

Optimizing Conservation of Nili Ravi Buffaloes in Southern Region of Khyber Pakhtunkhwa: A Comprehensive Evaluation of Phenotypic, Morphometric, and Productive Traits

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Recommended Citation

Khan, A., Din, I. u., Majid, H. A., Ali, A., Ali, A., Rizwan, M., Ullah, W., Zeb, S., Ullah, S., Khan, I., & Ullah, A. (2023). Optimizing Conservation of Nili Ravi Buffaloes in Southern Region of Khyber Pakhtunkhwa: A Comprehensive Evaluation of Phenotypic, Morphometric, and Productive Traits, *Journal of Bioresource Management*, 10 (4).

ISSN: 2309-3854 online

(Received: May 8, 2023; Accepted: Sep 29, 2023; Published: Dec 26, 2023)

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Cover Page Footnote

The Government of Khyber Pakhtunkhwa provided regular budget grant for this study.

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OPTIMIZING CONSERVATION OF NILI RAVI BUFFALOES IN SOUTHERN REGION OF KHYBER PAKHTUNKHWA: A COMPREHENSIVE EVALUATION OF PHENOTYPIC, MORPHOMETRIC, AND PRODUCTIVE TRAITS

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ABSTRACT

In Pakistan, Nili Ravi buffalo is highly valued breed due to its high milk yield. Native to Punjab province, the breed is now extensively distributed throughout the country. To assess and optimize the conservation strategies for Nili Ravi buffaloes in southern Khyber Pakhtunkhwa by evaluating their phenotypic, morphometric, productive, and reproductive characteristics. Buffaloes were maintained at Livestock Research and Development Station in Paharpur, Dera Ismail Khan from 2010-2023, under breed conservation program. A sum of 298 records Nili Ravi buffaloes were used for the investigation. The research examined physical attributes such as coat color, horn morphology, and other qualitative characteristics. In addition to body weight, body length, withers height, chest circumference, and hip breadth, morphometric measurements were recorded. Mean body weight of Nili Ravi buffaloes was recorded was 677 ± 213 Kg, produced 1387.12 ± 32.87 liters milk during their lactation period of 292.87 ± 32.40 days that was substantially impacted by year ($p < 0.05$) and calving season. Calving interval was 480.18 ± 22.09 days, whereas parity year and calving season bear considerable ($p < 0.05$) impact on their characteristic. Dry period was 181.2 ± 18.01 days. This research aided in the development of conservation and enhancement strategies for Nili Ravi buffalo in the region. Nili Ravi buffaloes in the region have excellent milk yield but the study disclosed morphometric variations within the breed, demonstrating the need for targeted breeding and management practices.

KEYWORDS: Breed Conservation; Khyber Pakhtunkhwa, lactation length; morphometric analysis; Nili Ravi.

INTRODUCTION

Pakistan is flourished in well-adapted, internationally renowned animal genetic resources. Kundhi and Nili-Ravi are two well-known breeds in Pakistan (Khan et al., 2014). Nili Ravi buffalo is greatly valued breed, predominantly due to its high milk yield and meat production (Islam et al., 2020). Originally from Punjab province, this breed is now extensively distributed throughout Pakistan, including southern region of

Khyber Pakhtunkhwa (Ahmed et al., 2017). In 2022-23 periods, the total buffalo population in Pakistan was reported to be around 45 million. Out of this, Khyber Pakhtunkhwa accounted for approximately 1.4 million buffaloes (Pakistan Bureau of Statistics, 2023). However, a comprehensive understanding of Pakistan's animal genetic resources is still lacking, and their fundamental features have not been sufficiently described. Hence, it is crucial to assess indigenous breeds in terms of phenotypic

traits, as well as their productivity and reproductive capabilities, particularly within their respective regions and under current management conditions (Khan et al., 2014).

The domestic buffalo is vital to the agricultural economies of numerous tropical and subtropical nations. Due to their higher milk fat content than cattle, most buffaloes in Pakistan are raised on small-scale farms that ingest majority of their own milk and sell the surplus to supplement the family's income. In recent years, the number of commercial dairy farms in Pakistan has tremendously exploded. Additionally, buffalo are a significant source of meat, primarily through the slaughter of mature females, males, and male calves (Tariq et al., 2013).

Phenotypic characterization of domestic animals involves documenting their external features. This description includes the measurement of each animal's body structure using suitable instruments, which helps analyze morphometric traits related to biometric characteristics. The primary goal of conducting research on animal morphometric measurements is to assess individual conformation, enabling the classification and differentiation of populations based on breed. Furthermore, this characterization allows for comparisons within and between genetic groups, establishing a link between an animal's conformation and its function. These traits also play a crucial role in the selection process by identifying animals with superior morphology and eliminating undesirable characteristics (Melo et al., 2018; Zapata et al., 2023).

Efficient enhancement of traits with high heritability can be accomplished through appropriate selective breeding practices. Heritability allows for the prediction of breeding outcomes, indicating that numerous traits are likely to exhibit favorable responses in subsequent generations. One such trait that has

demonstrated positive associations with several lifetime characteristics, such as milk yield, herd life, productive life, and productive days, is the age at which buffaloes first calve. Minimizing the age at first calving is desirable for reducing the cost of milk production and can contribute to extended productive lives for animals. Consequently, dairy farmers prioritize understanding the correlation between various production and reproduction parameters and lifetime traits. To optimize the genetic potential of buffaloes, it is crucial to acquire knowledge about the genetic and phenotypic parameters associated with early and lifetime performance traits, thereby facilitating the development of profitable selection and breeding systems (Tamboli et al., 2021).

However, little is known about the phenotypic and morphometric characteristics of Nili Ravi buffaloes in Khyber Pakhtunkhwa, which may have an effect on their productivity and conservation. It provided a thorough comprehension of the breed's characteristics to identify potential avenues for enhancing its productivity. This research would help breeders, farmers, and policymakers in the southern region of Khyber Pakhtunkhwa make informed decisions regarding breeding, conservation, and management practices for Nili Ravi buffaloes. Understanding the phenotypic and morphometric features of Nili Ravi in this region is essential for increasing their market value and productivity. The findings of this study could contribute to the regional economic growth and support the long-term management of this valuable breed.

METHOD AND MATERIALS

Between 2010 and 2023, the Livestock Research and Development Station in Paharpur, Dera Ismail Khan managed a herd of Nili Ravi buffaloes, comprising a total of 68 buffalo heads.

Over this period, 298 separate performance records were analyzed for these animals. Each record pertained to different aspects or time points of the buffalo's performance, hence the larger number of records compared to the number of buffaloes were shown (Figure 1).

Dera Ismail Khan has a very distinct climate in comparison to the buffalo's native range. At 174 meters above sea level and in a dry, desert region, the district of Dera Ismail Khan is balmy and humid. The highest temperature in the summer is 50 °C, while the lowest temperature in the winter is 10 - 50 °C. The annual precipitation average is only 249 mm. Professionals maintain weekly recordings of milk production and reproductive factors in specially designed herd frames. The milk was measured using scales. 68 buffaloes' milk and reproductive parameters were recorded for analysis. Maintaining weekly data and entry into the database containing all information regarding the farm's operations was ensured. On the basis of weekly output and the duration of each animal's lactation, lactation's worth of milk was determined for each animal.

Physical and morphometric characteristics of Nili Ravi buffaloes were examined (Figure 2). Physical characteristics such as coat color, cranium, and horn were documented for each animal. Morphometric measurements, including heart circumference, body length, and wither height, were taken using appropriate measuring tools while the animals stood on a flat surface. In the case of buffaloes, these measurements were collected 2 - 3 months after calving. Milk output was recorded from the time of parturition until the 305th day of lactation, with the first record taken within the first two weeks after giving birth. The daily milk yield for each individual buffalo was determined by combining the morning and

evening milk quantities. Reproductive traits, including puberty age, postpartum estrus interval, first service, conception rate, number of services per conception, calving interval, and dry period, were obtained from the records maintained by the Farm Manager.

Given the depth and variety of data, our methodology was structured to focus on the following:

- i. **Phenotypic Parameters:** Phenotypic attributes like coat color, horn type, body structure, muzzle, face color, and hoof color were critically evaluated. These characteristics gave insights into the physical distinctions within the breed, impacting management and conservation decisions.
- ii. **Morphometric Parameters:** Metrics including body weight, height at withers, pectoral girth, body length, and horn length were statistically significant. These parameters offered a nuanced understanding of the breed's physical stature and health.
- iii. **Reproductive and Productive Performance:** Understanding reproductive traits like birth weight, weaning weight, puberty age, breeding commencement age, and calving intervals provided critical insights for effective breeding and management practices. Similarly, productive data, including milk yield, elucidated the breed's performance in different conditions and over the years.
- iv. **Milk Production Analysis:** Detailed yearly analyses on milk production allowed us to gauge the performance of the Nili Ravi buffaloes, both within and across groups. This helped in assessing

the factors influencing milk yield and strategizing for optimization.

- v. **Descriptive Statistics:** This facilitated a multi-year analysis to track variations in the selected variable, shedding light on trends and patterns over time.
- vi. **Multiple Comparisons:** Duncan's multiple range tests were utilized post-ANOVA to discern which specific year pairs exhibited significant mean differences.



Figure 1: Labeled map of Khyber Pakhtunkhwa



Figure 2: Morphometric characteristics of Nili Ravi buffaloes (Source: RF Roy)

Statistical analysis was conducted using SPSS Version 24.0, with results presented as Mean \pm SD. Both One-way

ANOVA and Chi-square tests were utilized as the primary statistical tools in this investigation.

RESULTS

The buffalo's native habitats include Punjab province, yet this breed is now extensively distributed throughout Pakistan, including Khyber Pakhtunkhwa. Local communities work to preserve the breed. However, its population is declining, making it increasingly difficult for the breed's guardian communities to protect it. Therefore, Government of Pakhtunkhwa took the initiative to preserve this precious buffalo breed and enhance its productivity through scientific methodologies.

The results of an investigation on phenotypic parameters of Nili Ravi buffalo (Table 1). It contains frequencies and p-values for six distinct breed characteristics, including coat colour, horns, body structure, muzzle, face colour, and hoof colour. The preponderance of Nili Ravi buffaloes studied (77 %) had black coats ($p < 0.05$), followed by brown (19 %) and grey (4 %), majority of them (83 %) had upwardly curved horns ($p < 0.05$), while 13% had straight horns and 5 % had downwardly curved horns, 86 % had a massive body ($p < 0.05$) with a broad chest and powerful back, while 14 % had a slim body with a narrow chest and weak back. The majority of buffaloes (79 %) had grey muzzles ($p < 0.05$) with black pigmentation, whereas 21 % had white muzzles. The overwhelming majority of Nili Ravi buffaloes (92 %) had black faces ($p < 0.05$), while only 8 % had black faces with white markings. The preponderance of Nili Ravi buffaloes (68 %) had dark brown hooves ($p < 0.05$), whereas 32 % had black hooves. Understanding the physical characteristics of Nili Ravi buffaloes is essential for their

management, breeding, and conservation, as evidenced by these findings.

Morphometric parameters of Nili Ravi buffaloes, including body weight, height at withers, pectoral girth, body length, and horn length, were also significant statistically ($p < 0.05$). Males had mean body weight between 750 and 1200 Kg, female was between 550 and 750 Kg ($p < 0.05$). They had comparable withers heights, spanning from 135 to 160

cm for males and 135 to 140 cm for females. Males had larger chest circumference, ranging from 195 to 210 cm, than females, whose chest girth is between 180 and 198 cm. Males had body length extending from 170 to 200 cm, while females had body length between 160 and 180 cm. Males had longer horns than females, varying from 50 to 75 cm and 50 to 60 cm, respectively (Table 2).

Table 1: Phenotypic parameters of Nili Ravi buffalo

S. No	Character	Frequency (%)	p-value
1	Coat Color		0.00001*
	Black	77	
	Brown	19	
2	Horns		0.00001*
	Upward curved	83	
	Straight	12	
3	Body shape		0.00001*
	Massive, broad chest, strong back	86	
	Lean, narrow chest, weak back	14	
4	Muzzle		0.00001*
	Grey with black pigmented	79	
5	White	21	0.00001*
	Face color		
6	Black with white marks	92	0.00001*
	Black	08	
6	Hoof color		0.00001*
	Black	32	
	Dark brown	68	

**indicated that value is significant ($p < 0.05$)*

Table 2: Morphometric parameters of Nili Ravi buffalo

S. No	Character	Male	Female	χ^2	p-value
1	Body weight (Kg)	750-1200	550-750	19.70	0.00001*
2	Height at withers (cm)	135-160	135-140	0.0193	0.8895
3	Chest girth (cm)	195-210	180-198	0.2421	0.6226
4	Body length (cm)	170-200	160-180	0.111	0.7390
5	Horns length (cm)	50-75	50-60	0.3589	0.5490

**indicated that value is significant ($p < 0.05$)*

Reproductive and productive performance of Nili Ravi buffaloes can be utilised to optimise their breeding and management practises. We found average birth weight of a Nili Ravi buffalo calf as 33 - 50 Kg, weaning weight 65 to 85 kg.

Average puberty age of 550 - 720 days, average breeding commencement age of 900 - 1200 days. Maximum calving interval was 510 days and average was 430 days. Average duration of gestation was 285 - 305 days. They typically conceived

after a single natural service, though it also took up to 1.5 services in some instances. Age at first calving was 1200 - 1500 days. They produced 1524 to 1680 litres of milk per lactation with 260 and 380 days lactation length (Table 3).

Descriptive statistics for variable of interest across multiple years are presented in Table 4. Average value of variable across all years is 5.6633 indicated that, across all years of observation, the variable falls within this range on average. Mean value varied from 4.7590 (in year 1) to 6.3507 (in year 6). This indicated that variable had fluctuated over time, with the highest mean value occurring in year 6 and the lowest occurring in year 1. Minimum and maximum values for each year

indicated observed range of data variability. In the first year, the variable's minimum value was 4.05 and its greatest value was 5.83. This indicates that the variable had a limited range of variation in that year (Figure 3). Overall, it provided a summary of the descriptive statistics for the variable of interest across multiple years, allowing us to comprehend the data's trends and patterns over time (Table 4). Our findings indicated significant difference between means of variable of interest across years ($p < 0.05$). This information can be used to determine the years with the highest and lowest mean values of the variable, as well as the factors that may have contributed to the observed variability over time (Table 5).

Table 3: Productive and reproductive parameters of Nili Ravi buffalo

S. No.	Parameters	Average data of the parameters		Reference Ranges
		Min. value	Max. value	
1	Birth Weight of Calf (kg)	33	50	36.3 Kg (Ahmad et al., 2002)
2	Weaning Weight of Calf (kg)	65	85	66.4 Kg (Ahmad et al., 2002)
3	Age at Puberty (days)	550	720	1110 days (Anjum et al., 2012)
4	Age at 1 st Service (days)	900	1200	1291 days (Naqvi and Shami, 1999)
5	Calving Interval (days)	430	510	471-585 (Shah, 2007)
6	Gestation Length (days)	285	305	308 days (Usmani et al., 1987)
7	Natural Services per Conception (n)	1.0	1.5	1.3 (Anjum et al., 2012)
8	Age at 1 st Conception (days)	750	1020	976 days (Naqvi and Shami, 1999)
9	Age at 1 st Calving (days)	1200	1500	1291 days (Naqvi and Shami, 1999)
10	Milk per Lactation (litres)	1524	1680	1702 Litres (Cady et al., 1983)
11	Lactation Length (days)	260	380	250-350 days (Cady et al., 1983)

Table 4: Descriptive Statistics of Milk Production

Year	N	Mean	Minimum	Maximum
1.00	8	4.7590	4.05	5.83
2.00	12	5.3537	3.76	6.00
3.00	14	5.7251	5.26	6.58
4.00	9	5.8899	4.82	6.74
5.00	7	6.0271	4.47	8.02
6.00	8	6.3507	5.28	7.65
Total	58	5.6633	3.76	8.02

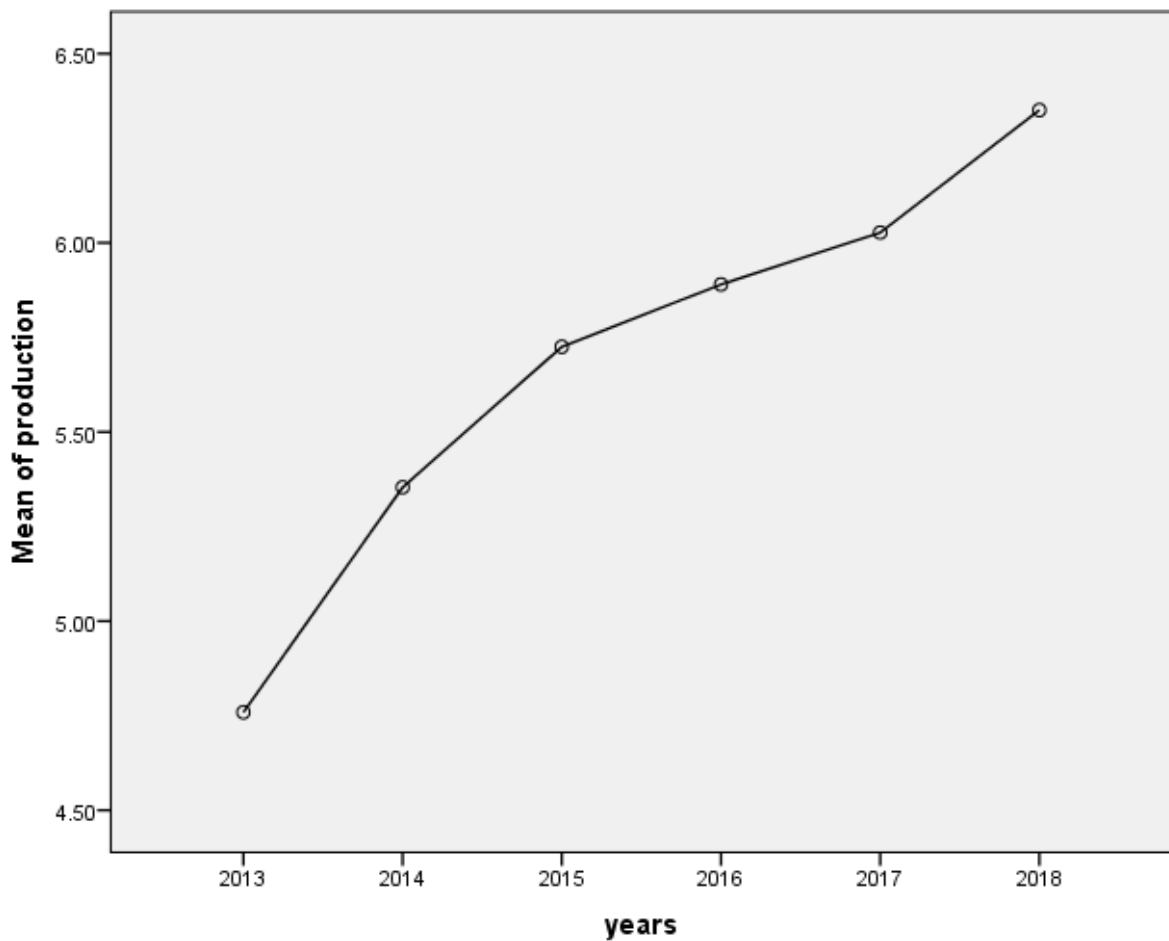


Figure 3: Graphical Display of Means of production

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	12.914	5	2.583	5.495	.000
Within Groups	24.441	52	.470		
Total	37.356	57			

Table 6: Duncan's Multiple Range Test

Years	N	Subset for alpha = 0.05		
		1	2	3
1.00	8	4.7590		
2.00	12	5.3537	5.3537	
3.00	14		5.7251	5.7251
4.00	9		5.8899	5.8899
5.00	7		6.0271	6.0271
6.00	8			6.3507
Sig.		.070	.059	.080

Table 07: Analysis of Milk production of Nili Ravi buffaloes

Year	N	Mean	Minimum	Maximum	Groups	F	Sig.
1.00	8	4.7590	4.05	5.83	Between the		.000
2.00	12	5.3537	3.76	6.00	Animals of	5.495	
3.00	14	5.7251	5.26	6.58	Same groups		
4.00	9	5.8899	4.82	6.74			
5.00	7	6.0271	4.47	8.02			
6.00	8	6.3507	5.28	7.65	Between the		
7.00	10	6.4240	5.30	7.65	Animals of		
8.00	12	6.3209	5.29	7.25	Different		
9.00	9	6.4736	5.30	7.76	groups		
10.00	12	6.4876	5.40	7.73			
Total	101	5.98116	4.893	7.121	Total		

Table 8: Descriptive Statistics of Body Weight and Weaning Weight of the animals

		N	Mean	Minimum	Maximum
Bw	2104	7	25.2857	23.00	28.00
	2015	3	26.3333	25.00	27.00
	2016	12	31.0833	29.00	33.00
	2017	10	32.3000	30.00	36.00
	2018	15	34.2667	32.00	38.00
	Total	47	31.1915	23.00	38.00
Ww	2104	7	53.8571	50.00	56.00
	2015	3	58.6667	57.00	60.00
	2016	12	61.2500	42.00	67.00
	2017	10	63.7000	59.00	67.00
	2018	15	65.3333	60.00	70.00
	Total	47	61.8085	42.00	70.00

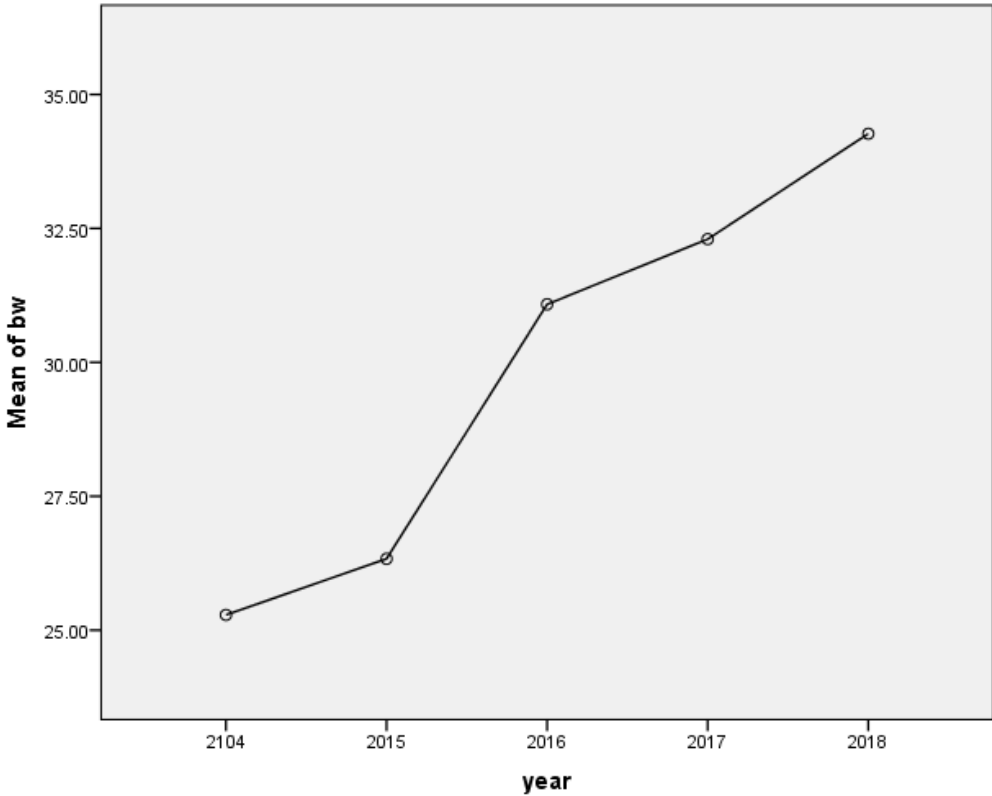


Figure 4: Graphical Display of Mean body weight

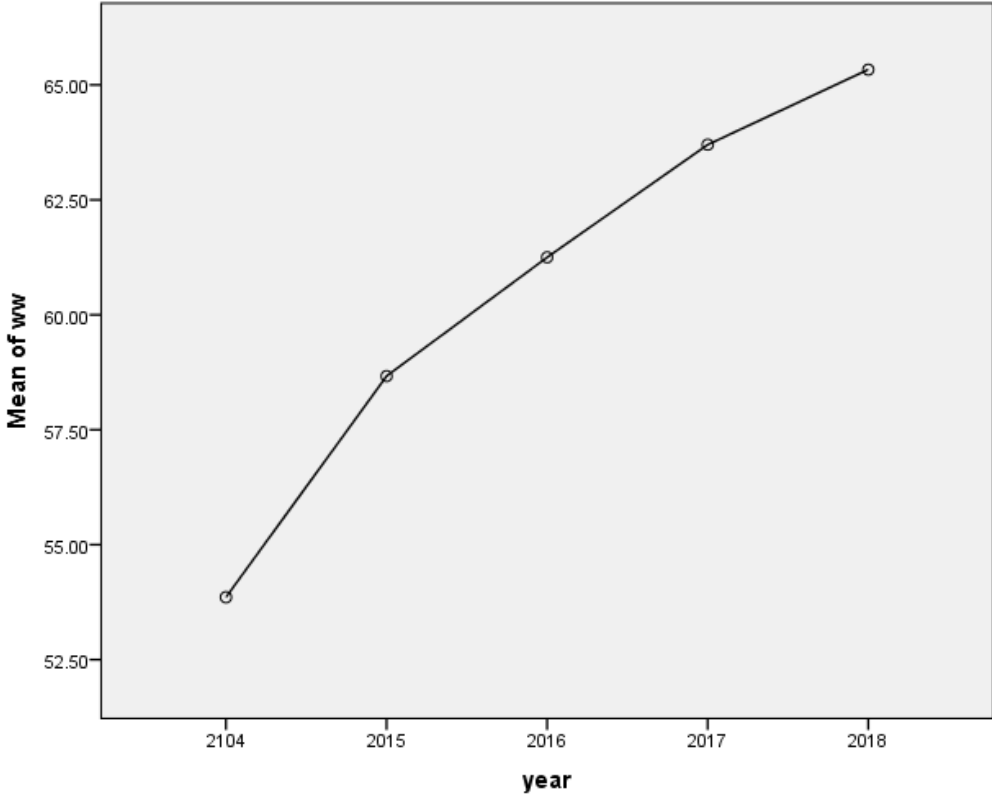


Figure 5: Mean of weaning weight

Table 9: Year-wise Comparison Through ANOVA for Body Weight and Weaning Weight of the animals

		Sum of Squares	df	Mean Square	F	Sig.
Bw	Between Groups	469.231	4	117.308	45.601	.000
	Within Groups	108.045	42	2.573		
	Total	577.277	46			
Ww	Between Groups	698.069	4	174.517	9.311	.000
	Within Groups	787.207	42	18.743		
	Total	1485.277	46			

Table 10: Duncan's Multiple Range Test for Birth Weight

Year	N	Subset for alpha = 0.05		
		1	2	3
2104	7	25.2857		
2015	3	26.3333		
2016	12		31.0833	
2017	10		32.3000	
2018	15			34.2667
Sig.		.232	.167	1.000

Table 11: Duncan's Multiple Range Test for Weaning Weight

Year	N	Subset for alpha = 0.05		
		1	2	3
2104	7	53.8571		
2015	3		58.6667	
2016	12		61.2500	61.2500
2017	10			63.7000
2018	15			65.3333
Sig.		1.000	.275	.105

Means and number of observations (N) for each year (1–6) were also displayed. Means for year 1 (4.7590) and year 2 (5.3537) were not significantly distinct from one another when examining the results. Similarly, means for year 3 (5.7251) and year 4 (5.8899) were not substantially different from one another due to the fact that they belong to the same subset. As they belong to the same subset, the means for years 5 (6.0271) and 6 (6.3507) were not significantly distinct

from one another. With a significance level of 0.059, however, the means for the second and third years are markedly different from one another. The means for the first and third years, as well as the first and fourth years, were not substantially different from one another, although the significance level was higher at 0.070 and 0.080, respectively. Overall, results of Duncan's multiple range tests found significant variations between some means of variable across various years, but not

between others. In our study, the ANOVA did indicate a significant difference in the means of our groups across years. Consequently, we applied Duncan's test to identify specifically which year pairs had significantly different means. While some pairs showed significant differences, others did not. This information can be used to determine the years with the highest and lowest mean values of the variable, as well as the factors that may have contributed to the observed variability over time (Table 6).

The results of milk analysis are presented in Table 7, including number of observations (N), mean milk production, minimum and maximum values, and groupings for each year. There is significant difference in milk production ($p < 0.05$) between animals of different groups and between animals of the same group ($p < 0.05$). Group effect was significant because the sum of squares between groups (24.44) is greater than the sum of squares within groups (12.91), indicating that differences in milk production cannot be attributed to random variation. Classifications of animals according to their average milk production are evident. In the first year, for instance, all animals belong to the same group because their mean milk production values are not substantially different. In the second year, animals are divided into five groups, with group 1 having the maximum mean milk production and group 5 the lowest. Similarly, livestock in years 3 to 5 are categorised according to their average milk production values. Overall, the results indicate that milk production among Nili Ravi buffaloes is highly variable, both within and between groups. This data can be used to identify the factors that contribute to this variability and to devise strategies for enhancing the milk production of Nili Ravi buffaloes (Table 7). Similarly the means of body weight,

weaning and birth weights are shown in Tables 8 - 11, and Figure 4 - 5, respectively.

DISCUSSION

The traits having high heritability can be efficiently improved through appropriate selective breeding. The heritability provides an opportunity to forecast the outcome of breeding strategy. Framing for profitable selection and breeding systems requires knowledge of genetic and phenotypic parameters of early and lifetime performance traits to enhance genetic potential of buffaloes. Our findings revealed that average body weight of Nili Ravi buffaloes was 677 ± 213 kg, and they produced 1387.12 ± 32.87 liters milk per lactation during their lactation period of 292.87 ± 32.40 days, which was significantly affected ($p < 0.05$) by year and season of calving. Calving interval was 480.18 ± 22.09 days, while parity year and calving season had a significant ($p < 0.05$) influence on their characteristic. 181.2 ± 18.01 days comprised the dry period.

The findings from our study align closely with the observations made by Ullah et al. (2021) concerning Nili Ravi buffalo calves. This agreement accentuates the robustness and consistency of the phenotypic characteristics inherent to this buffalo breed across different study parameters and conditions. Specifically, the dominant presence of a black coat with white markings in both studies underscores a definitive trait within this breed. While the measurements regarding cardiac circumference, body length, and withers height show slight variations, they are within the expected margins of error and variability seen in livestock studies (Ullah et al., 2021). One of the key determinants of milk yield in dairy animals is their genetic makeup. Advancements in genomic tools have paved the way to better understand and exploit this genetic

potential. The identification of SNPs related to milk yield and body weight, as highlighted by Bashir et al. (2007), offers a profound glimpse into the genetic architecture driving these traits. By pinpointing these genetic markers, breeders and farmers can make informed breeding decisions that may enhance milk production in subsequent generations. Furthermore, the genotyping-by-sequencing approach, as demonstrated by Islam et al. (2020), has proven effective in discerning the genomic regions crucial for the dairy traits of the Pakistani Nili-Ravi population. Not only does this provide a broader understanding of the breed's genetic landscape, but it also presents a pathway to optimize breeding programs. By leveraging these insights, there's potential to select animals with superior genetic markers, thereby potentially elevating the average milk production across the herd. The economic implications for the dairy industry are substantial. Enhanced milk production, driven by a genetically superior breed, can translate to increased profitability. As the Nili-Ravi buffalo continues to be a cornerstone in the Pakistani dairy sector, such genomic insights will be invaluable in sustaining and elevating the industry's productivity (Bashir et al., 2007). The effectiveness of the genotyping-by-sequencing approach was showcased in identifying genomic regions that reveal additional demographic complexities and have the potential to enhance the complex dairy traits of the Pakistani Nili-Ravi population. This development holds significant economic benefits for the dairy industry (Islam et al., 2020).

Our findings were also supported by Melo et al. that morphometry contributes to the understanding of animal conformation and reported the average values were 43.72 for BW, 143.07 for BL, 130.80 for HH, 33.00 for SW, 3.79 for LW and 201.30 for TP. The correlations with

highest values were 0.74 for SW/CHW, 0.69 for HIW/TW and 0.68 for TP/RH. These correlation coefficients between body morphometric measurements can be utilized in selection programs (Melo et al., 2018). Comparable results were reported that buffaloes produced between 7.30 and 9.58 liters milk daily during first six months of lactation, with optimum production occurring in third month (9.58 liters). They reached puberty age at 1147.93 ± 13.05 days. The manifestation of heat signs within 90 days postpartum in 50 % of the buffaloes, a first service conception rate of 64 %, a number of services per conception of 1.55 ± 0.04 , and a mean calving interval of 489.16 ± 5.8 days were the most prominent reproductive characteristics of the breed (Afzal et al., 2007; Khan et al., 2014).

CONCLUSION

The study illuminated the phenotypic and morphometric traits of Nili Ravi buffaloes in southern Khyber Pakhtunkhwa, Pakistan. The breed has a considerable body weight and chest girth, according to study. The study also showed regional breed morphometric variances. Nili Ravi buffaloes in the region have excellent milk yield potential. Lactation length and productivity were affected by parity and calving season. The study also disclosed morphometric variations within the breed, demonstrating the need for targeted breeding and management practices. These discoveries may improve breed management and output. The study aids in Nili Ravi buffalo conservation and enhancement. Breeders, farmers, and policymakers in southern Khyber Pakhtunkhwa, Pakistan, may use the findings to make breeding, conservation, and management policies for Nili Ravi buffaloes.

AUTHORS' CONTRIBUTION

All authors contributed significantly in this study

CONFLICT OF INTEREST

None.

ACKNOWLEDGEMENT

The Government of Khyber Pakhtunkhwa provided regular budget grant for this study.

AUTHORS' CONTRIBUTION

All the authors contributed significantly in this research.

CONFLICT OF INTEREST

None.

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