cell membrane damage, low protein degradation and lipid peroxidation; (Sneha, Rishi, and Chandra, 2014: Anjum *et al.*, 2016;). Meat quality is affected by increase in lipid oxidation causing foul smell, off-flavour, and discoloration of meat. This decline in quality affects the nutritional and functional value of meat triggering injurious effects to human health (Ahn Min, 2005 and McMillin, 2008).

Current study revealed that PM meat cut of MG exhibited significantly (P≤0.05) higher (145.99±4.01) SOD activity followed by As (113.08±1.92) among male groups which is lower than the findings of Li *et al.* (2018) who observed SOD activity in PM meat of broiler fed on sodium selenite and selenium-enriched yeast diets. Same trend was observed among female groups i.e., MG (121.93±3.07) and As (120.63±1.36) recording significantly (P≤0.05) higher SOD activity. SOD activity of BF (dark) meat cut was observed significantly (P≤0.05) higher in MG both in male (168.16±1.84) and female (165.73±4.27) studied chicken breeds. Current study results are in line with the results of Utama *et al*., (2016) where higher SOD activity was observed in BF (dark) meat cut as compared to PM (white) meat cut in MG among both male and female birds. On the contrary, SOD activity in As was higher in PM (white) meat cut than that of BF (dark) meat cut among both male and female birds. On the other hand, SOD activity in PM (83.74±1.53) and BF (88.61±1.39) meat cut of male NN was documented almost double than the female groups i.e., PM (58.40±1.60) and BF (47.71±2.28) of same breed, respectively. This difference in antioxidant activity of different meat cuts between different breeds has also been observed by Muhlisin *et al*. (2016) who said that various meat cut outs and chicken breeds exhibit varied SOD activity. Current study suggests that such disparity exists even among genders.

CAT activity was observed significantly (P≤0.05) higher in PM (white) meat cut of NN (385.00±5.00) among male groups and Fa (495.50±5.00) among female groups while in BF (dark) meat cut, Fa (505.00±5.00) in male groups and As (467.00±7.00) in female groups reported significantly (P≤0.05) higher CAT activity among other groups. Current study revealed that CAT activity of domestic backyard poultry is not only 3 to 4 times more than broiler as observed by Muhlisin *et al*. (2016) but also reaches the level of beef meat reported by Mei *et al*. (1994) & Pradhan *et al*. (2000). Comparatively, a trend of more CAT activity was observed in BF (dark) meat cut than PM (white) meat cut which is the same as observed by Lee *et al*. (1996), Renerre *et al*. (1996) and Pradhan *et al*. (2000) in chicken, turkey and beef, respectively. Current study domestic poultry breeds of Pakistan also exhibited 2 to 3 times the CAT activity than the Korean native poultry breeds as observed by Utama *et al*. (2016). Although, both CAT and SOD are associated enzymes yet they didn't expressed similar trends of activity which was also noted by Descalzo *et al.* (2007).

Catalase (CAT) and Ascorbate peroxidase (APX) are metabolic heme-enzymes responsible for controlling potential impacts of stress-provoked reactive oxygen species (ROS) (Anjum *et al.*, 2016). APX activity was observed maximum in PM meat of Fa (2075.00±75.00) among male groups and in MG (967.50±7.50) among female groups. BF meat of As (805.00±5.00) among male groups while Fa (1507.50±12.50) among female groups reported significantly higher APX activity. APX activity as strong antioxidant has been studied in plants by Anjum *et al*. (2016) and Fábián *et al*. (2018) and but current study is first of its kind in poultry meat. Further studies on APX activity in detoxification of reactive oxygen species (ROS) and its related oxidative stress in poultry products are also recommended to further evaluate domestic poultry breed potential.

Peroxidase (POD) plays the most important role in important physiological processes of innate immunity like apoptosis and cell signalling (Vlasova, 2018). POD activity in PM meat of NN (2972.20±41.80) among male groups and in MG (6723.60±69.60) among female groups was observed significantly (P≤0.05) higher. BF meat of As (4074.00±78.00) among male groups and NN (2204.70±59.700) among female groups showed significantly (P≤0.05) higher POD activity. Current study results of POD activity in indigenous poultry breeds are multiple folds higher than that of observed by (Aparna and Karunakaran, 2016) in broiler fed on selenium diet for boosted activity. Higher activity of POD in current study poultry groups may be the cause of less frequency of occurrence of diseases in them (Haunshi *et al*., 2022).

Polyphenolic secondary metabolites of plants known as flavonoids are abundant in wide variety of edibles and are absorbed at pH of 5.0-6.8 in the ileum (Kamboh *et al.*, 2019; Rafiei and Khajali, 2021). Being an antioxidant, flavonoid has shown improved serviceable life of poultry meat (Goliomytis *et al.*, 2014). Their primary antioxidant activity is attributed to free radical scavenging (Kamboh *et al.*, 2018). Current study has reported highest TFC contents in MG male breast muscles (150.86±1.28) and thigh muscles of female (132.81±2.32) as compared to other breeds under study. Kishawy *et al.* 2019 have reported comparatively higher values of flavonoid contents in breast muscle of broiler. The difference in flavonoid contents can be explained due to the use of different feed additives and different method of estimation.

Total antioxidant capacity against pro-oxidants in muscles tissue is the combined display of enzymatic i.e., SOD, CAT, POD and APX etc and non-enzymatic either hydrophilic or lipophilic compounds like vitamins (E and C), polyphenols, carotenoids and ubiquinols etc either in slaughtered animals or living (Chan and Decker, 1994; Decker *et al*., 2000). Meat of different species may show diverse TAC even among the animals of same specie (Pradhan *et al*., 2000; Descalzo *et al.*, 2007) not to mention the muscle type.

Bicep femoris (thigh) meat of Fa showed highest TAC (15.12±0.12µM/g) among male groups while pectoralis major (breast) meat of male group of same breed showed mean value of 12.93±0.33µM/g f. wt TAC. Both of which are lower than the observations of Wang *et al.* (2017) & Li *et al.* (2018) who observed higher TAC in thigh and breast meat, respectively. Wang *et al.* (2017) observed TAC (0.2-0.3±0.01units/mg) of broiler pectoralis major fed on marigold extract while Li *et al.* (2018) used sodium selenite (1.97±0.11units/mg) and selenium-enriched yeast (2.20±0.11units/mg) dietary supplementation. The difference between both may be a result of difference in housing and diets provided (Hernández, Park, and Rhee, 2002: Hernández *et al.*, 2004). Although, results of both male and female groups of Fa of current study are not so different as both were nurtured the same i.e., scavengers (Descalzo *et al.*, 2007).

Lipid oxidation in meat can also be estimated by its biproduct, malondialdehyde (MDA) content, in either free or conjugated (covalent bounded with proteins) form (Tsikas,

2017; Bertolín *et al*., 2019). It is believed to be the major marker for estimation of lipid oxidation (Domínguez *et al.*, 2019) in meat due to the production of stinking aroma, even at reduced quantity (Jones, 2017). Accepted limit of MDA i.e., 2-2.5mg per Kilogram of meat is believed where you won't see rancidity in meat and meat products (Campo *et al*., 2006; Zhang *et al.*, 2019). Statistically insignificant ($P \geq 0.05$) difference was observed in male groups of both PM (breast) meat and BF (thigh) meat, where lowest (197.55±2.45), (176.71±3.29) MDA content was observed in PM meat NN male birds and BF meat of As male birds. On the contrary, female birds of MG expressed lowest (279.53±5.47), (171.39±2.61) in PM and BF meat, respectively. Observations in current study are much higher than the observations of previous studies (Vaitukaityte *et al.*, 2013; Jung *et al*., 2016; Kayode *et al.*, 2018; Li *et al.*, 2022) which can be due to overestimation of MDA content by the procedure used for this study (Reitznerová *et al.*, 2017) or the evaluation of frozen samples rather than fresh meat cuts (Saeed and Howell, 2002; Kayode *et al.*, 2018). Results can be enhanced using methods like (infrared spectroscopy, Raman spectroscopy, fluorescence emission, chemiluminescence and/or magnetic resonance) but they are applicable only in very specific conditions, not straightforwardly adaptable in laboratory conditions, painstaking work, require a very precise knowledge for result interpretation and expensive (Barriuso *et al*., 2013; Yang and Boyle, 2016; Shahidi *et al*., 2017; Domínguez *et al.*, 2019). However, dietary supplementation with natural or synthetic compounds have been believed to reduce MDA content in meat and meat products (Santi *et al*., 2015; Rossi *et al.*, 2022).

Most of the rural households prefer to rear indigenous chicken breeds for income generation due to constrained finance resources and inability to fulfil exotic chicken breed requirement (Kuma and Gata, 2022). With more than double the SOD antioxidant activity, 3 to 4 times more CAT activity than broiler reaching up to the level of red meat, and higher APX and POD activity as observed in current study groups. Current study revealed a higher oxidative stability in studied chicken breeds upon which we can easily recommend the domestic backyard poultry breeds (DBPB) due to its higher quality than its counterparts. It contains the potential for a favourable choice to rural small households for domestic consumption and cheap income generation, even as a reserve cash with lowest input.

In addition, domestic backyard poultry has demonstrated promising results, even in our scavenger farming system with zero external input (no special feed or supplementation). Their meat can also be used in local as well as international dishes providing an advantage of oxidative stability other than their counterparts. Encouragement of farmers towards DBPB farming can help in poverty alleviation as well as women empowerment at grass root level.

Further studies may be conducted with reference to meat being preserved before evaluation of antioxidant activity as temperature and time favours the oxidation process (Saeed and Howell, 2002; Chaijan and Panpipat, 2017). Freezing, either rapid or slow, causes huge cell disruption due to the formation of ice crystals leading to let go of prooxidant composites hence, promoting oxidation (Domínguez *et al.*, 2019).

Conclusions: In conclusion, chicken breeds like MG and Fa can prove to be a potential antioxidant enriched source of protein and provide substantial health benefits along with income generation for small rural households in developing countries. Additionally, genetic studies and selection-based breed improvement is endorsed to take full advantage of these genomic gold mines. Provided insights pave the way for future research and practical applications in poultry farming. Identifying potential genetic basis of antioxidant traits in tested chicken breeds would be particularly valuable for future research. Further studies can be planned targeting the diverse markets to better understand the consumer acceptance and preference while consuming chicken meet.

Ethical approval: All procedures performed in this study which involves animals were carried out in compliance to the guidelines, ethical standards and recommendations of the Office of Research, Innovation and Commercialization and Animal Ethical Committee of University of Agriculture, Faisalabad.

Consent to participate: Not applicable

Consent for publication: Not applicable

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