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How Climate Change is Affecting Migration of Birds



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The climate on earth is changing drastically. Some birds adapt to changing climate patterns while others are on the verge of extinction. Migratory birds provide food to other wildlife and help in controlling pests and pollinating birds. The climate change and global warming we are experiencing is human induced, which makes it difficult to cope up for the birds. Other than global warming, pollution, increasing population, depletion of natural resources, sea level shifts, lower water tables, higher draught frequency, all collectively impact habitat destruction which affect migration of birds. Short distance migratory birds are less affected by changing climate patterns, while long distance birds are struggling to keep up with their migration rhythm.

During migration, birds need to refuel to continue their long journey. Habitat loss because of increasing temperatures causes flooding, drought, or desertification. Change in topography of landscape can affect migration route and food resources and can make bird feel exhausted at the end of the journey. Extreme weathers and intense storms can kill birds during migration due to thermals, and water and food scarcity.

Migratory birds use coastal wetlands as resting area and for foraging. Climate change in Sahel region is an example of loss of habitat and resulted in desertification and depletion of resources. This also affected rainfall and made the climate worse for migratory birds. Similarly, Sahara expansion and habitat loss has made it difficult for African Eurasian migratory birds to cross Sahara region because of no possibility of resting. Climate change is also affecting vegetation structure of Siberian region. The rise in temperature has caused forest expansion in the region to Tundra. Researchers have reported that forest expansion in Siberian region has caused loss of breeding habitat for more than 90% species of arctic goose.

The climate change is disturbing ecological balance. Some migratory birds cancel their journey and prefer to stay in their breeding places because of inhospitable stop over sites or changed weather pattern. This also affects food web for other animals and insects. Actions should be taken for successful breeding and survival of these birds. To fight climate change, more awareness should be given to reduce pollution and carbon emissions. Government should also take important steps to reduce the energy use and develop more sustainable future to conserve migratory birds and their habitat. Bird conservation programs should establish and protect migratory areas for birds. Migratory bird species and all other species on planet earth should be protected. This is the only they will perish.





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Original Article



Development and Implementation of an Optimized Rearing Protocol for Peafowl and Pheasant Chicks

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ABSTRACT

Maintaining high fertility and hatchability is essential for producing healthy chicks. **Objectives:** To identify factors affecting hatchability and fertility rates to develop effective rearing practices. **Methods:** The investigated factors responsible for lower fertility and lower hatchability rates were improper male-to-female ratios, the age of breeder flocks, egg storage duration, sand bedding, remnants of dead shells in incubators, rectangular brooders, and the absence of vaccinations and deworming. Data analysis involved the use of descriptive statistics and hypothesis testing. The study was based on one year of experimental data (January 2023 - September 2023) and six years of records from the Jallo Park Lahore captive breeding center (2017-2022). This research focused on two bird groups from the Galliformes order: peafowl and pheasant. This study analyzed 1,400 hatching eggs from six different peafowl breeder flocks and 1,480 hatching eggs from five pheasant breeder flocks. **Results:** Hatchability ratio in peafowl improved from 16% in 2017 to 60% in 2023, while fertility rates increased from 26% to 82% in 2023. For pheasant, hatchability rose from 34% in 2017 to 60% in 2023, with fertility rates reaching a peak of 86% in 2023. **Conclusions:** It was concluded that implementing a new protocol can improve both fertility and hatchability. Key components of this protocol for improving fertility include male-to-female ratio, timely egg collection, and proper floor bedding. To improve hatchability, clean the incubators, use circular brooders to reduce mortality, and apply vaccination and deworming, all of which significantly impact outcomes.

INTRODUCTION

The peafowl's great beauty makes it one of the world's most attractive animals. It is part of the *Phasianidae* family and falls under the order Galliformes within the class Aves, which includes 38 genera and around 138 species [1]. Peafowl typically engage in breeding activities during the rainy season, with the breeding period spanning from April to August [2]. Peafowl normally arrive at sexual maturity at two to three years old. The peahen lays between three to six

eggs, which are buff white, one egg each day, with an incubation period of 28-30 days [3]. Peafowl possess similar anatomical and physiological features to birds of the common order Galliformes, such as pheasants, but they also have unique anatomical and physiological characteristics [4]. The main distinction between peafowl and pheasants lies in their feather coloration. There are 49 recognized pheasants globally, with Pakistan hosting five

of these species [5]. Generally, pheasants are considered game birds and are often raised for hunting in many countries. Wild birds are kept in captivity to promote optimal growth, create a suitable environment for conservation and research, and enhance reproduction rates [6]. Although it is commonly believed that captivity comes with various challenges, including the study of early mortality and the resolution of captivity-related issues [7] such as proper nutrition, suitable housing, male-to-female ratios, external stressors, internal factors due to sudden environmental changes, feeding methods, and unfavorable microclimate conditions [8]. Numerous factors impact the successful hatching of a fertilized egg, including the age of the breeder, the shape index of the egg, the weight of the egg, the color and mottling of the shell, the initial egg mass, the amount of mass lost, and the yolk sac index [9]. The age of the flock directly causes early mortality. The mating sex ratio in captivity is a significant issue that greatly impacts egg quality, fertility, hatchability, and weight [10] was carried out to assess the influence of two mating ratios (8:1 and 12:1 female to males) on the health of birds, egg production and quality, fertility, and hatchability in pheasants. The fertility of eggs produced by birds in the 8:1 mating ratio was consistently and significantly 4% higher than that of eggs from the 12:1 mating ratio. Lack of fertilization (due to insufficient sperm) was twice as high in the 12:1 enclosure, accounting for 13.7% of the eggs [11]. Storing eggs for an extended period decreases eggshell weight, eggshell thickness, yolk weight, albumen index, yolk index, Haugh unit, shell percentage, albumen percentage, and yolk percentage ($p < 0.05$). By day 8, hatchability was significantly diminished, reaching its lowest value of 41.59% [12]. The causes of high embryo mortality in the hatching process can be caused by bacterial and fungal contamination and poor egg storage conditions (storage room temperature). Dickens et al., found that bacteriological investigation of dead-in-shell embryos has shown that different bacterial isolates may be causing embryonic mortalities, including *Salmonella* spp., *Escherichia coli* (*E. coli*), *Staphylococcus* spp., *Streptococcus* spp., *Pseudomonas* spp., *Proteus* spp., *Bacillus subtilis*, *Klebsiella* spp., *Micrococcus* spp. and *Mycoplasma* spp [13]. It is crucial to provide meticulous care and attention, as neglecting these essential factors can lead to increased mortality rates in captivity [8].

This study aims to examine the impact of a new rearing protocol on the successful production of eggs, fertility, and hatchability in peafowl and pheasants, as well as to contrast it with the conventional method. By highlighting the notable difference in mortality rates, hatchability, and fertility rates with pre- and post-rearing, this paper focuses on the importance of implementing novel rearing techniques to enhance the productivity and profitability of

peafowl and pheasant breeding programs under captivity.

METHODS

The cohort study was conducted at the Department of Wildlife and Parks, Lahore, Pakistan. The study lasted from January 1, 2022, to September 30, 2023. We used data from 2017 to 2022 as a historical control group to compare with the current study results. This approach allowed us to observe and interpret changes more accurately by establishing a reference baseline, similar to previous studies [14, 15]. The selected species of peafowl are *Pavo cristatus* (common peacock/Indian peacock), pied peacock, *Pavo cristatus* Linn. (black shoulder peacock), and *Pavo muticus* (Java green peacock). The selected species of pheasants are *Phasianus versicolor* (green pheasant), *Lophura nycthemera* (silver pheasant), *Chrysolophus pictus* (golden yellow pheasant), *Crossoptilon crossoptilon* (white pheasant), and *Phasianus colchicus* (ring-necked pheasant). The research conducted from January 2023 to September 2023 was segmented into five distinct phases. Each phase provided a concise overview of the breeding activities, following a new rearing protocol (Table 1).

Table 1: Phases of Study Plan Presenting Bird Type and Different Phases for the Year 2022-2023

Bird Types	Year	Phase A	Phase B	Phase C	Phase D	Phase E
Peafowls	2022-2023	(January-February)	(March - April)	(May-June)	(July-August)	(September)
Pheasants	2022-2023	(January-February)	(March - April)	(May-June)	(July-August)	(September)

In the initial phase A, from January to February (2022-2023), the selection of peafowl and pheasant pairs was based on their physical fitness and reproducibility. A total of 20 peafowl pairs were purposively selected, with a male-to-female ratio of 1:2, and 40 pheasant pairs were chosen, with a male-to-female ratio of 1:3 purposively selected present at Jallo Wildlife Park, Lahore, Pakistan. The birds were fed a standard breeder diet called "breeder layer," supplemented with legumes, seeds, peas, beans, and leafy green vegetables, including carrot tops, green scraps, and grasses [16]. In the second phase B, from February to March (2023), the 20 peafowl pairs were kept in individual breeding rooms, with a male-to-female ratio of 1:2, and the 40 pheasant pairs were housed with a male-to-female ratio of 1:3. It has been established that providing chicks with sufficient feed, particularly protein, is necessary for modern, promising, engaging, and productive farming [8]. Offering the best diet to the separated pairs is a priority, as diet directly affects their reproductive system and egg-laying capacity [17]. The selected birds were kept in captivity, with the floor bedding of paddy husk because sand, rice husk, or wheat can lead to conditions that help form diseases during decomposition, resulting in higher carbon dioxide and ammonia gases in the microclimate air.

In the second month of Phase B (March), eggs were gathered from separate cages to maintain their breeding environments. To ensure the hygiene of the eggs, freshly laid ones were collected three times daily and organized into sets. In Phase C, from April to May 2023, disinfected eggs were transferred to an incubator asset. The incubator was maintained at a constant temperature of 99°F and a humidity level between 55-60 g.m-3 [18]. Peafowl eggs require an incubation period of 28-29 days, while pheasant eggs hatch successfully after 22-24 days. After ten days of incubation, the critical process of candling began. Candling involves rotating the eggs hourly. This process continues until the incubation is complete. The rotation of the eggs ensures proper orientation for effective heat distribution. The automatic system positions each egg with its broad face on the upper side and the pointed end on the lower side. Three days before hatching, the incubation and candling processes were completed. In the second month of Phase C (May), three days before the anticipated hatching date, the eggs were successfully moved from the incubator to a hatcher, where the temperature was maintained between 97-96°F and the humidity level was around 75 g.m-3. The hatcher provides a more suitable environment for hatching by maintaining lower temperatures and higher humidity levels. In Phase D, which ran from June to July 2023, the newly hatched chicks were transferred to specialized enclosures called circular brooders. The brooders were designed in a circular shape to provide enough space for the chicks to move around without getting stuck in corners, preventing overcrowding and accidents. To maintain a warm environment within the brooder, 100-volt bulbs were used. During the brooding period, proper nutrition was provided; in the first week, chicks were fed a starter feed specially formulated to meet their nutritional needs [19]. Flushing occurred naturally when the chicks were introduced to regular (starter) feed. The vaccination protocol spanned one month and required repetition of certain medications as per standard guidelines. Therefore, the repeated entries reflect actual practice and cannot be altered without compromising data integrity. A mixture of Amoxicillin, Tyloxox, Lincomycin, Spectinomycin, and Amino Wit was administered on the first day to prevent infections. This regimen continued for one and a half months. After seven days, the Newcastle Disease (ND) vaccine was regularly sprayed to control viral infections such as influenza and colds, with this routine repeated every seven days for a month until the chicks reached three months of age [20]. On day 21, the chicks received CNC, NOCOX, and ADEK medication. This included C.N.S 200 powdered antibacterial agents (chlortetracycline HCL, neomycin sulfate, colistin sulfate) to manage diseases like coccidiosis, along with a combination of vitamins (ADEK) and NOCOX. Adding amino

acids enhanced nutrient absorption from the feed, while the antibiotics provided protection. The floor bedding in the brooder was made of corrugated material, which was changed daily. The chicks remained in the brooder for one and a half months, consuming 10 to 30 grams of feed during this period [21]. Medication and feeding protocol from zero-day until 1.5-month-old fancy birds rearing are analyzed (Table 2).

Table 2: Medication and Feeding Protocol from Zero-Day Until 1.5-Month-Aged Fancy Birds Rearing

Age (Day)	Vaccination Program (Peacocks and Pheasants)	Feed Activity	Method
00	Flushing with 4% sugar solution, Amino wit	Nil	At Hatchery
01	(Amoxicillina/Tyloxox/ Linkomycin/Spectinomycin) + Amino wit	Starter feed + Promotor L (Amino Wits) nutrition	Water
02	(Amoxicillina/Tyloxox/ Linkomycin/Spectinomycin) + Amino wit	Starter feed + Promotor L (Amino Wits) nutrition	Water
03	(Amoxicillina/Tyloxox/ Linkomycin/Spectinomycin) + Amino wit	Starter feed + Promotor L (Amino Wits) nutrition	Water
04	(Amoxicillina/Tyloxox/ Linkomycin/Spectinomycin) + Amino wit	Starter feed + Promotor L (Amino Wits) nutrition	Water
05	(Amoxicillina/Tyloxox/ Linkomycin/Spectinomycin) + Amino wit	Starter feed + Promotor L (Amino Wits) nutrition	Water
07	N.D vaccine	Starter feed + Promotor L (Amino Wits) nutrition	Spray
08	Amino wit	Starter feed + Promotor L (Amino Wits) nutrition	Water
09	Amino wit	Starter feed + Promotor L (Amino Wits) nutrition	Water
14	N.D vaccine	Starter feed + Promotor L (Amino Wits) nutrition	Spray
15	Amino wit	Starter feed + Promotor L (Amino Wits) nutrition	Water
16	Amino wit	Starter feed + Promotor L (Amino Wits) nutrition	Water
20	N.D vaccine	Starter feed + Promotor L (Amino Wits) nutrition	Spray
21	CNC + NOCOX + ADEK	Starter feed + Promotor L (Amino Wits) nutrition	Water
22	CNC + NOCOX + ADEK	Starter feed + Promotor L (Amino Wits) nutrition	Water
23	CNC + NOCOX + ADEK	Starter feed + Promotor L (Amino Wits) nutrition	Water
27	N.D vaccine	Starter feed + Promotor L (Amino Wits) nutrition	Spray

Amino wit: Two days a week for six weeks. Deworming: At the age of 1.5 months, after 15 days, then after one month. N.D vaccine: After every month

Phase E commenced in August and continued through September 2023. The chicks were carefully transferred to the rearing section. The diet and medication plans were maintained, consisting of starter feed combined with Promotor L (Amino Wits) for nutrition and CNC, NOCOX, and ADEK for three months. Deworming procedures were also implemented in the rearing section to ensure the cleanliness of the internal parasites in the stomach. This process began at one and a half months of age, followed by a repeat after fifteen days and again after one month. The floor bedding in the rearing section consisted of rice husk and wood shavings. After three months in the rearing section, the chicks matured successfully. A one-way ANOVA was conducted to evaluate the variation in egg production across all years. Protocols for the yearly rearing of peafowl are shown (Figure 1).

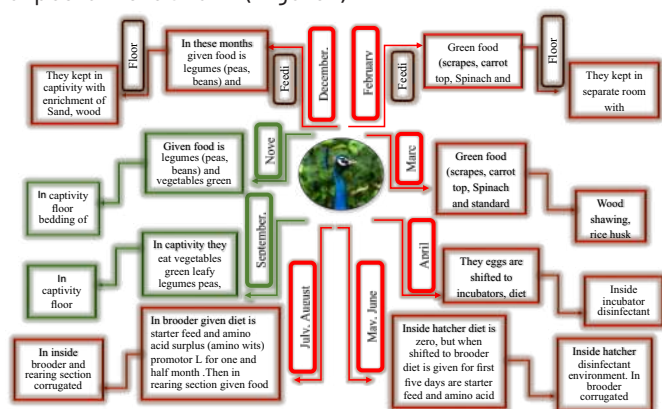


Figure 1: Yearly Rearing Protocol of Peafowl

Protocols for the yearly rearing of pheasants are shown (Figure 2).

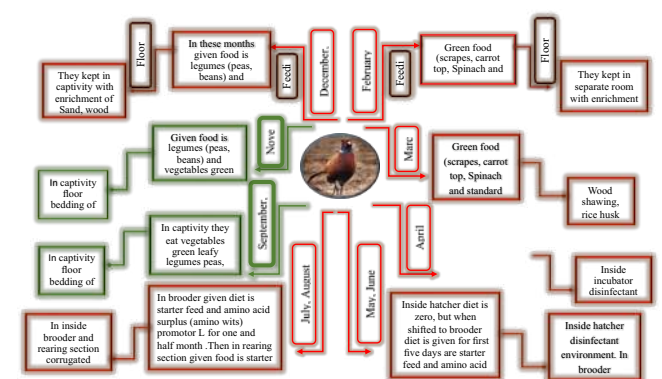


Figure 2: Yearly Rearing Protocol of Pheasants

RESULTS

By implementing established protocols in captivity on peafowl and pheasant selected species, the following percentages of fertility and hatchability and total number of eggs from 2017 to 2023 were analyzed using one-way

ANOVA. The study compared fertility and hatchability rates in peafowl and pheasants from 2017–2023 using a one-way ANOVA. Statistical analysis revealed significant differences in hatchability across years ($F(6, 28) = 8.34$, $p = 0.001$, 95% CI [12.3, 28.7]), with post-hoc tests confirming higher rates in 2023 versus baseline years (all $p < 0.05$) (Figure 3). According to previous records, there has been a notable variation over the years—the number of eggs produced by peafowls recorded year-wise. As shown in Figure 3A, egg production varied from 860 in 2017 to a low of 680 in both 2018 and 2019. Recovery started in 2020 with 980 eggs, then significant growth to 1,200 in 2021. After a decline to 737 in 2022, production increased to 1,400 in 2023. In 2023, a significant peak in egg production of peafowls was observed, with a total of 1,400 eggs following the implementation of an established rearing protocol. The fertility rates of peafowl eggs have steadily increased over the years, as shown in Figure 3C. In 2017, the fertility percentage was 26%, rising to 31% in 2018 and 30% in 2019. After slightly declining to 27% in 2020, the fertility rate improved to 34% in 2021 and 48% in 2022. By 2023, the fertility rate reached 82%, indicating a substantial enhancement due to the new protocol. For peafowl, hatchability improved from 16% (2017) to 60% (2023) ($F(4, 18) = 5.22$, $p = 0.006$) (Figure 3E). Hatchability percentages were 16% in 2017, 34% in 2018, and 47% in 2019. During 2020, the rate decreased slightly to 46%, then dropped to 33% in 2021, recovering to 38% in 2022. The hatchability rate peaked at 60% in 2023, demonstrating the effectiveness of the new rearing practices. Pheasant fertility, hatchability rates, and egg output have all changed significantly. The total number of eggs produced by pheasants is shown in Figure 3B. In 2017, a total of 860 eggs were recorded. This number saw a significant increase in 2018, reaching 1,580 eggs, and continued to rise to 1,720 eggs in 2019. In 2020, egg production slightly decreased to 1,680 eggs and then dropped to 1,180 eggs in 2021. In 2022, production was 1,260 eggs, and by 2023, it increased to 1,480 eggs. In Figure 3D, the fertility rates of pheasants have also shown notable changes over the years. The fertility percentage was 33% in 2017, increasing to 56% in 2018 and 77% in 2019. In 2020, the fertility rate was 71% and 70% in 2021. There was a significant improvement in 2022, with a fertility rate of 86% in 2023, marking the highest rate observed. Pheasants showed similar gains in hatchability (34% to 60%; $F(4, 18) = 4.91$, $p = 0.008$) (Figure 3F). Hatchability percentages for pheasants were 34% in 2017 and remained the same in 2018. In 2019, hatchability improved to 47%, while in 2020, it was 46%. The hatchability rate dropped to 33% in 2021 but improved to 38% in 2022 and peaked at 60% in 2023.



Figure 3: Year-Wise Comparison of Peafowl and Pheasant's Total Eggs, Hatchability, And Fertility Ratio

DISCUSSION

This study investigates the effects of established rearing protocols on the hatchability and fertility rates of peafowl and pheasant eggs at the Jallo Breeding Center in Lahore, Pakistan. The protocol was established by visiting the Breeding Center. The most highlighted factor which directly affects hatchability and fertility is improper male-to-female ratios, the age of breeder flocks, egg storage duration, lack of supplements during breeding and chick growth stages, sand bedding, remnants of dead shells in incubators, rectangular brooders, and the absence of vaccinations and deworming. All problematic factors that lead to low fertility and hatchability rates and including embryonic mortality, are addressed by consulting with the veterinary officers at Jallo Breeding Center. Furthermore, a new rearing protocol was established and implemented in 2023. The current study compares the outcomes from 2023, during which the new protocols were implemented, with the previous records from 2017 to 2022, when standard rearing practices were followed. In the case of peafowls and pheasants, a significant peak was observed in 2023. According to Fukuhara et al., hatchability is closely linked to egg fertility and embryonic mortality throughout the hatching process [22]. All factors that influence egg fertility and contribute to embryonic mortality during incubation are addressed through innovative methods. The established rearing protocol showed a very positive ratio of hatchability and fertility rates. The most effective change which improves fertility rate was proper male-to-female

ratios. For peafowls, a ratio of 1:2 was maintained, while for pheasants, a ratio of 1:3 was implemented. The healthiest individuals were carefully chosen as breeding pairs to ensure the propagation of desirable traits. Eggs were gathered three times daily; proper management of egg storage conditions influences the developmental conditions for embryos. To avoid bacterial infection, a modification to the breeding environment, including the replacement of sand bedding with rice husk. The hatchability level improved by thoroughly cleaning the incubators and removing dead shells and dead embryos. The rectangular brooder design was replaced with a circular brooder to reduce mortality rates. A comprehensive vaccination and deworming program was initiated to protect the birds against common diseases and parasitic infestations. It was shown that fertility rates improved by a male-to-female sex ratio, as well as the health and genetics of birds [23]. Storage of eggs with proper care increases development and consequently results in lower total embryo mortality and higher hatchability [24]. Research has demonstrated that embryonic mortality during incubation is caused by the presence of bacteria, which infiltrate the eggshell and result in dead embryos. According to Ibrahim et al., disinfectants enhance hatchability [19]. Tainika et al., observed that the mating sex ratio and timely egg collection use of disinfectants before incubation help maintain hatchability and fertility rates [25]. The main source of contamination is floor bedding in the hatchery [26]. It was shown that hens kept on rice husk bedding produced more eggs and were contamination-free [27]. According to Saidane et al., it was shown that vaccination is essential for animal health; it provides individual protection and stimulates the production of antibodies that enhance survival rates [28]. Vaccination has successfully managed most infectious diseases in chickens. Vaccines lower the occurrence of diseases like Newcastle disease and infectious bursal disease, thereby enhancing animal welfare and the economic sustainability of poultry farming [29]. Hauck and Macklin emphasize that various management factors lead to differences in hatchability across breeder farms [20]. These factors encompass housing systems, egg handling practices, methods of egg collection, storage management, genetics, nutrition, and health. Furthermore, the level of microbial contamination in hatching eggs is affected by housing systems [30] and the laying nest utilized, which can significantly influence hatchability. According to Tomczyk et al., potential results of hatchability would be obtained by following the hatchery management [31]. The findings of this study suggest that implementing a well-designed hatchery protocol can positively impact fertility rates and hatchability outcomes in poultry production. By optimizing factors such as

breeder selection, nutrition, egg handling, incubation conditions, and chick management, hatcheries can enhance the efficiency of their operations and ensure the availability of high-quality chicks for subsequent rearing stages.

CONCLUSIONS

It was concluded that the implementation of the revised rearing protocol significantly enhances fertility rates, hatchability rates, and overall chick quality in both peafowl and pheasants. The optimization of male-to-female ratios, improved egg collection practices, and stringent hygiene measures (incubators, floor bedding, vaccination, deworming) have proven to be critical factors in achieving these advancements. These findings highlight the vital role of effective management practices in hatchery operations for achieving successful breeding outcomes. Based on the quantitative results, it was concluded that targeted interventions can lead to substantial improvements in poultry production. These findings provide a practical framework for wildlife breeding centers and aviculture programs to optimize rearing protocols to improve the health, growth, and survival of their peafowl and pheasant chicks.

Authors Contribution

Conceptualization: BNK

Methodology: MNF, BNK, MRK

Formal analysis: HMMH, ZT, MRK, MA

Writing review and editing: MNF, BNK, HR, FB, HMMH, ZT, MRK

All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

All the authors declare no conflict of interest.

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REFERENCES

- [1] Kabir A and Hawkeswood TJ. Captive breeding and rehabilitation of Peafowls (Aves: Phasianidae) in Bangladesh. *Calodema*.2021; 890: 1-4.
- [2] Giersberg MF, Molenaar R, de Jong IC, da Silva CS, van den Brand H, Kemp B et al. Effects of hatching system on the welfare of broiler chickens in early and later life. *Poultry Science*.2021Mar;100(3):100946.doi:10.1016/j.psj.2020.12.043.
- [3] Wickramasuriya SS, Park I, Lee Y, Lillehoj HS. Effect of dietary organic selenium on growth performance, gut health, and coccidiosis response in broiler chickens. *Animals*.2023May;13(9):1560.doi:10.3390/ani13091560.
- [4] Muniz EC, Freitas CM, Godoi BC, Barbosa CC, Aoki SM, Salles GB et al. Evaluation of the Performance of a New Pentavalent Vaccine in Poultry. *Brazilian Journal of Poultry Science*.2024May;26:eRBCA-2023.doi: 10.1590/1806-9061-2023-1771.
- [5] Chousalkar KK, Khan S, McWhorter AR. Microbial quality, safety and storage of eggs. *Current Opinion in Food Science*.2021Apr;38:91-5.doi:10.1016/j.cofs.2020.10.022.
- [6] Ashrafzadeh MR, Khosravi R, Fernandes C, Aguayo C, Bagi Z, Lavadinović VM et al. Assessing the origin, genetic structure and demographic history of the common pheasant (*Phasianus colchicus*) in the introduced European range. *Scientific Reports*.2021 Nov; 11(1): 21721. doi: 10.1038/s41598-021-00567-1.
- [7] Salamon A. Fertility and hatchability in goose eggs: A review. *International Journal of Poultry Science*. 2020 Jan; 19(2): 51-65. doi: 10.3923/ijps.2020.51.65.
- [8] Fathi M, Abou-Emera O, Al-Homidan I, Galal A, Rayan G. Effect of genotype and egg weight on hatchability properties and embryonic mortality pattern of native chicken populations. *Poultry Science*.2022Nov;101(11): 102129. doi: 10.1016/j.psj.2022.102129.
- [9] Miazi OF, Das A, Shaha M, Khan MM, Hassan MM, Shahadat MA et al. Disease Conditions and Different Abnormalities of Indian Peafowl and its Treatment in Captivity. *International Journal of Advanced Research in Biological*. 2024 Apr; 11(4): 1-4.
- [10] Khalid S, Ashraf S, Khan S, Khalid W, Chamba MV. Comparative study of egg quality parameters of different breeds of peacock. *International Journal of Food Properties*.2023Sep;26(1):1749-59.doi:10.1080/10942912.2023.2230378.
- [11] Meissner W, Wardecki Ł, Antczak J, Betleja J, Częstkwicz D, Czechowski P et al. Zimowanie ptaków wodnych na obszarach miejskich w Polsce w roku 2023. *Ornis Polonica*. 2024; 65(1).
- [12] Khan MT, Niazi AS, Arslan M, Azhar M, Asad T, Raziq F et al. Effects of selenium supplementation on the growth performance, slaughter characteristics, and blood biochemistry of naked neck chicken. *Poultry Science*.2023Mar;102(3):102420.doi:10.1016/j.psj.2022.102420.
- [13] Dickens MJ, Earle KA, Romero LM. Initial transference of wild birds to captivity alters stress physiology. *General and Comparative Endocrinology*. 2009Jan;160(1): 76-83.doi:10.1016/j.ygcen.2008.10.023.
- [14] Shahid B, Ihsan M, Khan MI. A Study of Egg Quality, Fertility and Hatchability in Kashmiri Rhode Island Red Chicken Breed. *Proceedings of the Pakistan Academy of Sciences: B. Life and Environmental*

- Sciences.2024Sep;61(3):259-72.doi:10.53560/PPASB(61-3)839.
- [15] Subagja H, Nadia ZA, Zubaidah S. Effect of hygiene on hatching performance of duck eggs (*Anas platyrhynchos domesticus*). *Advances in Animal and Veterinary Sciences*.2025;13(5):1060-5.doi:10.17582/journal.aavs/2025/13.5.1060.1065.
- [16] Al-Obaidi FA and Al-Shadeedi SM. Effect of age and seasons on egg production and egg morphology of peafowl reared outdoors in Baghdad. *Biochemical and Cellular Archives*. 2020 Apr; 20(1): 791-5.
- [17] Lin MJ, Chang SC, Lin LJ, Peng SY, Lee TT. Effect of laying parity and sex ratio on reproduction performance and biochemical parameters of White Roman geese kept in an environmentally controlled house. *British Poultry Science*.2025Mar;66(2):266-74. doi: 10.1080/00071668.2024.2403490.
- [18] Schmidt A, Beane G, Martin JA. Source population and time spent in captivity affect survival and reproduction of long-distance translocated northern bobwhites. *Animal Conservation*.2024Aug.doi:10.1111/acv.12985.
- [19] Ibrahim HM, Salem HM, Alamoudi SA, Al-Hoshani N, Alkahtani AM, Alshammari NM et al. Evaluating the Bactericidal Activity of Various Disinfectants against *Pseudomonas aeruginosa* Contamination in Broiler Chicken Hatcheries. *Pakistan Veterinary Journal*. 2024 Jul; 44(3).
- [20] Hauck R and Macklin KS. Vaccination against poultry parasites. *Avian Diseases*.2024Jan;67(4):441-9.doi: 10.1637/aviandiseases-D-23-99989.
- [21] Wang Q, Wang Q, Wang C, Sun C, Yang N, Wen C. Genetic improvement of duration of fertility in chickens and its commercial application for extending insemination intervals. *Poultry Science*. 2024Mar;103(3):103438.doi:10.1016/j.psj.2024.103438.
- [22] Fukuhara R, Agarie J, Furugen M, Seki H. Nesting habitats of free-ranging Indian peafowl, *Pavo cristatus*, revealed by sniffer dogs in Okinawa, Japan. *Applied Animal Behaviour Science*.2022Apr;249: 105605. doi: 10.1016/j.applanim.2022.105605.
- [23] Wegner M, Kokoszyński D, Kotowicz M, Krajewski K. Effect of housing system on carcass composition, meat quality, digestive morphometry, and leg bone dimensions of Ross 308 parent broilers. *Poultry Science*.2024Mar;103(3):103384.doi:10.1016/j.psj.2023.103384.
- [24] Tariq M, Butt NS, Mansha M, Bhinder MA. 34. Breeding performance and disease profile of six peafowl species in captivity at Jallo breeding center, Lahore. *Pure and Applied Biology*.2019 Feb; 8(1): 312-20.
- [25] Tainika B, Abdallah N, Damaziak K, Waithaka Ng'ang'a Z, Shah T, Wójcik W. Egg storage conditions and manipulations during storage: effect on egg quality traits, embryonic development, hatchability and chick quality of broiler hatching eggs. *World's Poultry Science Journal*.2024Jan;80(1):75-107.doi:10.1080/00439339.2023.2252785.
- [26] Prabowo MC, Sayekti I, Astuti S, Nursaputro ST, Supriyati S. Development of an IoT-based egg incubator with PID control system and web application. *JOIV: International Journal on Informatics Visualization*.2024Mar;8(1):465-72.doi: 10.62527/joiv.8.1.2044.
- [27] Yenilmez F. Peafowl Production. *Turkish Journal of Agriculture-Food Science and Technology*.2020 Apr;8(4):945-8.doi:10.24925/turjaf.v8i4.945-948.3198.
- [28] Saidane Z, Dahou AA, Daoudi M, Dahmouni S, Homrani A. Consequences of Technical and Sanitary Practices on Laying and Hatching Rates in the *Gallus gallus domesticus* Meat Sector in the Mostaganem Region (Algeria). *Acta Veterinaria Eurasia*.2024Jan;50(1). doi: 10.5152/actavet.2024.23032.
- [29] Fijalovych LM, Kyrlyiv YI, Barylo BS, Paskevych GA, Lunyk JM, Petryshak OI et al. Peculiarities of keeping pheasants. *Scientific Messenger of LNU of Veterinary Medicine and Biotechnologies. Series: Agricultural Sciences*.2023Mar;25(98):125-31.doi:10.32718/nvlvet-a9821.
- [30] Hardy IC, Wajnberg E, editors. *Jervis's insects as natural enemies: practical perspectives*. Cham, Switzerland: Springer.2023Nov.doi:10.1007/978-3-031-23880-2.
- [31] Tomczyk G, Niczyporuk JS, Kozdruń W, Sawicka-Durkalec A, Bocian Ł, Barabasz M et al. Probiotic supplementation as an alternative to antibiotics in broiler chickens. *Journal of Veterinary Research*. 2024 Mar; 68(1): 147. doi: 10.2478/jvetres-2024-0009.



Original Article

Diversity of Insect Pollinators Visiting Rohi Sarson (*Brassica Juncea L.*) in Southern Punjab, Pakistan

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ABSTRACT

Rapeseed is an important oilseed crop in Pakistan, and its yield can be significantly improved through insect pollination. **Objective:** To explore the diversity of insect pollinators visiting the rapeseed crop (locally known as Rohi Sarson, *Brassica juncea*) in Panjgrain, District Bhakkar, Southern Punjab, Pakistan. **Methods:** This Descriptive ecological survey was conducted in Panjgrain, District Bhakkar, Southern Punjab, Pakistan. The crop was grown in October 2024 and flowered from February to April 2025, which coincided with the period of pollinator activity. During this time, data were collected using a cone-type hand net at regular intervals from 10:00 AM to 4:00 PM, when insect activity was at its peak. Captured specimens were preserved and later identified using standard taxonomic methods and online identification tools. **Results:** A total of 18 insect species were recorded visiting the rapeseed flowers. These species were represented by four major insect classes, namely: Hymenoptera, Diptera, Lepidoptera, and Coleoptera. The best group was Hymenoptera, which comprised different species of honeybee and carpenter bee, mining bee, and paper wasp. It was also found that the dominant pollinators were the honeybees, which defined the production level and quality of the crop. Insects, such as hover flies, butterflies, and beetles, also contributed complementarily to pollination. The pollinators were affected by environmental factors, including temperature, sunlight, and time of day. **Conclusions:** The study concluded the significant role of insect pollinators in rapeseed production and indicated that sustainability in pollinator diversity on the farm enhances crop output with the help of pollinator-friendly farming systems.

INTRODUCTION

In the plant family Cruciferae, rapeseed is one of the significant plant crops, grown as oilseed in Pakistan. Rapeseed has been produced primarily to use oil, which serves both cooking and industrial needs [1]. The rapeseed also forms Rapeseed oil and Canola oil, where it contains very low quantities of erucic acids (less than 2 per cent) and glucosinolates (less than 30 U mole/g of oil) to be fit to utilize in humans [2]. Rapeseed is grown in all provinces of Pakistan over an area of 26.02 thousand hectares with an annual yield of 812 kg/hectare [3]. Attock, Rawalpindi, Jehlum, Chakwal and Faisalabad are the major rapeseed growing areas in Upper Punjab while in Lower Punjab, the major rapeseed growing areas are Multan, Muzaffargarh, Bahawalpur, Rahim Yar Khan and Bhakkar. In Pakistan, the

primary cultivated rapeseed species are *Brassica napus* (gobhi sarson), *Brassica juncea* (raya), and *Eruca sativa* (taramira). Within these species, various cultivars are grown, including Khanpur Raya, Anmol Raya, Punjab Canola, and others [4]. "Rohi Sarson" refers to Rohi, a new high-yielding rapeseed variety released for irrigated areas in central and south Punjab (lower Punjab), Pakistan. It is also known as a type of mustard seed. The variety is characterized by its high yield, early maturity, and good quality oil. Rapeseed is largely self-fertilized; however, the presence of insect pollinators greatly enhances its yield, seed quality, and oil content. Insect pollination plays a crucial role in crop production, as it impacts both the quality and quantity of yields [5,6,7]. Among pollinators,

honeybees (*Apis mellifera*) [8] are the most effective agents. Studies have shown that fields with active bee visitation can experience up to a 20–30% increase in seed yield compared to non-pollinated or wind-pollinated crops [9]. The mutual benefit between rapeseed crops and pollinators is significant. While bees enhance crop productivity, the rapeseed flowers provide nectar and pollen during early spring when floral resources are limited [10, 11, 12, 13]. The insect pollinators diversity in the region of Southern Punjab is, however, incomplete, in particular, in regions such as Panjgrain, District Bhakkar. The literature has concentrated more on other areas, and the contribution that non-bee pollinators in rapeseed farming is quite insignificant. The research study bridged this gap by recording the available species of pollinators in the area that were critical to enhancing food production and popularizing pollinator-friendly agricultural strategies. This study aimed to explore the diversity of insect pollinators visiting the rapeseed crop in Panjgrain, District Bhakkar, Southern Punjab, Pakistan.

METHODS

The current research was carried out at a rural area called Panjgrain in Southern Punjab in District Bhakkar, Pakistan, located at the coordinates W6F9+QXW, Shah Alam, Panjgrain. Description: The study was an ecological survey that was conducted during the flowering season of *Brassica juncea* (February–April 2025) in Panjgrain, Bhakkar, to document the insect pollinator diversity and the abundance. The region is agricultural land because of these productive plains and climatic properties of the land, which result in high production of oilseed crops like Canola (*Brassica juncea*), commonly referred to as Rohi Sarson. The flowering season of Rohi Sarson is between February and April, and the weather in the area is usually moderate with a temperature of not more than 25°C and low humidity, and sufficient daylight that is suitable to be pollination by insect pollinators and the flowering of crops. In the present research study, the Rohi Sarson crop was planted in October 2024. Pollination started in early February and lasted up to April 2025, what is coincided with the research time. These months have been chosen cautiously to make pollinator observations because the floral resources are plentiful at this time, and the insects are active in their foraging behavior. The agro-climatic condition of the area, along with Rohi Sarson's flowering stage, offered a suitable environment to study the diversity in insect pollinators of Rohi Sarson (Figure 1).



Figure 1: Rohi Sarson fields in Panjgrain, District Bhakkar, Punjab, Pakistan

The insect pollinators observed during the flowering period at the Rohi Sarson (*Brassica juncea*) crop in the month of February to April 2025 had been recorded. The cone-type hand net, which is a common entomology tool used to sample flower visiting insects, was on offer to sample the flower-visiting insects since active pollinators can be captured without causing much havoc to the plants. The insect pollinator was sampled in the form of random transect walks every two hours between 1000 and 1600 hours (10:00 AM to 4:00 PM), the period at which this insect pollinator is active at the highest level given optimal light and temperature conditions. Within each of the periods, systematic sweep netting was done in the sites of the crop field which were flowering. Caught insects were stored precisely into the killing jars with ethyl acetate and subsequently pinned, spread and labeled in an ordinary entomological tradition. Insect collection boxes containing naphthalene were used, after which the specimens were placed. The specimens that had been gathered were put into entomological boxes to be used in the lab to identify the taxon and measure the diversity further. This technique enabled an effective sampling of a vast number of insect pollinators, hence the validity of data that could be utilized in the analysis of their diversity and abundance in the Rohi Sarson crop ecosystem. Identification and classification of insect samples collected were done through available online taxonomic sources and also morphology. Specimens were grouped into large insect orders and families pertinent to pollination, such as, Hymenoptera (bees and wasps), Diptera (flies), Lepidoptera (butterflies and moths), and Coleoptera (beetles). In order to identify correctly, standard keys and field guides on insect identification and entomology were used. Furthermore, a range of online databases and websites of good quality were consulted to help in identifying species at the lowest level. Of these sites, BugGuide.net, iNaturalist and the Integrated Taxonomic Information System (ITIS) offered the most value because of the detailed pictures coupled with a hierarchy of taxonomy and a community of experts who can help. Such an integration of the morphological analysis and the digital tools of identification made it possible to make a dependable classification of the insect pollinators visiting the Rohi Sarson crop, and gave the basis to assess the perceived diversity of the pollinators in the

study region. Data were collected on 12 field trips, and each field trip was taken in regular intervals between 10:00 AM and 4:00 PM in the time of flower maturity. To obtain consistency, three replicates of sweep net sampling were done in each visit. The relative abundance and frequency of each species of insect were noted. The analysis of data was performed with IBM SPSS Statistics, version 27.0. The descriptive statistics were used, and $p < 0.05$ was assigned.

RESULTS

The main aim of the data analysis in this research study was to list and describe the various insect species that would visit the flowers of Rohi Sarson (*Brassica juncea*) when it is in a flowering phase between February to April 2025. Identification of the variety of insect species was vital because they visit flowers, but not their frequency or population patterns. Each collected specimen was examined and identified using morphological features and verified through reliable insect identification resources. The analysis involved determining the common name, scientific name, and taxonomic classification (Order, Family, Genus, and Species) of each visitor species. Specifically, the average temperatures, humidity, and high sunlight levels in the period of flowering provided comfortable circumstances of foraging and floral visitation. Identified species were organized systematically and presented in tabular form for clarity (Table 1).

Table 1: Insect Pollinators on Rapeseed Crop in Panjgrain

Sr. No.	Common Name	Scientific Name	Order	Family
1	Eastern honeybees	<i>Apis cerana</i>	Hymenoptera	Apidae
2	European honeybees	<i>Apis mellifera</i>	Hymenoptera	Apidae
3	Giant honeybees	<i>Apis dorsata</i>	Hymenoptera	Apidae
4	Dwarf's honeybees	<i>Apis florea</i>	Hymenoptera	Apidae
5	Indian honeybee	<i>Apis indica</i>	Hymenoptera	Apidae
6	Stingless honeybee	<i>Melipona marginata</i>	Hymenoptera	Apidae
7	Carpenter bee	<i>Xylocopa fenestrata</i>	Hymenoptera	Apidae
8	Mining bee	<i>Andrena aegyptiaca</i>	Hymenoptera	Andrenidae
9	Paper wasp	<i>Polistes olivaceus</i>	Hymenoptera	Vespidae
10	European Hover fly	<i>Eristalis tenax</i>	Diptera	Syrphidae
11	Syrphid fly	<i>Eupeodes bacculatus</i>	Diptera	Syrphidae
12	Marmalade hoverfly	<i>Episyrphus balteatus</i>	Diptera	Syrphidae
13	Cabbage butterfly	<i>Pieris brassica</i>	Lepidoptera	Pieridae
14	Blue butterfly	<i>Zizina Otis</i>	Lepidoptera	Lycaenidae

15	Painted lady butterfly	<i>Vanessa cardui</i>	Lepidoptera	Nymphalidae
16	Monarch butterfly	<i>Danaus chrysippus</i>	Lepidoptera	Nymphalidae
17	Lady bird beetle	<i>Coccinella septempunctata</i>	Coleoptera	Coccinellidae

During the flowering period of Rohi Sarson (*Brassica juncea*) from February to April 2025 in Panjgrain, Southern Punjab, a variety of insect species were observed visiting the crop. A total of 18 distinct insect species belonging to four major insect orders—Hymenoptera, Diptera, Lepidoptera, and Coleoptera—were identified as flower visitors.

A total of 18 insect species were documented in 4 insect orders with Hymenoptera (61 %), Diptera (17 %), Lepidoptera (11 %) and Coleoptera (11 %). These included the most predominant honeybees of the genus *Apis* (about 52 percent of all bees counted) and honeybees of the genus *Melophorus* (about 48 percent) (Figure 2).



■ Hymenoptera ■ Diptera ■ Lepidoptera ■ Coleoptera

Figure 2: Taxonomic Diversity of Insect Pollinators Rohi Sarson (*Brassica Juncea*)

Among these, the order Hymenoptera was the most represented, with ten species recorded. These included five species of honeybees: *Apis cerana* (Eastern honeybee), *Apis mellifera* (European honeybee), *Apis dorsata* (Giant honeybee), *Apis florea* (Dwarf honeybee), and *Apis indica* (Indian honeybee). Additionally, *Melipona marginata* (Stingless honeybee), *Xylocopa fenestrata* (Carpenter bee), *Andrena aegyptiaca* (Mining bee), and *Polistes olivaceus* (Paper wasp) were also noted, reflecting the strong presence and diversity of pollinators from the Apidae, Andrenidae, and Vespidae families. The order Diptera was represented by three hoverfly species: *Eristalis tenax* (European hover fly), *Eupeodes bacculatus* (Syrphid fly), and *Episyrphus balteatus* (Marmalade hoverfly), all from the family Syrphidae. These flies were commonly seen hovering over and landing on Rohi Sarson flowers during the day. From the order Lepidoptera, four butterfly species were recorded: *Pieris brassica* (Cabbage butterfly), *Zizina otis* (Blue butterfly), *Vanessa cardui* (Painted lady), and *Danaus chrysippus* (Monarch butterfly), belonging to three different families—Pieridae, Lycaenidae, and Nymphalidae. One beetle species from the order Coleoptera, *Coccinella septempunctata* (Lady bird beetle), was also identified, indicating occasional floral visitation by beetles. The

predominance puts them at the focus of pollination of rapeseed (*Brassica juncea*) in the region (Figure 3).



Figure 3: Honeybees Gathering Nectar Over Rohi Sarson Flowers

DISCUSSION

The present study focused on identifying insect pollinators visiting Rohi Sarson (*Brassica juncea*) flowers in the agricultural region of Panjgrain, Southern Punjab, during the flowering season from February to April 2025. The identified pollinators belonged to four major insect orders: Hymenoptera, Diptera, Lepidoptera, and Coleoptera. This diversity indicates the ecological adaptability of Rohi Sarson flowers in attracting a wide range of pollinators [14]. The dominance of bees (Hymenoptera), particularly species from the genus *Apis*, is consistent with findings from earlier studies on mustard and Rohi Sarson crops [14, 15, 16]. Species such as *Apis mellifera*, *A. cerana*, and *A. dorsata* are known to be highly efficient pollinators due to their flower fidelity and morphological adaptations that enhance pollen transfer [17]. Similar results were reported by researchers in various regions of South Asia, confirming the central role of honeybees in mustard-family crop pollination [15, 16]. Hoverflies (Diptera: Syrphidae) and butterflies (Lepidoptera) also contributed notably to floral visitation. Although these groups are generally considered less efficient than bees, their high mobility and regular presence at flowers enhance overall pollination [18, 19]. Studies have shown that flies and butterflies complement bee activity, especially under variable weather conditions when bee foraging may decline [19]. The observed pollinator diversity and activity were influenced by several environmental factors. Weather conditions, particularly temperature, sunshine, and wind, played a key role in determining daily insect activity [20]. In addition, floral density and the availability of nectar and pollen likely influenced the attractiveness of Rohi Sarson plants to different insect groups. Pollinators were most active during clear, sunny periods between 10:00 AM and 4:00 PM, corresponding with optimal foraging conditions [17]. From an agricultural perspective, the presence of a wide range of pollinators has important implications. It suggests that Rohi Sarson production in the region benefits not only from managed bee colonies but also from wild insect

populations. Conserving this pollinator diversity is essential for maintaining crop yields, seed quality, and ecosystem health [21]. The findings emphasize the need for pollinator-friendly farming practices, such as reduced pesticide use and the preservation of natural habitats around crop fields. This study was based on visual observation and hand-net collection, so some very small or night-active (nocturnal) pollinators might not have been recorded. Also, changes in weather like unusual rain or temperature can affect insect activity. The research was done only in one area and during one flowering season, so the findings may not fully represent all of Southern Punjab.

CONCLUSIONS

In summary, the study highlights the ecological importance of diverse insect pollinators in Rohi Sarson cultivation and reinforces the need to protect these beneficial organisms for sustainable agricultural development. This study was also narrowed to a single season in a single location, which is a limitation to pollinator diversity in terms of space and time. The study must be extended across seasons and sites of study in the future. The superiority of the *Apis* species raises the importance of the conservation of the native pollinators, via minimizing the use of pesticides and implementing pollinator-friendly agriculture to support sustainable crop production.

Authors Contribution

Conceptualization: MSK

Methodology: MP

Formal analysis: MSK, MP

Writing review and editing: MSK

All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

All the authors declare no conflict of interest.

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REFERENCES

- [1] Mohamed IA, Shalby N, El-Badri AM, Awad-Allah EF, Batool M, Saleem MH et al. Multipurpose uses of rapeseed (*Brassica napus* L.) crop (food, feed, industrial, medicinal, and environmental conservation uses) and improvement strategies in China. *Journal of Agriculture and Food Research*. 2025; 101794. doi: 10.1016/j.jafr.2025.101794.
- [2] Eskin MN, Iassonova DR, Rempel CB. High-oleic canola oil. In: *High Oleic Oils*. AOCS Press. 2022; 89-108. doi: 10.1016/B978-0-12-822912-5.00001-0.
- [3] Zada H. Yield and yield attributes of rapeseed cultivars as influenced by sulfur level under Swat

- Valley conditions. Pure and Applied Biology. 2015.
- [4] Abrol DP. Insect pollination and crop production in Jammu and Kashmir. *Current Science*. 1993;65:265-9.
- [5] Khalifa SA, Elshafiey EH, Shetaia AA, El-Wahed AA, Algethami AF, Musharraf SG et al. Overview of bee pollination and its economic value for crop production. *Insects*. 2021;12(8):688. doi:10.3390/insects12080688.
- [6] Srivastava KS, Sharma DS, Singh SS, Ahmad HA. Foraging behaviour of honeybees in seed production of *Brassica oleracea* var. *italica* Plenck. 2017.
- [7] Fikadu Z. The contribution of managed honey bees to crop pollination, food security, and economic stability: Case of Ethiopia. *The Open Agriculture Journal*. 2019;13(1). doi: 10.2174/1874331501913010175.
- [8] Bommarco R, Marini L, Vaissière BE. Insect pollination enhances seed yield, quality, and market value in oilseed rape. *Oecologia*. 2012;169(4): 1025-32. doi:10.1007/s00442-012-2271-6.
- [9] Stanley DA, Gunning D, Stout JC. Pollinators and pollination of oilseed rape crops (*Brassica napus* L.) in Ireland: ecological and economic incentives for pollinator conservation. *Journal of Insect Conservation*. 2013; 17(6): 1181-9. doi: 10.1007/s10841-013-9599-z.
- [10] Haldhar SM, Kumar R, Samadia DK, Singh B, Singh H. Role of insect pollinators and pollinizers in arid and semi-arid horticultural crops. *Journal of Agriculture and Ecology*. 2018;5:1-25. doi:10.53911/JAE.2018.5101.
- [11] Hung KL, Kingston JM, Albrecht M, Holway DA, Kohn JR. The worldwide importance of honey bees as pollinators in natural habitats. *Proceedings of the Royal Society B: Biological Sciences*. 2018; 285(1870): 20172140. doi: 10.1098/rspb.2017.2140.
- [12] Shakeel M, Ali H, Ahmad S, Said F, Khan KA, Bashir MA et al. Insect pollinators' diversity and abundance in *Eruca sativa* Mill. (Arugula) and *Brassica rapa* L. (Field mustard) crops. *Saudi Journal of Biological Sciences*. 2019;26(7):1704-9. doi:10.1016/j.sjbs.2018.08.012.
- [13] Yadav S, Yadav SS, Jat MK, Kumar H. Diversity and abundance of insect visitors/pollinators on different genotypes of *Brassica* spp. *Journal of Agriculture and Ecology*. 2022; 13: 112-20. doi: 10.53911/JAE.2022.13111.
- [14] Badenes-Perez FR. Benefits of insect pollination in Brassicaceae: A meta-analysis of self-compatible and self-incompatible crop species. *Agriculture*. 2022;12: 446. doi:10.3390/agriculture12040446
- [15] Gomez JM, Navarro L, Gonzalez-Megias A, Armas C, Perfectti F, Caravantes A et al. Pollination effectiveness affects the level of generalisation of a plant species with phenotypically plastic flowers. *Annals of Botany Plant*. 2025;17:plae065. doi:10.1093/aobpla/plae065
- [16] Klecka J, Hadrava J, Biella P, Akter A. Flower visitation by hoverflies (Diptera: Syrphidae) in a temperate plant-pollinator network. *PeerJ*. 2018;6: e6025. doi: 10.7717/peerj.6025
- [17] Jauker F and Wolters V. Hover flies are efficient pollinators of oilseed rape. *Oecologia*. 2008;156: 819-23. doi: 10.1007/s00442-008-1034-x
- [18] Bansal A and Abrol DP. Diversity and abundance of insect pollinators affecting seed production in Mustard (*Brassica napus* L.). *Journal of Apiculture*. 2023;38:315-23. doi:10.17519/apiculture.2023.38.4.315
- [19] Goswami VI and Khan MS. Impact of honey bee pollination on pod set of mustard (*Brassica juncea* L.: Cruciferae) at Pantnagar. *The Bioscan*. 2014; 9: 75-8.
- [20] Yadav R, Kaushal BR, Singh D, Kumar N. Diversity and abundance of pollinating insects in mustard crops of Northern India. *Pollination Research*. 2022;12(1): 33-40.
- [21] Stanley J, Sah K, Subbanna AR. How efficient is the Asian honey bee, *Apis cerana*, in pollinating mustard, *Brassica campestris* var. *toria*? Pollination behavior, pollinator efficiency, pollinator requirements and impact of pollination. *Journal of Apicultural Research*. 2017;56:439-51. doi:10.1080/00218839.2017.1329796.



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Original Article

Relationship of Udder Morphometric Traits with Milk Production in Dairy Cattle and Buffaloes

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ABSTRACT

Pakistan's dairy industry depends on cattle and buffaloes for milk production. Subclinical Mastitis (SCM), on the other hand, is a serious restriction that lowers milk quality and quantity.

Objective: To examine the udder and teat morphometric characteristics of cattle and buffaloes and evaluate how they relate to Somatic Cell Count (SCC), milk output, composition, and mineral content. **Methods:** A total of 150 lactating animals (75 cattle and 75 buffaloes) were sampled from five farms in Faisalabad. Milk yield was recorded over three consecutive mornings using a digital meter, while composition and SNF were analyzed. with a Lactoscan SLP SCC was quantified, and minerals were measured by atomic absorption spectrophotometry. Udder and teat dimensions were taken using a Vernier caliper, tape, and visual observation. **Results:** The findings showed that, funnel/cylindrical teats and pendulous udders had significantly higher width and depth ($p < 0.05$), as well as significantly larger teat diameter and length ($p < 0.05$). Pendulous and spherical udders ($p < 0.01$) and funnel, pear, and cylindrical teats ($p < 0.05$) were substantially linked to higher SCC. While several teat measurements, such right front teat length and left front teat diameter, exhibited moderately negative relationships ($r = -0.31$, $p = 0.02$, $r = -0.26$, $p = 0.04$), there was a positive link between milk yield and udder width ($r = 0.45$, $p = 0.003$). Additionally, morphology had a significant impact on the mineral content and composition of milk ($p < 0.05$). **Conclusion:** These results suggested that, selecting dairy animals with favorable udder and teat characteristics could improve udder health and milk productivity.

INTRODUCTION

Milk is a vital source of nourishment and a vital commodity in the cattle industry. However, mastitis negatively impacts milk composition and processing qualities, which frequently compromises milk supply and quality [1, 2]. While buffaloes can have up to ~300,000 cells/mL before being deemed infected, healthy cattle usually have SCC <200,000 cells/mL, whereas SCM, which is a common and expensive constraint, lacks obvious indications but boosts SCC [3]. Although SCC testing and the California Mastitis Test (CMT) are crucial for the diagnosis of SCM, they are still

reactive procedures that are employed after infection symptoms manifest [4, 5]. According to recent research, udder shape (globular, pendulum, or bowl) and teat conformations (conical, pear, funnel, or cylindrical) affect milking efficiency and hygiene and are also associated with increased susceptibility to mastitis and SCC [6, 7]. The early identification of these anatomical risk factors would enable farmers to implement preventative measures or targeted screenings programs. However, there are few studies in Pakistan that integrate diagnostic markers with

morphometric and visual features. Few research has examined the relationship between udder and teat morphometric features and milk yield, composition, mineral levels, and udder health, despite their practical significance. It may be possible to improve early identification of SCM, guide selection methods, and increase dairy yield in field settings by combining visual morphology with measurements and biochemical diagnostics [8, 9]. This study aims to investigate the relationship between udder and teat morphometric traits (visual categorization. and physical measurements) and milk yield, composition, mineral content, and SCC in dairy cattle and buffaloes. Therefore, by integrating anatomical, biochemical, and mastitis related data, this research evaluate the morphological indicators of udder health and milk productivity, thereby addressing a critical knowledge gap and offering tools for improved selection, early diagnosis, and management in Pakistan's dairy sector.

METHODS

The study was approved by the ethical review committee, Department of Clinical Medicine and Surgery, Faculty of Veterinary Science, University of Agriculture, Faisalabad (Ref No: Approval No/Diary No 1483). All research. procedures complied with institutional animal care guidelines. Five dairy farms were purposively selected across intensive and semi-intensive systems in Faisalabad, and total 150 lactating animals, comprising 75 cattle and 75 buffalo were selected. Within each farm, 15 lactating cattle and 15 buffalo were randomly selected using simple random sampling. Farms were stratified by management, and animals were randomly chosen via random number tables. To control for environmental variation, housing conditions, temperature, and humidity were recorded at sampling time. **Milk Sampling:** Animals were rested for at least 12 hours and sampled early in the morning (07:00–09:00), under standardized SOPs on three consecutive days to standardized timing, and reduced circadian variation in milk composition and SCC. This ensure the consistent sampling and aligns with study showed pronounced variation in milk yield, fat, and SCC between morning and evening milking [10]. **California Mastitis Test (CMT):** CMT was conducted with. standard kits, labeling quarters A–D. Approximately two milk strips (~2 mL each) were collected per teat, mixed with equal volume of CMT reagent, and gently swirled for. 10 seconds. Results were scored after. 20 seconds as Negative (no reaction), Trace. (slight slime), 1 (distinct slime), 2 (light gel), and 3 (strong gel). Grades ≥ 1 were considered indicative of SCM [11]. **Udder and Teat Morphometry:** Udder shape (bowl, goaty, pendulous, round. and globular) while teats shape (cylindrical, bottle, funnel, pear, conical) were categorized based on standardized visual scoring system,

noting symmetry, attachment, and teat orientation. Categories followed prior morphometric study. Physical measurements were obtained using a flexi-tape and. Vernier caliper (precision $\pm 0.01\text{cm}$). Measurements included Udder Length (UL), Udder Depth (UD), Udder Width (UW), Teat Length (TL), Teat Diameter (TD), Distance between Fore Teats (DBET), Distance between Hind Teats (DBHT), and Distance between Fore-Hind Teats (DBFHT) [12]. **Milk Yield Measurement:** Milk yield was recorded using a calibrated electronic meter during the morning milking. Daily yield for each animal was averaged over three consecutive sampling days to minimize variation due to milking time or environmental factors [13]. **Milk Composition and SCC Analysis:** Milk samples from healthy and. SCM-affected animals were analyzed at NIFSAT, University of Agriculture, Faisalabad. Milk sample were analyzed using a Lactoscan SLP. Milk Analyzer (Milkotronic Ltd., Bulgaria; Catalogue. **No.13070102**) which utilized ultrasonic biochemical detection to quantify fat, protein, lactose, and. SNF level. Daily milk yield was recorded using a calibrated electronic meter, averaged over three consecutive morning (07:00–9:00) milking sessions to minimize variability SCC was quantified using the SCC kit (PortaCheck, USA)., validated with ~80–90% agreement against standard lab counters and cross-checked against. CMT results. SCC $< 100,000\text{cells/mL}$ was considered normal, but the threshold for SCM include, SCC $> 200,000\text{cells/mL}$ in cattle and $> 280,000\text{cells/mL}$ in buffaloes indicated SCM [14]. **Mineral Analysis:** Milk aliquots. (10mL) were acid-digested, and concentrations of. Ca, and Mg were determined by atomic absorption spectrophotometry. Procedures followed published methods with certified standards [15]. Primary outcomes included physical udder and teat morphometric measurements, milk yield. (L/day), milk composition (fat, protein, lactose, SNF), mineral concentrations. (Ca, Mg), and SCC (cells/mL). Secondary variables included visual shape classification and CMT grade. Data were analyzed using SPSS v26. Associations between categorical traits. (udder/teat shape vs SCM status) were tested using chi-square tests, while continuous trait differences (e.g. udder dimensions, milk yield, composition, SCC) among groups were evaluated by one-way ANOVA with Tukey's post-hoc test. Pearson correlation and multivariate regression accounted for farm, breed, and lactation stage. Statistical significance was set at. $p < 0.05$.

RESULTS

Udder Morphometry and Shape Distribution

The majority of cattle had round udders (50%), followed by bowl (17.1%) and globular (15.7%) shapes at moderate rates, and goaty and pendulous forms were less common. 8.6% apiece. The most prevalent udder style in buffaloes was

bowl-shaped (28.5%), followed by round (22.9%), globular (20.0%), and pendulous (11.4%). Significant differences in the distribution of udder shapes between the two species were confirmed by chi-square analysis (χ^2 test, $p < 0.001$), revealing different morphological patterns (see Fig. 1a-b). The most common teats in cattle were cylindrical (67.1%), followed by conical (1.4%), funnel (7.1%), bottle (4.3%), and pear (20.0%). According to Fig. 1c-d, funnel teats were the least common in buffaloes, while cylindrical teats were the most common (29.0%). Significant interspecies variations in the distribution of teat shapes were validated by chi square analysis. (χ^2 , $p < 0.001$).

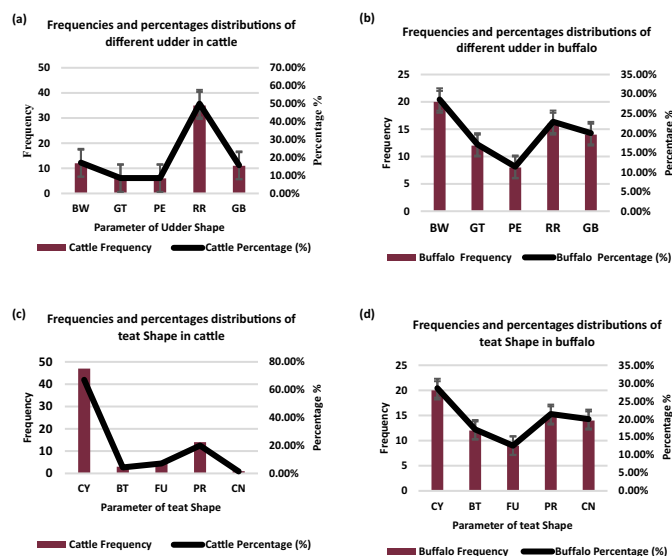


Figure 1: Percentages and frequencies distributions of different udder and teat shapes in cattle and buffalo, BW= Bowl, GT= Goaty, PE= Pendulous, RR= Round, GB= Globular, CY= Cylindrical, BT= Bottle, FU=Funnel, PR= Pear, CN=Conical

Morphometric Evaluation of Udder Dimensions Across Different Udder Shapes in Cattle and Buffaloes

Morphometric analysis revealed that pendulous and round udders exhibited the largest dimensions, with mean udder widths of 35.2 ± 3.2 cm vs 34.8 ± 3.0 cm. ($p = 0.02$) and depths of 29.5 ± 2.8 cm vs 29.0 ± 2.7 cm ($p = 0.03$), respectively. In contrast, goat (cattle) and globular (buffalo) udders had the smallest measurements (udder width: 28.1 ± 3.4 cm; udder depth as 24.8 ± 2.5 cm, $p < 0.01$ compared to pendulous). These differences were statistically significant via ANOVA with Tukey's post-hoc ($p < 0.05$), illustrating clear morphometric variations across shape categories (Figure 2a-b). Mean udder dimensions were UL= 32.4cm (SD 3.5), UW =31.2 cm (SD 3.2), and UD=27.1cm (SD2.8). ANOVA showed significant differences among udder shapes ($p < 0.01$). Specific p values are indicated in Figure 2 and 3.

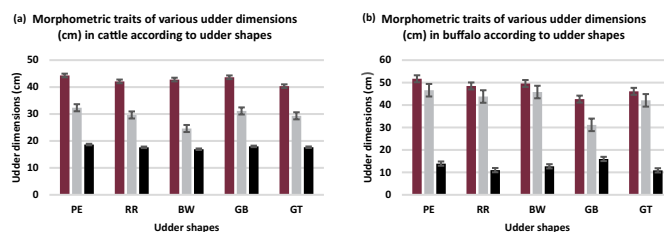


Figure 2: According to udder shapes, morphometric characteristics of different udder dimensions (cm) in cattle and buffalo are as follows: UD = udder depth, UL = udder length, UW = udder width, PE = pendulous, RR = round, GB = globular, and BW = bowl

Morphometric Evaluation of Teat Traits and Their Association with Teat Shapes in Cattle and Buffaloes

Buffalo teats were markedly longer and wider than cattle teats. For bottle-type buffalo teats, mean length was 9.02 ± 0.7 cm and diameter 3.82 ± 0.4 cm, significantly larger than cattle bottle teats (6.50 ± 0.6 cm length, 3.90 ± 0.3 cm diameter as $p < 0.001$). Additionally, cylindrical teats remained the most common in both species (cattle .67.1%, buffalo .29%), with significant interspecies. distribution differences (χ^2 , $p < 0.01$) (see Figure 3a-b).

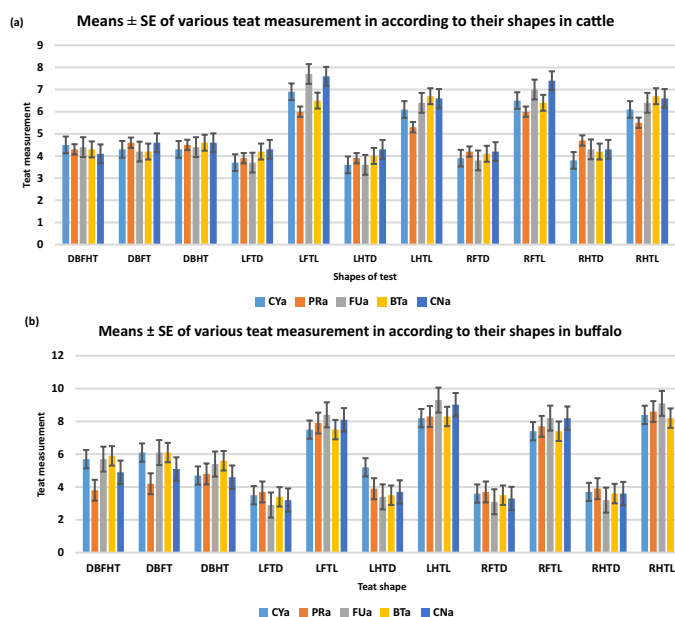


Figure 3: Means $\pm$ SE for different teat measurements based on their morphologies DBHFT= Distance between fore and hind teat, DBFT=Distance between fore teats, DBHT=Distance between hind teats, LFTD=Left for teat diameter, LFTL=Left for teat length, LHTD=Left hind teat diameter, LHTL=Left hind teat length, RFTD=Right for teat diameter, RFTL=Right for teat length.

Milk Composition Analysis of Healthy and SCM Milk

In both species, SCM markedly changed the content of milk. In cattle, mean fat percentage decreased from $4.20 \pm 0.30\%$ to $3.60 \pm 0.40\%$ ($p < 0.001$), protein declined from $3.50 \pm 0.20\%$ to $3.00 \pm 0.30\%$ ($p < 0.001$), and lactose dropped from $4.50 \pm 0.20\%$ to $3.98 \pm 0.25\%$ ($p < 0.001$). SNF also decreased ($p = 0.005$) from $7.05 \pm 0.40\%$ to $6.50 \pm$

0.45%. In buffaloes, fat fell from $5.80 \pm 0.50\%$ to $5.20 \pm 0.60\%$ ($p = 0.002$), protein from $3.10 \pm 0.30\%$ to $2.60 \pm 0.40\%$ ($p < 0.001$), while lactose slightly decreased from $4.10 \pm 0.20\%$ to $4.03 \pm 0.22\%$ ($p = 0.04$). SNF change was minimal and non-significant ($p = 0.12$). These results underscore the detrimental impact of SCM on key milk constituents (Figure 4a-b).

Somatic Cell Count by Udder and Teat Morphology

Buffaloes with round and pendulous udder shapes exhibited significantly higher SCC levels compared to bowl and globular udders ($p < 0.01$, Fig. 4c). Similarly, pear, conical, and cylindrical teat morphologies were associated with elevated SCC ($p < 0.05$) (Figure 4d).

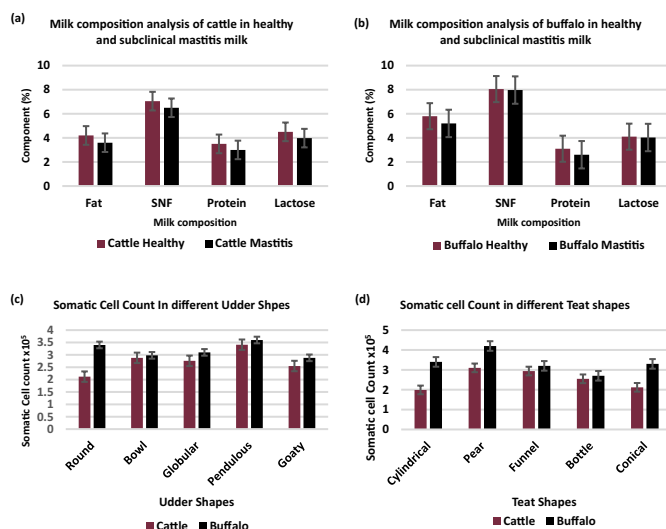


Figure 4: Analysis of the composition of SCM and healthy milk, as well as SCC in various udder and teat shapes

Correlations: Morphometry, Milk Yield, Composition, Minerals, and SCC

Table 1: Association of Morphometric Traits of Udder and Teats with Related Factors

Variables	Udder			Teats Length				Teat Diameter				Milk Composition					Mineral		SCC
	UL	UW	UD	RF	LF	RR	LR	RF	LF	RR	LR	Yield	Protein	Fat	Lactose	SNF	Ca	Mg	
Udder Length	1.0	0.35	0.39	0.06	0.03	0.02	0.01	0.1	0.35	0.03	0.10	-0.24*	-0.13	0.21*	-0.11	-0.08	0.1*	0.20*	0.2*
	1.0	0.43	0.29	0.06	0.07	0.04	0.05	0.2	0.52	0.05	0.24	-0.31*	-0.15	-0.31	-0.021	-0.05	-0.2	0.26	0.8
Udder Width	-	1	0.46	0.22	0.01	0.24	0.31	0.34	0.27	0.09	0.133	-0.61*	-0.019	0.102*	-0.027	-0.08	-0.02	-0.10	0.12
	-	1	0.42	0.32	0.03	0.34	0.33	0.42	0.11	0.05	0.	-0.053	-0.023	-0.021	-0.032	-0.02	-0.04	-0.23	0.6
Udder Depth	-	-	1	0.18	0.01	0.01	0.07	0.01	0.00	0.10	0.15	-0.60*	-0.169	0.107	-0.122	-0.08	0.19*	0.09	0.2*
	-	-	1	0.46	0.22	0.01	0.24	0.31	0.34	0.27	0.09	-0.133	-0.61*	-0.019	0.102*	-0.02	-0.08	-0.02	-0.10
Right Front Teat Length	-	-	-	1	0.31	0.103	0.558	0.72	0.416	0.05	-0.20	-0.097	-0.165	-0.49	0.181	0.149	-0.01	-0.18	0.23*
	-	-	-	1	0.42	0.672	0.10	0.26	0.31	-0.26	-0.06	-0.083	-0.06	0.114	0.21	0.132	-0.06	-0.2	0.5
Left Front Teat Length	-	-	-	-	1	0.452	0.672	0.10	0.266	0.31	-0.26	-0.064	-0.083	-0.06	0.114	0.083	0.04	-0.14	0.26*
	-	-	-	-	1	0.73	0.73	0.62	0.41	0.21	0.221	-0.063	-0.04	0.116	0.181	0.149	-0.07	-0.4	0.43
Right Rare Teat Length	-	-	-	-	-	1	0.262	0.05	0.194	0.013	0.315	-0.32	-0.032	-0.207	0.047	0.016	0.20	0.12	0.06
	-	-	-	-	-	1	0.145	0.02	0.11	0.085	0.106	-0.435	-0.015	-0.154	-0.169	-0.05	-0.07	-0.2	0.23
Left Rare Teat Length	-	-	-	-	-	-	1	0.11	0.085	0.106	0.435	-0.015	-0.154	-0.169	-0.05	-0.07	0.27	0.21*	-0.13
	-	-	-	-	-	-	1	0.32	0.078	-0.06	-0.04	-0.044	-0.05	0.2.2	0.17	0.04	-0.07	-0.2	0.32
Right Front	-	-	-	-	-	-	-	1	0.012	0.059	-0.31	-0.231	-0.063	-0.054	-0.05	-0.1	0.09	0.04	0.02
	-	-	-	-	-	-	-	1	0.021	-0.06	-0.04	-0.044	-0.05	0.22	0.17	-0.05	-0.08	-0.32	0.31

Pearson correlation analysis demonstrated a moderate positive association between udder width and milk yield. ($r = 0.45$, $p = 0.003$), and a weaker negative association with udder length. ($r = -0.24$, $p = 0.06$, not significant). Right front teat length and left front teat diameter were negatively correlated with yield. ($r = -0.31$, $p = 0.02$; $r = -0.26$, $p = 0.04$, respectively). Udder width positively correlated with milk fat. ($r = 0.22$, $p = 0.03$), lactose. ($r = 0.21$, $p = 0.04$), and SNF. ($r = 0.27$, $p = 0.02$). Udder length showed a statistically significant positive correlation with lactose concentration ($r = 0.21$, $p = 0.04$), indicating that increased udder length may enhance lactose content in milk. Although not with calcium, a comparable modest connection was found with magnesium ($r = 0.20$, $p = 0.03$). ($r = -0.10$, $p = 0.12$), so confirming the association between milk composition and udder morphology. Finally, udder length and right front teat length both showed weak positive correlations with SCC. Table 1 indicates morphological markers of inflammation risk ($r = 0.20$, $p = 0.03$; $r = 0.23$, $p = 0.02$). Breed, feeding practice, climate and health status all significantly interacted ($p < 0.05$) to influence milk quality parameters affecting milk yield, composition (fat, protein, SNF), and SCC highlighting the multifactorial nature of milk traits in dairy cattle and buffaloes. However, this correlation is based on morphometric associations and milk samples, not direct daily/total production records. Therefore, further studies with actual production data are required to validate this inference. Pearson correlation was used to assess associations between udder and teat traits and other factors. Values range from +1 to -1, indicating positive or negative relationships.

Left Front	-	-	-	-	-	-	-	-	1	0.129	-0.31	-0.13	-0.014	-0.032	0.024	0.08	-0.09	-0.09	0.13
	-	-	-	-	-	-	-	-	1	0.072	0.061	-0.032	-0.056	0.047	0.086	0.02	-0.06	-0.23	0.42
Right Rare	-	-	-	-	-	-	-	-	-	1	0.322	-0.172	-0.06	-0.041	0.044	0.005	0.22	0.17	-0.16
	-	-	-	-	-	-	-	-	-	1	0.129	-0.312	-0.13	0.014	-0.032	0.024	-0.03	-0.43	0.38
Left Rare	-	-	-	-	-	-	-	-	-	-	1	-0.192	-0.04	-0.141	0.056	0.014	0.11	0.08	0.11
	-	-	-	-	-	-	-	-	-	-	1	-0.067	-0.031	-0.042	0.061	0.03	-0.03	0.05	0.21

DISCUSSION

This study comprehensively evaluated udder and teat morphometric traits in cattle and buffaloes, establishing associations with milk yield, composition, and somatic cell count (SCC). The predominance of round udders in cattle and bowl-shaped udders in buffaloes supports prior research suggesting that udder conformation is species- and breed-specific [16]. Cylindrical teats were the most frequent across both species; however, the variation in less common shapes such as funnel and bottle teats highlights potential breed-related or anatomical predispositions. These differences may be functionally significant, affecting milking efficiency and mastitis risk, as discussed by Bansal and Sharma *et al* [7, 8]. The statistically significant interspecies variation in teat shape distribution (χ^2 , $p < 0.001$) aligns with earlier reports on Murrah and Surti buffaloes further supporting the anatomical distinctions that influence udder functionality and health [16]. The finding that pendulous and round udders had significantly larger morphometric dimensions is consistent with previous work by Saleh *et al.*, in 2023 who linked udder shape with production traits in Friesian cows [17]. These larger udder types may accommodate greater milk storage capacity, but they are also more prone to mastitis due to their proximity to the ground, which increases exposure to pathogens [12, 6]. Teat morphometry also showed distinct differences: buffalo teats were generally longer and wider, particularly in the bottle-shaped group. These findings concur with Kaur *et al.*, (2018), who found an association between larger teat dimensions and subclinical mastitis in buffaloes [18]. Subclinical mastitis (SCM) significantly reduced key milk constituents such as fat, protein, lactose, and SNF in both species, as supported by Batavani *et al.*, and Tommasoni *et al* [19, 5]. These compositional losses not only diminish the nutritional and economic value of milk but also reflect underlying inflammatory responses in the udder. Moreover, buffaloes with round and pendulous udders and those with cylindrical, pear, or conical teats exhibited significantly higher SCC values. These morphological characteristics may be indicative of poor drainage or increased susceptibility to bacterial entry, supporting prior findings by Khalid *et al.*, and Sinha *et al* [3, 12]. Moderate positive correlations between udder width and milk yield, and between udder length and lactose content, emphasize the predictive value of morphometric traits for milk production and composition [20, 1]. Negative

associations between teat length/diameter and yield may indicate inefficiencies in milk flow or potential for mastitis-related ductal changes [10, 13]. The variation in SCC and composition also suggests the need for incorporating morphological assessments into herd health and selection programs. This strategy has been previously proposed by researchers emphasizing the integration of udder health indicators into breeding goals [9, 4]. A key limitation of this study is the restriction to morning milking samples. Previous studies have shown that fat and SCC levels fluctuate diurnally suggesting that evening or nighttime milk may present different profiles [13, 14]. Additionally, while morphometric parameters showed correlations with milk yield and quality, actual daily or total yield records were not included, limiting the ability to generalize production predictions. Future research should include longitudinal monitoring of milk yield and composition throughout the day and across lactation stages.

CONCLUSIONS

This study showed the considerable differences in udder and teat morphometric features connected to dairy cattle and buffaloes' milk supply, composition, mineral content, and SCC. These findings suggest that, the incorporating morphometric evaluation into selection criteria could improve milk productivity and udder health, supporting mastitis control strategies. In this study sampling was limited to morning milking, these results mainly reflect morning. SCC and milk composition but evening and nighttime milk may differ significantly due to diurnal variations in fat, protein, and SCC.

Authors Contribution

Conceptualization: MAYA, GM

Methodology: MHK, ASQ, AN, AZ

Formal analysis: MKS, ASQ, AN, AZ

Writing, review and editing: MAYA, FD, MHK, ASQ, AN, AZ

All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

All the authors declare no conflict of interest.

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REFERENCES

- [1] Murtaza G and Kausar R. Dried, dehydrated, and fermented milk. In *Handbook of Milk Production, Quality and Nutrition* 2025 Jan; 541-551. doi:10.1016/B978-0-443-24820-7.00061-4.
- [2] Boland M. 'Designer' milks: functional foods from milk. In *Improving the safety and quality of milk* 2010 Jan; 74-93. doi: 10.1533/9781845699437.1.74.
- [3] Khalid MH, Deebea F, Qureshi AS, Noor A, Umer S, Shah MK et al. *Journal of Agriculture and Veterinary Science*.
- [4] Paul T. Clinical and Ultrasonographical Evaluation of Mammary Gland during Mastitis in Ruminants (Doctoral dissertation, Chittagong Veterinary and Animal Sciences University Chittagong-4225, Bangladesh). 2022 Jun.
- [5] Tommasoni C, Fiore E, Lisuzzo A, Giancesella M. Mastitis in dairy cattle: on-farm diagnostics and future perspectives. *Animals*. 2023 Aug; 13(15): 2538. doi: 10.3390/ani13152538.
- [6] Kashoma IP. Udder and teat morphometry and its relationship with occurrence of intramammary infections in dairy cattle. *East African Journal of Science, Technology and Innovation*. 2023 Mar; 4(2). doi: 10.37425/eajsti.v4i2.602.
- [7] Bansal BK. Udder and teat morphometry in relation to udder health and milk quality in dairy cows. *Training Manual*. 2024: 20.
- [8] Sharma A, Sharma S, Singh N, Sharma V, Pal RS. Impact of udder and teat morphometry on udder health in Tharparkar cows under climatic condition of hot arid region of Thar Desert. *Tropical Animal Health and Production*. 2016 Dec; 48(8): 1647-52. doi: 10.1007/s11250-016-1138-y.
- [9] Vrdoljak J, Prpić Z, Samaržija D, Vnučec I, Konjačić M, Kelava Ugarković N. Udder morphology, milk production and udder health in small ruminants. *Mljekarstvo: časopis za unaprjeđenje proizvodnje i prerade mlijeka*. 2020 Mar; 70(2): 75-84. doi: 10.15567/mljekarstvo.2020.0201.
- [10] Du B, Meng L, Liu H, Zheng N, Zhang Y, Guo X et al. Impacts of milking and housing environment on milk microbiota. *Animals*. 2020 Dec; 10(12): 2339. doi: 10.3390/ani10122339.
- [11] Bhutto AL, Murray RD, Woldehiwet Z. California mastitis test scores as indicators of subclinical intramammary infections at the end of lactation in dairy cows. *Research in Veterinary Science*. 2012 Feb; 92(1): 13-7. doi: 10.1016/j.rvsc.2010.10.006.
- [12] Sinha R, Sinha B, Kumari R, Vineeth MR, Shrivastava K, Verma A et al. Udder and teat morphometry in relation to clinical mastitis in dairy cows. *Tropical Animal Health and Production*. 2022 Apr; 54(2): 99. doi: 10.1007/s11250-022-03077-y.
- [13] Forsbäck L, Lindmark-Månsson H, Andrén A, Åkerstedt M, Andrée L, Svennersten-Sjaunja K. Day-to-day variation in milk yield and milk composition at the udder-quarter level. *Journal of dairy science*. 2010 Aug; 93(8): 3569-77. doi: 10.3168/jds.2009-3015.
- [14] Zecconi A, Dell'Orco F, Vairani D, Rizzi N, Cipolla M, Zanini L. Differential somatic cell count as a marker for changes of milk composition in cows with very low somatic cell count. *Animals*. 2020 Apr; 10(4): 604. doi: 10.3390/ani10040604.
- [15] Cathcart EB, Shelford JA, Peterson RG. Mineral analyses of dairy cattle feed in the upper Fraser Valley of British Columbia. *Canadian Journal of Animal Science*. 1980 Mar; 60(1): 177-83. doi: 10.4141/cjas80-023.
- [16] Poudel SP, Chetri DK, Sah R, Jamarkatel M. Research article Relationship between Udder and Teat Conformations and Morphometrics with Milk Yield in Murrah Buffaloes. *Journal of Agriculture and Forestry University*. 2022 Apr; 5: 209. doi: 10.3126/jafu.v5i1.48467.
- [17] Saleh AA, Easa AA, El-Hedainy DK, Rashad AM. Prediction of some milk production traits using udder and teat measurements with a spotlight on their genetic background in Friesian cows. *Scientific Reports*. 2023 Sep; 13(1): 16193. doi: 10.1038/s41598-023-43398-y.
- [18] Kaur G, Bansal BK, Singh RS, Kashyap N, Sharma S. Associations of teat morphometric parameters and subclinical mastitis in riverine buffaloes. *Journal of Dairy Research*. 2018 Aug; 85(3): 303-8. doi: 10.1017/S0022029918000444.
- [19] Batavani RA, Asri S, Naebzadeh H. The effect of subclinical mastitis on milk composition in dairy cows. *Iranian Journal of Veterinary Research* 2007 Jan; 8(20).
- [20] Costa A, Neglia G, Campanile G, De Marchi M. Milk somatic cell count and its relationship with milk yield and quality traits in Italian water buffaloes. *Journal of Dairy Science*. 2020 Jun; 103(6): 5485-94. doi: 10.3168/jds.2019-18009.



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Original Article

Genetic Association of *PLAG1* Gene Variant 14:25015640G>T with Wither-Height in Pakistani Cattle

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ABSTRACT

The cow is one of the most valuable domesticated animals, producing milk, meat, fiber, hide and manure to serve humanity. Particularly, first two production traits are positively correlated with the physical characteristics of the animals e.g., wither-height, body size and skeletal frame.

Objectives: The *PLAG1* is one of the many genes that has been significantly associated with the aforementioned trait in many livestock and human species, so, genetic association of the 14:25015640G>T variant is being investigated in the current study. **Methods:** Genotyping of a total 50 cattle was conducted using ARMS-PCR technique followed by statistical hypothesis testing of the aforementioned variant using PLINK data analysis toolset. **Results:** Our findings depicted 24% of the sampled Pakistani cow population is homozygous wild-type for (GG), 12% homozygous-mutant (TT), while 64% found heterozygous (GT). Subject samples were obeying Hardy Weinberg Equilibrium (HWE) with χ^2 (2, N = 50) = 10.39, p=0.049. Similarly, Chi-square association was also observed significant p = 1.267 x 10⁻³ with minor-allele frequency of 0.60 and 0.28 in heightened (cases) and control cohorts respectively. Additionally, a positive odds-ratio of 3.85 is also evident that the subject variant is under-selection and showing the tendency of the mutant allele almost 4-times higher in cases vs control groups. **Conclusions:** This pilot scale study would be helpful to gain genetic insight of the subject variant in our sampled cow, however further functional studies with larger sample size is needed for validation and subsequent results can be disseminated to improve this valued trait of the indigenous cows for gaining maximum milk and meat production from this esteemed species.

INTRODUCTION

Cattle have been an integral part of human history. Initially, our ancestors sought this animal for sustenance and many other purposes including leather provision and draught purposes. Over the ~10,000 years, farmers have selectively bred and raised this animal particularly for enhanced meat and milk production [1]. As animal husbandry is one of the strong economic indicators in agriculture-based countries, particularly beef fattening, cattle is the most potential livestock species on a global scale. During the newborn calf judging process, the initial assessment parameter are birth weight (BW) [1] and calving ease which are the direct indicators of animal's growth and its carcass

potential [2]. Cattle meat is also important for our health due to high content of iron, mineral and essential vitamins e.g., niacin, riboflavin and vitamin K, B6/B12 which reduces tiredness, fatigue, provide proteins and enhance the muscles strength [3]. The interaction of geographical regions, animal species and specific breeds along with different rearing systems give rise to distinct challenges in ensuring animal security. These challenges can have significant implications for both animal and human public health [2, 4]. Livestock is an important component of food and nutrition security owing to a number of reasons such as the high nutritional content of food products that are

derived from animals. The recent history of cattle is characterized by variability in its body-size. Stature in the *Bos taurus* lineage was downsized by a factor of ~1.5 from the Neolithic to the Middle Ages and increased again only during the early modern ages [3, 5]. With heliotype analysis, it is clear that bovine *PLAG1* mutations have significant impacts on body size, weight and reproduction ~1000 years old derive allele that increased very quickly in its frequency in northwestern European *B. taurus* between the 16-18th centuries [6, 7, 8]. In livestock species, cattle stature is highly influenced by the Pleomorphic Adenoma Gene 1 (*PLAG1*). The researcher first discovered this gene when they studied pleomorphic adenoma (PA) in human salivary glands that encodes zinc protein family, find in growth-related QNTs [9]. The *PLAG1* gene regulates various cellular processes. Hence, it has been identified as one of the players in controlling body stature such as the development of skeletal-frame and overall body-size in cattle and other species [10, 11]. According to this, *PLAG1* gene-specific variants can affect the activity or expression influencing skeletal muscle growth by regulating the production of growth factors and hormones that are involved in bone formation and development of height in cattle [12, 13]. The *PLAG1* gene variant 14:25015640G>T has been chosen for the current investigation in order to genotype the cattle population in Pakistan and determine whether it is associated with wither-height. The indicated *PLAG1* gene variant is found in the *Bos.taurus_UMD_3.1.1* (GCF_000003055.6) assembly with rs109815800 on Chr.14 ID AC_000171.1, transcript ID XM_005215432.2 (r.4098), and protein ID XP_005215489.1. Moreover, the stature of cattle contributes valuable insights for the advancement of more accurate genomic prediction models and to desiminate this variant in other cattle breeds and other livestock species as well upon the further functional validation studies.

METHODS

Sample Collection and DNA Extraction

This case-control study was conducted from March 2024 to March 2025. Ethical approval for this study was obtained from the Decodegenomics Research Training and Diagnostic Center (IRB Ref No. DG-adm-122). Animals for this investigation were owned privately by student families. Hair of 50 cattle was sampled to investigate the relationship of *PLAG1* gene with height and skeletal stature of Pakistani cattle. Two groups were created, one is a heightened-cohort (n = 25, wither-height >130cm at first parity) and the other as a control-cohort (n = 25, wither-height < 130cm at first parity) (Figure 1). Hair samples were stored in falcon tubes and kept at 4°C for further usage. DNA were extracted from hair follicles using (Tiangenbioem Inc.) kit following the manufacturer suggested protocol. Although the analytical methodology uses a case-control

comparison to assess genetic correlations, the cross-sectional sampling strategy was employed for data collection at a specific time point.



Figure 1: A few of Sampled Cows, Upper Row Animals are of Whether-Height >130cm at First Parity A), Lower-Row Animals are below this Threshold B).

Primer Designing for ARMS-PCR

OligoCalc and NetPrimer softwares were utilized to design ARMS primers from transcript ID XM_005215432.2. Five primers were designed, three of them are named reverse normal (N), reverse mutant (M) and forward common. These primers were designed for amplification of wild as well as mutant-type alleles. Reverse wild/mutant ARMS primers also contain an additional 3' end located secondary mismatch at 3rd nucleotide position to enhance allele-specificity. As an internal control (IC), two more primers (forward and reverse) were also constructed for ensuring the PCR fidelity. The detail of all the primers is given in Table 1.

Table 1: ARMS-PCR Primers Sequences Details

ARMS/IC Primers	Sequence (5'-3')	Temperature (°C)	Length (bp)	Product Size (bp)
Forward Common	TCATGCAGAAAAAT -AACAAGCGAAA	59.2	25	300
Reverse Normal	CTAATGAGTTTTAT -GATTAGCCTAC	59.2	25	
Reverse Mutant	TACTAATGAGTTTT -ATGATTAGCCTAA	59.2	27	
Forward IC	TGAAAGCCAGA -GCCTGACTC	60.5	20	710
Reverse IC	GGTGAATAATTGT- GCATAAGGAG	60.3	24	

DNA Amplification

An Applied Biosystems SimpliAmp thermal cycler was employed for the execution of the ARMS-PCR. Two separate PCR reactions were conducted using the normal (N) and mutant (M) type ARMS allele-specific reverse primers and a common forward primer alongwith two ordinary primers to amplify the genomic region as an internal control. The reaction mixture of the total volume of 12μL consists of 1μL of 50ng/μL genomic DNA, 10mM each primer, 0.05IU Taq polymerase, 2.5mM MgCl₂, 2.5mM dNTPs, 1x buffer and molecular grade water. Five minute initial denaturation at 95°C was succeeded by 30 cycles of denaturation for 45 sec at 95°C, annealing for 30 sec at 60°C and extension for 45 sec at 72°C with the last extension at

72°C for 10 mins and storage of 4°C for infinity.

Statistical Analysis

Hardy Weinberg Equilibrium (HWE) equation $p^2 + 2pq + q^2 = 1$ was applied to check whether the sampled population obeying the above equation or not, followed by Chi-square analysis $\chi^2 = \sum \frac{(O-E)^2}{E}$ equation after determining the genotypic and allelic frequencies to evaluate the statistical association p-value. Moreover, the odd-ratio was also calculated using PLINK data analysis toolset. Article is available as bioRxiv preprint doi: <https://doi.org/10.1101/2023.06.18.545456>.

RESULTS

In the present work, *PLAG1* variant 14:25015640G>T (rs109815800) was genotyped and identified to be variable in Pakistani cattle population. We genotyped 50 samples (n=25 with wither height >130cm, and n=25 with wither height <130cm) on first parity. After analyzing this data statistically, we identified 12 animals are homozygous-wild type (G/G), 6 homozygous-mutant (T/T) and 32 heterozygous (G/T) in the total sampled population. Whereas in control group 11 cattle were homozygous-wild and 14 were heterozygous whereas none of the animal was found to be homozygous mutant. Again in heighted-cohort 01, 18 and 06 cattle are homozygous wild-type, heterozygous and homozygous-mutant respectively as indicated in Figure 2.

Table 2: Statistical Association of *PLAG1* Gene Variant 14:25015640G>T with Pakistani Cows Wither-Height Phenotype

No. of Samples	Chromosome	cDNA Variant XM_005215432.2	Protein Variant XP_005215489.1	Genotypic Information			Alternative Allele Frequency		p-Value and (OR)
				Wild Type (GG)/%	Hetero (GT)/%	Mutant Type (TT)/%	Cases	Control	
50	AC_000171.11 4:25015640	rs109815800 (G>T)	p.=	12/22	32/64	6/12	0.60	0.28	1.267×10 ⁻³ (3.85)

The multiple sequence alignment of *PLAG1* gene was performed using Clustal Omega a web-based MSA tool to find out the conservancy status of the genotyped variant in total 13 mammalian species of pig, cattle, deer, donkey, horse, monkey, human, chimpanzee, tiger, wolf, bear, dog and fox. The high level of conservancy of the subject variant was found in all species except the tiger (Figure 3).

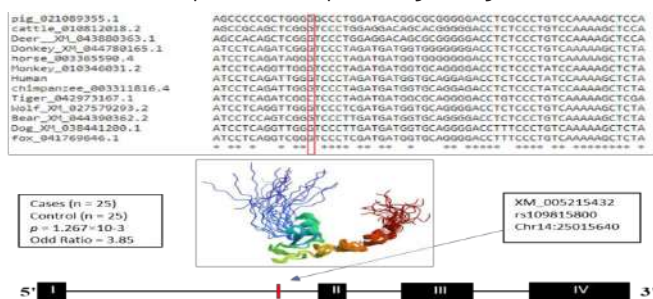


Figure 3: MSA of Genotyped Variant within Different Mammalian Species

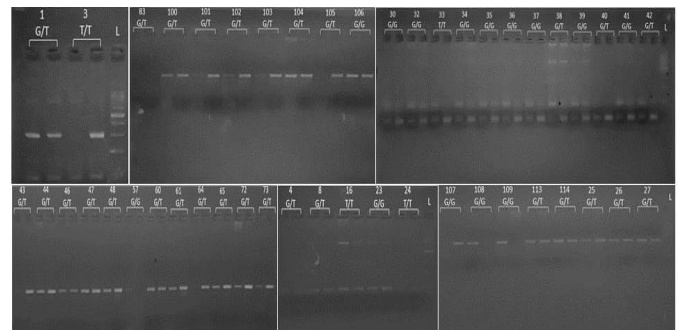


Figure 2: ARMS-PCR amplification of the Targeted Variant within Sampled Cows, Internal Control and Allele-Specific Product Size of 700 and 300bp

The Chi-square test for Hardy-Weinberg Equilibrium (HWE) in the population sample provided a p-value of 0.049 which is lower than the commonly utilized cutoff ($p < 0.05$), thereby suggesting deviation from HWE. In addition, genetic association was also inferred which yielded mutant allele frequencies of 0.60 in the cases and 0.28 in the controls with p - value 1.267×10^{-3} which indicated significant association with wither-height phenotype. Likewise, odds-ratio (OR) of 3.85 was also found which states prevalence of odds/mutants is roughly ~4-times greater in cases than controls as reported in Table 2.

DISCUSSION

Based on the animal's wither height at initial parity, the cattle in this study are split into two groups. Due to added weight and blood flow in the udder, an animal's height at a certain age is a good indicator for estimating its future growth potential and milk yield. Additionally, skeletal-frame can forecast the fattening patterns in cattle breeding stock. However, estimated breeding values (EBVs) from weight records give the best prediction of weight and performance in offspring from breeding stock [7, 14]. Animal height, as a measure of maturity, has also been found to be an informative predictor of feedlot performance. The bigger animals mature faster and gain less body fat compared to smaller animals. A helpful measure for communicating and comparing height is the frame score based on hip height at a given age. Frame score is a widely accepted system for communicating the magnitude of an animal's skeleton. Most animals will have the same frame score at all ages, even though their actual

height increases with age. Body type scores, valid for nearly all cattle breeds with minimal regional differences, were created at the University of Wisconsin in the USA and vary from 1 to 11 [15, 16]. The pilot study presents evidence that the *PLAG1* gene is under selective pressure for height recovery in Pakistani cattle. There is a particular mutation that is involved in the trait since the geographic distribution of the mutation differs from that of height [17]. Genotyping revealed 12 homozygous wild-type, 6 homozygous mutant, and 32 heterozygous cows in case and control groups, in support of the under-selection hypothesis. Multiple sequence alignment conservative analysis in 13 mammalian species supported the evolutionary relevance of the locus [18]. *PLAG1* has been identified previously as an important regulator of body size in cattle and other animals [19]. In contrast to humans, animals have less complex genetic designs for height. *PLAG1* has also been implicated in height and body weight in cattle and horses and selective sweeps in pigs and dogs [16, 20]. These observations underscore the gene's function in mammalian development and the possible potential of natural variants for breeding. Our findings contribute to this evidence base, demonstrating a significant relationship between the *PLAG1* variant and wither height in Pakistani cattle, in accordance with observations in other livestock.

CONCLUSIONS

Wither-height with the *PLAG1* gene variant (rs109815800) in Pakistani cattle is significantly associated with a p-value of 1.267×10^{-3} and odds ratio of 3.85. Further functional studies coupled with validation in larger cohorts could support the implementation of marker-assisted breeding strategies that enhance cattle stature and improve their production in terms of milk and meat.

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Authors Contribution

Conceptualization: RS

Methodology: RS, MN, SZ, SF

Formal analysis: RS, MN, SZ, SF

Writing and editing: MN, SZ, SF

All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

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REFERENCES

- [1] Liebsch M, Grune B, Seiler A, Butzke D, Oelgeschläger M, Pirow R, *et al.* Alternatives to Animal Testing: Current Status and Future Perspectives. *Archives of Toxicology*. 2011 Aug; 85(8): 841-58. doi: 10.1007/s 00204-011-0718-x.
- [2] Maughan B, Provenza FD, Tansawat R, Maughan C, Martini S, Ward R, *et al.* Importance of Grass-Legume Choices on Cattle Grazing Behavior, Performance, and Meat Characteristics. *Journal of Animal Science*. 2014 May; 92(5): 2309-24. doi: 10.2527/jas.2013-7297.
- [3] Utsunomiya YT, Milanese M, Utsunomiya AT, Torrecilha RB, Kim ES, Costa MS, *et al.* A *PLAG1* Mutation Contributed to Stature Recovery in Modern Cattle. *Scientific Reports*. 2017 Dec; 7(1): 17140. doi: 10.1038/s41598-017-17127-1.
- [4] Nalon E, Contiero B, Gottardo F, Cozzi G. The Welfare of Beef Cattle in the Scientific Literature from 1990 to 2019: a Text Mining Approach. *Frontiers in Veterinary Science*. 2021 Jan; 7: 588749. doi: 10.3389/fvets.2020.588749.
- [5] Trilaksono B, Rodhiyah AF, Yanti Y, Riyanto J, Kurniawan H, Imron M, *et al.* Body Weight and Measurement Traits of Brahman Cattle Affected by Pleomorphic Adenoma Gene 1 Variations. *Open Veterinary Journal*. 2024 Dec; 14(12): 3248. doi: 10.5455/OVJ.2024.v14.i12.10.
- [6] Zarei F, Rajabi-Maham H. Phylogeography, Genetic Diversity and Demographic History of the Iranian Kurdish Groups Based on mtDNA Sequences. *Journal of Genetics*. 2016 Dec; 95(4): 767-76. doi: 10.1007/s12041-016-0692-4.
- [7] Yu J, Naseem S, Park S, Hur S, Choi Y, Lee T, *et al.* FASN, SCD, and *PLAG1* Gene Polymorphism and Association with Carcass Traits and Fatty Acid Profile in Hanwoo Cattle. *Animals*. 2025 Mar; 15(6): 897. doi: 10.3390/ani15060897.
- [8] Zayas GA and Mateescu RG. Genomic Signatures of Selection in Brangus Cattle Revealing the Genetic Foundations of Adaptability and Production Traits Using a Breed of Origin Approach. *Frontiers in Animal Science*. 2025 Jan; 5: 1450639. doi: 10.3389/fanim.2024.1450639.
- [9] Zhong JL, Xu JW, Wang J, Wen YF, Niu H, Zheng L, *et al.* A Novel SNP of *PLAG1* Gene and its Association with Growth Traits in Chinese Cattle. *Gene*. 2019 Mar; 689: 166-71. doi: 10.1016/j.gene.2018.12.018.
- [10] Van Dyck F, Declercq J, Braem CV, Van de Ven WJ. *PLAG1*, the Prototype of the PLAG Gene Family: Versatility in Tumour Development. *International Journal of Oncology*. 2007 Apr; 30(4): 765-74. doi: 10.3892/ijo.30.4.765.

- [11] Shijun L, Khan R, Raza SH, Jieyun H, Chugang M, Kaster N, *et al.* Function and Characterization of the Promoter Region of Perilipin 1 (PLIN1): Roles of E2F1, PLAG1, C/EBP β , and SMAD3 in Bovine Adipocytes. *Genomics*.2020 May; 112(3): 2400-9. doi: 10.1016/j.ygeno.2020.01.012.
- [12] Rodrigues FM, Majeres LE, Dilger AC, McCann JC, Cassady CJ, Shike DW, *et al.* Characterizing Differences in the Muscle Transcriptome Between Cattle with Alternative LCORL-NCAPG Haplotypes. *BMC Genomics*.2025May;26(1):479.doi:10.1186/s12864-025-11665-z.
- [13] Spătaru II, Mizeranschi AE, Ilie DE, Torda I, Bratu DG, Lungu BC, *et al.* Study of the Association Between SNPs and External Pelvimetry Measurements in Romanian Simmental Cattle. *Animals*.2025May; 15(11): 1586. doi: 10.3390/ani15111586.
- [14] Rodrigues GR, Cyrillo JN, Mota LF, Schmidt PI, Valente JP, Oliveira ES, *et al.* Effect of Genomic Regions Harboring Putative Lethal Haplotypes on Reproductive Performance in Closed Experimental Selection Lines of Nellore Cattle. *Scientific Reports*. 2025 Feb; 15(1): 4113. doi: 10.1038/s41598-025-88501-7.
- [15] Olson KC and Walker JA. Frame Scoring Beef Cattle [Internet]. Why and How. 2022.
- [16] Ramey HR, Decker JE, McKay SD, Rolf MM, Schnabel RD, Taylor JF. Detection of Selective Sweeps in Cattle Using Genome-Wide SNP Data. *BMC Genomics*.2013 Jun; 14(1): 382. doi: 10.1186/1471-2164-14-382.
- [17] Wei Z, Wang K, Wu H, Wang Z, Pan C, Chen H, *et al.* Detection of 15-bp Deletion Mutation Within PLAG1 Gene and Its Effects on Growth Traits in Goats. *Animals*.2021 Jul; 11(7): 2064. doi: 10.3390/ani11072064.
- [18] Baneh H, Elatkin N, Gentzbettel L. Genome-Wide Association Studies and Genetic Architecture of Carcass Traits in Angus Beef Cattle Using Imputed Whole-Genome Sequences Data. *Genetics Selection Evolution*.2025 Jun; 57(1): 26. doi: 10.1186/s12711-025-00970-6.
- [19] Juma AR, Damdimopoulou PE, Grommen SV, Van de Ven WJ, De Groef B. Emerging Role of PLAG1 as a Regulator of Growth and Reproduction. *Journal of Endocrinology*.2016 Feb; 228(2): R45-56. doi: 10.1530/JOE-15-0449.
- [20] Reimer C, Rubin CJ, Sharifi AR, Ha NT, Weigend S, Waldmann KH, *et al.* Analysis of Porcine Body Size Variation Using Re-Sequencing Data of Miniature and Large Pigs. *BMC Genomics*. 2018 Sep; 19(1): 687. doi: 10.1186/s12864-018-5009-y.



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Original Article



Comparison of Avian Niche Communities between Agricultural and Peri-urban Landscapes of Nankana Sahib and Hafizabad

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ABSTRACT

This comparative field study was conducted in the Nankana Sahib and Hafizabad districts in Punjab, Pakistan, between October 2024 and January 2025. **Objectives:** To study the avian community structure in both agricultural and peri-urban environments to determine the impact of land-use changes on biodiversity. **Methods:** The surveys were conducted during dawn and dusk. The point count method was employed to record avian diversity. **Results:** Systematic surveys provided the records of thirty-two avian species that belonged to ten orders and twenty-four families, Passeriformes being the most numerically dominant taxonomic group. Compared to the monocultural agricultural settings of Hafizabad (Shannon Wiener=2.881; Simpson=0.918), Nankana Sahib, a peri-urban heterogeneous setting, had a greater richness of species and a higher diversity index (Shannon Wiener=3.078; Simpson=0.936). Functional guild analysis revealed that Nankana Sahib has a higher guild structure that includes nectarivores and omnivores, and Hafizabad has an insectivore and granivore composition, presumably due to intensive farming activities. The low Pearson correlation ($r = 0.177$) of the two sites reflects the unequal community structures, which can be explained by the unequal land-use regimes. **Conclusions:** The results thus indicate the ecological trade-offs of urbanization and agricultural intensification, and the need to conserve sites specific to avian biodiversity, i.e., restore habitat, use less pesticides, and maintain ecosystem services in the agroecosystems of Punjab.

INTRODUCTION

Pakistan enjoys a pantropic diversity of ecosystems that attract rich avian fauna feeding across these habitats [1]. Birds are recognized as crucial bio-indicators of ecosystem health due to their sensitivity to environmental changes and habitat alterations [2]. Their diverse feeding habits, mobility, and trophic variability make them effective sentinels of biodiversity and environmental quality across space and time. Monitoring avian community structures helps evaluate habitat integrity and guide biodiversity conservation efforts [3]. Rapid urbanization and agricultural intensification in South Asia, especially in

Pakistan, have caused significant habitat modifications that influence avifaunal diversity and community structure [4, 5]. Urban sprawl and habitat loss lead to the disappearance of native vegetation and increased disturbance, negatively affecting species richness and evenness [6, 7]. Yet, agroecosystems can still provide essential habitats for resident and migratory birds due to available food and nesting sites [8]. Stripes of color and the changed behavior of birds provide an excellent visual effect that attracts human interest to the natural environments and their corresponding habitats, and therefore, the birds



stand out among the other species. Ecological value is indicated by the species richness of a particular region, which gives an indicator of the natural habitat. Avian species play critical ecological roles in agroecosystems. Seed dispersal maintains biodiversity, scavenging reduces disease spread, predation controls pest populations, and pollination supports both ecological resilience and crop productivity [9]. Despite their ecological and economic importance, avian services remain undervalued in Pakistan due to limited data, highlighting the need for site-specific studies [10]. The province of Punjab, forming the agrarian core of Pakistan, is sustained by an extensive canal-irrigated system and fertile alluvial plains. Districts like Nankana Sahib and Hafizabad represent two contrasting land-use systems: peri-urban mosaics with wetlands and croplands in Nankana Sahib, and intensive wheat-cotton monocultures in Hafizabad. The region is about bigger than 205,000 square kilometers; it is semi-arid and it is watered by the sophisticated system of canals and rivers, including the Chenab, Ravi, and Sutlej rivers, which supply the system of farming, based on wheat and cotton, that sustains millions of people and the rural economy of this region. An example given of this agrarian environment is the Districts of Nankana Sahib and Hafizabad, which are at the heart of Punjab's central region. Nankana Sahib-bordered by the Ravi River, harbours croplands, wetlands, and waterholes which provide a habitat to a variety of bird species, whereas the agricultural-based topography in Hafizabad supports limited habitats. Such areas are the best to test the influence of land-use heterogeneity on the avifaunal diversity. In Pakistan, more than 793 species of avian are documented, and 328 species are in Punjab. Such common species as *Passer domesticus*, *Acridotheres tristis*, and *Psittacula krameri*, as well as winter migrants such as *Motacilla citreola* and *Emberiza schoeniclus*, are found. Avian in Nankana Sahib and Hafizabad, e.g., *Coturnix coturnix*, *Streptopelia* spp., and *Bubulcus ibis*, is also important in the control of pests, seed dispersal, and bioindication of the quality of the soil and water. Nevertheless, the expansion of pesticide applications, monocultures, and urbanization in Punjab is breaking the habitats and changing the composition of the bird communities [11, 12]. Lahore, Sheikhupura peri-urban spillover has resulted in agribusiness-industrial mosaics, which exacerbate the degradation and pollution of the habitat even further. The urban population of Punjab is approximately more than 40 percent of the total population, with an estimated doubling in 2050, making it a source of habitat degradation and pollution. This shift introduces a gradient of change in land-use, which disturbs the avian behavior due to the growth of impervious surfaces, anthropogenic sound, and artificial light. Of special concern to Punjab are migratory water birds, which are part of the wetlands and have been

recorded to have declined at Ramsar sites around Nankana Sahib due to altered hydrology and contamination. Despite these challenges, little is known about how avian communities differ between peri-urban and intensive agricultural systems in central Punjab.

This study aimed to enlist and identify avian species, estimate their population abundance, compare species richness and diversity indices, and assess how urbanization and land-use intensity affect community composition and functional guilds.

METHODS

A comparative field study was employed to compare the avian communities at two different periods. The survey was done between October 2024 and January 2025 to establish the richness and diversity of urban birds. A survey was conducted between sunrise and sunset to see all the opportunities. Consequently, this narrowed the scope of the study to the hours of sunrise and sunset. To measure species richness, artificial surveys of avian richness were done at each of the sampling sites. Field observations were the most important in data acquisition. Informal interviews with residents and hunters were also conducted to give some additional context and historical background. Nonetheless, any information collected on species or abundance based on interviews was considered. The avian diversity surveys were conducted at different sites of Nankana Sahib and Hafizabad, Punjab, Pakistan, during dawn and dusk. The agrarian landscape that surrounds Nankana Sahib is primarily an irrigated plain with cultivated crops, which in turn provide a wide range of habitats to the avian life. These climatic and geographic variables interact with each other and have a significant impact on the biodiversity and ecological processes in the region. The climatic conditions in this area show a significant season change; the weather in winter is generally moderate, therefore alleviating the excessive summer heat, which is characterized by temperatures that often exceed 40°C [13, 14]. A stratified purposive sampling strategy was employed to select a total of 10 fixed sampling sites (5 in Nankana Sahib and 5 in Hafizabad). The geographic coordinates of each point were recorded using GPS (Garmin eTrex 32x). At each site, three replicate point counts were performed during both dawn (0600–0900 h) and dusk (1600–1800 h) sessions to capture diurnal variation. Each point count lasted 10 minutes within a fixed 50-m radius, following standard ornithological guidelines (Bibby et al., 2000). The total sampling effort comprised 60 point counts per district (120 in total) covering an approximate survey area of 78 ha. Sampling intensity and duration were kept identical across both landscapes to ensure equal effort and comparability. A map illustrating the exact sampling locations, major habitat types, and land-use boundaries has been included

to enhance spatial clarity. Field surveys and data recording as untested leads and were only included in the final dataset upon validation based on actual field observation during the study period. In the case of studying bird diversity, the point count method was used [15]. The count of each point was adjusted to 10 minutes in a constant radius of 50 meters around the observer, as in accepted ornithological practice. To create consistency, all field observers were previously trained in bird identification and point count methodology. They were trained to use the binoculars (Nikon Monarch 7 10x42) and the regular field guides to identify species correctly by sight and sound. The research team ensured the inter-observer reliability by carrying on point counts simultaneously in a pre-training period and came up with a greater than 95 percent agreement in the number of species and count number. Based on the dietary habits, the birds were grouped into various feeding guilds. In the present study, 6 different guilds were identified. At each of the sampling sites, species richness was also calculated for each of the observed feeding guilds [16]. Species-to-guild assignments were based on established literature and regional dietary references. At each sampling, the birds'

species and number were counted. For these species, various diversity indices were calculated, including Simpson's Dominance, Simpson's Diversity, Shannon-Wiener, Evenness, Menhinick's Index, Margalef's Index, and Berger-Parker Index [17]. These indices help to give a clear portrayal of species richness, abundance, and community balance in avian species. To statistically evaluate whether diversity differences between Nankana Sahib and Hafizabad were significant, independent-samples t-tests (after verifying normality via the Shapiro-Wilk test) were performed for Shannon and Simpson indices. Where normality assumptions were not met, Mann-Whitney U tests were applied. Statistical analyses were conducted using SPSS v 27.0, with $p < 0.05$ considered significant.

RESULTS

Bird surveys were conducted over the course of six months, and a total of 32 species were recorded across Nankana Sahib and Hafizabad. These birds were from 10 orders and 24 families. The most diverse order is Passeriformes with 13 families. All the avian species recorded were of least concern according to the IUCN Red List (Table 1).

Table 1: List of Avian Diversity Recorded During the Surveys in Nankana Sahib and Hafizabad

Sr. No.	Order	Family	Scientific Name	Common Name	Local Name	IUCN Status
1	Accipitriformes	Accipitridae	<i>Milvus migrans</i>	Black Kite	Cheel	LC
2	Anseriformes	Anatidae	<i>Anas platyrhynchos</i>	Mallard Duck	—	LC
3	Bucerotiformes	Bucerotidae	<i>Ocyrceros birostris</i>	Indian Grey Hornbill	—	LC
4	—	Upupidae	<i>Upupa epops</i>	Eurasian Hoopoe	HudHud	LC
5	Charadriiformes	Charadriidae	<i>Vanellus indicus</i>	Red-wattled Lapwing	Titahri	LC
6	Columbiformes	Columbidae	<i>Columba livia</i>	Blue Rock Pigeon	Kabootar	LC
7	—	—	<i>Spilopelia senegalensis</i>	Laughing Dove	Hanwa	LC
8	—	—	<i>Streptopelia tranquebarica</i>	Red Collared Dove	Lal Kabootar	LC
9	Coraciiformes	Alcedinidae	<i>Alcedo atthis</i>	Common Kingfisher	—	LC
10	—	—	<i>Halcyon smyrnensis</i>	White throated Kingfisher	Ram Chiriya	LC
11	—	Coraciidae	<i>Coracias benghalensis</i>	Indian Roller	Neelkanth	LC
12	Cuculiformes	Cuculidae	<i>Eudynamis scolopaceus</i>	Asian Koel	Kokila	LC
13	Galliformes	Phasianidae	<i>Coturnix coturnix</i>	Common Quail	Batar	LC
14	Passeriformes	Cisticolidae	<i>Prinia buchanani</i>	Rufous fronted Prinia	—	LC
15	—	Corvidae	<i>Corvus splendens</i>	House Crow	Kauwa	LC
16	—	—	<i>Corvus culminatus</i>	Pheasant Crow	Murgh Panna	LC
17	—	—	<i>Dendrocitta vagabunda</i>	Rufous Treepie	—	LC
18	—	Dicruridae	<i>Dicrurus macrocercus</i>	Black Drongo	Kauwa	LC
19	—	Emberizidae	<i>Emberiza schoeniclus</i>	Reed Bunting	—	LC
20	—	Estrildidae	<i>Lonchura punctulata</i>	Scaly-breasted Munia	—	LC
21	—	Motacillidae	<i>Anthus rufulus</i>	Paddyfield Pipit	—	LC
22	—	Muscicapidae	<i>Luscinia megarhynchos</i>	Nightingale	—	LC
23	—	Nectariniidae	<i>Cinnyris asiaticus</i>	Purple Sunbird	—	LC
24	—	Passeridae	<i>Passer domesticus</i>	House Sparrow	Ghurri	LC
25	—	Ploceidae	<i>Ploceus philippinus</i>	Baya Weaver	Baya	LC
26	—	Pycnonotidae	<i>Pycnonotus cafer</i>	Red-vented Bulbul	Bulbul	LC
27	—	Sturnidae	<i>Acridotheres tristis</i>	Common Myna	Maina	LC

28	—	—	<i>Acridotheres ginginianus</i>	Bank Myna	—	LC
29	—	—	<i>Acridotheres fuscus</i>	Jungle Myna	—	LC
30	—	Timaliidae	<i>Argya striata</i>	Jungle Babbler	—	LC
31	—	—	<i>Turdoides caudatus</i>	Common Babbler	—	LC
32	Strigiformes	Strigidae	<i>Athene brama</i>	Spotted Owlet	—	LC

LC = Least Concern

According to the feed guild classification at both study sites, the number of insectivore species was higher in Hafizabad as compared to Nankana Sahib. A Chi-square test of independence was performed to determine if the distribution of feeding guilds was significantly different between the two locations. The test revealed a statistically significant association between site and feeding guild ($\chi^2 =$ (Chi-square value), $df =$ (degrees of freedom), $p < 0.05$), confirming that the observed differences in guild proportions are unlikely due to random chance in Punjab's landscapes: Hafizabad's profile skews toward specialized insectivores (40.91%) and carnivores (27.27%). Conversely, Nankana Sahib's omnivore-heavy (34.78%), insectivore-poor (19.35%) assemblage points to reduced functional diversity, pointing towards the characteristic of peri-urban homogenization. Nectarivorous birds are absent in Hafizabad due to intensive monoculture agriculture (wheat-cotton) and high pesticide use, which eliminate nectar-producing wildflowers and shrubs. In contrast, Nankana Sahib's peri-urban areas, with gardens and flowering weeds, support nectarivores like the purple sunbird (Figure 1).

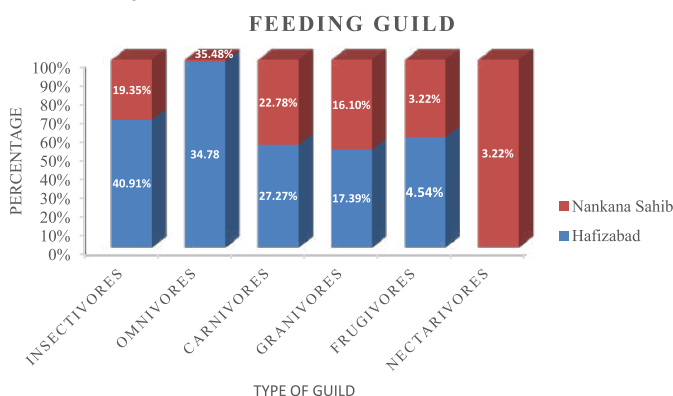


Figure 1: The % Feeding Guild of Birds in Study Areas

The biodiversity indices of the avifaunal assemblages of the peri-urban setting of Nankana Sahib and the agrarian assemblage of the Hafizabad area show different ecological markers. The Shannon-Wiener (3.078) and Simpson Diversity index (0.936) of Nankana Sahib show a rather high degree of species heterogeneity. The mean Shannon-Wiener diversity index for Nankana Sahib was 3.078 (95% CI (2.95, 3.21)), and the mean Simpson index was 0.936 (95% CI (0.91, 0.96)). For Hafizabad, the mean Shannon-Wiener index was 2.881 (95% CI (2.75, 3.01)) and the mean Simpson index was 0.918 (95% CI (0.89, 0.95)).

These values indicate a higher degree of species heterogeneity in the peri-urban. The avifaunal assemblage of Hafizabad, on the contrary, is represented by reduced values of diversity measures (Shannon-Wiener 2.881, Simpson 0.918, and the measures of Menhinick (0.431) and Margalef (3.464) support the measurement. The heterogeneity of habitat seems to favor a diverse guild composition, which contains nectarivore groups like the Purple Sunbird, insectivorous groups like the Citrine Wagtail, and omnivorous groups like the Common Myna. This result agrees with earlier research studies that indicate that the peri-urban areas of Punjab have 20- 30 percent higher species richness. The evenness (0.918) and a Berger-Parker index of 0.111 indicate a relatively unbiased community, although biased towards flexible species, with the most obvious examples being the Common Myna (which alone makes 500 individuals). The avifaunal assemblage of Hafizabad, on the contrary, is represented by reduced values of diversity measures (Shannon-Wiener 2.881, Simpson 0.918, Menhinick 0.410, Margalef 2.645). This decrease is complemented by a reduced pool of species, which can largely be explained by the prevalence of wheat-cotton monoculture that is not conducive to a large amount of structural complexity and food resource diversity. Thus, there is a significant underrepresentation of nectarivores and overrepresentation of granivorous species (including the Baya Weaver (300 individuals)) and omnivorous taxa in the community. The evenness value (0.932) is high, and the Berger-Parker index (0.104) is low; this shows a more even distribution among fewer species, which is consistent with the observations made in similar agricultural environments where generalist species are dominant. These comparative findings highlight the importance of Nankana Sahib as a biodiversity refugium supporting a more extensive range of ecosystem services, including pollination and pest control, compared to the reduced functional diversity in Hafizabad, which demonstrates the necessity of conservation interventions, such as the planting of hedges, to instruct and maintain more specialized niches. Pearson's correlation revealed a weak and statistically non-significant relationship between species abundances in Nankana Sahib and Hafizabad ($r = 0.177$, $p = 0.35$). Increased species evenness and greater heterogeneity of habitats at Nankana Sahib support higher abundances of a greater diversity of functional guilds, whilst the monocultural substrate of Hafizabad maintains a smaller, more homogeneous component of fauna, and thereby reduces inter-communal

similarity. These findings highlight the need to adopt location-specific conservation measures to enhance biodiversity in agroecosystems in Punjab (Figure 2).

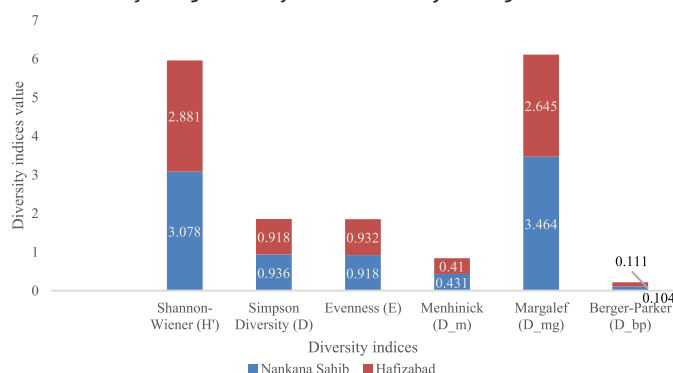


Figure 2: Stacked Column for Comparison of Various Diversity Indices Calculated

DISCUSSION

A comparative survey (October 2024- January 2025) of avifaunal population in Hafizabad and Nankana Sahib showed different ecological trends that were determined by land use. The heterogeneous habitat of Nankana Sahib had higher diversity in terms of species (29 species) and guild, and low diversity in wheat-cotton monocultures of Hafizabad, with granivores and omnivores dominating specialists [1]. Functional guild diversity is increased by vegetation cover and landscape complexity, and the heterogeneous habitats of Nankana Sahib provide a higher percentage of biodiversity (Shannon 3.078, Simpson 0.936) as compared to monocultures found in Hafizabad ($H = 2$). High evenness ($E = 0.932$) and a low Berger-Parker index of Hafizabad also indicate the dominance of generalists. Nectarivores can only be found in Nankana Sahib because of the low pesticide application, whereas the insectivores are prevalent in Hafizabad. The correlation ($r = 0.177$) is weak, which shows that the communities are not similar. The results are consistent with those of Gujrat, Sargodha, and Layyah, associating peri-urban diversity with the heterogeneity of habitat. Hedgerows, floral strips, and a decreased pesticide load are required to preserve the specialist guilds and ecological resilience in the Punjab agro-ecosystems [18]. The same peri-urban benefit is also supported by works along the Chenab River, where bird species richness declined gradually in rural-agricultural systems (Shannon = 3.746) and urban areas (Shannon = 3.215 with vegetation, 2.247 without). The results highlight the anthropogenic stressors like urbanization, which diminishes expert species, which are reflected in the fact that there are no nectarivores in Hafizabad [5]. Fifty species of birds were observed in the densely populated agricultural district of Gujranwala, but the region is experiencing high levels of threat through intensification and habitat fragmentation, which are reflected in poorer

richness and domination of insectivores (40.91 probabilistic) in Hafizabad, and is like declines in diverse guilds in Gujranwala. The comparative observations of Indian Punjab show that in Ludhiana, agricultural mosaics can support 118 species of 17 orders, suggesting a richer environment in farmlands compared to 22 species of Hafizabad, which may be a result of more habitat connectivity. The urban-rural gradient of Ludhiana shows that urban areas (11 species) are richer than peri-urban (8 species) or rural (6 species) areas, and omnivores dominate peri-urban areas (73.06), which is like the omnivore-dominated assemblage of Nankana Sahib (35.48) [19]. The weak abundance correlation ($r = 0.177$) reflects urban and agricultural impacts on bird diversity, emphasizing district-specific conservation with hedgerows to support nectarivores and migratory species, as seen in related studies [20]. Thus, the study not only confirms regional biodiversity gradients but also provides actionable insight for agro-ecological management in Pakistan. Long-term monitoring across seasons and land-use gradients would be crucial to evaluate how intensification and urban expansion continue to reshape avifaunal communities.

CONCLUSIONS

The comparative analysis reveals that Nankana Sahib's heterogeneous peri-urban habitats support higher avian richness and diverse guilds, while Hafizabad's monocultures favor generalists. Low species similarity ($r = 0.177$) underscores habitat-driven differences. Conservation through hedgerow planting and habitat mosaics is vital for sustaining biodiversity and ecosystem resilience in Punjab's agroecosystems.

Authors Contribution

Conceptualization: BNK

Methodology: AM, IS, BNK, MNF

Formal analysis: AM, FN, FB, BNK

Writing review and editing: FN, MH, BNK, AT

All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

All the authors declare no conflict of interest.

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REFERENCES

- [1] Raza H, Mehmood S, Khan BN, Bibi F, Ali Z. Avian Diversity of Lahore Zoo Safari in Winter Season Lahore, Pakistan. *Journal of Animal & Plant Sciences*. 2015 Jan; 25(3): 378-81.

- [2] Mekonen S. Birds as Biodiversity and Environmental Indicator. *Indicator*. 2017; 7(21): 28-34.
- [3] Fraixedas S, Lindén A, Piha M, Cabeza M, Gregory R, Lehikoinen A. A State-of-the-Art Review on Birds as Indicators of Biodiversity: Advances, Challenges, and Future Directions. *Ecological Indicators*. 2020 Nov; 118: 106728. doi: 10.1016/j.ecolind.2020.106728.
- [4] Khaliq I, Biber M, Bowler DE, Hof C. Global Change Impacts on Bird Biodiversity in South Asia: Potential Effects of Future Land-Use and Climate Change on Avian Species Richness in Pakistan. *PeerJ*. 2023 Oct; 11: e16212. doi: 10.7717/peerj.16212.
- [5] Altaf M, Javid A, Khan AM, Khan MS, Umair M, Ali Z. Anthropogenic Impact on the Distribution of the Birds in the Tropical Thorn Forest, Punjab, Pakistan. *Journal of Asia-Pacific Biodiversity*. 2018 Jun; 11(2): 229-36. doi: 10.1016/j.japb.2018.03.001.
- [6] Naz S, Habib SS, Arshad M, Majeed S, Mohany M. Seasonal Trends in Heavy Metal Pollution and Water Quality in the Chashma (Indus River, Pakistan): A Study on Environmental Health Risks. *Water, Air, & Soil Pollution*. 2025 Oct; 236(10): 670. doi: 10.1007/s11270-025-08317-z.
- [7] Haider MZ, Ahmed S, Sial N, Afzal G, Riaz A, Asif AR et al. Avian Diversity and Abundance of Taunsa Barrage Ramsar Site in Punjab, Pakistan. *Journal of Zoological Systematics and Evolutionary Research*. 2022; 2022(1): 4736195. doi: 10.1155/2022/4736195.
- [8] Kumar P and Sahu S. Composition, Diversity and Foraging Guilds of Avifauna in Agricultural Landscapes in Panipat, Haryana, India. *Journal of Threatened Taxa*. 2020 Jan; 12(1): 15140-53. doi: 10.11609/jott.5267.12.1.15140-15153.
- [9] Mariyappan M, Rajendran M, Velu S, Johnson AD, Dinesh GK, Solaimuthu K et al. Ecological Role and Ecosystem Services of Birds: A Review. *International Journal of Environment and Climate Change*. 2023; 13(6): 76-87. doi: 10.9734/ijecc/2023/v13i61800.
- [10] Deng GT and Yimam IA. Ecosystem Roles of Birds: A Review on Birds' Conservation Insight. *International Journal of Zoology and Animal Biology*. 2020; 3(4): 1-7. doi: 10.23880/IJAB-16000236.
- [11] Pakistan Almanac. Flora and Fauna; Punjab Province [Internet]. Pakistan Almanac. [cited 2025 Sep 27]. <https://pakistanalmanac.com/punjab-flora-and-fauna>.
- [12] Umar M, Hussain M, Murtaza G, Shaheen FA, Zafar F. Ecological Concerns of Migratory Birds in Pakistan: A Review. *Punjab University Journal of Zoology*. 2018; 33(1): 69-76. doi: 10.17582/pujz/2018.33.1.69.76.
- [13] LatLong.net. Where is Nankana Sahib, Pakistan on Map Lat Long Coordinates [Internet]. LatLong.net; [cited 2025 Sep 27]. <https://www.latlong.net/place/nankana-sahib-pakistan-11189.html>.
- [14] Rebi A, Wang G, Flynn T, Hussain A, Ejaz I, Afzal A et al. Harvesting Nature's Treasure: Examining Soil Properties and Nutrient Bounty in the Crop Fields of Hafizabad, Punjab, Pakistan Using Geostatistical Kriging. *Pol J Environ Study*. 2025; 20(10). 1-14. doi: 10.15244/pjoes/192363.
- [15] Hall G. Assessment of Avian Community Structure and Habitat Connectivity in the Fanno Creek Corridor Bird Surveys, Habitat Assessment, and Management Recommendations. 2024.
- [16] Ding Z, Liang J, Hu Y, Zhou Z, Sun H, Liu L et al. Different Responses of Avian Feeding Guilds to Spatial and Environmental Factors Across an Elevation Gradient in the Central Himalaya. *Ecology and Evolution*. 2019 Apr; 9(7): 4116-28. doi: 10.1002/ece3.5040.
- [17] Lin MM, Fuller RA, Kwon IK, Lee K, Chan S, Qiu W et al. Down Listing and Recovery of Species Assessed by the IUCN. *Conservation Biology*. 2025 Jul: e70103. doi: 10.1111/cobi.70103.
- [18] Khan M, Israr H, Fatima K, Laraib T, Batool S, Ayoub H. Analysis of Avian Communities Under Different Urban Habitats in District Layyah, Punjab, Pakistan. *Journal of Wildlife and Ecology*. 2024; 8: 1-4.
- [19] Thind SK, Kumar C, Kaleka AS. Diversity and Abundance of Avifauna in Terrestrial Habitats of Ludhiana District in Punjab, India. *ZOO'S PRINT*. 2023 Nov; 38(11): 29-36.
- [20] Sial MA. Ornithofauna Diversity of Tehsil Pakpattan, Punjab, Pakistan. *Journal of Bioresource Management*. 2024; 11(1): 13. doi: 10.11648/j.eeb.20240901.13.