

Assessment of the Expression of Phenotypic Traits in Male and Female Indigenous Chickens (*Gallus gallus domesticus* L.) in Rajshahi, Bangladesh

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ABSTRACT

This study aimed to identify and describe phenotypic traits of indigenous chicken (*Gallus gallus domesticus* L.) populations in five market sites in Rajshahi City, Bangladesh: Shaheb Bazar (S₁), Court Bazar (S₂), Vodra Bazar (S₃), Binodpur Bazar (S₄), and Katakhal Bazar (S₅). A total of 496 chickens (205 males and 291 females) were evaluated for qualitative phenotypic traits, including feather type, head shape, plumage color, shank color, comb type, and ear lobe color. Descriptive statistics and chi-square (χ^2) test were used to analyze the data. In the male chickens, significant differences were found in traits such as normal feathering, cock's comb head shape, black plumage, white shank, black shank, brown shank, single comb, and red ear lobe ($p < 0.001$); multicolor plumage, white and black plumage, white ear lobe color ($p < 0.01$), and white plumage ($p < 0.05$) among the different sites. Similarly, in females, significant differences prevailed in normal feather, plain head, black plumage, multicolor plumage, golden mixed plumage, white plumage, white shank, black shank, single comb, and red and white earlobe ($p < 0.001$); brown shank ($p < 0.01$), and cock's comb, yellow shank ($p < 0.05$) within the different sites. In total, the male and female showed significant differences in the plain head, cock's comb head, black plumage, black shank, single comb, and white ear lobe ($p < 0.001$); white plumage ($p < 0.01$), and white and black plumage and white shank ($p < 0.05$). The high phenotypic diversity in indigenous chickens is major evidence of high genetic variability at the population level. These findings highlight the broad spectrum of phenotypic traits among the studied populations and provide a foundation for developing conservation and selection strategies for indigenous chickens in the region.

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Introduction

The poor villagers prefer to rear the indigenous chickens because of their potential for disease resistance, heat stress, and environmental adaptation, helping them to survive with low feed input with low productivity (Hamid, 2019; Tadele *et al.*, 2018; Negassa *et al.*, 2014, FAO, 2008). Despite these, they have certain attributes of economic and cultural importance (Mengesha and Tsega, 2011), like meat tenderness, leanness, flavor, and special taste of meat and nutritious

eggs (Chowdhury, 2012; Halima *et al.*, 2007). Thus, they are very popular with rural, peri-rural, and urban people (Chowdhury, 2012) all over the world (Dessie *et al.*, 2012). In Bangladesh, chickens are indigenous or native, exotic, crossbred, and commercial hybrids (Hamid, 2019). The indigenous chicken is comprised of a number of breeds, such as Non-descript Desi (ND), naked neck (NN), Aseel (AS), Hilly (H), Native Dwarf, Jungle Fowl, and Frizzled Plumage (Das *et al.*, 2008; Bhuiyan *et al.*, 2005).



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The nondescript deshi chickens are distributed throughout the country, and the frequency of naked necks is very low (Hamid, 2019).

To meet the demand for animal protein for the increasing human population in Bangladesh, many efforts have been expended to sustain commercial hybrid broilers and layer chicken for their high-yield attributes. The demand for indigenous chickens continues to rise sharply because exotic hybrids do not maintain the expected standards in rural village conditions, creating a big gap between demand and supply (Islam *et al.*, 2015). So, their conservation not only provides benefits to be self-reliant but also generates income, which facilitates job opportunities for the people of these areas under study. Indigenous chickens contribute 90% of the total poultry population in some developing countries (Guèye, 1998), and in Bangladesh, they provide about 78% of poultry meat and 75% of eggs for domestic consumption (Bhuiyan *et al.*, 2005). However, the global use of highly productive breeds leads to the genetic erosion of indigenous chickens resulting from the continuous indiscriminate crossing with improved exotic stock (Melesse and Negesse, 2011; Bhuiyan *et al.*, 2005) and due to poor characterization of their phenotypes (Brown *et al.*, 2017; Negassa *et al.*, 2014). So, the improvement and conservation programs of indigenous chickens are very important to reduce the genetic loss in the populations, a way for maintaining the existing indigenous chickens suited to the regional environment. The information should make the identification and characterization of chicken phenotypic traits of phenotypic characters of current or future value (Weigend and Romanov, 2001) where it is used as first step to find out the phenotypic variation within and between breeds (Maharani *et al.*, 2021; Maharani *et al.*, 2019) or differentiate breeds or species (Moges *et al.*, 2009; Halima *et al.*, 2007). Attention has been paid to improving indigenous chickens for consumers' demands and economic potentials, *i.e.*, more expensive than the exotic, crossbred, and commercial hybrids (Hamid, 2019) and to conserve to resist genetic loss from the populations (Bhuiyan *et al.*, 2005).

Phenotypic characterization means identifying and recording diversity within and between

distinct breeds based on their observable attributes (FAO, 2012). The assessment of phenotypic characters is used as a marker for selecting breeds for econometric traits (Onasanya *et al.*, 2018; Sarker *et al.*, 2014). Given the demand for and probable environmental changes in the future resulting in genetic variations and for the sustainable use of indigenous chickens, the evaluation and monitoring of the phenotypic characteristics are highly recommended (Maharani *et al.*, 2021).

The phenotypic characterization is a useful approach because it is simple, easy, fast, and cost-effective (Maharani *et al.*, 2021). It provides data on present and future potential uses of those indigenous chicken populations and establishes their current state as distinct breed and their risk status (Tadele *et al.*, 2018). In Bangladesh, the improvement's major challenge is the lack of adequate information on the genetic potentials of the available indigenous chicken populations. Assessment of phenotypic traits is crucial in designing breeding strategies, and implementation of development programs can conserve them from genetic loss. The information on the phenotypic characteristics of indigenous chickens in this region is inadequate. To sustainably utilize and conserve them, the phenotypic characteristics of indigenous chickens need to be determined. Very limited information on phenotypic diversity is available in this region, and a recent study performed by Rahman *et al.* (2024) described phenotypic diversity in different localities, but the incidences of traits in separate sexes, *i.e.*, male and female, are yet to be done. Thus, the present study was undertaken to assess the phenotypical characteristics of the male and female indigenous chicken populations at five different market sites such as Shaheb Bazar (S₁), Court Bazar (S₂), Vodra Bazar (S₃), Binodpur Bazar (S₄), and Katakhal Bazar (S₅) at Rajshahi City in Bangladesh.

Materials and Methods

Selection of the study area

The study was carried out at five different market sites in Rajshahi City, including Shaheb Bazar (S₁), Court Bazar (S₂), Vodra Bazar (S₃), Binodpur Bazar (S₄), and Katakhal Bazar (S₅).

Indigenous chickens with different phenotypes were available in these markets, forming large chicken populations from a wider range of villages adjacent to each site and covering a broader regional diversity. The study of a large population would provide significant qualitative trait diversity in each site of this area. Getting more phenotypic data from the market sites with less effort and shorter duration was easy. The research work was conducted from January to June 2022.

Experimental animals and their management

A total of 496 indigenous chickens (205 males and 291 females) were used to conduct the study. They were from the five marketplaces of Rajshahi City, Bangladesh. These chickens were kept in bamboo baskets covered made by nylon nets in market places for sale by farmers. Food and water pots were inside the baskets for the chickens' feeding (commercial) and watering *ad libitum*.

Data collection

Data on phenotypes were collected from male and female indigenous chickens of five different market sites by using field observation, and photographs were taken with a digital camera (Canon IXUS, 16.1 MEGA PIXELS). Six qualitative traits classified as feather type (normal, naked neck), head shape (plain, crested, cock's comb), plumage color (black, brown and black, multicolor, golden mixed, white, white and black), shank color (white, black, yellow, brown), comb type (single, pea, rose cushion), and earlobe color (red, white) were recorded in this study.

Measurement of phenotypic traits

All male and female chickens were individually assessed and scored for six qualitative traits (feather type, head shape, plumage color, shank color, comb type, and earlobe color). The qualitative traits for each chicken were visually appraised and scored from a total of 205 male and 291 female individual chickens.

Data analysis

Data on phenotypic traits were entered into a computer using Microsoft Office Excel 2007 and were analyzed by descriptive statistics. The

percentages of males or females were calculated from the presence of respective traits found in respective chickens, divided by the total number of chickens surveyed in each market site and similarly overall from the summation of individuals of five market sites. A chi-square (χ^2) test was performed to determine the differences in qualitative traits among five different market sites for male or female chickens individually and between overall (total) males and females. To justify the differences, the levels of significance 5%, 1%, and 0.1% were used.

Results

The phenotypic traits (Fig. 1) recorded from male and female indigenous chickens were surveyed from five different market sites covering broader areas to obtain more diversified information at a time. The data on variability present in the indigenous chickens are represented by male (Table 1), female (Table 2), and overall male and female (Table 3).

Five sites were used to collect phenotypic traits from indigenous chicken populations selected from Shaheb Bazar (S_1), with 83 males and 139 females; Court Bazar (S_2), with 31 males and 34 females; Vodra Bazar (S_3), with 29 males and 28 females; Binodpur Bazar (S_4), with 25 males and 45 females; and Katakhal Bazar (S_5), with 37 males and 45 females.

Feather type

The percentage of normal feather type in male indigenous chickens was 93.98%, 96.77%, 93.10%, 92.00%, and 83.78% in S_1 , S_2 , S_3 , S_4 , S_5 market sites, respectively (Table 1; Fig. 2) which were significantly different ($\chi^2 = 55.72$; $p < 0.001$) (Table 1). In female chickens, this trait was 97.12%, 97.06%, 96.43%, 95.56%, and 97.78% in five stations, respectively (Table 2; Fig. 2), showing significant differences among the five sites ($\chi^2 = 140.45$; $p < 0.001$) (Table 2). The overall percentage of normal feather traits in males was 92.20% and in females 96.91%, which were not significantly different (Table 3). The naked neck feather types were not significantly different in males (6.02%, 3.23%, 6.90%, 8.00%, and 16.22% among stations, respectively), which shows in Table 1 and Fig. 2. In females, this trait was 2.88%, 2.94%, 3.57%,

4.44%, and 2.22% in five market sites (S_1 , S_2 , S_3 , S_4 , S_5), which as shown in Table 2 and Fig. 2, did not show significant differences among them (Table 2). The overall percentage of this

phenotypic trait in males and females was 7.80% and 3.09%, respectively, which were not significantly different (Table 3).



Fig. 1. Phenotypic traits of indigenous chickens: The image illustrates indigenous chickens' different phenotypic traits, highlighted by black or white arrows within the images. These traits represent variations in physical characteristics.

Table 1. Occurrence of phenotypic (qualitative) traits of male indigenous chickens from different market sites in Rajshahi City.

Trait	Phenotypes	S_1 (N=83)	S_2 (N=31)	S_3 (N=29)	S_4 (N=25)	S_5 (N=37)	χ^2
Feather type	Normal	78(93.98)	30(96.77)	27(93.10)	23(92.00)	31(83.78)	55.72***
	Naked neck	5(6.02)	1(3.23)	2(6.90)	2(8.00)	6(16.22)	5.87 ns
Head shape	Plain	10(12.05)	5(16.13)	6(20.69)	4(16.00)	5(13.51)	3.64 ns
	Crested	3(3.61)	2(6.45)	1(3.45)	1(4.00)	2(5.41)	1.54 ns
Plumage color	Cock's comb	70(84.34)	24(77.42)	22(75.86)	20(80.00)	30(81.08)	52.64***
	Black	12(14.46)	3(9.68)	1(3.45)	1(4.00)	2(5.41)	22.82***
Shank color	Brown+ Black	8(9.64)	4(12.90)	5(17.24)	4(16.00)	5(13.51)	2.06 ns
	Multicolor	29(34.94)	14(45.16)	12(41.38)	11(44.00)	12(32.43)	14.68**
Comb type	Golden mixed	16(19.28)	5(16.13)	8(27.58)	7(28.00)	14(37.83)	9.00 ns
	White	7(8.43)	1(3.23)	1(3.45)	1(4.00)	2(5.41)	11.30*
Earlobe color	White +Black	11(13.25)	4(12.90)	2(6.90)	1(4.00)	2(5.41)	15.50**
	White	48(57.83)	16(51.61)	20(68.97)	14(56.00)	6(16.22)	49.44***
Shank color	Black	19(22.89)	2(6.45)	2(6.90)	3(12.00)	9(24.32)	30.56***
	Yellow	15(18.07)	10(32.26)	6(20.69)	6(24.00)	10(27.03)	5.83 ns
Comb type	Brown	1(1.21)	3(9.68)	1(3.44)	2(8.00)	12(32.43)	22.82***
	Single	77(92.77)	28(90.32)	25(86.20)	22(88.00)	30(81.08)	57.59***
Earlobe color	Pea	2(2.41)	1(3.23)	2(6.90)	1(4.00)	1(2.70)	0.83 ns
	Rose	2(2.41)	1(3.23)	1(3.45)	1(4.00)	5(13.52)	6.00 ns
Shank color	Cushion	2(2.41)	1(3.22)	1(3.45)	1(4.00)	1(2.70)	0.65 ns
	Red	65(78.31)	22(70.97)	21(72.41)	22(88.00)	21(56.76)	50.14***
Comb type	White	18(21.69)	9(29.03)	8(27.59)	3(12.00)	16(43.24)	13.95**

S_1 , Shaheb Bazar; S_2 , Court Bazar; S_3 , Vodra Bazar; S_4 , Binodpur Bazar, S_5 , Katakali Bazar. N = Number of sampled chickens. Number outside the parenthesis is for male chicken count and number in the parenthesis for percentages of phenotypic traits. χ^2 = Chi-square; *** = $p < 0.001$; ** = $p < 0.01$; * = $p < 0.05$; ns = non-significant.

Table 2. Occurrence of phenotypic (qualitative) traits of female indigenous chickens from different market sites in Rajshahi City.

Trait	Phenotypes	S ₁ (N=139)	S ₂ (N=34)	S ₃ (N=28)	S ₄ (N= 45)	S ₅ (N= 45)	χ^2
Feather type	Normal	135(97.12)	33(97.06)	27(96.43)	43(95.56)	44(97.78)	140.45***
	Naked neck	4(2.88)	1(2.94)	1(3.57)	2(4.44)	1(2.22)	4.82 ns
Head shape	Plain	124(89.21)	30(88.24)	26(92.86)	39(86.67)	35(77.78)	133.73***
	Crested	5(3.60)	2(5.88)	1(3.57)	1(2.22)	4(8.89)	5.05 ns
	Cock's Comb	10(7.19)	2(5.88)	1(3.57)	5(11.11)	6(13.33)	10.30*
Plumage color	Black	30(21.58)	11(32.35)	4(14.29)	16(35.56)	9(20.00)	28.12***
	Brown+ Black	12(8.63)	8(23.53)	3(10.71)	7(15.56)	7(15.56)	5.54 ns
	Multicolor	50(35.97)	6(17.65)	5(17.86)	3(6.67)	5(11.11)	119.02***
	Golden mixed	23(16.55)	4(11.76)	5(17.86)	10(22.22)	6(13.33)	25.52***
	White	15(10.79)	1(2.95)	3(10.71)	4(8.88)	8(17.78)	19.80***
	White+Black	9(6.48)	4(11.76)	8(28.57)	5(11.11)	10(22.22)	3.70 ns
Shank color	White	73(52.52)	18(52.94)	14(50.00)	24(53.34)	14(31.11)	88.47***
	Black	35(25.18)	8(23.53)	4(14.29)	10(22.22)	17(37.78)	40.44***
	Yellow	18(12.95)	7(20.59)	7(25.00)	6(13.33)	5(11.11)	13.13*
	Brown	13(9.35)	1(2.94)	3(10.71)	5(11.11)	9(20.00)	14.95**
Comb type	Single	125(89.92)	30(88.24)	24(85.72)	37(82.22)	39(86.67)	136.96***
	Pea	5(3.60)	1(2.94)	1(3.57)	5(11.11)	3(6.67)	5.32 ns
	Rose	7(5.04)	2(5.88)	2(7.14)	2(4.44)	2(4.44)	6.65 ns
	Cushion	2(1.44)	1(2.94)	1(3.57)	1((2.23))	1(2.22)	0.65 ns
Earlobe color	Red	80(57.55)	28(82.35)	24(85.71)	37(82.22)	14(31.11)	71.77***
	White	59(42.45)	6(17.65)	4(14.29)	8(17.78)	31(68.89)	103.00***

S₁, Shaheb Bazar; S₂, Court Bazar; S₃, Vodra Bazar; S₄, Binodpur Bazar, S₅, Katakali Bazar. N = Number of sampled chickens. Number outside the parenthesis is for male chicken count and number in the parenthesis for percentages of phenotypic traits. χ^2 = Chi-square; *** = p< 0.001; ** = p< 0.01; * = p< 0.05; ns = non-significant.

Table 3. Occurrence of phenotypic (qualitative) traits of overall male and female indigenous chickens from different marketplaces in Rajshahi City.

Trait	Distribution	Overall Male (N= 205)	Overall Female (N= 291)	χ^2
Feather type	Normal	189(92.20)	282(96.91)	1.78 ns
	Naked neck	16(7.80)	9(3.09)	0.98 ns
Head shape	Plain	30(14.63)	254(87.29)	176.66***
	Crested	9(4.39)	13(4.46)	0.72 ns
	Cock's comb	166(80.98)	24(8.25)	106.12***
Plumage color	Black	19(9.27)	70(24.06)	29.22***
	Brown+ Black	26(12.68)	37(12.71)	1.92 ns
	Multicolor	78(38.05)	69(23.71)	0.54 ns
	Golden mixed	50(24.39)	48(16.50)	0.04 ns
	White	12(5.85)	31(10.65)	8.38**
	White+ Black	20(9.76)	36(12.37)	4.56*
Shank color	White	104(50.73)	143(49.14)	6.14*
	Black	35(17.07)	74(25.43)	13.94***
	Yellow	47(22.93)	43(14.78)	0.16 ns
	Brown	19(9.27)	31(10.65)	2.88 ns
Comb type	Single	182(88.78)	255(87.63)	12.18***
	Pea	7(3.41)	15(5.15)	2.90 ns
	Rose	10(4.88)	15(5.15)	1.00 ns
	Cushion	6((2.93)	6(2.07)	0.00 ns
Earlobe color	Red	151(73.66)	183(62.89)	3.06 ns
	White	54(26.34)	108(37.11)	18.00***

N = Number of overall chickens (male and female). Number outside the parenthesis is for chicken count and number in the parenthesis for percentages of phenotypic traits. χ^2 = Chi-square; *** = p< 0.001; ** = p< 0.01; * = p< 0.05; ns = non-significant.

Head shape

The plain head trait in male indigenous chicken was 12.05%, 16.13%, 20.69%, 16.00%, and 13.51% in stations and there were no significant differences among them (Table 1; Fig. 3). The crested head shape trait was fewer percentages 3.61%, 6.45%, 3.45%, 4.00%, and 5.41% were statistically similar (Table 1; Fig. 3). The cock's comb trait value (percentage) was found to be highest ($S_1 = 84.34\%$, $S_2 = 77.42\%$, $S_3 = 75.86\%$, $S_4 = 80.00\%$, $S_5 = 81.08\%$) among the other head shape traits and they were different with high significance with $\chi^2 = 52.64$; $p < 0.001$ (Table 1; Fig. 3). In female chickens the plain head trait was 89.21%, 88.24%, 92.86%, 86.67%, and 77.78% in five market places and showed significant differences with $\chi^2 = 133.73$; $p < 0.001$

(Table 2; Fig. 3). The percentage values of crested and cock's comb phenotypic traits were 3.60%, 5.88%, 3.57%, 2.22%, 8.89%, 7.19%, 5.88%, 3.57%, 11.11%, and 13.33%, where the crested head shape was insignificant, and cock's comb showed significant difference with $\chi^2 = 10.30$; $p < 0.05$ (Table 2; Fig. 3).

The overall percentage of plain head phenotypic traits in males was 14.63% and in females 87.29%, showing a significant difference with $\chi^2 = 176.66$ and $p < 0.001$ (Table 3). The overall crested head shapes in males and females were 4.39% and 4.46%, which was not significantly different. On the other hand, cock's comb trait had a frequency for males at 80.98% and for females at 8.25%, which were significantly different $\chi^2 = 106.12$ and $p < 0.001$ (Table 3).

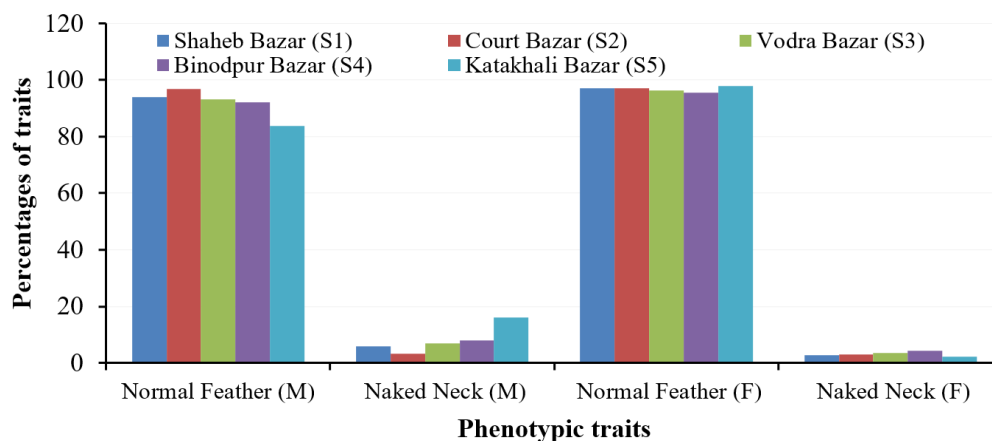


Fig. 2. Distribution of feather types in indigenous chickens: The graph presents the distribution of feather types in male and female indigenous chickens across five different sites (S_1 , S_2 , S_3 , S_4 , S_5); M= male and F= female.

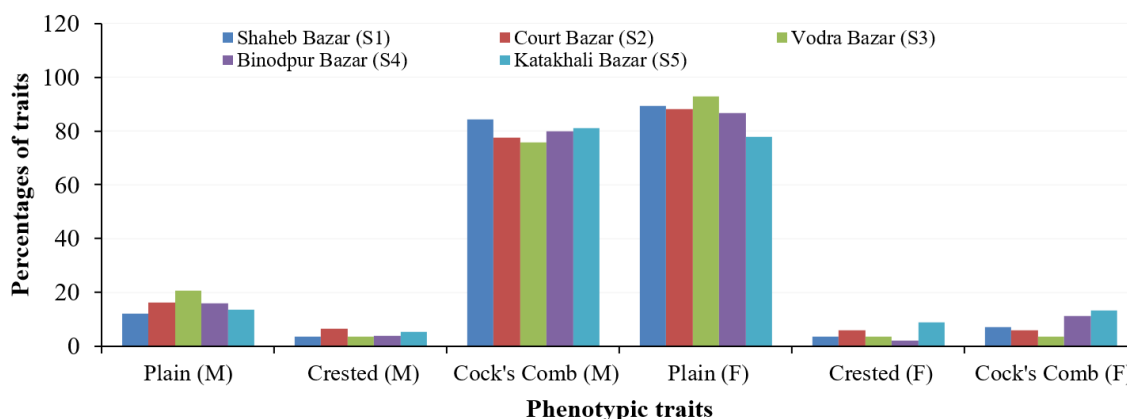


Fig. 3. Distribution of head shape traits in indigenous chickens: The graph presents the distribution of head shape traits in male and female indigenous chickens across five different sites (S_1 , S_2 , S_3 , S_4 , S_5); M= male and F= female.

Plumage color

As shown in Table 1 and Fig. 4, the black plumage color in male indigenous chicken was 14.46%, 9.68%, 3.45%, 4.00%, and 5.41% ($\chi^2=22.82$; $p<0.001$); brown + black was 9.64%, 12.90%, 17.24%, 16.00%, and 13.51%; multicolor was 34.94%, 45.16%, 41.38%, 44.00%, and 32.43% ($\chi^2=14.68$; $p<0.01$); golden mixed was 19.28%, 16.13%, 27.58%, 28.00%, and 37.83% (not statistically significant); white was 8.43%, 3.23%, 3.45%, 4.00%, and 5.41% ($\chi^2=11.30$; $p<0.05$); and white + black was 13.25%, 12.90%, 6.90%, 4.00%, and 5.41% ($\chi^2=15.50$; $p<0.01$) in five market sites. Similarly, as shown in Table 1 and Fig. 4, the black plumage color in female indigenous chicken was 21.58%, 32.35%, 14.29%, 35.56%, and 20.00% ($\chi^2=28.12$; $p<0.001$); brown + black was 8.63%, 23.53%, 10.71%, 15.56%, and 15.56% (not significantly different); multicolor was 35.97%, 17.65%, 17.86%, 6.67%, and 11.11% ($\chi^2=119.02$; $p<$

0.001); golden mixed was 16.55%, 11.76%, 17.86%, 22.22%, and 13.33% ($\chi^2=25.52$; $p<0.001$); white was 10.79%, 2.95%, 10.71%, 8.88%, and 17.78% ($\chi^2=19.80$; $p<0.001$); and white + black was 6.48%, 11.76%, 28.57%, 11.11%, and 22.22% in five market places were statistically indifferent (Table 2; Fig. 4). The overall percentages of plumage color are presented in Table 3, where black plumage color traits in total males was 9.27%, and females 24.06%, showing a significant difference ($\chi^2=29.22$; $p<0.001$). The brown + black trait in overall males was 12.68% and females 12.71%; multicolor in males was 38.05% and females 23.71%; golden mixed in males was 24.39% and females 16.50%, which were not significantly different. The white plumage color trait in total males was 5.85%, and in females was 10.65%, showing a difference ($\chi^2=8.38$; $p<0.01$). The white and black traits of male, 9.76%, and female, 12.37%, were also statistically significant ($\chi^2=4.56$; $p<0.05$).

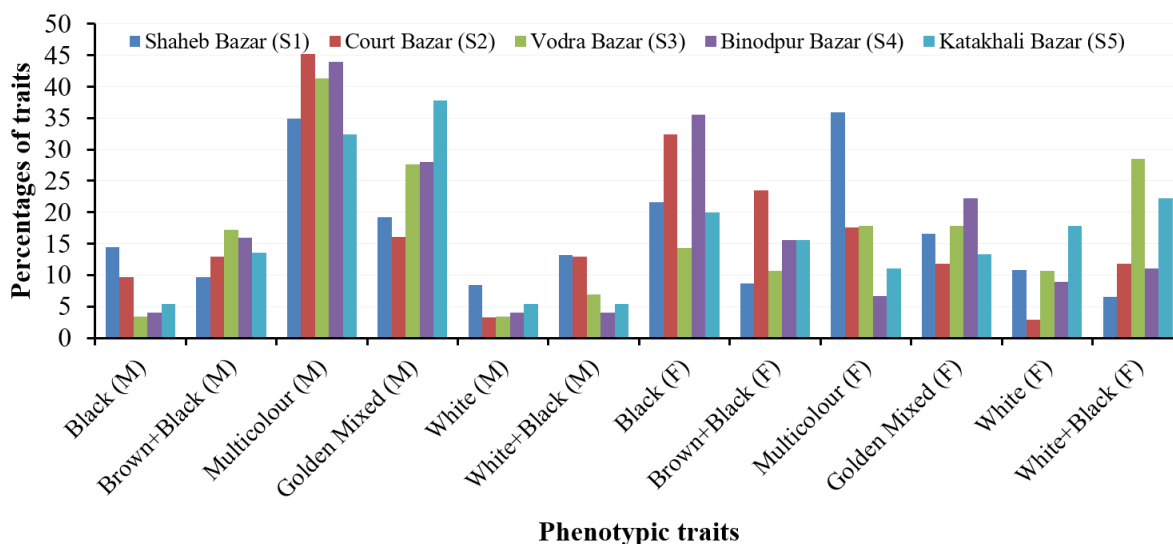


Fig. 4. Distribution of plumage color traits: The graph presents the distribution of plumage color traits in male and female indigenous chickens across five different sites (S₁, S₂, S₃, S₄, S₅); M = male and F = female.

Shank color

The white shank color of male indigenous chicken was 57.83%, 51.61%, 68.97%, 56.00%, and 16.22%, which were significantly different ($\chi^2=49.44$; $p<0.001$); black was 22.89%, 6.45%, 6.90%, 12.00%, and 24.32% and the values were significantly different ($\chi^2=30.56$;

$p<0.001$); yellow was 18.07%, 32.26%, 20.69%, 24.00%, and 27.03%, insignificantly varied; and brown was 1.21%, 9.68%, 3.44%, 8.00%, and 32.43%, which showed significant difference with $\chi^2=22.82$ and $p<0.001$ (Table 1; Fig. 5). In female indigenous chicken the white shank color was 52.52%, 52.94%, 50.00%, 53.34%, and

31.11% ($\chi^2 = 88.47$; $p < 0.001$); black was 25.18%, 23.53%, 14.29%, 22.22%, and 37.78% ($\chi^2 = 40.44$; $p < 0.001$); yellow was 12.95%, 20.59%, 25.00%, 13.33%, and 11.11% ($\chi^2 = 13.13$; $p < 0.05$); brown was 9.35%, 2.94%, 10.71%, 11.11%, and 20.00% with $\chi^2 = 14.95$ and $p < 0.01$ (Table 2; Fig. 5).

The total (overall) males and females showed significant differences in their phenotypic expressions. The percentage of the white shank

color of overall male chickens was 50.73%, while in females, it was 49.14%, and they were statistically different ($\chi^2 = 6.14$; $p < 0.05$); black in males was 17.07% and females was 25.43%, which were significantly different ($\chi^2 = 13.94$; $p < 0.001$); yellow in males was 22.93% and in females was 14.78%, having no difference; and brown in males was 9.27% and in female was 10.65%, which were not statistically different (Table 3).

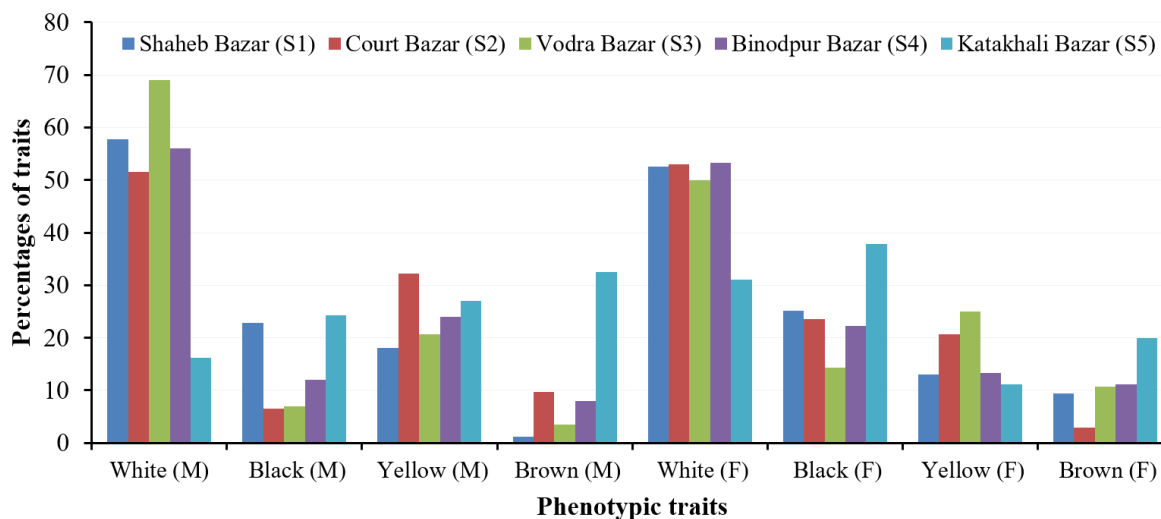


Fig. 5. Distribution of shank color traits in indigenous chickens: The graph presents the distribution of shank color traits in male and female indigenous chickens across five different sites (S₁, S₂, S₃, S₄, S₅); M= male and F= female.

Comb type

The single comb percentages in five different marketplaces of male chickens were 92.77%, 90.32%, 86.20%, 88.00%, and 81.08%, which were significantly different ($\chi^2 = 57.59$; $p < 0.001$). In the case of pea comb type, the frequencies 2.41%, 3.23%, 6.90%, 4.00%, and 2.70%; rose type 2.41%, 3.23%, 3.45%, 4.00%, and 13.52%; and cushion type 2.41%, 3.22%, 3.45%, 4.00%, and 2.70% were insignificantly varied (Table 1; Fig. 6).

In females, the single comb type showed different percentages in five different market places, and the values 89.92%, 88.24%, 85.72%, 82.22%, and 86.67% were significantly different ($\chi^2 = 136.96$; $p < 0.001$). The pea trait was 3.60%, 2.94%, 3.57%, 11.11%, and 6.67%; the rose was 5.04%, 5.88%, 7.14%, 4.44%, and 4.44%; and the cushion was 1.44%, 2.94%, 3.57%, 2.23%,

and 2.22%, which were not significantly different (Table 2; Fig. 6).

The phenotypic expressions of single comb in total males and females were 88.78% and 87.63%, showing a significant difference ($\chi^2 = 12.18$; $p < 0.001$). On the other hand, the pea was at 3.41% in males and at 5.15% in females; the rose was at 4.88% in males and at 5.15% in females; the cushion was at 2.93% in males, and in females it was at 2.07%. All the values of traits (pea, rose, and cushion) in males and females were statistically insignificant (Table 3).

Earlobe color

The percentages of the red earlobe in male chickens at five market places were 78.31%, 70.97%, 72.41%, 88.00%, and 56.76%, which were significantly different ($\chi^2 = 50.14$; $p > 0.001$). The white earlobe showed the

percentages as 21.69%, 29.03%, 27.59%, 12.00%, and 43.24%, which were statistically different with $\chi^2=13.95$ and $p < 0.01$ (Table 1; Fig. 7). On the other hand, the percentages of the red earlobe in female indigenous chickens at five sites were 57.55%, 82.35%, 85.71%, 82.22%, and 31.11%, showing a significant difference ($\chi^2=71.77$; $p < 0.001$). The percentages of white earlobe color in females were 42.45%, 17.65%,

14.29%, 17.78%, and 68.89%, showing a difference with $\chi^2=103.00$ and $p < 0.001$ (Table 2; Fig. 7). The red earlobe percentages of total male and female 73.66% and 62.89% were not different and in case of white earlobe the overall male and female showed the trait as 26.34% and 37.11% were statistically significant with $\chi^2=18.00$ and $p < 0.001$ (Table 3).

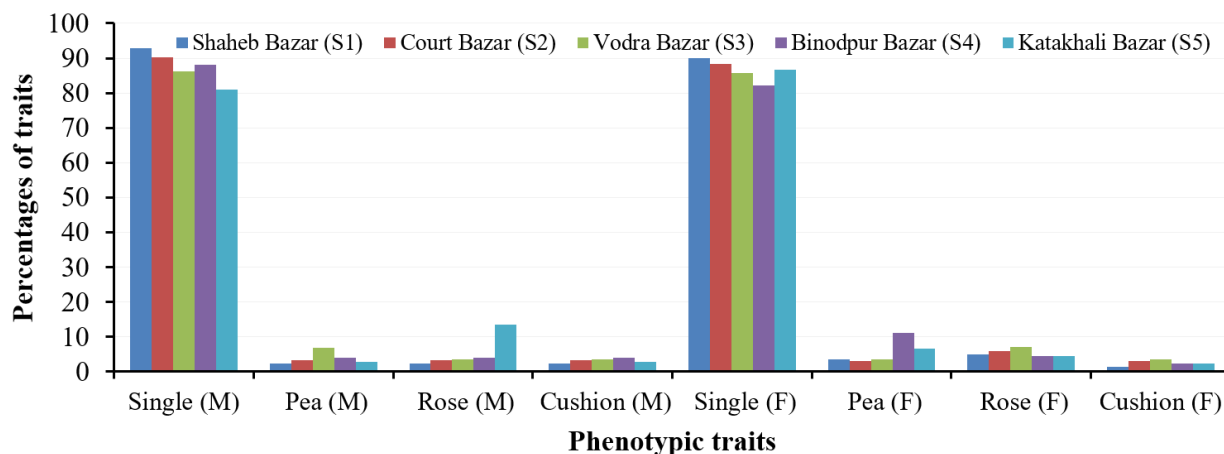


Fig. 6. Distribution of comb-type traits in indigenous chickens: The graph presents the distribution of comb-type traits in male and female indigenous chickens across five different sites (S₁, S₂, S₃, S₄, S₅); M= male and F= female.

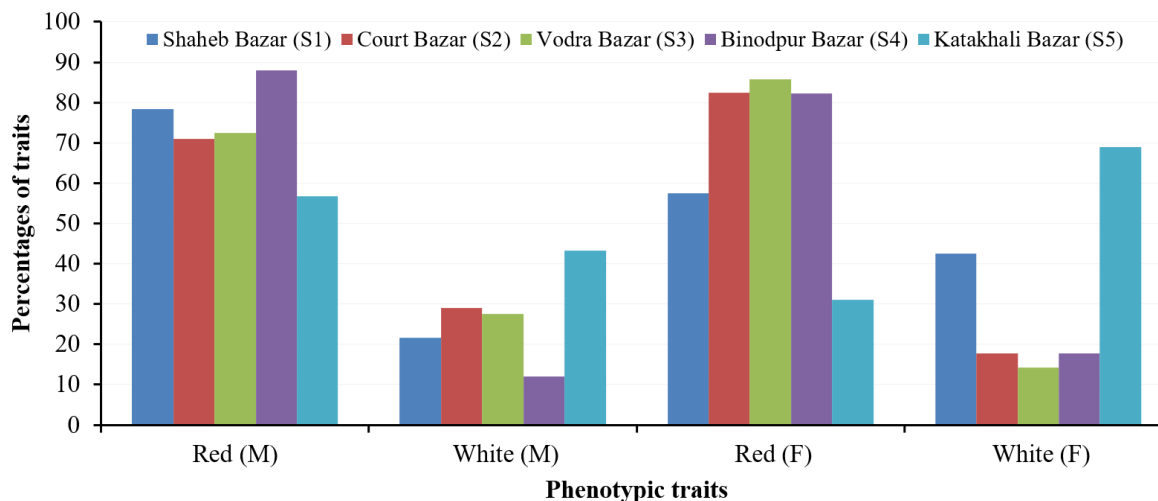


Fig. 7. Earlobe color trait distribution in indigenous chickens: The graph illustrates the distribution of earlobe color traits in male and female indigenous chickens across five different sites (S₁, S₂, S₃, S₄, S₅); M= male and F= female.

Discussion

The diversity of phenotypic traits of indigenous chickens from five different sites and overall male and female chickens are elucidated as follows.

In the present study, the normal feather type in indigenous male and female chickens separately showed higher percentages in different marketplaces, which is similar to the reports of previous workers (Rahman *et al.*, 2024; Beckle *et al.*, 2022; Wario *et al.*, 2021; Ekeocha *et al.*, 2021; Brown *et al.*, 2017; Tabassum *et al.*, 2014) whereas Falculan (2023) found 100% normal feather in native Philippine's male and female chickens. The current study described that overall, males and females had 92.20% and 96.91% normal feathers, which was dissimilar with the reports of Tadele *et al.* (2018) where they advocated that male percentages in three districts (Decha, Chena, Gimbo) were 87.9, 92.5, and 82.5 and female were 71.1, 78.8, and 78.7 and overall male was 87.7 and female 75.2. This study's naked neck percentage was similar to earlier findings (Beckle *et al.*, 2022; Musa, 2022; Brown *et al.*, 2017; Tabassum *et al.*, 2014). The normal feather distribution was statistically significant ($p < 0.001$) in male and female chicken populations separately, which was similar ($p < 0.01$) to that of Tadele *et al.* (2018). The naked neck was not different, and similarly, in males and females, the normal feather was insignificant, which was in agreement with the reports of Wario *et al.* (2021).

Plain head shape (51.18%) was found to be dominant over crest type (48.82%) in Ethiopia (Halima *et al.*, 2007). The overall occurrence of the plain head of Botswana indigenous chickens was 56.4%, and the crest was 43.6% (Machete *et al.*, 2021). In Ethiopia, 78.1% plain head was dominant over barred 21.9% (Musa, 2022). Rahman *et al.* (2024) found that the dominant plain head was 69.34%, followed by cock's comb at 26.42% and the crested head at 4.24%. In a study, roosters and hens had 78.3% and 70.7% flat heads, whereas 21.6% and 29.3% had snake-like heads (Falculan, 2023). The plain head was dominant in males and females over crested head in two districts of Ethiopia (male 97.22%, 100%; female 97.62%, 95.83%), though these two head types were statistically

insignificant (Wario *et al.*, 2021). The results of this study described those plain and crested heads of males were not different in five sites, whereas cock's combs were statistically different ($p < 0.001$). On the other hand, in females, the plain and cock comb heads were different in five sites ($p < 0.001$, $p < 0.05$), and the total males and females showed a statistical difference ($p < 0.001$) in these two traits. So, plain head (overall) was more common in females while cock's comb (overall) was in males. Recently, Rahman *et al.* (2024) found that the plain head shape trait was dominant over others and might result from a male-female combined survey.

Plumage color showed great variations in indigenous chickens. The indigenous chickens in three districts of Bangladesh showed that the black, brownish plumage was 35%, brown with black stripes at 30%, completely black at 30%, and golden red at 5% (Monira & Hussain, 2018). Ferdaus *et al.* (2016) reported that the black color was the predominant type with a percentage of 41.11 indigenous chickens in Bangladesh, which was similar (black 21.8%) to Liyanage *et al.* (2015) in Sri Lanka. In a study conducted in Pakistan by Bibi *et al.* (2021), the different plumage colors showed differences with the highest values in different native chicken populations (green 36.8%; red 32%; white 35.6%; multicolor 65.7% in male, 65.50% in female). Halima *et al.* (2007) studied eight different types of plumage color in Ethiopian indigenous chickens and found 25.49% as white with the highest value. The highest plumage color was black and mottled (17.07%) in Nigerian indigenous chickens. The environment keeps impact on it (Apuno *et al.*, 2011), and the diversity indicates many genes govern the traits and resulted from random mating (Aklilu *et al.*, 2013). Multicolor was dominant (25%) over others (golden mixed 19.81%, brownish-black 16.51%, white and black 16.04%, black 15.56%, white 7.08%) (Rahman *et al.*, 2024). There were seven plumage colors distributed in the neck, eight in the back, and seven in the wing in six breeds of indigenous chickens where different frequencies (4.55% to 100%) were present (Yaemkong *et al.*, 2024). The phenotypic trait white/blk/br was dominant over others in three study areas (20%, 26.67%, and 30%) (Ekeocha *et al.*, 2021). The brown color prevailed as

dominant in the breast, back, and neck (54.4%, 56.7%, 49.0%) regions of six Tswana chickens over others, while in the tail region, black was dominant (Machete *et al.*, 2021). The majority of male (97.06%) and female (96.15%) chickens had color plumage, whereas only 2.94% of males and 3.85% of females had white plumage (Maharani *et al.*, 2021). There were two districts as survey sites, and the white plumage color expressed as dominant in males (Yabello 29.17%, Dire 26.39%) and females (Yabello 28.57%, Dire 29.76%) over other colors and showed a non-significant difference (Wario *et al.*, 2021). The overall male showed 57.3% of red plumage as the highest value, and females had 35.8% of reddish-brown color as dominant phenotypic traits over others, and the difference among ten surveyed traits in three districts was significant ($p < 0.01$) (Tadele *et al.*, 2018). In the current study, males had the highest multicolor plumage, and females had the highest black plumage, and four (black, multicolor, golden mix, and white) out of six plumage colors showed a significant difference in five sites ($p < 0.001$) in male chickens. The female chickens had four traits, *i.e.* black, multicolor, golden mix, and white, which were statistically significant ($p < 0.001$) among five sites. On the other hand, total males and females showed a difference in black ($p < 0.001$), white ($p < 0.01$), and white and black ($p < 0.05$) plumage color.

The shank color of native chickens showed variability all over the world. The highest values showed as yellow in all localities individually (33.3%, 46.1%, 24.5%, 30.5%, and 40.3%) in Pakistan (Bibi *et al.*, 2021), which was in agreement with Liyanage *et al.* (2015) in Sri Lanka (yellow 51%), Musa (2022) (47.1% yellow) in Ethiopia, Halima *et al.* (2007) (yellow 64.42%) in another study in Ethiopia. On the other hand, the Nigerian indigenous chickens showed their shank color as pink, which had the highest value (38.80%) (Apuno *et al.*, 2011). Bekele *et al.* (2022) reported that yellow (85.33%) had the highest value among others (grey, 11.6%; red, 3%) in Ethiopian indigenous chickens. Ninety percent of indigenous chickens had white shanks, and 10% had black shanks (Monira & Hussain, 2018), whereas Ferdaus *et al.* (2016) found black (27.78%) as dominant among others, and white was dominant as

38.21% followed by black 24.53%, yellow 23.58%, and brown 13.68% (Rahman *et al.*, 2024) in Bangladesh. Similar results with white dominant characters were reported by Moreda *et al.* (2014) in Ethiopia (white 33.73%, yellow 32.48%, brown 11.4%, and black 7.75%). There were six distinct breeds, and male chickens showed specific characteristics where the black shank was dominant in Khiew Palee (100%), blackish-green in Pra Dhu Hang Dam (66.67%), grey in Jae (100%), and white-yellow in Thao Thong, Lueng Hang Khao, and Chee (100%) (Yaemkong *et al.*, 2024). The yellow shank color values (54.24%, 65%, 61.67%) from a randomly sampled population (180; 90 male and 90 female) were highest among others in three study areas Ekeocha *et al.* (2021). The grey shank color was found to be dominant in three strains (naked neck, frizzled, dwarf), blue in two (normal, frizzled), and khaki in two (frizzled, rumples), leaving the highest overall mean value (32.9%) as grey (Machete *et al.*, 2021). Roosters constituted 45.9% as the superior white shank, whereas hens showed the same black color (39.0%), as reported by Falculan (2023). The white shank color of male chickens was dominant (45.83% and 36.11%), and in female chickens, it was with the same pattern (48.81% and 40.47%), resulting from two districts, and they were significantly varied ($p < 0.05$) (Wario *et al.*, 2021). The yellow shank color of male chickens from three districts showed all dominant features as 69.3%, 55%, and 52.5%, while females showed white at 32.2% and 40.6% as dominant, and another 40% yellow as dominant like males and the significant variation ($p < 0.01$) was prevailed in overall male and female chickens in surveyed areas (Tadele *et al.*, 2018). The male shank color of the present findings revealed that white was dominant in four sites and brown in only one site, 32.43% over the other colors. A significant variation was found in five sites in white, black, and brown colors ($p < 0.001$), whereas yellow was non-significant. In females, the white shank color was dominant in four sites and black in only one site over the other colors. The variation was found to be significant in five sites: white, black ($p < 0.001$), brown ($p < 0.01$), and yellow ($p < 0.05$). However, the black ($p < 0.001$) and white ($p < 0.05$) shank colors significantly differed in

overall male and female indigenous chickens. Thus, the present results showed similarity with the findings of Wario *et al.* (2021) and Tadele *et al.* (2018).

In our previous study, we found a high proportion (93.87%) of single comb and lower proportion of rose (2.83%), pea (2.36%), and very low cushion type (0.94%) (Rahman *et al.*, 2024), which were similar with the reports of Bibi *et al.* (2021) (92.5% in Pakistan), Monira and Hussain (2018) (99% single comb and others 1% in Bangladesh), Tabassum *et al.* (2014) (99% single comb in Bangladesh), Bhuiyan *et al.* (2005) in Bangladesh (97% single comb), Apuno *et al.* (2011) in Nigeria (96.45% single comb and 0.44% pea comb), and Badubi *et al.* (2006) in Botswana (90% single comb and 1% pea comb), whereas Ferdaus *et al.* (2016) reported 100% indigenous chickens had single comb in BD chickens. Similarly, the comb type of present study showed consistency with the reports of Beckle *et al.* (2022) (Ethiopia: 80.83% single, 16.33% double, 1.67% strawberry and 1.17% rose), Machete *et al.* (2021) (Botswana, all five strains had single comb type as dominant rendering overall mean 81.7%), Moreda *et al.* (2014) (Ethiopia), Liyanage *et al.* (2015) (Srilanka), and Banerjee (2012) (India). However, Halima *et al.* (2007) (single comb, 13.34% only) and Musa (2022) (single, 19%; double, 81%) found exceptional results, which were different from other workers. The single comb showed 100% frequency in only one native chicken breed (Jae), whereas the other five breeds with walnut shape of comb possessed 100% each (Yaemkong *et al.*, 2024); more than 75% single comb was found in three study areas (90%, 78.33%, and 76.67%) (Ekeocha *et al.*, 2021), roosters had single comb as 59.5% and hens had 82.9% as dominant trait in both sexes (Falculan, 2023), males 82.35% and females 80.22% with overall 80.80% (Maharani *et al.*, 2021). In a study, Wario *et al.* (2021) found the traits of 68.06% and 72.22% in males and 73.23% and 68.45% in females as dominant in Yabello and Dire districts, and these values were not significantly different. Males and females both had the highest value (%) in the three districts, leaving an overall male of 59.7% and a female of 72.2% with a statistical difference ($p < 0.01$) (Tadele *et al.*, 2018). The results of the

current study described that the females possessed the single comb trait as dominant and were different ($p < 0.001$) among sites, and males were also different ($p < 0.001$) in five sites. The overall males and females for this trait were also significantly different ($p < 0.001$). Thus, the present study's results conformed to previous workers' research reports, which showed that the single comb was dominant over any other comb types.

In Bangladesh, 53.33% of indigenous chickens had red color earlobes, and the rest had white earlobes (Monira and Hussain, 2018); this was similar to (red/reddish dominant over white) Ferdaus *et al.* (2016) and Liyanage *et al.* (2015) in Sri Lanka. In Ethiopia, 54.17% of indigenous chickens were yellow in color, and others were as red (22.33%), white and red 15.33%, and white 8.17% (Beckle *et al.*, 2022), whereas in the same country Ethiopia white and red ear lobe color was found 49.3% as the highest value among others (red 24.3%, white 18.6%, and yellow 7.9%) (Musa, 2022).

Our previous study indicated that the red ear lobe percentage (63.68%) was dominant over white colored ear lobe (26.32%) (Rahman *et al.*, 2024). This trait showed the distribution as dominant red 53.7% over white 51.4% in hens (Falculan, 2023) in the Philippines; similarly, red was dominant in all indigenous strains- normal 78%, naked neck 66.7%, frizzled 54.6%, rumpless 75%, and dwarf 63.5%, leaving an overall mean 67.6% but the black, yellow, and white ranged from 0-27.6% in five strains with overall means as 11.8% were black, 11.1% were yellow, and 9.6% were white in Botswana (Machete *et al.*, 2021). Maharani *et al.* (2021) reported six Indonesian indigenous chicken breeds individually all had red earlobes as the dominant trait, where overall males had 73.53% and females 79.12% red over white (male 26.47%, female 13.19%) or black (male 0%, female 7.69%). The red trait was superior in male (38.89% and 56.94%) and female (44.64% and 40.48%) chickens, whereas white, as the second value, was 38.89% and 22.22% in males and 37.50% and 35.71% in females. White and red mix, yellow, and yellow with red mix earlobe colors had fewer percentages in the two districts of Ethiopia, and the values were statistically insignificant (Wario *et al.*, 2021).

In a study of three districts of the Kaffa Zone in Ethiopia, Tadele *et al.* (2018) reported that red earlobe was dominant in male chickens (82.8%, 82.5%, and 75%; overall 80.7%) over females (51.1%, 37.5%, and 38.8%; overall 44.2%), with a significant variation ($p < 0.01$). The current study found that the red earlobe was dominant in male chickens at all five sites and in females at four sites out of five. In male indigenous chickens, the red and white earlobe color showed a significant difference in five sites ($p < 0.001$; $p < 0.01$), and in females, both red and white color rendered difference at 0.01% level of significance ($p < 0.01$), whereas overall males and females with red color were insignificant and with white, they were statistically different ($p < 0.01$).

The characteristics need conservation because some traits are of future importance for being vigorous and adapting to harsh environments (Wario *et al.*, 2021). The performances of local chickens can be enhanced greatly with improvement in rearing systems, which will enhance their responsiveness to genetic improvement for increased body weight (meat) and egg production (Ekeocha *et al.*, 2021). On the other hand, the aesthetic characteristics bear testimony because of their valued preferences by consumers. The respective variation can be improved for the specific needs of farmers (Yaemkong *et al.*, 2024) because there is a positive relationship between qualitative and quantitative traits (Wiyabot and Kiattinarueyut, 2022; Tadele *et al.*, 2018). The variations are resulted from unimproved local chickens (Faruque *et al.*, 2010). The variability is caused by both genetic and environmental factors (Alebachew *et al.*, 2020), suggesting a good opportunity for genetic improvement through selection. Qualitative traits are often governed by one or a few genes, indicating that they are inherited traits, and the gene and genotypic frequencies could be estimated from the phenotypic traits (Rahman and Islam, 2002). Thus, the database serves as a guide for conservation and improvement programs facilitating appropriate characteristics within the breeds. Advanced molecular characterization is recommended to assert the advantage of maintaining genetic diversity regarding adaptability, productivity, and preference.

Conclusion

This study estimates variation in phenotypic traits in male and female indigenous chicken populations from five different sites and revealed distinct differences between males and females between the sites, individually or comprehensively. Both genetic and environmental factors cause this variability. The high phenotypic diversity in indigenous chickens is major evidence of high genetic variability at the population level. This variability may provide an opportunity to do research for conservation through selection and breeding improvement strategies.

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Conflicts of interest

The authors declare no conflicts of interest.

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