Characterization of Punjab Brown chicken

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Summary

A survey was conducted in the native tract of the Punjab Brown breed of chicken to study management practices, as well as morphological, performance and egg quality parameters. The study covered the three districts of Gurdaspur district in Punjab, and Ambala and Yamunanagar districts in Haryana, and included 532 birds and 61 families. Twenty-six microsatellite loci were used to assess genetic variability. The Punjab Brown is a multi-purpose breed, yielding good quality meat and eggs. Birds are reared in the backyard system and shelter is provided only during the night in the form of small enclosures mostly made up of mud and sometimes of wood. Average flock size is 8.7. Plumage colour is mostly brown and the pattern is usually solid but is sometimes spotted or striped. Males in particular have black spots/stripes on their neck, wings and tail. The comb is red, of single type and erect in position. The average weight of cocks and hens is 2.15±0.94 and 1.57±0.04 kg respectively. Hens start laying eggs at the age of about five to six months. Clutch size is about four to five. Average egg production is around 60-80 eggs per year. Eggshell colour is mostly light brown and average egg weight is 46.0±1.91g. The average weight of shell, albumin and yolk were 5.4±0.21, 24.4±0.63 and 16.2±0.48g respectively. Yolk index, albumin index and Haugh units were 0.41±0.005, 0.10±0.006 and 82.80±0.98 respectively. A total of 218 alleles were observed. The number of alleles per locus varied from 4-14. The mean PIC value for all the loci was 0.744. Twenty-four loci were found to be neutral (P<0.05) using Ewens Watterson test of neutrality. The exact test revealed that 15 loci deviated from Hardy Weinberg Equilibrium. The population has not undergone any recent bottleneck as revealed by quantitative and graphical qualitative tests.

Resumen

Se ha llevado a cabo una encuesta en la zona originaria de la raza avícola Punjab Brown para estudiar las prácticas de manejo y los parámetros morfológicos y de rendimiento y calidad de los huevos. El estudio se realizó en tres zonas: Gurdaspur (Punjab), y Ambala y Yamunanagar (Haryana) con 532 aves y 61 familias. Se utilizaron 26 microsatélites de loci para averiguar la variabilidad genética. La raza Punjab Brown es tanto de carne como de huevos. Las aves se crían en sistema de corral y se encierran solo durante la noche en jaulas fabricadas la mayoría de las veces con barro y a veces de madera. La media de los grupos es de 8,7 animales. El plumaje es mayormente marrón y casi siempre uniforme, aunque a veces puedes tener manchas o estrías. Los machos suelen tener manchas negras en el cuello, las alas y la cola; la cresta suele ser rojiza, de forma única y en posición erecta. La media de peso de los machos y hembras resultó de 2,15±0,94 y 1,57±0,04 kg, respectivamente. Las hembras empiezan a poner huevos a los 5-6 meses de edad y el tiempo de incubación es de 4-5 días. La media de producción de huevos es de 60-80 huevos por año. El color de la cáscara suele ser marrón. La media del peso de los huevos es de 46,0±1,91 gr. El peso medio de la cáscara, albumen y yema fueron de 5,4±0,21, 24,4±0,63 y 16,2±0,48 gr, respectivamente. El índice de yema, albumen

y de unidades Haugh fueron de 0,41±0,005, 0,10±0,006 y 82,80±0,98, respectivamente. Se estudiaron un total de 218 alelos. El número de alelos por locus varió entre 4 y 14. La media del valor PIC para todos los loci fue de 0,744. Se encontraron 24 loci neutrales (P<0,05) utilizando el test de neutralidad Ewens Watterson. El test reveló que 15 loci se desviaban del Hardy Weinberg Equilibrium. La pobalción no ha encontrado recientemente ningún problema, tal como demuestran la cantidad y calidad gráfica de los tests.

***Keywords**: Chicken, Punjab Brown, Management practices, Morphological characters, Performance traits, Egg quality parameters, Microsatellites.*

Introduction

Rural poultry were the major source of production of eggs and meat in India about two to three decades ago. Backyard poultry farming was an important practice in rural areas, using indigenous or desi birds. These birds are of a scavenging type and require very little input for their survival and production. Growth rate and egg production are very low, but the birds are hardy and adapted to low input conditions. Increasing demand for poultry products and the consequent commercialization of the poultry industry has resulted in a rapid decline in the number as well as the purity of local breeds of birds. Rearing of few birds (5-20) in the backyard system adds significantly to the nutrition and economy of rural people. The eggs and meat of indigenous chicken are preferred over that of commercial birds due to their characteristic flavor, and consequently they fetch a higher price. Native chickens are known to be good foragers, efficient mothers, require less care to grow and are, therefore, most suited for raising under village conditions. These birds do however need special attention to be paid to their conservation and improvement. Many of the Indian chicken breeds exist as names in the literature but there is no information on their characteristics and performance. There is a need to define existing chicken populations/breeds and to develop improvement and conservation programs so as to benefit rural people. The Punjab Brown is one such breed, and is found in northern India. Although Mahapatra and Panda (1981), Bhat *et al*. (1981), Acharya and Bhat (1984), Ayyagari (2000), Singh and Johari (2000) and Singh and Singh (2000) have reported on this breed, no detailed information exists. Therefore, an attempt has been made in this study to characterize and evaluate Punjab Brown breed in its native tract.

Materials and Methods

A survey was conducted in the Gurdaspur district of Punjab, and the Ambala and Yamunanagar districts of Haryana covering a total of 15 villages (10 in Punjab and 5 in Haryana). 532 birds (336 in Punjab and 196 in Haryana) maintained by 61 families (34 in Punjab and 27 in Haryana) were observed. Data on management practices, morphological characteristics and body weights were recorded. Performance parameters were recorded by interviewing the farmers. Eggs were collected for the purpose of studying quality parameters. Eggs were weighed and then broken out onto a level surface. The height of the thick albumen and yolk were measured with a spherometer. Egg yolks were weighed after separation from albumen. Shell thickness was measured with micrometer. Average body weight, and egg, albumin, yolk and shell weights were measured. Student's t – test was used to study the differences between birds of Punjab and Haryana area. Albumin index, yolk index and Haugh units were estimated as:

- Albumen index=Height of albumen/Average width of albumen.
- Yolk index = Height of yolk/Average width of yolk.
- Haugh unit = 100 log (H + 7.57 - 1.7 $W^{0.37}$)

where H = Height of albumen and
W = Weight of egg.

Blood samples were collected from 44 unrelated birds from the breeding tract. The DNA was isolated using standard laboratory protocol (Sambrook *et al*., 1989). The 26 microsatellite primers were selected based on their location, size and polymorphic information content (Table 1). The primers were tagged with Hex and Fam dyes. The genotyping was performed using ABI Avant 3100 Automated DNA Sequencer and Gene Mapper software version 3.0. The statistical analysis was performed using Popgene software (Yeh *et al*., 1999). The mutation drift equilibrium test was applied using all the three models of microsatellite evolution using Bottleneck software version 1.2.02 (Cornuet and Luikart, 1999). The exact test for deviation from HWE was also carried out as implemented in Genepop software version 3.4 (Raymond and Rousset, 1995).

Results and Discussion

Distribution

Birds of the Punjab Brown breed are found in rural areas of Punjab and Haryana (Figure 1). They are used for both meat and egg production. In Punjab, these birds are maintained by progressive farmers as well as by poor families. While the former keep the birds for home consumption, the latter sell live birds/chicks and eggs as part of their livelihood. In Haryana, birds are generally maintained as a cash reserve by a few low-income families located in one part of the village. These birds are also found in the slums on the outskirts of cities with their owners doing good business because of the readily available market.

Flock size and composition

The number of birds per household mostly varied from 3 to 15. Average flock size was

Figure 1. Distribution of Punjab Brown chicken.

Table 1. Details of microsatellite primers.

Locus	Forward and reverse primers	Annealing temperature	Dye	Chr. no.
HUJ 002	CATCTCACAgAgCAgCAgTg gAATCCTggATgTCAAAgCC	55	FAM	17
HUJ003	gACAgCAAggATTAACCTgAg gTCTTggAgACTgTTAgTTgg	55	FAM	1
LEI120	CgTAACACATgCAACTCAATg TTAgAATgAAAAggCTgTTCC	55	FAM	15
LEI122	AATCCCTATAgAACTTTgTgC gATCTTACTggATTACCATTC	55	HEX	4
LEI147	TCAggCCTCTTgAACTCAgg gCTATTAAgATACCTCAgCTC	55	HEX	2
LEI155	gTACgTgTAgCTCggCTCACC gTCCgTgCATggCTCCgCTC	55	FAM	24
LEI166	AAgCAAgTgCTggCTgTgCTC TCCTgCCCTTAgCTACgCAC	55	HEX	3
LEI174	ATCATACATgTTCTAgggCTg AAAgggCATTCCCgCATgAg	55	HEX	1
LEI64	TggTTgTCTCAATACAACggTC CTgTAAAgATTTCTCAgAAACAg	55	HEX	7
LEI74	AAACgTCTgCCTTCATgCgAg CATCAATTAgAgCgAAgCCTC	55	FAM	26
LEI80	gTTAgAgCCATACAgAAACTTC ATCACACAAgCTTTCTTCCTg	55	HEX	E46C08W18
LEI82	TATCCATACAgTACCCTCCTg CCTTAgCTggCTCAgTggATg	55	HEX	5
LEI90	TAgTgCAgCCCTATggAgCg ggTgAgTgTgCgTTACACgC	55	HEX	23
LEI98	CAgTTAgCAgAgATTTTCCTAC TgCCACTgATgCTgTCACTg	55	FAM	14
MCW176	AAAgAgAAgTATAAAACATgCC TCCATTCTTggCAgTgCATAg	55	HEX	6
MCW213	CTgTTCACTTTAAggACATgg gACAAgTCAACAACTTgCCAg	55	FAM	13
MCW217	gATCTTTCTggAACAgATTTC CTgCACTTggTTCAggTTCTg	50	FAM	18
MCW228	gATCTCTgCATTACAAgCATg TTgCTgACCTgCTCATgCAAg	55	FAM	10
MCW250	CAgAATTTAgAgACTgTCTAC ATACggTAgCTCTgTTgCAAg	55	FAM	6
MCW261	gTAgTAgCAgCTACACCAgAg gAgCAgTTCATATgAAgTgCAg	55	FAM	3
MCW262	gATCCAggCTTTAAgAAgAgg gATCTTgTACATgCCAgCAC	55	HEX	E46C08W18
MCW266	gATCCCCATgCgCACAC TTgCTACACTTCCACCTTTgg	55	HEX	19
MCW305	TCAgAAACAAAgCAggAgCTg TgACATCTTTCAAACgAgACC	55	FAM	8

(Table 1 to be continued ....)

(... Table 1 to be continued)

Locus	Forward and reverse primers	Annealing temperature	Dye	Chr. no.
MCW317	ACTTgTTggCTgCTTgAgATg ATgCATgCATTCACAgAAAgC	55	HEX	E46C08W18
MCW328	ATggAAACAgATggAgCTggC CTCCAATCCCAggCTCCAAC	55	FAM	27
MCW84	TTTgAAgggATgCTgCATgCA CTgATTTgCAgCTTggCTgAg	50	HEX	9

8.7 and was larger in Punjab (9.8) than in Haryana (7.3). On average, a flock in Punjab consisted of 24.7% chicks, 17.6%cocks and 57.7%hens while that in Haryana consisted of 49.5, 9.7 and 40.8 % chicks, cocks and hens respectively. Most of the flocks were of mixed type consisting of indigenous birds of varying colors. About 60 to 70 % of birds were of Punjab Brown type. Flocks of pure Punjab Brown birds were very few (2-3%).

Management practices

The birds are reared in a backyard system. Shelter is provided mostly during night. About 10% of farmers keep the birds confined both during the day and at night. Enclosures are small, mostly made of mud (68%). About 30% were made of bricks and 2% of wood. Most of the enclosures were single storied and only about 2% were

Figure 3. Punjab Brown hen.

multistoried. Some of the farmers have even made provision for birds below the mangers of cattle or buffalo. Chicks are kept under a basket made of bamboo sticks.

Birds are set free in the morning and scavenge the whole day in the vicinity of the farmer's house. The birds return to their enclosures in the evening. Hens come in as required to lay eggs. Farmers feed whole grains (wheat, broken rice, etc.) in morning and evening only. Kitchen waste is fed to the birds in a routine manner. Eggs for hatching are put on paddy husk on the floor or in a basket. About 40% of farmers usually and 15%sometimes set eggs for hatching at home while others purchase chicks. The broody hen is made to sit on the eggs, and then it is covered with a basket. Sometimes the hen is made to sit in an earthen pot or '*Hara*' which is covered with a stone and placed in one corner of the room meant for family members. During the hatching period of 21-22 days, the hen is regularly fed grains and water. Birds are not vaccinated against any disease.

Morphological characteristics

The plumage colour is mostly brown (Figures 2 to 6). Some black or white coloured birds with a golden colour on their neck, wings and tail are also available. The pattern is usually solid but sometimes it is spotted or striped. Males in particular have black spots/stripes on their neck, wings and tail. The neck is darker in colour (brown/golden) than the rest of the body. The skin is white and the shanks are yellow. The ear lobes are mostly brown but sometimes white or grey depending upon the plumage colour. The wattles are red, large sized in males and small in females. The eye ring is red as is the comb which is single type and erect in position. Very few hens have a floppy comb. The beak is yellow but in many birds the upper part of the beak turns black with age.

Performance

The overall average weight of cocks and hens was 2.15±0.94 and 1.57±0.04 kg, respectively (Table 2) and the differences were statistically significant ($P<0.001$). These weights were on the low side of those reported by Mahapatra and Panda (1981). The weights of cocks as well as of hens in the two regions were not significantly different.

Hens start laying eggs at the age of about five to six months. Clutch size is about four to five. They lay about 15-20 eggs in one laying period of around 25-30 days, then the hen becomes broody and incubates eggs for 21-22 days. After hatching, it broods the chicks for 30-45 days and then the hen enters its next laying cycle. Each laying cycle takes about three months and in a year a bird undergoes a maximum of four laying cycles. Average egg production is around 60-80 eggs per year. Hatchability is about 60-80 % on a total egg basis and is lower in summer compared to that in winter. Mortality up to one month ranges from 10 to 30 %.

Table 2. Adult body weights (kg).

		Punjab Area	Haryana Area	Overall*
Cock	Mean	2.18±0.11 (36)	2.09±0.10 (15)	2.15±0.94 (51)
	Range	1.2 – 4.5	1.5 – 3.0	
Hen	Mean	1.70±0.04 (67)	1.40±0.05 (56)	1.57±0.04 (123)
	Range	1.2 – 2.7	0.8 – 2.8	

( ) No. of birds.
*Differences significant $P \leq 0.001$.

Table 3. Egg quality parameters.

Parameter	Haryana Area (29)	Punjab Area (8)	Overall (37)
Egg wt (g)	44.79±1.5	49.02±1.5	46.00±1.2
Shell wt (g)*	5.02±0.3	6.39±0.3	5.41±0.2
Albumin wt (g)	23.99±0.8	25.28±1.2	24.36±0.6
Yolk wt. (g)	15.79±0.6	17.36±0.7	16.24±0.5
Shell thickness (mm)**	0.32±0.0	0.35±0.0	0.33±0.0

( ) No. of eggs.
*Differences significant $P \leq 0.01$.
** Differences significant $P \leq 0.05$.

Egg characteristics

A light brown shell colour was most frequent (60.7%) followed by brown (25%) and dark brown (14.3%). Average egg weight was 46.0±1.19g (Table 3) and did not differ significantly in birds from the Punjab and Haryana areas. Average egg weight of Punjab Brown birds was more than the egg weight of 30-35g generally found in desi birds and was similar to that of the Brown Nicobari (45g, Padhi *et al*., 2004) and Kadaknath (44.2g, Annual Report, 2001-2002) breeds. Average weight of shell, albumin and yolk were 5.4±0.21, 24.4±0.63 and 16.2±0.48g respectively. The shell was strong and average thickness was 0.33±0.007mm**.** Eggshell weight and thickness were significantly higher in the Punjab area (6.4g and 0.35mm) than those of the Haryana area (5.0g and 0.32mm) while albumin and yolk weights were not different. The consistency of albumin was thick. Yolk was yellow in colour in the majority of eggs (53.6%) followed by deep yellow (39.3%) and light yellow (7.1%). Yolk index, albumin index and Haugh units were 0.41±0.005, 0.10±0.006 and 82.80±0.98 respectively. Blood spots were absent in both albumin and yolk. Meat spots were absent in yolk but were present in albumin of about 18% of eggs.

Figure 4. Punjab Brown cock.

Egg composition

On average, an egg contained 35.3, 52.9 and 11.8 % yolk, albumin, and shell and shell membranes respectively. The percentage of yolk, albumin and shell ranged

from 31 to 42, 45 to 58 and 9 to 17 respectively in different eggs.

Multi-locus genotyping

A total of 218 alleles were observed in 26 microsatellite loci. The number of alleles per locus varied from 4-14, with a maximum (14) in locus LEI 120 and LEI 82, and a minimum (4) in LEI 90, MCW 84 and LEI 174. The minimum effective number of alleles was found to be 1.8 in HUJ 002 (Table 4). The microsatellite loci used in the study were hypervariable and to test the deviation from Hardy-Weinberg proportions, Exact test (Guo and Thompson, 1992; Charkraborty and Zhong, 1994) was employed. The analysis revealed 15 out of 26 microsatellite loci deviated from Hardy Weinberg proportions. The observed heterozygosity was less than the expected heterozygosity in 20 of the 26 loci. The mean overall heterozygosity over all loci was quite high (0.602) as compared to the commercial strains of poultry, which is usually 0.54 and 0.26 for broiler and layers respectively (Croojmans *et al*., 1996). The mean F_{IS} value was found to be 0.191, which was significantly different from zero. This suggests that the Punjab Brown population is a closed population with little gene flow. The deviation from the Hardy-Weinberg equilibrium can be attributed to non-random mating among the individuals of the population and or due to selection. The Ewens Watterson test of Neutrality (Watterson, 1978) revealed 24 out of 26 loci to be neutral and thus selection as the cause of a decrease in observed heterozygosity is ruled out. Thus the only plausible reason for the difference between observed and expected heterozygosity is non-random mating among the individuals of the population.

To test the null hypothesis of mutation drift equilibrium, three quantitative tests viz. Sign, Standardized difference and Wilcoxon Rank (Cornuet and Luikart, 1996) were applied using all three models of

Figure 5. Punjab Brown chickens in front of enclosure.

Table 4. Number of alleles, heterozygosity and Fis.

Locus	No. of observations	No. of alleles observed	Effective no. of alleles	Allele size	Observed heterozygosity	Expected heterozygosity	PIC	Wright's Fixation Index (Fis)
HUJ002	84	5	1.7827	121-137	0.3810	0.4443	0.4391	0.1323
HUJ003	88	8	4.6538	147-182	0.8636	0.7941	0.7851	-0.1000
LEI120	88	14	7.5922	274-314	0.6591	0.8783	0.8683	0.2409
LEI122	86	8	4.6869	285-301	0.6047	0.7959	0.7866	0.2314
LEI147	62	11	6.3642	255-285	0.2903	0.8567	0.8429	0.6556
LEI155	88	7	3.7924	92-104	0.9545	0.7448	0.7363	-0.2964
LEI164	88	9	3.9111	255-263	0.4545	0.7529	0.7443	0.3893
LEI166	88	5	3.3236	230-263	0.5000	0.7072	0.6991	0.2848
LEI174	88	11	4.4506	290-304	0.5455	0.7842	0.7753	0.2965
LEI74	88	6	3.4883	301-313	0.5227	0.7215	0.7133	0.2672
LEI80	86	8	6.7359	185-207	0.8605	0.8616	0.8515	-0.0105
LEI82	86	14	4.2751	246-284	0.8140	0.7751	0.7661	-0.0625
LEI90	88	4	2.0135	204-214	0.4091	0.5091	0.5034	0.1873
LEI98	88	8	2.3740	152-168	0.5909	0.5854	0.5788	-0.0210
MCW176	66	8	4.5756	257-270	0.1212	0.7935	0.7815	0.8449
MCW213	82	8	5.0030	290-316	0.7073	0.8100	0.8001	0.1160
MCW217	86	9	3.5489	149-175	0.6512	0.7267	0.7182	0.0934
MCW228	86	11	5.6286	218-248	0.7907	0.8320	0.8223	0.0385
MCW250	84	8	4.3717	222-236	0.4762	0.7806	0.7713	0.3826
MCW261	82	8	5.3112	243-257	0.8537	0.8217	0.8117	-0.0517
MCW262	88	9	5.2752	61-77	0.9773	0.8197	0.8104	-0.2059
MCW266	88	7	3.7159	159-187	0.7045	0.7393	0.7309	0.0360
MCW305	64	9	3.4020	258-268	0.0938	0.7173	0.7061	0.8672
MCW317	82	11	7.4711	229-255	0.8049	0.8768	0.8662	0.0707
MCW328	82	8	4.3776	249-263	0.5122	0.7811	0.7716	0.3362
MCW84	88	4	2.9854	93-99	0.5000	0.6727	0.665	0.2482
Mean	83.61	8.38	4.4270	-	0.6017	0.7532	0.7441	0.1914

Figure 6. Hen brooding chicks.

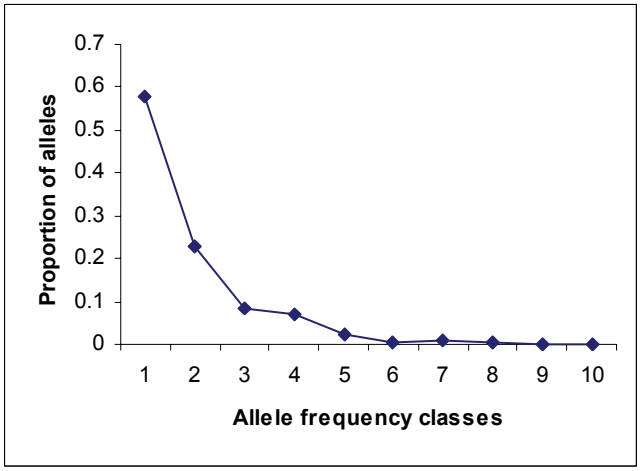

Figure 7. Normal L-shaped curve of alleles (proportion and frequency) in the Punjub chicken.

Table 5.Test for Null Hypothesis under three microsatellite evolution models.

Test		Model of microsatellite evolution		
		IAM	TPM	SMM
Sign Test (No. of loci with heterozygosity excess)	Expected	15.49	15.44	15.27
	Observed	22*	19	6
Standardized differences test (T_2 values)		3.401*	0.499	-5.323
Wilcoxon Rank Test (Probability of heterozygosity Excess)		0.00001*	0.15158	0.99853

*Bottleneck (rejection of null hypothesis of mutation drift equilibrium).

microsatellite evolution. The Infinite Allele Model (IAM) revealed heterozygosity excess and rejected the null hypothesis. The null hypothesis was accepted for both two-phase model (TPM) and stepwise mutation model (SMM). The values for these tests are given in **Table 5**. The TPM and SMM are the most suitable models for microsatellite evolution and the population can be considered in mutation drift equilibrium. The mode shift graphical test (Luikart *et al*., 1998) also accepted the null hypothesis and the population showed normal L shaped distribution (**Figure 7**). Thus, the Punjab Brown has not experienced any recent genetic bottleneck.

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